

Molecular taxonomy of ascomycetes and basidiomycetes: unveiling fungal diversity and evolution

Edited by

Sinang Hongsanan, Ahmed M. Abdel-Azeem and Steven Miller

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Molecular taxonomy of ascomycetes and basidiomycetes: unveiling fungal diversity and evolution

Topic editors

Sinang Hongnanan — Shenzhen University, China

Ahmed M. Abdel-Azeem — Suez Canal University, Egypt

Steven Miller — University of Wyoming, United States

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EDITED BY

Sinang Hongsan,an,
Shenzhen University, China

REVIEWED BY

Mustafa Rüstemoğlu,
Şırnak University, Türkiye
Danielle Rutkowski,
University of California, Davis, United States

*CORRESPONDENCE

Nuttapol Noirungsee
✉ nuttapol.n@cmu.ac.th
Terd Disayathanoowat
✉ terd.dis@cmu.ac.th

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Distinct fungal microbiomes of two Thai commercial stingless bee species, *Lepidotrigona terminata* and *Tetragonula pagdeni* suggest a possible niche separation in a shared habitat

Diana C. Castillo^{1,2,3}, Chainarong Sinpoo^{1,3,4},
Patcharin Phokasem^{1,3,4}, Rujipas Yongsawas^{1,3},
Chakriya Sansupa¹, Korrawat Attasopa^{3,5,6},
Nakarin Suwannarach^{3,4,6}, Sahutchai Inwongwan^{1,3,6},
Nuttapol Noirungsee^{1,3,6*} and Terd Disayathanoowat^{1,3,6*}

¹Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ²Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, ³Research Center of Deep Technology in Beekeeping and Bee Products for Sustainable Development Goals (SMART BEE SDGs), Chiang Mai University, Chiang Mai, Thailand, ⁴Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand, ⁵Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, ⁶Center of Excellence in Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Stingless bees, a social corbiculate bee member, play a crucial role in providing pollination services. Despite their importance, the structure of their microbiome, particularly the fungal communities, remains poorly understood. This study presents an initial characterization of the fungal community associated with two Thai commercial stingless bee species, *Lepidotrigona terminata* (Smith) and *Tetragonula pagdeni* (Schwarz) from Chiang Mai, Thailand. Utilizing ITS amplicon sequencing, we identified distinct fungal microbiomes in these two species. Notably, fungi from the phyla Ascomycota, Basidiomycota, Mucoromycota, Mortierellomycota, and Rozellomycota were present. The most dominant genera, which varied significantly between species, included *Candida* and *Starmerella*. Additionally, several key enzymes associated with energy metabolism, structural strength, and host defense reactions, such as adenosine triphosphatase, alcohol dehydrogenase, β -glucosidase, chitinase, and peptidylprolyl isomerase, were predicted. Our findings not only augment the limited knowledge of the fungal microbiome in Thai commercial stingless bees but also provide insights for their sustainable management through understanding their microbiome.

KEYWORDS

fungi, corbiculate bee, next-generation sequencing, stingless bee, *Lepidotrigona terminata*, *Tetragonula pagdeni*

1 Introduction

Pollination by insects is crucial for the conservation of ecosystems' natural balance and maintenance of biodiversity. Agricultural crops rely on vectors such as insects, wind, and water for the transmission of their pollen during cross-pollination (MacInnis and Forrest, 2020; Zeng and Fischer, 2020). It has been estimated that animals pollinate 87.5% of flowering plant species (Ollerton et al., 2011). Bees (Apoidea) are considered the most efficient animal pollinators due to their body composition and consistent flower-visiting pollination services (Batra, 1995). The consistent visits and aggregate effects of various bee species influence both the quality and quantity of crops (Hall et al., 2020). Further, pollination by multiple species of bees, such as honeybees, carpenter bees, stingless bees, and bumblebees, results in better pollination and vegetation processes (Khalifa et al., 2021). Although regarded as vital pollinators, bee populations are declining as they face increasing threats from many risk factors such as pesticides, bee diseases, agricultural intensification, among others (Potts et al., 2016).

Stingless bees, belonging to the Meliponini Tribe, are eusocial bees found in tropical and subtropical areas (Leonhardt, 2017; Gonçalves et al., 2018; Vale et al., 2021). Before the introduction of honeybees from Europe, stingless bees were the primary pollinators of plants in the Americas (Toledo-Hernández et al., 2022). Beekeeping practices with stingless bees have been popular in tropical regions due to their ease of management. In contrast to honeybee harvesting, where safety gear is necessary, harvesting honey, pollen, and propolis from stingless bees is simpler as they do not sting (Abd Jalil et al., 2017). The honey and geopropolis produced by stingless bees possess various beneficial properties such as antimicrobial, anti-inflammatory, wound-healing, and anticancer effects (Abd Jalil et al., 2017; Seabrooks and Hu, 2017; Alvarez et al., 2018).

The associations of microbes within insects significantly impact their health. Various microorganisms and insects engage in symbiotic relationships that range from mandatory mutualism to specialized parasitism (Menegatti et al., 2021). For instance, insects rely on bacteria for the degradation of plant materials, regulation of pH, and vitamin synthesis (Dillon and Dillon, 2003). Some insects, such as ants, termites, and beetles, cultivate fungi as their food source (Beaver, 1989; Mueller et al., 1998; Aanen et al., 2002). Yeasts like *Starmerella bacillaris*, *St. etchellsii*, *Candida californica*, *Pichia membranifaciens*, *P. occidentalis*, and *Zygosaccharomyces bailii* have been identified as beneficial symbionts in *Drosophila melanogaster* microbiome (Dmitrieva et al., 2023). Fall armyworm (*Spodoptera frugiperda*) harbors fungal symbionts such as *Fusarium oxysporum* and *Cladosporium* sp (Watson et al., 2023). Like other insects, bees depend on a symbiotic relationship with microbes, ranging from pathogenic to mutually beneficial (de Paula et al., 2023; Rutkowski et al., 2023). Common fungal symbionts include yeasts from the taxa of *Starmerella*, *Metschnikowia*, *Zygosaccharomyces*, and *Candida*, which are found across various tribes and species (Rutkowski et al., 2023). Wherein these microbes in honeybee larvae, adults, food, and honeycombs are important for digestion, pollination, and exerting antagonistic effects on various pathogens.

Recent research has shown that bee microbiome plays a critical role in determining the health status of both social and solitary bees (Lozo et al., 2015; Engel et al., 2016; McFrederick and Rehan, 2016; Kwong et al., 2017). In addition, the choice of habitat location of honeybees may affect the core gut microbiome; human pathogens were detected in the gut of honeybee (Rüstemoglu, 2023). The benefits of fungal symbionts in honeybees include aiding in pollen degradation and assisting in the maturation of royal jelly; additionally, fungi may also serve as food sources (Yun et al., 2018; de Paula et al., 2021). Filamentous fungi are also present in bees, with species such as *Aspergillus* spp. potentially competing with other pathogenic and mycotoxigenic strains of *Aspergillus* (Bhandari et al., 2020). These species may enhance the honeybee's resistance to xenobiotics through detoxification and stabilize pollen and bee bread by producing vitamins and minerals (Berenbaum and Johnson, 2015; Kieliszek et al., 2018).

Various researchers have reported mutualism between stingless bees and fungi. For example, larvae of one species of stingless bee have been found to depend on fungi for growth (Ravoet et al., 2014; Maxfield-Taylor et al., 2015; Rutkowski et al., 2023). The yeast *Zygosaccharomyces* aids in the development of larvae from several species, including *Scaptotrigona bipunctata*, *Sc. postica*, *Sc. tubiba*, *Tetragona clavipes*, *Melipona quadrifasciata*, *M. fasciculata*, *M. bicolor*, and *Partamona helleri* (de Paula et al., 2023). Additionally, Liu et al. (2023) have found an association between the gut microbiome composition and the flight traits of stingless bees, though not a causative one. This association was specifically observed in bacterial gut communities. In Brazil, species of *Talaromyces* and a new species of *Penicillium* were discovered in *M. scutellaris* (Barbosa et al., 2018). A recent study has shown that *C. apicola*, *Starmerella* spp., and *Zygosaccharomyces* constitute the core fungal microbiome of *M. quadrifasciata* gut (Haag et al., 2023). This evidence strongly indicates that the fungal microbiome significantly influences the health of stingless bees, offering potential contributions to the sustainable management of these vital pollinators. However, investigations into fungal microbiomes associated with Thai commercial stingless bees, particularly those in Northern Thailand, are scarce. In this study, we examined the fungal microbiome of two species of stingless bees found in Northern Thailand: *Lepidotrigona terminata* and *Tetragonula pagdeni*. Increasing our understanding of the fungal microbiome in these stingless bee species could pave the way for innovative strategies to fortify their health and, in turn, enhance their crucial role in pollination services and ecosystem sustainability.

2 Materials and methods

2.1 Stingless bee species and sampling

Tetragonula pagdeni is a common species found throughout Southeast Asia (Wongsa et al., 2023). The average body length ranges from 3.4 mm to 3.9 mm, and the body color varies from black to blackish brown (Sakagami, 1978; Vijayakumar and Jeyaraaj, 2020). The nest are typically situated in tree crevices, exhibiting colors from black to blackish brown, with a texture that is reminiscent of waxy material and resin (Supplementary Figure 1A).

Lepidotrigona terminata is an abundant species distributed across Southeast Asia (Attasopa et al., 2020). The body is brownish in color and ranges in size from 4.0 mm to 5.5 mm. Nest entrance tubes are softer and exhibit a paler texture, resembling thin-walled, cylindrical funnels. Their color ranges from light yellow to dark brown (Supplementary Figure 1B) (Li et al., 2021).

Insect specimens were collected from a total of eighteen wild hive colonies, comprising nine colonies each of two stingless bee species, *L. terminata* and *T. pagdeni*. These colonies were nested in tree trunks within 3–5 km geographical radius on the campus of Chiang Mai University in Northern Thailand. Collections took place from January to April 2023, during the time of the day when the temperature was around 25°C. Simultaneously, all nine colonies of each species were collected at the same time on the same day.

Specimen collection was carried out with the method described by (Leonhardt and Kaltenpoth, 2014). We placed 50 mL sterilized centrifuged tubes at the hive entrance to catch the worker stingless bees, as illustrated in Supplementary Figures 1C, D, to collect twenty live adult stingless bees from each colony. The specimens were then transported to the SMART Bee Research Center Laboratory at the Faculty of Science, Chiang Mai University, for further processing. The taxonomic status of the colony of each species was principally based on hive-building characteristics (entrance of the hives), notably to separate the two species with their colony identity. Consequently, we further preferred to identify the two species by their morphological characteristics using the dichotomous keys from (Schwarz, 1939) and (Sakagami, 1978).

2.2 Stingless bee processing and DNA extraction

In a sterile environment, stingless bee samples were surfaced-sterilized following the method of (Pakwan et al., 2018), with some modifications. The samples were immersed in 7% (v/v) sodium hypochlorite for 1 min and 70% (v/v) ethanol for 3 min, then rinsed three times in sterile distilled water and dried on sterile paper towels. The sterilized samples were then placed in bead-beating tubes and lysed for 20 minutes. For DNA extraction, we prepared 18 separate samples in total, with each sample consisting of ten individual stingless bees. Nine samples contained stingless bees from *L. terminata* and another nine from *T. pagdeni*. Total genomic DNA was extracted from each of these samples by the manufacturer's protocol provided with the ZymoBIOMICS DNA Miniprep Kit (ZYMO Research, Germany). The DNA concentration was determined using a NanoDrop UV-vis spectrophotometer.

2.3 ITS amplicon sequencing and processing

The ITS region was amplified using the forward primer (CTTGGTCAATTTAGAGGAAGTA) and reverse primer

(GCTGCGTTCTTCATCGATGC). The resulting DNA was sequenced on an Illumina MiSeq platform with paired-end reads. These reads were then imported into QIIME2 version 2019.10 for processing (Bolyen et al., 2019). The primer sequences were trimmed from the reads. The reads underwent quality filtering, truncating at positions where the Phred score fell below 30 (Supplementary Figure 2). Quality filtering and denoising were performed using DADA2 (Callahan et al., 2016). Then the singletons were removed (Unterseher et al., 2011). After generating rarefaction curves to assess the appropriate depth, the datasets were rarefied to a consistent depth of 2801 sequences per sample (Mbareche et al., 2020). Taxonomic classification was carried out using the UNITE (version 8.3) database (Abarenkov, et al., 2020), employing a Naive-Bayes classifier to assign taxonomy to the ITS sequences.

2.4 Data analyses

Diversity of fungal microbiome was determined with 'vegan' package in R. Alpha diversity was assessed using indices including Shannon (Shannon, 1948), Simpson (Simpson, 1949) and Chao-1 (Chao and Chiu, 2016). A Mann Whitney U Test was computed to compare the alpha diversity of the two species of stingless bee. The fungal community composition between the two species of stingless bees was compared using the Bray-Curtis dissimilarity (Jones et al., 2018). The differences were assessed using analysis of group similarities (ANOSIM) and visualized through a non-metric multidimensional scaling (NMDS) using PAST software version 4.03 (Hammer et al., 2001). Fungal taxa correlations were assessed using Spearman's correlation via the 'Hmisc' package in R. Only significant correlations ($p < 0.05$) and those with strong coefficients ($p > 0.7$) were imported into Gephi 0.9.2 (Bastian et al., 2009) and visualized in Fruchterman-Reingold layout. Functional pathways were predicted using the ENZYME nomenclature database (Bairoch, 2000) PICRUSt2 software (Douglas et al., 2020). A heatmap was produced to visualize the hierarchical clustering of each predicted gene using R through RStudio (Allaire, 2011; Wickham et al., 2019).

3 Results

3.1 Sequence processing and amplicon sequence variants inference

A pooled sample of whole stingless bees, comprising 10 individuals (~0.1 gram) from each colony of each species, was used to sequence the ITS region using the Illumina MiSeq platform. This process resulted in 817,614 raw reads with median depth of 44,523.5 (Supplementary Table S1). After the quality filtering, denoising, and removal of chimeric sequences, we obtained 730,651 amplicons (Supplementary Table S2). Subsequently, after removing singletons and rarefying, the datasets contained 547 unique Amplicon Sequence Variants (ASVs), with a total count of 50,418 ASVs (Supplementary Table S2). Alpha rarefaction curves plateaued and reached saturation, indicating comprehensive diversity capture (Supplementary Figure S3).

3.2 Fungal community

The rarefied datasets were classified into 137 different genera across 5 phyla, 18 classes, 42 orders, and 80 families. The phylum Ascomycota was the most dominant, representing 94.35% of ASVs. In *L. terminata*, the most abundant genus was *Candida* (Supplementary Table S3), comprising 38.09% of the community, followed by *Moniliella* (6.54%), *Cladosporium* (4.35%), and *Starmerella* (3.25%). Conversely, in *T. pagdeni*, *Starmerella* was the most abundant genus, accounting for 87.86% of the community, with the next most abundant being *Cladosporium*, *Cutaneotrichosporon*, and *Candida*. Genera such as *Acremonium*, *Agaricus*, *Aspergillus*, *Clitopilus*, *Coprinellus*, *Gymnopus*, *Hymenochaete*, *Hypoxylon*, *Malassezia*, *Nigrospora*, *Penicillium*, *Phlebiopsis*, *Pseudozyma*, *Pyrrhoderma*, *Strelitziana*, *Vishniacozyma*, and *Wickerhamiella* were common to both species.

Shannon, Simpson, and Chao-1 indices were used to determine the alpha diversity at the genus level of fungal microbiomes of *L. terminata* and *T. pagdeni*. Mann Whitney U Test of Shannon Diversity ($p = 0.001$) and Simpson Diversity ($p = 0.002$) showed that the fungal community of the two species of stingless bees differed significantly. However, Chao - 1 was not statistically significant ($p = 0.136$). In addition, alpha diversity was illustrated using a boxplot (Figure 1). The relative abundance of fungal community members at the genus level in each species of stingless bees is depicted in Figure 2.

Similarly, we analyzed for beta diversity by comparing the fungal community of the two stingless bees. Results showed that there were overall differences ($p = 0.001$, $R = 0.969$) as the output of one-way ANOSIM at the permutation of 999 based on Bray - Curtis similarity matrix. As such, we visualized the data through Non-metric Multi-Dimensional Scaling (NMDS) with a stress value of 0.07 (Figure 3).

3.3 Network analysis

In Figure 4, the fungal communities' correlation network is displayed. The fungal microbiome of *L. terminata* is represented with 106 nodes and 397 edges. Each fungal genus exhibits distinct interactions. Negative interactions were found among the genera. For instance, negative interactions occur between *Candida* and *Tinctoporellus*, as well as, between *Candida* and *Marasmius*. Conversely, the fungal microbiome in *T. pagdeni* is depicted as an

undirected graph with 56 nodes and 371 edges. Within this network, *Starmerella* shows negative interactions with *Acremonium*, *Candida*, *Cladosporium*, *Cutaneotrichosporon*, *Penicillium*, and *Wickerhamiella*. Notably, a positive interaction exists between *Penicillium* and *Cladosporium*.

3.4 Predicted functional pathways

The fungal genera were used to predict their respective functional pathways, which were generated based on the ENZYME nomenclature database through PICRUSt2 (Supplementary Figure 4). A total of 425 functional pathways were identified within the fungal microbiome. Several key enzymes were detected, including glucose-6-phosphatase, alcohol dehydrogenase, beta-glucosidase, chitinase, and peptidylprolyl isomerase. These enzymes are associated with flight behaviors/muscle function, metabolism, energy sources, structural integrity, and host defense mechanisms. Additionally, the majority of the indicated functional enzymes prominent in bee-associated microbiomes were related to carbohydrate metabolisms, specifically to the import of sugars.

4 Discussion

Our study revealed that the fungal microbiomes of *L. terminata* and *T. pagdeni* have radically distinct structures and interactions despite being collected in a similar geographical radius with identical conditions and weather parameters. However, studies have shown an increase in distance decreases the gut microbial community similarity in stingless bees due to its dispersion limitation (Soininen et al., 2007; Liu et al., 2023). On the other hand, Kwong et al. (2017) suggest that the host species has a significantly greater influence on the gut microbial populations than geographic factors. Thus, this study found several distinct genera including *Acremonium*, *Agaricus*, *Aspergillus*, *Clitopilus*, *Coprinellus*, *Gymnopus*, *Hymenochaete*, *Hypoxylon*, *Malassezia*, *Nigrospora*, *Penicillium*, *Phlebiopsis*, *Pseudozyma*, *Pyrrhoderma*, *Strelitziana*, *Vishniacozyma*, and *Wickerhamiella*. Ascomycota was the most dominant phylum among the identified species. Similarly, Liu et al. (2023) identified Ascomycota and Basidiomycota as the most dominant phyla, along with

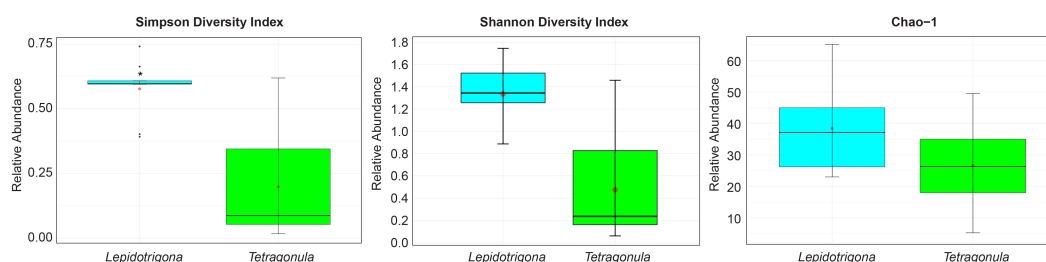
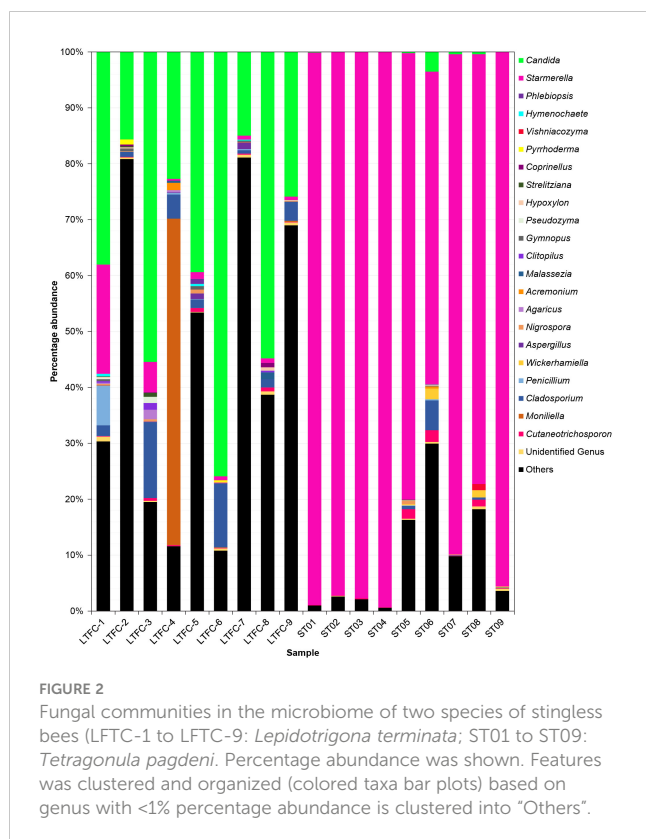


FIGURE 1

Alpha diversity plots. Boxplot corresponding to Simpson diversity index, Shannon diversity index and Chao -1. Asterisks (*) indicate significant differences ($p < 0.05$, Mann Whitney U test).

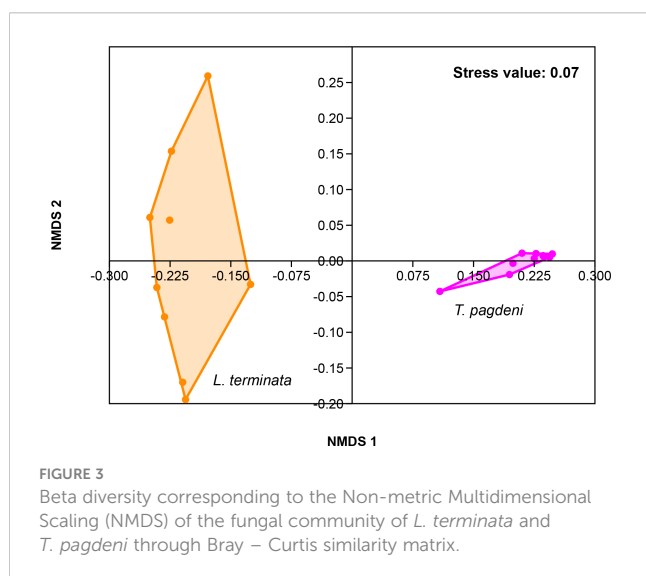


Chytridiomycota and Mucoromycota, in Australian stingless bees (*Tetragonula carbonaria* and *Austroplebeia australis*). Further, *Candida* and *Starmerella* were also determined as the dominant fungal genera in *L. terminata* and *T. pagdeni*, respectively. Such genera were also observed in the larval food of Brazilian native stingless bees (Santos et al., 2023) since *Candida* and *Starmerella* were usually isolated from stingless bees (de Paula et al., 2021), along with *Saccharomyces*.

Generally, gut communities differ among host species despite the closest kinship of *T. pagdeni* with *Lepidotrigona* species, wherein the

main differentiators of these species were the abundance of *Candida* in *L. terminata* and *Starmerella* in *T. pagdeni*. Kwong et al. (2017) have also reported the ability of the microbiota of social bees to evolve and undergo alteration dynamically thus the variation in microbial composition. Further, these variations may also have an environmental origin, whether from hive materials or foraging, which may lead to different fungal microbiomes among the two stingless bee species. For instance, Keller et al. (2021) reported a shared microbiome between bees and flowers, wherein the floral visitation shapes bee microbiome assembly given that majority of bee species mostly obtain their nourishment from floral resources (Rutkowski et al., 2023). Rothman et al. (2018) discovered that additional floral resources can influence the gut microbiota of *A. mellifera*. Therefore, floral visitation, through the collection of nectar and pollen from various plants, may be a significant factor in the differences observed in the fungal microbiome between the two species of stingless bees. Similarly, each species of stingless bee exhibits unique foraging habits based on their dietary requirements (Salatnaya et al., 2023). However, it remains unclear whether preferences for specific floral resources contribute to the distinct microbiomes of each species. Sawatthum and Kumler (2015) found that both species of stingless bees, *L. terminata* and *T. pagdeni*, preferred to visit plants belonging to the families Leguminosae, Poaceae and Amaranthaceae. Consistent with the findings of Rutkowski et al. (2023), other fungal genera found in both species, including *Candida* and *Starmerella*, as well as *Aspergillus*, *Cladosporium*, *Penicillium*, *Wickerhamiella*, and others, were also found in floral nectar and pollen. Our results highlight an intriguing observation that nesting in the same geographic location, the two species exhibit distinct fungal microbiomes. Nevertheless, the relationship between floral resources and the bee microbiome requires further elucidation. This suggests that the foraging food sources may marginally overlap, indicating a separation of niches. However, this requires further behavioral studies to pinpoint the cause of this distinction in bees. Thus, the exploration of fungal microbiome patterns within Thai commercial stingless bees offers a valuable initial understanding of their host ecology. However, the current study's scope does not allow for conclusive determinations regarding the consistent association of these two stingless bee species with the identified fungal community. Further investigations encompassing a broader environmental spectrum and longitudinal studies are necessary to better comprehend potential fluctuations in fungal community abundance attributed to environmental factors.

Conversely, body size is another factor to consider regarding microbiome composition. We found that *L. terminata* fungal microbiome was more diverse than *T. pagdeni*. *L. terminata* has a larger body size compared to *T. pagdeni*, which contrasts with the findings of Kueneman et al. (2023), who reported a negative correlation between bee body size, tongue length, and microbial richness. However, Liu et al. (2023) stated that bees with larger wings tend to have larger bodies, potentially providing a large area for bacteria colonization. Although these findings pertain to bacterial colonization, we may speculate that a similar mechanism applies to fungal colonization. In bees, larger individuals forage disproportionately farther than smaller ones, according to a power function with $b > 1$ (Greenleaf et al., 2007). Layek et al. (2021) also



Our study uncovered anotable negative correlation between *Starmerella* and other fungal members of the community (Figure 4D). This phenomenon may be attributed to the antifungal properties of sophorolipids, which are known to decrease surface tension, destabilizing and rupturing microbial membranes, thereby increasing (Valotteau et al., 2017). Such actions can induce high levels of oxidative stress, leading to necrosis and apoptosis (Haque et al., 2019). *Starmerella*, a yeast associated with bees, produces sophorolipids that can inhibit the growth of various bacteria and fungi, particularly those associated with floral resources and bee hives (Hipólito et al., 2020; De Clercq et al., 2021; Alfian et al., 2022). For instance, sophorolipids derived from *St. bombycola* inhibited all tested fungi (Hipólito et al., 2020). Similarly, sophorolipids from the newly identified species *St. riodocensis* may prevent the growth of *C. albicans* hyphae (Alfian et al., 2022). This suggests that these secondary metabolites function as an antifungal agent and totally inhibit fungal development (Hipólito et al., 2020). Consequently, we propose that sophorolipids might account for the observed negative correlation of *Starmerella* to other fungal genera, although a more comprehensive analysis is needed to confirm this relationship.

5 Conclusion

Our study provides the first in-depth investigation into the fungal microbiome associated with Thai commercial stingless bees *L. terminata* and *T. pagdeni*. The results revealed that the fungal microbiomes of these two species are significantly distinct despite sharing a geographical habitat. Specifically, *Candida* dominates the microbiome of *L. terminata*, while *Starmerella* prevails in that of *T. pagdeni*. Although both stingless bee species share some aspects within their fungal microbiome, the diversity and abundance of these components markedly differ. Furthermore, the interactions observed among fungal members within these microbiomes display unique patterns for each species. These discoveries contribute to our comprehension of the microbiomes intrinsic to these essential pollinators, providing insights into the intricate ecology of these remarkable species.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/genbank/>, PRJNA1060844.

Ethics statement

The requirement of ethical approval was waived by Laboratory Animal Center, Chiang Mai University, Thailand for the studies involving animals because Insect model was waived in this institute due to it is not a vertebrate. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

DC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. CSi: Conceptualization, Validation, Writing – review & editing. PP: Conceptualization, Validation, Writing – review & editing. RY: Conceptualization, Validation, Writing – original draft. CSa: Conceptualization, Validation, Writing – original draft. KA: Validation, Writing – review & editing. NS: Validation, Writing – review & editing. SI: Validation, Writing – review & editing. NN: Validation, Writing – review & editing. TD: Funding acquisition, Project administration, Supervision, Writing – review & editing, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2024.1367010/full#supplementary-material>

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EDITED BY

Asha Janadaree Dissanayake,
University of Electronic Science and
Technology of China, China

REVIEWED BY

Subashini Chathumini Jayasiri,
Mae Fah Luang University, Thailand
Kasun Thambugala,
University of Sri Jayewardenepura, Sri Lanka

*CORRESPONDENCE

Nakarin Suwannarach
✉ suwan_461@hotmail.com

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Insights into the molecular phylogeny and morphology of three novel *Dothiora* species, along with a worldwide checklist of *Dothiora*

Chanokned Senwana^{1,2}, Sinang Hongsanan^{1,2,3},
Surapong Khuna^{1,2}, Jaturong Kumla^{1,2}, Manzura Yarasheva⁴,
Yusufjon Gafforov^{5,6}, Aziz Abdurazakov⁷
and Nakarin Suwannarach^{1,2*}

¹Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand, ²Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai, Thailand, ³Guangdong Provincial Key Laboratory for Plant Epigenetics, Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen, China, ⁴Department of Education and Training Management, Tashkent International University of Education, Tashkent, Uzbekistan, ⁵Central Asian Center for Development Studies, New Uzbekistan University, Tashkent, Uzbekistan, ⁶Mycology Laboratory, Institute of Botany, Academy of Sciences of Republic of Uzbekistan, Tashkent, Uzbekistan, ⁷Department of Ecology and Botany, Faculty of Natural Sciences, Andijan State University, Andijan, Uzbekistan

Most species of *Dothiora* are known from the dead parts of various host plants as saprobic fungi in terrestrial habitats occurring in tropical and temperate regions. In the present study, samples of *Dothiora* were collected from dead twigs and branches of *Capparis spinosa*, *Rhaponticum repens*, and an unknown angiosperm plant from the Tashkent and Jizzakh regions of Uzbekistan. Multi-gene phylogenetic analyses based on a combined ITS, LSU, SSU, *TEF1*, and *TUB2* sequence data revealed their taxonomic positions within the Dothideaceae. Three new species of *Dothiora*, namely, *Dothiora capparis*, *Dothiora rhapontici*, and *Dothiora uzbekistanica* were proposed by molecular and morphological data. Likewise, the phylogenetic relationship and morphology of *Dothiora* are discussed. In addition, we provide a list of accepted *Dothiora* species, including host information, distribution, morphology descriptions, and availability of sequence data, to enhance the current knowledge of the diversity within *Dothiora*.

KEYWORDS

Asia, Dothideomycetes, Dothideales, fungal taxonomy, new species, saprobic fungi

Introduction

Dothideales Lindau is an order in the class Dothideomycetes that comprises four families, including Dothideaceae, Neocelosporiaceae, Saccoteliaceae, and Zalaraceae (Hongsanan et al., 2020; Wijayawardene et al., 2022). Members of this order are saprobic and occasionally pathogenic to plants in terrestrial habitats and humans, including house dust (Thambugala et al., 2014; Crous et al., 2018a; Hongsanan et al., 2020). In addition, some species have been used as potential biocontrol for pest management (Zajc et al., 2019; Cignola et al., 2023). To date, 26 genera were accepted in Dothideales, while six genera were proposed in Dothideales genera *incertae sedis* (Wijayawardene et al., 2022). Dothideaceae Chevall. was introduced to accommodate the genus *Dothidea*, with *D. gibberulosa* as the type species (Chevallier, 1826; Fuckel, 1869). Currently, 14 genera are accepted in Dothideaceae, viz., *Delphinella* (Sacc.) Kuntze, *Dictyodothis* Theiss. & Syd., *Dothidea* Fr., *Dothiora* Fr., *Endoconidioma* Tsuneda, *Endodothiora* Petr., *Kabatina* R. Schneid. & Arx, *Neocylindroseptoria* Thambug. & K.D. Hyde, *Neodothiora* Crous, G.C. Adams & Winton, *Phaeocryptopus* Naumov, *Plowrightia* Sacc., *Stylodothis* Arx & E. Müll., *Sydowia* Bres., and *Uleodothis* Theiss. & Syd (Wijayawardene et al., 2022).

Dothiora was established by Fries (1894), typified by *D. pyrenophora* Berk. ex Sacc. The sexual morph of *Dothiora* is characterized by immersed to erumpent ascostromata, lacking pseudoparaphyses, eight or more spored, bitunicate asci, hyaline to yellow or pale brown, and one septate or muriform ascospores (Thambugala et al., 2014; Crous and Groenewald, 2017). *Dothiora* has dothichiza-like asexual morph via culture studies, which is characterized by pycnidial conidiomata, phialidic conidiogenous cells, hyaline, aseptate conidia, forming a hormonema-like synasexual morph (Thambugala et al., 2014; Crous and Groenewald, 2016; Crous and Groenewald, 2017). Earlier, *Dothiora* was treated in different families, such as Dothideaceae and Dothioraceae, by several authors (Barr, 1972; Barr, 1979; Barr, 1987; Froidevaux, 1972; Luttrell, 1973; von Arx and Müller, 1975; Hawksworth et al., 1995; Lumbsch and Huhndorf, 2010); however, Dothioraceae was synonymized under Dothideaceae based on its phylogenetic placement within Dothideales (Thambugala et al., 2014). Although 89 epithets are listed in Index Fungorum (accessed on 13 March 2024), most species have not been well studied since their introduction, and many species have been transferred to other genera such as *Dothiorella*, *Myriangium*, *Protoscypha*, and *Saccothecium*. Furthermore, only 28 *Dothiora* species have been sequenced and confirmed their phylogenetic placements (Hyde et al., 2020; Boonmee et al., 2021). *Dothiora* species are distributed worldwide on woody plants in terrestrial habitats as saprobes and pathogens causing leaf spots or possibly weak pathogens on stressed plant tissues (Crous and Groenewald, 2016; Crous and Groenewald, 2017; Hyde et al., 2020). Not only *Dothiora infusans* Rodr.-Andr., Stchigel, Guarro & Cano is reported on the blackened wall of an industrial warehouse (Crous et al., 2018a), but also *Dothiora* sp. is recorded as an endophytic fungus that produces compounds with cytotoxic activity against cancer cell lines (Pérez-Bonilla et al., 2017).

Most of the *Dothiora* species are reported from Europe and North America (Hyde et al., 2020). Targeting underexplored

regions such as Central Asia, including Uzbekistan, might be helpful for the discovery of new fungi (Gafforov, 2017; Cheek et al., 2020). Recent studies have led to the discovery of several new genera and species of ascomycetous microfungi in Uzbekistan (Gafforov and Rakhimov, 2017; Pem et al., 2018; Pem et al., 2019a; Pem et al., 2019b; Gafforov et al., 2019; Abdurazakov et al., 2021; Appadoo et al., 2021; Htet et al., 2021; Lestari et al., 2021; Aluthmuhandiram et al., 2022; Dong et al., 2023). However, *Dothiora* is still poorly known in Asia including Central Asian regions. The aim of the present study was to clarify the taxonomic position of *Dothiora* and to identify new taxa through multi-gene phylogeny and morphological examination. Fresh specimens collected from Uzbekistan were examined and their DNA sequence data were obtained for use in multi-gene phylogenetic analyses. Moreover, an updated list of *Dothiora* species worldwide is also provided.

Materials and methods

Sample collection and specimen examination

Specimens were collected from *Capparis spinosa*, *Rhaponticum repens*, and an unknown angiosperm plant from the Tashkent and Jizzakh regions of Uzbekistan. The collected specimens were brought to the laboratory in small plastic bags. Ascospores were sectioned by hand, examined, and captured under a Nikon SMZ800N stereomicroscope. The slides were prepared by mounting the materials in double-distilled water (ddH₂O), lactophenol, and Indian ink stain. The micro-morphological characters were examined and captured using a Nikon DS-Ri2 camera connected with a Nikon ECLIPSE Ni (Tokyo, Japan) compound microscope. The measurement of structures was done by the Tarosoft® Image Framework program (v.0.9.0.7). Adobe Photoshop Version: 22.4.2 (Adobe Systems U.S.A.) was used to make the photographic plates. The specimens were deposited in the Herbarium of the Department of Biology (CMUB), Faculty of Science, Chiang Mai University, Thailand, and the Tashkent Mycological Herbarium (TASM) of the Institute of Botany, Uzbekistan Academy of Sciences, Uzbekistan.

DNA extraction, PCR amplification, and sequencing

To obtain pure cultures, single ascospore isolation was carried out following the methods of Senwanna et al. (2019) and Senanayake et al. (2020). However, no germinated ascospores were found on Petri dishes containing 2% malt extract agar (MEA; Gibco, Life Technologies Corporation, USA), 2% water agar (WA), and potato dextrose agar (PDA; BD Difco™, Becton, Dickinson and Company, USA) after incubation at 25°C to 30°C in the dark for 24–96 h. Fungal fruiting bodies, thus, were picked up and placed in a 1.5-mL sterilized tube. Genomic DNA was directly extracted, using E.Z.N.A.® Genomic DNA Isolation Kits (OMEGA

Bio-Tek, Georgia) following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification was carried out using the primer pairs as follows: ITS5 and ITS4 (White et al., 1990) to amplify the partial gene regions of internal transcribed spacers (ITS); LR0R and LR5 (Vilgalys and Hester, 1990) to amplify the 28S large subunit (LSU); NS1 and NS4 (White et al., 1990) to amplify the 18S small subunit (SSU); and EF1-728F (Carbone and Kohn, 1999) and EF2 (O'Donnell et al., 1998) to amplify the protein coding region for the translation elongation factor 1-alpha gene (*TEF1*). The PCR mixture contained 6 µL of double-distilled water (ddH₂O), 10 µL of 2 × Quick Taq™ HS DyeMix (TOYOBO, Japan), 2 µL of genomic DNA, and 1 µL of each forward and reverse primer. The PCR thermal cycle programs for ITS, LSU, and SSU amplification were as follows: initial denaturing step of 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 45 s, elongation at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR thermal cycle programs for *TEF1* amplification were as follows: initial denaturing step of 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 1 min, and final

extension at 72°C for 10 min. PCR products were examined on 1% agarose electrophoresis gels under UV light. PCR products were purified using the PCR clean-up Gel extraction NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. PCR fragments were performed and sequenced at 1st BASE Company (Kembangan, Malaysia).

Phylogenetic analyses

The generated sequence data were assembled using SeqMan 5.00, and the consensus sequences were subjected to BLASTn searches of the NCBI nucleotide database (<http://blast.ncbi.nlm.nih.gov/>; accessed on 2 November 2023) to determine their most probable closely related taxa. The representative taxa used in the analyses were selected from GenBank based on the BLASTn searches and recently published data (Boonmee et al., 2021; Gao et al., 2021) (Table 1). Each gene alignment was carried out with MAFFT version 7 (Katoh et al., 2019; <http://mafft.cbrc.jp/alignment/server/>; accessed on 6

TABLE 1 Taxa names, strain numbers, and GenBank accession numbers of sequences used in the phylogenetic analyses of this study.

Fungal Taxa	Strain	GenBank Accession Numbers					References
		ITS	LSU	SSU	<i>TEF1</i>	<i>TUB2</i>	
<i>Coniozoma leucospermi</i>	CBS 114035	AY720707	N/A	AY720711	N/A	N/A	Lennox et al. (2004)
<i>Coniozoma leucospermi</i>	CBS 111289	EU552113	EU552113	N/A	N/A	N/A	Marincowitz et al. (2008)
<i>Delphinella balsameae</i>	DJO-B-080615-A4	KY997059	N/A	N/A	KY997060	MF034404	Guertin et al. (2019)
<i>Delphinella strobiligena</i>	CBS 735.71	MH860318	MH872074	DQ471029	N/A	N/A	Spatafora et al. (2006); Vu et al. (2019)
<i>Dothidea berberidis</i>	CBS 186.58	EU167601	EU167601	EU167601	N/A	N/A	Simon et al. (2009)
<i>Dothidea hippophaeos</i>	AFTOL-ID 919	N/A	DQ678048	N/A	DQ677887	N/A	Schoch et al. (2006b)
<i>Dothidea insculpta</i>	CBS 189.58	AF027764	DQ247802	DQ247810	DQ471081	N/A	Jacobs and Rehner (1998); Schoch et al. (2006a)
<i>Dothidea mueller</i>	CBS 191.58	EU167593	EU167593	EU167593	N/A	N/A	Simon et al. (2009)
<i>Dothidea ribesia</i>	CPC 30638	KY929140	KY929173	N/A	KY929192	KY929205	Crous and Groenewald (2017)
<i>Dothidea ribesia</i>	CPC 30689	KY929141	KY929174	N/A	KY929193	KY929206	Crous and Groenewald (2017)
<i>Dothidea sambuci</i>	AFTOL-ID 274 ^T	AY883094	AY544681	AY544722	DQ497606	N/A	Lutzoni et al. (2004); Shoemaker and Hambleton (2005)
<i>Dothiora agapanthi</i>	CPC 20600 ^T	KU728498	KU728537	N/A	KU728578	KU728617	Crous and Groenewald (2016)
<i>Dothiora aloidendri</i>	CPC 38535	MW175347	MW175387	N/A	MW173123	MW173138	Crous et al. (2020)
<i>Dothiora buxi</i>	MFLU 15-3404 ^T	KX765294	KX765295	N/A	N/A	N/A	Hyde et al. (2016)
<i>Dothiora bupleuricola</i>	CBS 112.75 ^T	KU728499	KU728538	N/A	KU728579	KU728618	Crous and Groenewald (2016)
<i>Dothiora cactacearum</i>	CBS 142492 ^T	KY929143	KY929176	N/A	KY929195	KY929208	Crous and Groenewald (2017)
<i>Dothiora cactacearum</i>	CPC 15587	KY929144	KY929177	N/A	KY929196	KY929209	Crous and Groenewald (2017)
<i>Dothiora cannabinae</i>	AFTOL-ID 1359 ^T	AJ244243	DQ470984	DQ479933	DQ471107	N/A	Spatafora et al. (2006); De Hoog et al. (1999)
<i>Dothiora capparis</i>	TASM 6169 ^T	PP086677	PP086685	PP086692	PP084937	N/A	This study

(Continued)

TABLE 1 Continued

Fungal Taxa	Strain	GenBank Accession Numbers					References
		ITS	LSU	SSU	TEF1	TUB2	
<i>Dothiora capparisi</i>	CMUB40036	PP086678	PP086686	N/A	PP084938	N/A	This study
<i>Dothiora capparisi</i>	CMUB40037	PP086679	PP086687	PP086693	PP093832	N/A	This study
<i>Dothiora capparisi</i>	CMUB40038	PP086680	PP086688	PP086694	PP093833	N/A	This study
<i>Dothiora ceratoniae</i>	CBS 477.69 ^T	KF251151	KF251655	N/A	KF253111	KF252649	Quaedvlieg et al. (2013)
<i>Dothiora coronillae</i>	MFLU 17-0005	MF443252	N/A	N/A	N/A	N/A	Hyde et al. (2017)
<i>Dothiora coronillicola</i>	MFLUCC 17-1007	MZ571207	MZ571206	N/A	N/A	N/A	Boonmee et al. (2021)
<i>Dothiora cytisi</i>	MFLUCC 14-0970 ^T	KU248848	KU248849	KU248850	N/A	N/A	Li et al. (2016)
<i>Dothiora elliptica</i>	CBS 736.71 ^T	KU728502	KU728541	N/A	GU349013	N/A	Schoch et al. (2009) ; Crous and Groenewald (2016)
<i>Dothiora europaea</i>	CBS 740.71	MH860322	MH872078	N/A	N/A	N/A	Vu et al. (2019)
<i>Dothiora infusans</i>	FMR 16326 ^T	LT993342	LT993345	N/A	N/A	N/A	Crous et al. (2018a)
<i>Dothiora laureolae</i>	CBS 744.71 ^T	KU728503	KU728542	N/A	N/A	N/A	Crous and Groenewald (2016)
<i>Dothiora mahoniae</i>	CBS 264.92 ^T	MH862357	MH874022	N/A	N/A	N/A	Vu et al. (2019)
<i>Dothiora maculans</i>	CBS 299.76	KU728504	KU728543	N/A	KU728582	KU728621	Crous and Groenewald (2016)
<i>Dothiora maculans</i>	CBS 301.76	KU728505	KU728544	N/A	KU728583	KU728622	Crous and Groenewald (2016)
<i>Dothiora maculans</i>	CBS 302.76	KU728506	KU728545	N/A	KU728584	KU728623	Crous and Groenewald (2016)
<i>Dothiora oleae</i>	CBS 152.71	KU728508	KU728547	N/A	KU728586	KU728625	Crous and Groenewald (2016)
<i>Dothiora oleae</i>	CBS 235.57	KU728509	KU728548	N/A	KU728587	KU728626	Crous and Groenewald (2016)
<i>Dothiora oleae</i>	CBS 472.69	KU728510	KU728549	N/A	KU728588	KU728627	Crous and Groenewald (2016)
<i>Dothiora omaniana</i>	SQUCC 13293	MT077213	MT077209	N/A	MT081204	MT081205	Hyde et al. (2020)
<i>Dothiora phaeosperma</i>	CBS 870.71	KU728512	KU728550	N/A	N/A	N/A	Crous and Groenewald (2016)
<i>Dothiora phillyreae</i>	CBS 473.69 ^T	KU728513	EU754146	N/A	KU728590	KU728629	De Gruyter et al. (2009) ; Crous and Groenewald (2016)
<i>Dothiora prunorum</i>	CBS 933.72 ^T	AJ244248	KU728551	N/A	N/A	N/A	De Hoog et al. (1999) ; Crous and Groenewald (2016)
<i>Dothiora pyrenophora</i>	CPC 30632 ^T	KY929145	KY929178	KY929125	KY929203	KY929210	Crous and Groenewald (2017)
<i>Dothiora pyrenophora</i>	CPC 30634	N/A	KY929179	N/A	KY929204	KY929211	Crous and Groenewald (2017)
<i>Dothiora rhamni-alpinae</i>	CBS 745.71	MH860327	MH872082	N/A	N/A	N/A	Vu et al. (2019)
<i>Dothiora rhapontici</i>	TASM 6170 ^T	PP086681	PP086689	PP086695	PP084939	N/A	This study
<i>Dothiora rhapontici</i>	CMUB40040	PP086682	PP086690	PP086696	PP084940	N/A	This study
<i>Dothiora rhapontici</i>	CMUB40041	PP086683	N/A	N/A	PP084941	N/A	This study
<i>Dothiora schizospora</i>	CBS:189.55	MH857439	MH868980	N/A	N/A	N/A	Vu et al. (2019)
<i>Dothiora sorbi</i>	CBS 742.71	KU728514	KU728552	N/A	N/A	N/A	Crous and Groenewald (2016)
<i>Dothiora spartii</i>	MFLU 15-3469	MF443250	MF443253	N/A	N/A	N/A	Hyde et al. (2017)
<i>Dothiora uzbekistanica</i>	TASM 6171 ^T	PP086684	PP086691	PP086697	PP084936	N/A	This study
<i>Dothiora viburnicola</i>	CBS 274.72 ^T	KU728515	KU728554	N/A	KU728591	N/A	Crous and Groenewald (2016)
<i>Dothiora viticola</i>	CBS 140676	NR_137620	MH878164	N/A	N/A	N/A	Vu et al. (2019)

(Continued)

TABLE 1 Continued

Fungal Taxa	Strain	GenBank Accession Numbers					References
		ITS	LSU	SSU	TEF1	TUB2	
<i>Endoconidioma rosae-hissaricae</i>	MFLUCC 17-0821 ^T	MG828898	MG829008	MG829119	N/A	N/A	Wanasinghe et al. (2018)
<i>Endoconidioma populi</i>	UAMH 10297	AY604526	EU981287	AY604526	N/A	N/A	Tsuneda et al. (2004)
<i>Endoconidioma populi</i>	UAMH 10902	HM185487	HM185488	N/A	N/A	N/A	Tsuneda et al. (2010)
<i>Hormonema carpetanum</i>	TRN31	AY616206	N/A	N/A	N/A	N/A	Bills et al. (2004)
<i>Hormonema dematioides</i>	C1-07/07/98	AJ278927	N/A	N/A	N/A	N/A	Tsuneda et al. (2010)
<i>Hormonema macrosporum</i>	CBS 536.94 ^T	AJ244247	MH874128	N/A	N/A	N/A	De Hoog et al. (1999); Vu et al. (2019)
<i>Kabatina juniperi</i>	CBS 466.66	AY616212	N/A	N/A	N/A	N/A	Bills et al. (2004)
<i>Kabatina thujae</i>	CBS 238.66	MH858786	MH870424	N/A	N/A	N/A	Vu et al. (2019)
<i>Neocylindrosetoria corymbiae</i>	CBS 145060 ^T	MK047431	MK047482	N/A	N/A	N/A	Crous et al. (2018b)
<i>Neocylindrosetoria pistaciae</i>	CBS 471.69 ^T	KF251152	KF251656	N/A	KF253112	KF252650	Quaedvlieg et al. (2013)
<i>Neodothiora populina</i>	CPC 39399	MW175365	MW175405	N/A	MW173127	MW173142	Crous et al. (2020)
<i>Phaeocryptopus nudus</i>	CBS 268.37	EU700371	GU301856	GU296182	GU349034	EU747283	Park et al. (2006); Schoch et al. (2009)
<i>Plowrightia obietis</i>	ATCC 24339	N/A	EF114703	EF114727	N/A	N/A	Winton et al. (2007)
<i>Plowrightia periclymeni</i>	178096	N/A	FJ215702	FJ215709	N/A	N/A	Li and Zhuang (2009)
<i>Pseudoseptoria obscura</i>	CBS 135103	KF251219	KF251722	N/A	KF253175	KF252708	Quaedvlieg et al. (2013)
<i>Pseudosydowia eucalypti</i>	CPC 14028	GQ303296	GQ303327	N/A	N/A	N/A	Cheewangkoon et al. (2009)
<i>Rhizosphaera kalkhoffii</i>	ATCC 26605	N/A	EF114706	EF114731	N/A	N/A	Winton et al. (2007)
<i>Rhizosphaera oudemansii</i>	rhoubc	N/A	EF114707	EF114732	N/A	N/A	Winton et al. (2007)
<i>Rhizosphaera pini</i>	rhpirs	N/A	EF114708	EF114733	N/A	N/A	Winton et al. (2007)
<i>Stylodothis puccinioides</i>	CBS 193.58	MH857753	MH869286	N/A	N/A	N/A	Vu et al. (2019)
<i>Sydowia polyspora</i>	CBS 116.29	MH855019	MH866487	N/A	N/A	N/A	Vu et al. (2019)

AFTOL-ID, Assembling the Fungal Tree of Life; ATCC, American Type Culture Collection, Manassas, VA, USA; CBS, Culture collection of the Westerdijk Fungal Biodiversity Institute, the Netherlands; CPC, Culture collection of Pedro Crous, housed at CBS; FMR, Culture collection of the Faculty of Medicine at the Rovira i Virgili University, Reus, Spain; MFLU, MFLUCC, Mae Fah Luang University, Chiang Rai, Thailand; SQUCC, the Sultan Qaboos University culture collection, Oman; UAMH, UAMH Centre for Global Microfungal Biodiversity, the Dalla Lana School of Public Health at the University of Toronto; Type and reference collections are denoted with a superscripted “T”; N/A, no information available.

November 2023) and was improved manually where necessary. The phylogenetic tree was carried out using the maximum likelihood (ML). The single gene datasets were then combined using BioEdit v.7.0.9.1 (Hall, 1999). The final alignments of the combined ITS, LSU, SSU, *TEF1*, and beta-tubulin (*TUB2*) datasets were analyzed, and the phylogenetic trees were inferred based on ML and Bayesian inference (BI) analyses.

The ML tree was accomplished using the RAXML-HPC2 on XSEDE (v. 8.2.12) (Stamatakis, 2014) under the GTRGAMMA substitution model of nucleotide substitution with 1,000 bootstrap (BS) iterations. For BI analyses, the best-fit model of the sequence evolution of each locus was estimated using the Akaike information criterion (AIC) in MrModeltest v. 2.3 (Nylander, 2008) implemented in PAUP v. 4.0b10 (Swofford, 2002). The GTR+G+I substitution model was the best-fit model for all loci. The BI tree was executed with MrBayes v. 3.2.6 (Ronquist et al., 2012) to

evaluate posterior probabilities (PP) (Rannala and Yang, 1996; Zhaxybayeva and Gogarten, 2002) by Markov Chain Monte Carlo sampling (BMCMC). Four simultaneous Markov chains were run for 5,000,000 generations, with the trees sampled every 100th generation resulting in 50,000 trees. The run was stopped when the standard deviation of split frequencies reached below 0.01. The first 12,500 trees were discarded as the burn-in phase of the analyses, while the remaining 37,500 trees were calculated for PP in the majority rule consensus tree. The resulting phylogenetic trees were drawn using FigTree v1.4.0 (Rambaut, 2016) and edited using Adobe Illustrator Version 25.2.3 and Adobe Photoshop Version 22.4.2 (Adobe Systems, U.S.A.). ML bootstrap values $\geq 50\%$ and Bayesian PP ≥ 0.95 were placed above each node (Figure 1). The new nucleotide sequence data are deposited in GenBank (Table 1). The final alignment and tree were deposited in TreeBASE (<http://www.treebase.org/>) under the accession number S31239 and URL

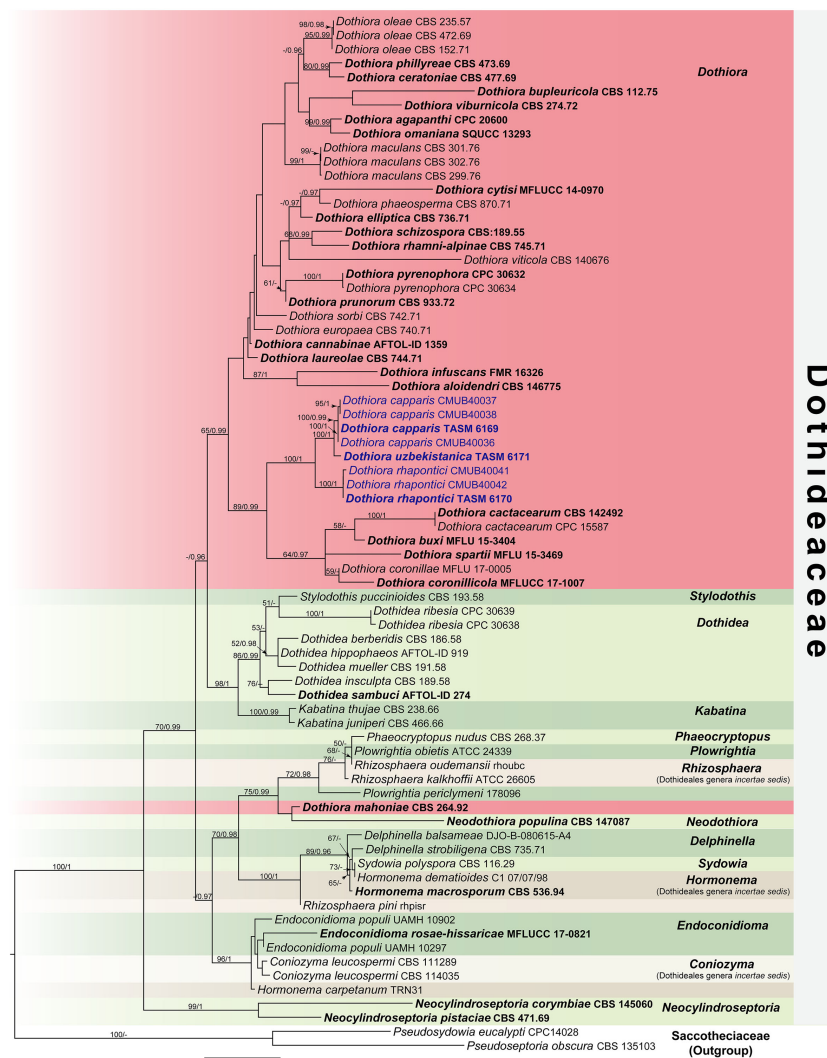


FIGURE 1

RAXML tree generated by maximum likelihood analysis of combined LSU, ITS, SSU, *TEF1*, and *TUB2* sequence data representing Dothideaceae. Bootstrap support values for maximum likelihood (ML, left) $\geq 50\%$ and Bayesian posterior probabilities (PP, right) ≥ 0.95 are indicated above the nodes. Hyphens (-) represent support values $< 50\%$ ML/0.95 PP. The tree is rooted to *Pseudoseptoria obscura* (CBS 135103) and *Pseudosydowia eucalypti* (CPC14028). The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

<http://purl.org/phylo/treebase/phylows/study/TB2:S31239?x-access-code=12b27b6543ffd8301eed3a89eb09aed8&format=html>.

Results

Phylogenetic analyses

The combined dataset of LSU, ITS, SSU, *TEF1*, and *TUB2* sequence data comprises 74 taxa, which represent strains from Dothideaceae and two outgroup taxa in Saccoteciaceae, *Pseudoseptoria obscura* Quaedvl., Verkley & Crous (CBS 135103) and *Pseudosydowia eucalypti* (Verwoerd & du Plessis) Thambug. & K.D. Hyde (CPC14028) (Table 1). The combined sequence alignment consisting of 4,055 characters was analyzed by ML and BI. A best scoring RAXML tree with a final likelihood value of $-19,627.809539$ is presented in Figure 1. The matrix of the

combined dataset had 1,384 distinct alignment patterns and 53.70% of undetermined characters or gaps. Estimated base frequencies were A = 0.253918, C = 0.235773, G = 0.266686, T = 0.243623; substitution rates were AC = 1.637334, AG = 2.504185, AT = 1.806129, CG = 1.203612, CT = 7.247662, GT = 1.000000; and gamma distribution shape parameter $\alpha = 0.504007$. Bayesian posterior probabilities (PP) from MCMC were evaluated with the final average standard deviation of split frequencies = 0.009894. The Bayesian analysis resulted in a tree with similar topology and clades as the ML tree. Phylogenetic analyses of a combined LSU, ITS, SSU, *TEF1*, and *TUB2* sequence data (Figure 1) show that three novel species of *Dothiora* in this study form a clade within the Dothideaceae with high support (100% ML and 1 PP) and sister to the clade containing *Dothiora buxi* Jayasiri, Camporesi & K.D. Hyde, *D. cactacearum* Crous, *D. coronillae* Dissan., Camporesi & K.D. Hyde, *D. coronilicola* Dissan., Camporesi & K.D. Hyde, and *D. spartii* Dissan., Camporesi & K.D. Hyde.

Taxonomic descriptions

Dothiora capparis Senwanna, N. Suwannar., & Gafforov, sp. nov. (Figure 2).

Mycobank number: MB851612

Etymology: Name reflects the host genus *Capparis* from which it was isolated.

Saprobic on dead twigs and branches of *Capparis spinosa* L. Sexual morph: *Mycelium* partly immersed on the substrate, simple to branched, septate, smooth-walled, pale brown to brown hyphae. *Ascomata* 90–115 μm diam. $\times$ 55–100 μm high, semi-immersed to erumpent through the epidermis, solitary or clustered, scattered, globose, dark brown to black, with single locules. *Peridium* (13–) 17–28(–33) μm wide, composed of cells of *textura angularis*, an

outer layer dark brown to black, thick-walled, an inner layer hyaline, thin-walled. *Hamathecium* lacking pseudoparaphyses. *Asci* (47.5–)54–82(–88) $\times$ (16–)21–28(–34) μm ($\bar{x}$ = 66 $\times$ 24 μm , n = 40), 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, apically rounded, with a small ocular chamber. *Ascospores* (15–)19–28 $\times$ (6–)9–12 μm ($\bar{x}$ = 24 $\times$ 10 μm , n = 50), overlapping 1–2-seriate, fusoid to ovoid, one end narrower than other, hyaline, aseptate, smooth-walled with granular contents, surrounded by a distinct mucilaginous sheath, 4–10 μm wide at sides. Asexual morph: undetermined.

Material examined: UZBEKISTAN, Jizzakh Region, Forish District, Yangiqishloq village, dead twigs and branches of *Capparis spinosa* L. (Capparaceae), 05 May 2021, Y. Gafforov, M. Yarasheva, YG-F-2-1 (TASM 6169, holotype; CMUB40035,

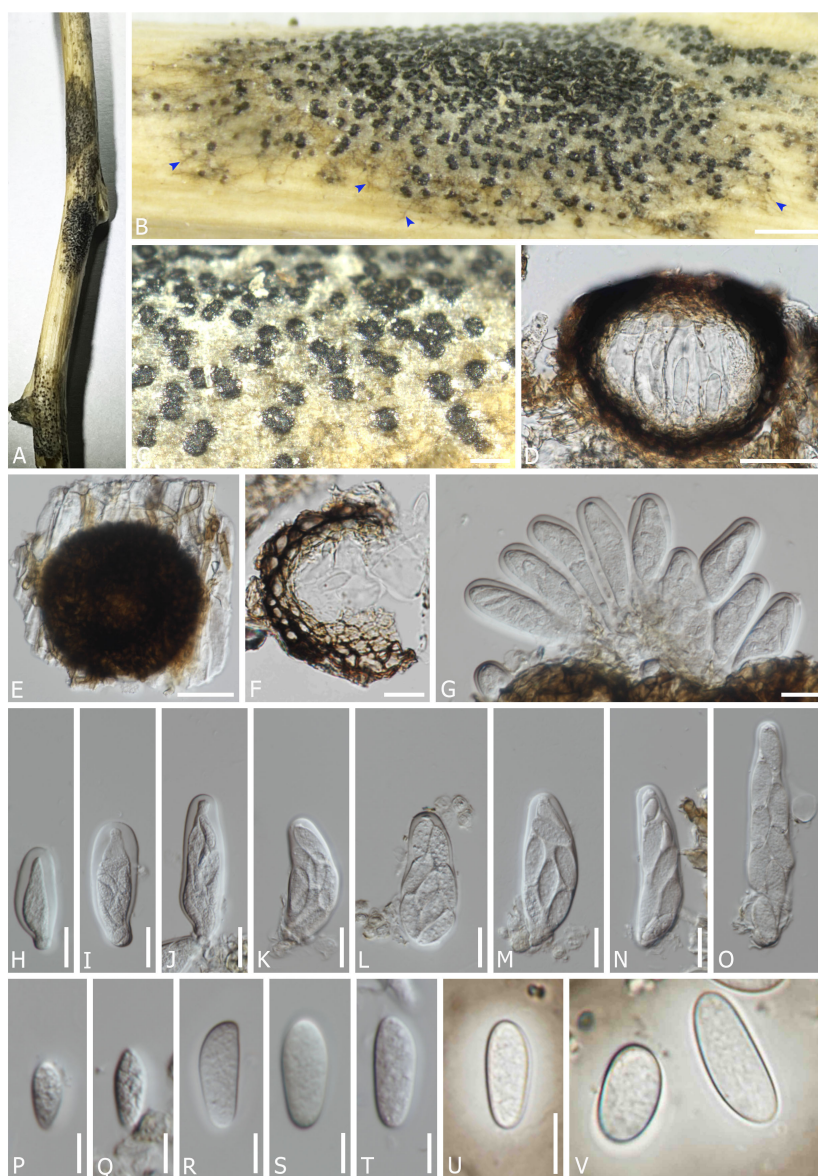


FIGURE 2

Dothiora capparis (TASM 6169, holotype). (A) Appearance of ascomata on host. (B, C) Fruiting bodies under a stereo microscope [superficial mycelia; blue arrows in panel (B)]. (D) Section through the ascoma. (E) Squash mount. (F) Peridium. (G–O) Asci. (P–T) Ascospores (mounted in ddH₂O). (U, V) Ascospore with mucilaginous sheath (mounted in Indian ink). Scale bars: (B) 1000 μm , (C) 200 μm , (D, E) 50 μm , (F–O) 20 μm , and (P–V) 10 μm .

paratype); *ibid*, *Capparis spinosa*, 05 May 2021, Y. Gafforov, M. Yarasheva, YG-F-2-2 (CMUB40036); *ibid*, Nurota District, Nurota, dead twigs and branches of *Capparis spinosa*, 07 May 2021, Y. Gafforov, YG-F-5-1 (CMUB40037); *ibid*, dead twigs of *Capparis spinosa*, 07 May 2021, Y. Gafforov, YG-F-5-2 (CMUB40038).

Notes: In a BLASTn search of NCBI GenBank, the closest match of the LSU sequence of *D. capparis* (TASM 6169, holotype) was *D. spartii* (strain MFLU 15-3469; MF443250) with 99.52% similarity; the closest match of the ITS sequence with 98.36% similarity was *D. coronillae* (MFLU 17-0005; NR157481); the closest matches of the SSU sequence with 100% similarity were *D. pyrenophora* (CPC 30632; KY929125), *D. prunorum* (C. Dennis & Buhagiar) Crous (CBS 933.72; EU707926), and *D. cannabinae* Froid. (CBS 737.71; NG062696), respectively; while the closest matches of the *TEF1* sequence with 97.77% similarity were *D. oleae* (DC.) Crous (SAG 68856-SF; KY613610, CBS 472.69; KU728588, CBS 235.57; KU728587, and CBS 152.71; KU728586) and *D. ceratoniae* (Quaedvl., Verkley & Crous) Crous (CBS 441.75; KU728581). Based on the multi-gene phylogenetic analyses, *D. capparis* (TASM6169, CMUB40036, CMUB40037, and CMUB40038) form a distinct lineage with 100% ML and 0.99 PP statistical support

(Figure 1) and is closely related to *D. uzbekistanica*. Albeit the phylogenetic relationships of those four strains clustering in two different clades, a comparison of ITS and *TEF1* nucleotides shows that strains TASM 6169 and CMUB40036 differ from strains CMUB40037 and CMUB40038 in 1/589 bp (0.17%) and 2/479 bp (0.42%), respectively. Moreover, *D. capparis* differs from *D. uzbekistanica* in having ascospores with a distinct mucilaginous sheath, while the latter lacks this character. A nucleotide comparison of the *TEF1* gene indicated that all strains of *D. capparis* differ from *D. uzbekistanica* by 20/414 bp (4.83%). According to Jeewon and Hyde (2016), a nucleotide comparison of reliable genes must reveal a difference of more than 1.5% to confirm the existence of a different species. Therefore, *D. capparis* and *D. uzbekistanica* are different species.

Dothiora rhapontici Senwanna, N. Suwannar., & Gafforov, sp. nov. (Figure 3).

Mycobank number: MB851613

Etymology: The name reflects the host genus *Rhaponticum* from where it was isolated.

Saprobic on dead twigs of *Rhaponticum repens* L. Sexual morph: *Ascomata* 80–155 μm diam. $\times$ 60–120 μm high, semi-immersed to

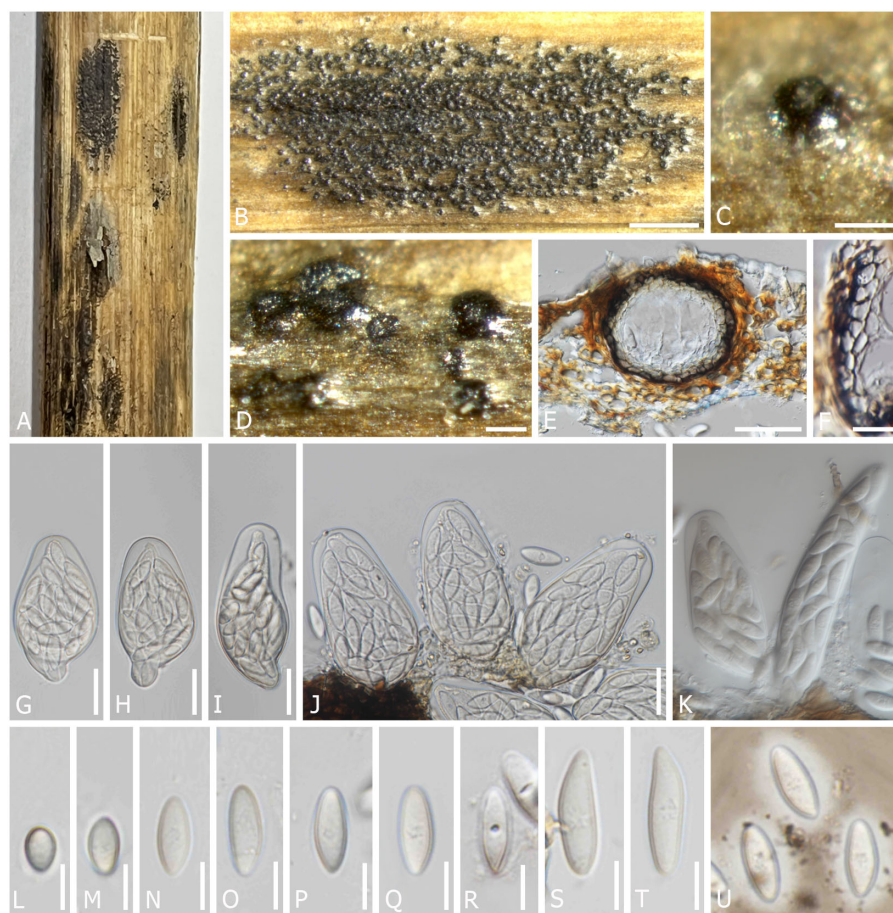


FIGURE 3

Dothiora rhapontici (TASM 6170, holotype). (A) Appearance of ascomata on host. (B–D) Fruiting bodies under a stereo microscope. (E) Section through the ascoma. (F) Peridium. (G–J) Asci (mounted in ddH₂O). (K) Asci (mounted in lactophenol). (L–T) Ascospores (mounted in ddH₂O). (U) Ascospores with mucilaginous sheath (mounted in Indian ink). Scale bars: (B) 1,000 μm , (C, D) 100 μm , (E) 50 μm , (F–K) 20 μm , and (L–U) 10 μm .

erumpent through the epidermis, solitary or clustered, scattered, globose to subglobose, dark brown to black, with single locules. *Peridium* 12–21(–23) μm wide, composed of cells of *textura angularis*, an outer layer dark brown to black, thick-walled, an inner layer hyaline, thin-walled. *Hamathecium* lacking pseudoparaphyses. *Asci* 63–80(–113) $\times$ (23–)29–39 μm ($\bar{x}$ = 73 $\times$ 34 μm , n = 20), polysporous (24 or more spores), bitunicate, cylindro-clavate, pedicellate, apically rounded. *Ascospores* (14–)16–26(–28) $\times$ 6–7(–8) μm ($\bar{x}$ = 19 $\times$ 7 μm , n = 40), multi-seriate, fusoid to ovoid, one end narrower than other, hyaline, aseptate, with a central concave depression, smooth-walled with granular contents, surrounded with a mucilaginous sheath, 2–4.5 μm wide at sides.

Material examined: UZBEKISTAN, Tashkent Region, Ugam-Chatkal National Park, Chimyon, Western Tien-Shan Mountains, dead twigs of *Rhaponticum repens* (L.) Hidalgo (Asteraceae), 21 July 2019, Y. Gafforov, M. Yarasheva, YG-S-22-4 (TASM 6170, holotype; CMUB40039, paratype); Jizzakh Region, Zaamin District, Zaamin National State Park, dead branches of *Capparis spinosa* L. (Capparaceae), 27 August 2020, Y. Gafforov, A. Abdurazakov, YG-ZMB-40-1 (CMUB40040); *ibid.*, dead branches of *Capparis spinosa*, 27 August 2020, Y. Gafforov, A. Abdurazakov, YG-ZMB-40-2 (CMUB 40041).

Notes: In a BLASTn search of NCBI GenBank, the closest match of the LSU sequence of *D. rhapontici* (TASM6170, holotype) was *D. cannabinae* (AFTOL-ID 1359; MF443250) with 99.66% similarity; the closest match of the ITS sequence with 98.16% similarity was *D. coronillae* (MFLU 17-0005; NR157481); the closest matches of the SSU sequence with 100% similarity were *D. pyrenophora* (CPC 30632; KY929125), *D. prunorum* (CBS 933.72; EU707926), and *D. cannabinae* (CBS 737.71; NG062696), respectively; while the closest matches of the *TEF1* sequence with 96.09% similarity was *D. phillyreae* (CBS 473.69; KU728590). The phylogenetic analysis reveals that *D. rhapontici* formed a distinct sister clade with *D. capparis* and *D. uzbekistanica*, which is statistically supported (100% ML and 1 PP) (Figure 1). *Dothiora rhapontici* shares similar morphological features of ascospores with related species; however, *D. rhapontici* can be distinguished from those latter species by having polysporous asci and having longer and wider ascospores.

Dothiora uzbekistanica Senwanna, N. Suwannar., & Gafforov, sp. nov. (Figure 4).

Mycobank number: MB851614

Etymology: The name refers to the country Uzbekistan, where it was collected.

Saprobic on dead twigs of unknown angiosperm perennial plants. Sexual morph: *Ascomata* 95–155 μm diam. $\times$ 80–135 μm high, semi-immersed to erumpent through the epidermis, solitary or clustered, globose to subglobose, black, with single locules. *Peridium* (10–)15–25(–30) μm wide, composed of cells of *textura angularis*, an outer layer dark brown to black, thick-walled, an inner layer hyaline, thin-walled. *Hamathecium* lacking pseudoparaphyses. *Asci* (50–)54–66(–70) $\times$ (19–)21–27 μm ($\bar{x}$ = 58 $\times$ 22 μm , n = 20), 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, apically rounded. *Ascospore* (16–)19–26 $\times$ (5–)9–11 μm ($\bar{x}$ = 22 $\times$ 9.5 μm , n = 45), overlapping one

to two-seriate, fusoid to ovoid, one end narrower than other, hyaline, aseptate, smooth-walled with granular contents, lacking a mucilaginous sheath. Asexual morph: undetermined.

Material examined: UZBEKISTAN, Jizzakh Region, Forish District, Yangiqishloq village, dead twigs of unknown angiosperm plants, 05 May 2021, Y. Gafforov, M. Yarasheva, YG-F-4-2 (TASM 6171, holotype; CMUB40042, paratype).

Notes: In a BLASTn search of NCBI GenBank, the closest matches of the LSU sequence of *D. uzbekistanica* (TASM 6171, holotype) is *D. spartii* (strain MFLU 15-3469; MF443250) with 99.52% similarity, the closest matches of the ITS sequence with 98.16% similarity, was *D. coronillae* (MFLU 17-0005; NR157481), the closest matches of the SSU sequence with 100% similarity was *D. pyrenophora* (CPC 30632; KY929125), *D. prunorum* (CBS 933.72; EU707926) and *D. cannabinae* (CBS 737.71; NG062696), respectively, while the closest matches of the *TEF1* sequence with 97.21% similarity was *D. oleae* (SAG 68856-SF; KY613610, CBS 472.69; KU728588, CBS 235.57; KU728587, and CBS 152.71; KU728586) and *D. ceratoniae* (CBS 441.75; KU728581). In the phylogenetic analysis, *D. uzbekistanica* forms a distinct lineage basal to *D. capparis* with 100% ML and 1 PP bootstrap support (Figure 1). Moreover, the different characteristics of the microscopic features and the nucleotide comparison data of *D. uzbekistanica* differ from *D. capparis* have been mentioned above.

Discussion

Historically, *Dothiora* has relied on morphological studies, and only a few sequences of species are available in GenBank. In this study, three novel species of *Dothiora* are introduced in the family Dothideaceae from the Central Asian region based on their morphological distinctiveness and phylogenetic analyses. Although a living culture from an isolated ascospore could not be obtained, the fungal DNA was extracted directly from the ascomata. The connection between sexual and asexual morphs is likewise unknown. The individual phylogenetic analyses of ITS or LSU separated *Dothiora* species from other genera in Dothideaceae, but their placement was otherwise unresolved (Crous and Groenewald, 2017; Crous et al., 2018a; Crous et al., 2018b). Therefore, the combination of LSU, ITS, and SSU sequence data was previously used to clarify the relationships among the species in Dothideaceae, although there is no strong statistical support (Thambugala et al., 2014; Hyde et al., 2017; Crous et al., 2020; Crous et al., 2022; Hongsanan et al., 2020; Boonmee et al., 2021). Gao et al. (2021) recommended using a combination of the nuclear ribosomal region (ITS, LSU, and SSU) and the protein-coding gene regions (*TEF1* and *TUB2*) to clarify the relationships of Dothideaceae. Our attempts to obtain *TUB2* sequence data for our new strains were unsuccessful; however, the data from the combined sequence analyses of the ITS, LSU, SSU, and *TEF1* loci are not well-resolved for most *Dothiora* species. Thus, a phylogenetic analysis based on a combination of five loci was generated for a better phylogenetic relationship within the family and genus. Our multigene phylogeny (Figure 1) revealed that the generic placement within Dothideaceae, comprising 14 genera and

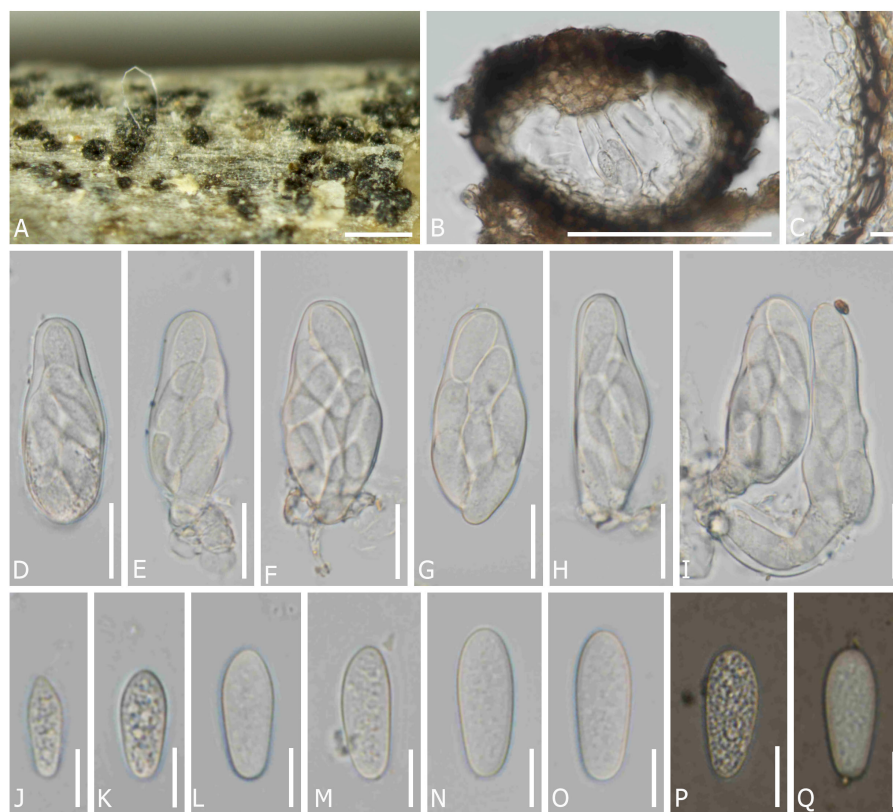


FIGURE 4

Dothiora uzbekistanica (TASM 6171, holotype). (A) Fruiting bodies under a stereo microscope. (B) Section through the ascoma. (C) Peridium.

(D–I) Asci. (J–O) Ascospores (mounted in ddH₂O). (P, Q) Ascospores (mounted in Indian ink). Scale bars: (A) 200 µm, (B) 100 µm, (C, J–Q) 10 µm, and (D–I) 20 µm.

three Dothideales genera *incertae sedis*, viz., *Coniozyma*, *Hormonema*, and *Rhizosphaera*, was similar to those of Hongsanan et al. (2020) and Gao et al. (2021). Most *Dothiora* taxa clustered together in its own clade with 65% ML, 1 PP statistical support, excepting *D. mahoniae* (A.W. Ramaley) Crous (strain CBS 264.92) (Figure 1).

A checklist of 69 accepted *Dothiora* species, including details of each species based on recorded from Index Fungorum (2024), MycoBank (2024), and published articles, is provided in Table 2. *Dothiora* have a cosmopolitan distribution and are mainly saprobic, found in decaying wood and plant litter in terrestrial environments. Members of *Dothiora* have been recorded on 36 host plant families, viz., Aceraceae, Adoxaceae, Amaryllidaceae, Apiaceae, Apocynaceae, Araliaceae, Asphodelaceae, Asteraceae, Berberidaceae, Buxaceae, Cactaceae, Capparaceae, Caprifoliaceae, Celastraceae, Convolvulaceae, Cupressaceae, Elaeagnaceae, Ericaceae, Fabaceae, Grossulariaceae, Lythraceae, Magnoliaceae, Oleaceae, Pinaceae, Plantaginaceae, Podocarpaceae, Rhamnaceae, Rosaceae, Rubiaceae, Salicaceae, Staphyleaceae, Symplococaceae, Tamaricaceae, Taxaceae, Thymelaeaceae, and Vitaceae. This study is the first record of *Dothiora* on Asteraceae and Capparaceae. The genus has mainly been reported in the USA (22 species), Italy (10 species), Canada (8 species), and Switzerland (7 species). The identification of *Dothiora* species was initially based on its sexual morph. A total of 37 species of *Dothiora* are known only for their

sexual morph, 15 species are known only for their asexual morph, and another eight species have no information available. There are only eight *Dothiora* species that have asexual-sexual morph connections, viz., *D. buxi*, *D. cytisi* (Wanas., Camporesi, E.B.G. Jones & K.D. Hyde) Crous, *D. lonicerae* Fuckel, *D. pyrenophora*, *D. schizospora* Luttr., *D. sorbi* (Wahlenb.) Fuckel, *D. sphaeroides* (Pers.) Fr., and *D. taxicola* (Peck) M.E. Barr. To date, 31 species have been reported based on molecular data, and only in four species have been proven the connectivity of sexual and asexual morphs through sequence data and culture studies.

Although the sexual morph of *Dothiora* species has been delimited according to morphological criteria by having one or more septate or muriform ascospores (Thambugala et al., 2014; Crous and Groenewald, 2017), many species with aseptate ascospores were also classified as *Dothiora* based on morphological characteristics and phylogenetic analyses (Hyde et al., 2016; Hyde et al., 2017; Boonmee et al., 2021). Most *Dothiora* species with hyaline to pale brown, one or more septate or muriform ascospores, form a separate clade without statistical support (Figure 1), while three new taxa, *D. capparis*, *D. rhapsontici*, and *D. uzbekistanica*, cluster with *D. buxi*, *D. coronillae*, *D. coronillicola*, and *D. spartii*, which also have hyaline, aseptate, and fusoid to ovoid ascospores with 89% ML and 0.99 PP statistical support in different clades, agreeing with previous studies (Boonmee et al., 2021). *Dothiora rhapsontici* and *D. buxi* differ

TABLE 2 Morphology, host information, locality, sequence data, and related references *Dothiora* reported worldwide based on the record of Species Fungorum and MycoBank database 2024.

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
<i>D. agapanthi</i>	On leaves of <i>Agapanthus</i> sp. (Amaryllidaceae)	South Africa	Sexual: Undetermined Asexual: <i>Conidiomata</i> pycnidial, globose with long neck, brown, to 250 µm diam, with central ostiole, exuding a creamy conidial mass; <i>Conidiophores</i> hyaline, smooth, ampulliform to doliiform, 5–7 × 5–6 µm, with central phialidic locus; <i>Conidia</i> hyaline, smooth, guttulate, subcylindrical, apex obtuse, tapering to a truncate hilum (8–)10–12(–13) × 3(–3.5) µm; <i>Hyphae</i> becoming brown, verruculose, and constricted at septa, giving rise to a Hormonema-like synasexual morph.	Available	Crous and Groenewald (2016)
<i>D. aloidendri</i>	On leaves of <i>Aloidendron dichotomum</i> (Asphodelaceae)	South Africa	Sexual: Undetermined Asexual: <i>Conidiomata</i> pycnidial, globose, black, glabrous, 200–350 µm diam, aggregated in dense clusters, exuding a creamy conidial mass; <i>Conidiophores</i> reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, ampulliform to doliiform, phialidic, 6–9 × 5–7 µm; <i>Conidia</i> solitary, straight, subcylindrical, aseptate, guttulate, hyaline, smooth, thin-walled, apex obtuse, tapering at base to truncate hilum, 1–1.5 µm diam, (10–)12–13(–14) × (3–)4 µm.	Available	Crous et al. (2020)
<i>D. amelanchieris</i>	In stems of <i>Amelanchier alnifolia</i> (Rosaceae)	Canada	Sexual: <i>Ascostromata</i> up to 500 µm wide, 300 µm high; Hamathecium lacking pseudoparaphyses; <i>Asci</i> 8-spored, bitunicate, fissitunicate, oblong, parallel from basal subhymenial layer, 75–90 × 12–15 µm; <i>Ascospores</i> hyaline, ellipsoid to somewhat obovoid, 20–30(–32) × 7–10 µm, 5–7 transversely septate, one longitudinal septum in one, two, or three of mid cells, hemispores somewhat unequal, upper longer, constricted at first-formed septum. Asexual: Undetermined	N/A	Barr (2001)
<i>D. bupleuricola</i>	On leaf spot of <i>Bupleurum fruticosum</i> (Apiaceae)	France	Sexual: Undetermined Asexual: <i>Conidiomata</i> pycnidial, globose with long neck, brown, to 250 µm diam, with central ostiole, exuding a creamy conidial mass; <i>Conidiophores</i> hyaline, smooth, globose to allantoid 5–7 × 4–6 µm, with central phialidic locus. <i>Conidia</i> hyaline, smooth, guttulate, subcylindrical, apex obtuse, tapering to a truncate hilum (8–)9–10(–12) × 2(–2.5) µm.	Available	Crous and Groenewald (2016)
<i>D. buxi</i>	On dead branch, dying leaves and twigs (hemibiotrophic) of <i>Buxus sempervirens</i> (Buxaceae)	Italy and Russia	Sexual: <i>Ascostromata</i> 500–1000 µm long, 220–250 µm high, 320–340 µm diam., erumpent through the epidermis, solitary or clustered, globose, brown to black, with single locules, with a central longitudinal slit-like opening. <i>Peridium</i> 32–83 µm wide; Hamathecium lacking pseudoparaphyses. <i>Asci</i> 100–115 × 14–21 µm, 32-spored, bitunicate, fissitunicate, cylindro-clavate, short pedicellate, apically rounded, with a small ocular chamber. <i>Ascospores</i> 11–15 × 5.4–7 µm, bi-seriate to multi-seriate, hyaline to very pale brown, aseptate, fusoid to ovoid, one end narrower than other, smooth-walled with granular contents, with a thin mucilaginous sheath. Asexual: <i>Conidiomata</i> 182–263 µm high × 383–447 µm diam., pycnidial, globose to subglobose, visible as brown to black, pustulate on the lower	Available	Hyde et al. (2016); Tibpromma et al. (2017)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			leaf surface; Peridium 21–43 μm wide; <i>Conidiogenous cells</i> 11–17 $\times$ 6–11 μm diam., phialidic, subglobose, hyaline. <i>Conidia</i> 10–16 $\times$ 6–19 μm diam., ellipsoid to obovoid, rounded at top, narrow at base, guttulate, smooth, 1-celled, hyaline.		
<i>D. cactacearum</i>	On phylloides of Cactaceae	USA (Texas)	Sexual: Undetermined Asexual: <i>Conidiomata</i> separate, erumpent, pycnidial, globose, medium brown, 150–300 μm diam., with a central ostiole, exuding a creamy conidial mass; <i>Conidiophores</i> reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, ampulliform to doliiform, 7–15 $\times$ 7–15 μm , phialidic, at times with percurrent proliferation and prominent collarette; <i>Conidia</i> hyaline, smooth, guttulate, subcylindrical to broadly ellipsoidal, apex obtuse, tapering to a truncate, protuding hilum, 2–3 μm diam., (12–) 14–17(–19) $\times$ (5–)6–7.5(–8) μm , conidia becoming brown, verruculose and constricted at the septa.	Available	Crous and Groenewald (2016)
<i>D. cannabinae</i>	On dead branches of <i>Daphne cannabina</i> (Thymelaeaceae)	India	N/A	Available	Froidevaux (1972)
<i>D. ceratoniae</i>	On dead leaves of <i>Nerium oleander</i> (Apocynaceae), on dead leaves of <i>Arbutus unedo</i> (Ericaceae), and on leaves of <i>Cerantonia siliqua</i> (Fabaceae)	Italy and Spain	Sexual: Undetermined Asexual: <i>Conidiomata</i> brown, cupulate, short-stipitate, rim up to 300 μm diam, 100–180 μm tall, tapering toward base, 20–50 μm diam; <i>Conidiogenous cells</i> hyaline, smooth, ampulliform, 7–12 $\times$ 4–6 μm ; apex 2 μm diam, with prominent periclinal thickening; <i>Conidia</i> hyaline, smooth, granular or not, cylindrical with obtuse apex, tapering at base to truncate scar 1 μm diam, aseptate, (10–)12–14(–16) $\times$ 3 (–3.5) μm .	Available	Quaedvlieg et al. (2013); Crous and Groenewald (2016)
<i>D. coronillae</i>	On dead aerial branches of <i>Coronilla emerus</i> (Fabaceae)	Italy	Sexual: <i>Ascostromata</i> 185–310 μm high $\times$ 220–250 μm diam., immersed or erumpent through the epidermis, solitary or clustered, globose, brown to black, with single locules; <i>Peridium</i> 32–83 μm wide; <i>Asci</i> 60–110 $\times$ 14–21 μm , 8-spored, bitunicate, fissitunicate, cylindro-clavate, short pedicellate, apically rounded, with a small ocular chamber; <i>Ascospores</i> 17–22 $\times$ 7–9 μm , bi-seriate to multi-seriate, hyaline, aseptate, fusoid to ovoid, one end narrower than other, smooth-walled with granular contents, lacking a mucilaginous sheath. Asexual: Undetermined	Available	Hyde et al. (2017)
<i>D. coronillicola</i>	On dead branches of <i>Coronilla emerus</i> (Fabaceae)	Italy	Sexual: <i>Ascomata</i> 215–430 $\times$ 240–285 μm , immersed or erumpent through the epidermis, solitary or clustered, globose, brown to black, with single locules; <i>Peridium</i> 39–76 μm wide; <i>Asci</i> 80–145 $\times$ 15–30 μm , 8-spored, bitunicate, fissitunicate, cylindro-clavate, pedicellate, apically rounded, with a small ocular chamber; <i>Ascospores</i> 21–25 $\times$ 8–11 μm , bi-seriate to multiseriate, hyaline, aseptate, fusoid to ovoid, one end narrower than the other, smooth-walled with granular contents, lacking a mucilaginous sheath. Asexual: Undetermined	Available	Boonmee et al. (2021)
<i>D. cytisi</i>	On dead and hanging branches of <i>Cytisus scoparius</i> (Fabaceae)	Italy	Sexual: <i>Ascostromata</i> 180–250 $\times$ 170–210 μm , superficial, semi-immersed to erumpent,	Available	Li et al. (2016)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			solitary, scattered, broadly oblong, dark brown to black, coriaceous, uniloculate; <i>Peridium</i> 35–45 µm wide at the base, 30–40 µm wide at the sides; <i>Hamathecium</i> lacking pseudoparaphyses; <i>Asci</i> 70–90 × 20–30 µm, 8-spored, bitunicate, fissitunicate, clavate to broadly-clavate, short pedicellate, thickened and rounded at apex, with an ocular chamber; <i>Ascospores</i> 25–35 × 7–10 µm, overlapping 1–2-seriate, hyaline, broadly fusiform, rounded at both ends, 1-septate, with a median septum, constricted at the septum, smooth-walled, lacking a mucilaginous sheath. Asexual: <i>Conidiomata</i> stromatic, immersed in agar to superficial, uni- to multi-loculate, globose to subglobose, glabrous, ostiole central, with minute papilla; <i>Conidiomata walls</i> composed of several layers of hyaline to dark brown, pseudoparenchymatous cells, organized in a <i>textura angularis</i> ; <i>Conidiophores</i> arising from basal cavity of conidiomata, mostly reduced to conidiogenous cells; <i>Conidiogenous cells</i> holoblastic, phialidic, discrete, ampulliform to cylindric-clavate, hyaline, aseptate, smooth-walled; <i>Conidia</i> 25–35 × 6–9 µm, solitary, 1-celled, fusiform to falcate, with narrowed ends, initially hyaline, becoming pale brown at maturity, aseptate, smooth and thin-walled, guttulate, contents granular.		
<i>D. dothideoides</i> ²	<i>Prunus emarginata</i> (Rosaceae), On dead twigs of <i>Shepherdia canadensis</i> (Elaeagnaceae) and on dead branch of <i>Populus tremuloides</i> (Salicaceae)	Canada and USA	Sexual: <i>Ascostromata</i> connected by and seated in dense subiculum; <i>Asci</i> polysporous; <i>Ascospores</i> (13–)16–18(–21) × 4–8 µm, hyaline, muriform. Asexual: Undetermined	N/A	Barr (2001)
<i>D. elegans</i> ²	On twig of <i>Pinus sylvestris</i> (Pinaceae)	USA	Sexual: Undetermined Asexual: Punctiform; <i>Spores</i> fenestrated, 25 µm long.	N/A	Saccardo (1889)
<i>D. elliptica</i>	On dry branches of <i>Vaccinium uliginosum</i> (Ericaceae) and on dead twigs of <i>Linaria vulgaris</i> (Plantaginaceae)	France and Switzerland	Sexual: <i>Ascomata</i> arranged in parallel, elliptical (hysteriiform), 1 mm. long, <i>Asci</i> 8-spored, elongate, 96 × 12 µm; <i>Ascospores</i> distichis, oblong-fusiform, 3-septate, hyaline, 16 × 7 µm. Asexual: Undetermined	Available	Fuckel (1873)
<i>D. ellisii</i> ²	On decayed needles of <i>Pinus contorta</i> var. <i>latifolia</i> (Pinaceae)	USA	Sexual: <i>Ascomata</i> compound to clustered in groups of 3–10, immersed becoming erumpent, appearing superficial, globose, depressed, glabrous, 100–150 µm wide, 150–200 µm high; <i>Beak</i> short, truncated-conical, terete, 20–25 µm long, 60–70 µm wide, composed of 2 or 3 layers of brown polygonal 7–10 × 6–8 µm cells around a 20–25 µm diam. Ostiole, without periphyses or surface setae; <i>Wall</i> in longitudinal section uniform in thickness, 15–20 µm thick, of 2 or 3 layers of 6–10 × 4–5 µm polygonal to rectangular thin-walled brown cells; <i>Hamathecium</i> not seen; <i>Asci</i> numerous, from a central base, bitunicate, broadly fusiform, 70–85 × 25–30 µm, with 8 tetraseriate ascospores; <i>Ascospores</i> broadly fusiform to obovoid, straight, 3-septate, in sequence 2:1:2, second cell from apex enlarged, first septum complete and slightly constricted, median (0.50), not constricted at additional full septa, brown, smooth, guttulate, with a conspicuous sharply delimited sheath constricted at first septum, 1.5–	N/A	Shoemaker and Babcock (1987)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			2 µm wide, or wider if exposed to water for a long time. Asexual: Undetermined		
<i>D. eunomia</i>	In dry branches of <i>Fraxinus excelsior</i> (Oleaceae)	Finland	Sexual: <i>Stromata</i> gregarious, covered with raised fissured periderm, usually rounded, unicellular, 0.3–0.5 mm wide; <i>Spermatia</i> elongate, straight or gently curved, elongate, 4–5 µm long, 0.5–1 µm thick. Asexual: Undetermined	N/A	Karsten (1884)
<i>D. europaea</i>	<i>Alnus</i> sp., on dead branches of <i>Salix elvetica</i> (Salicaceae)	Switzerland and USA	Sexual: <i>Asci</i> polysporous; <i>Ascospores</i> hyaline, 20–25 × 5–6 µm, upper hemispore shorter and wider than lower. Asexual: Undetermined	Available	Barr (2001)
<i>D. harknessii</i> ²	On dead stems of <i>Convolvulus californicus</i> (Convolvulaceae)	USA	Sexual: <i>Stromata</i> immersed, visible and slightly erumpent through splits in periderm, dull black, elongate oblong or ± rounded, 1 mm or more long, up to 550 µm wide, 275–300 µm high, apex plane or shallowly depressed; <i>Locule</i> single, up to 385 µm wide, or two and ± 180 µm wide, approximately 100 µm deep; <i>Walls</i> pseudoparenchymatous, cells thick walled, dark reddish brown externally, slightly tinged reddish or hyaline internally, base 80–100 µm deep, sides 30–40 µm wide, subhymenium small celled, hyaline, in a low arching cushion; <i>Asci</i> densely packed in broad fascicle from cushion, bitunicate, 60–80 × 8–11 µm, 8-spored; interthecial tissues sparse, visible at sides and above asci; <i>Ascospores</i> 13–16 × 5–7 µm, hyaline, or slightly reddish-brown in age, obovoid, asymmetric, broader and usually longer above, 3–5-septate, constricted at submedian primary septum, longitudinal septum in mid cells, at times obliquely in one end cell, smooth-walled, contents minutely guttulate, overlapping biserial or partially uniserial in the ascus. Asexual: Undetermined	N/A	Barr (1981)
<i>D. hederæ</i>	On dead branches of <i>Hedera helix</i> (Araliaceae)	Switzerland	N/A	N/A	Index Fungorum (2024)
<i>D. huræ</i>	N/A	N/A	N/A	N/A	Index Fungorum (2024)
<i>D. infuscans</i>	Isolated from the blackened wall of an industrial warehouse	Spain	Sexual: Undetermined Asexual: <i>Mycelium</i> composed of subhyaline, smooth-, thin-walled, septate hyphae, 5–7 µm wide, later becoming thick-walled, increasing the number of septa and the volume of their cells to give them a moniliform appearance, and finally the hyphae turn dark brown and produce chains of holothallic (chlamydospore-like) conidia of up to 20 µm diam, which also develop longitudinal/oblique secondary septa over time, giving consequently a ‘muriform’ aspect to these propagules; <i>Conidiophores</i> micronematous, reduced to conidiogenous cells, mostly intercalary, producing conidia on lateral, short to long conic-truncate denticles, with 1–3 per conidiogenous cell; <i>Conidia</i> holoblastic, solitary, but attached to one another by a mucilaginous substance, mostly aseptate, smooth- and thinto thick-walled, hyaline, becoming dark brown,	Available	Crous et al. (2018a)

(Continued)

TABLE 2 Continued

Dothiora species	Host	Locality	Morphology	Sequence data	References
			thick-walled, roughened and mostly 1-septate, occasionally 2–3-septate, globose, ellipsoid or irregularly-shaped, prominently constricted at septa when old, unicellular conidia 8–9 × 4–5 μm, 2-celled conidia 10–13 × 6–7 μm, multi-celled conidia 18–19 × 5–7 μm; <i>Microcyclic conidia</i> produced by budding of the hyaline or pigmented conidia, solitary or in chains of up to 5 elements on inconspicuous denticles when the conidiogenous cell is young, but on protruding conical-truncate denticles when old, at one or both ends but also laterally, being smaller than the primary conidia.		
<i>D. laureolae</i>	On dead branches of <i>Daphne laureola</i> (Thymelaeaceae)	Italy (Sicilia)	N/A	Available	Index Fungorum (2024)
<i>D. lepargyrea</i> ²	On dead twigs of <i>Shepherdia canadensis</i> (Elaeagnaceae)	USA	Sexual: <i>Ascomata</i> 330–440 μm diam., 275–330 μm high; <i>Wall</i> 52–65 μm thick at sides, up to 104 μm thick below; <i>Asci</i> 90–104 × 15.5–20 μm, more than 32-spored; <i>Ascospores</i> 14–18(–22) × 3.5–5 μm, hyaline, obovate, broadest above and tapered to the pointed base, (1–)3(4–7)-septate, with vertical septum in one or more of mid cells Asexual: Undetermined	N/A	Barr (1972) ; Barr, (2001)
<i>D. lonicerae</i> ¹	On dead corticate branches of <i>Lonicera alpigena</i> (Caprifoliaceae)	France and Germany	Sexual: <i>Ascomata</i> scattered, oblong or rounded, creeping flat on the edge; <i>Asci</i> ample, tapering toward the base, 8-spored, 112 × 18 μm; <i>Ascospores</i> disordered, irregularly oblong, obtuse on both sides, muriform, 6–8-septate, constricted at the septa, hyaline, 30–90 × 8–10 μm. Asexual: <i>Perithecia</i> spurious, scattered, acicular, sharp, black, with a white globule, very minute at the terminal; <i>Stylospores</i> very narrow, fusiform, curved, 4-guttulate, 32 × 2 μm, hyaline.	N/A	Fuckel (1870)
<i>D. maculans</i>	Leaf of <i>Acer pseudoplatanus</i> (Aceraceae), leaf litter of <i>Populus tremuloides</i> (Salicaceae)	Canada and Netherlands	Sexual: Undetermined Asexual: <i>Conidiomata</i> pycnidial, with central ostiole; <i>Conidiogenous cells</i> 1–2 loci, aggregated in pseudochains, encased in a thick, persistent mucoid layer; <i>Conidia</i> hyaline, smooth, subcylindrical to oblong, guttulate, apex obtuse, tapering to a truncate hilum (7–)10–12(–13) × (2.5–)3(–3.5) μm; <i>Hormonema-like synasexual morph</i> with ampulliform to doliiform, phialidic conidiogenous cells, 5–7 × 5–6 μm.	Available	Crous and Groenewald (2016)
<i>D. mahoniae</i>	On leaves of <i>Mahonia repens</i> (Berberidaceae)	USA	Sexual: Undetermined Asexual: <i>Mycelium</i> immersed, grey-black, branched, septate, 3–12 μm wide; <i>Conidiomata</i> acervular, somewhat pulvinate, erumpent, amphigenous but mostly hypophyllous, subepidermal to epidermal, separate or confluent, formed of gray-black rather thick-walled <i>textura angularis</i> at the base, changing to brown at the level of the conidiophores, 50–400 μm diam.; <i>Dehiscence</i> by irregular rupture of the cuticle and epidermis; <i>Conidial masses</i> white to sordid; <i>Conidiophores</i> smooth, brown, lighter colored toward the apex, septate, branched at any level along the length, up to 65 μm long; <i>Conidiogenous cells</i> integrated, pale brown, smooth, cylindrical to subclavate, periclinal thickening absent, collarette absent, 6–	Available	Ramaley (1992)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			19 × 3–6 µm; <i>Conidial ontogeny</i> holoblastic by apical wall building, maturation synchronous with conidial ontogeny, conidiogenous cell proliferation enteroblastic to produce additional conidia at ca same level or rarely, conidiogenous cell proliferation enteroblastic to produce additional conidia at successively higher levels; <i>Conidia</i> acrogenous, hyaline, aseptate, cylindrical-ellipsoidal, smooth, thin-walled, apex and base bluntly rounded or the base truncate at the indistinct scar, 6.5–12.5 × 2.5–3.5 µm.		
<i>D. meynae</i>	On dead branches of <i>Meyna</i> sp. (Rubiaceae)	India	Sexual: <i>Stroma</i> solitary or gregarious, dark brown to black, innate to widely erumpent through the bark, surrounded by vertically bent, more or less lobate edges of peridermis on lateral sides 1,000–1,500 µm long and 500–800 µm broad; <i>Asci</i> oblong to cylindrical, thick-walled, bitunicate, stipitate, rounded at the apex, parallel, densely clustered in one or a few locules of indefinite shape in the stroma 80–140 × 14–20 µm, situated on the thin walled hyaline tissue of isodiametric cells; <i>Ascospores</i> fusoid, muriform, 34–40 × 6–9 µm, 6–7 transverse septa, 1 or 2 incomplete longitudinal septa through one or two or few of the thickest cells, distinctly constricted in the middle, thickest slightly above the middle septum, hyaline. Asexual: Undetermined	N/A	Rao (1971)
<i>D. moravica</i>	On dead stems of <i>Evonymus europaeus</i> (Celastraceae)	Czech Republic	Sexual: <i>Stromata</i> covered by the usually evenly black or black-brown colored, often sheer shiny epidermis, not breaking out, often several densely packed together and then more or less fused together, cushion-shaped from a rounded to elliptical base, with a fairly flat apex, usually 200–300 µm in diameter, 100–200 µm high, completely closed when young, tearing quite irregularly at the apex when ripe; <i>Hamathecium</i> not seen; <i>Asci</i> polysporous, 80–120 × 18–24 µm, sessile, broadly rounded at the top, downwards only slightly tapered; <i>Ascospores</i> elongated spindle-shaped or almost club-shaped, usually with 3, rarely 5 transverse walls, usually strong in the middle, barely constricted on the remaining transverse walls, in 1–2 of the middle cells later with 1 longitudinal wall, straight or slightly curved, hyaline, usually only at the top, more tapered downwards, bluntly rounded at both ends, 15–24 × 4.5–7 µm. Asexual: Undetermined	N/A	Petrak (1919)
<i>D. oleae</i>	On fallen leaf, leaves and rotting fruit of <i>Olea europaea</i> (Oleaceae), <i>Olea cuspidate</i> , and <i>Olea</i> sp.	Greece, India, Israel, Italy, Pakistan, Spain, Turkey, and USA	Sexual: Undetermined Asexual: Follicolous. <i>Mycelium</i> immersed, composed of branched, septate, pale brown to dark brown, thin- or thick-walled, smooth hyphae; <i>Conidiomata</i> pycnidial, globose, subcylindrical, or flattened at the base, dark brown, black, unilocular, 100–250 µm diam., up to 350 µm high; Ostiole single, central, circular, often becoming wide late in development; <i>Conidiophores</i> absent or poorly developed; <i>Conidiogenous cells</i> discrete, determinate, terminal or intercalary within conidiophores, phialidic, ampulliform to lageniform or subcylindrical, hyaline, smooth, thin-walled, channel and collarete minute, occasionally with	Available	Duan et al. (2007); Crous and Groenewald (2016)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			a percurrent proliferation, 8–15 × 3–5 µm; <i>Conidia</i> hyaline, aseptate, cylindrical, apex obtuse, base truncate, thin-walled, smooth, with several large guttules, 15–23 × 3–5 µm.		
<i>D. omaniana</i>	Leaf spot on leaves of <i>Punica granatum</i> (Lythraceae)	Oman	Sexual: Undetermined Asexual: <i>Conidiomata</i> pycnidial, to 250 µm diam; <i>Conidiophores</i> hyaline, smooth, aseptate, ampulliform 4–10 × 3–4 µm, with central phialidic locus; <i>Conidia</i> solitary, hyaline, smooth, subcylindrical to oblong, guttulate, apex obtuse, aseptate, (4–)6–8(–9) × 2–4 µm; <i>Hyphae</i> brown, verruculose, and constricted at septa.	Available	Hyde et al. (2020)
<i>D. petrakiana</i> ²	On dry, hanging branches of <i>Crataegus oxyacantha</i> (Rosaceae)	Germany	Sexual: <i>Ascomata</i> scattered or gregarious, circular or elliptical, approximately 300–600 µm long, 200–400 µm wide, 150–250 µm thick or approximately 250–400 µm diam., completely closed, breaking off from the center of the apex when mature, finally irregularly and more or less widely open; <i>Wall</i> 30–40 µm at sides, 50–60 µm at the top, rarely up to 70 µm thick; <i>Asci</i> quite numerous, arranged more or less parallel, cylindraceo-clavate, anteriorly broadly rounded, posteriorly often a little saccate and contracted into a short, thickly nodular stalk, thickly sheathed, 8-spored, p. sp. 70–90 µm, rarely up to 110 µm long, 12–20 µm thick; <i>Ascospores</i> more or less di-rarely indistinctly tristic, fusoid or inequilateral, rarely curved, hyaline, 7–rarely 5–6-septate, pocket in the middle often divided by a longitudinal septum, around the middle a little but usually distinctly, otherwise not or very gently constricted, 20–30 µm rarely up to 32 µm long, 6–10 µm wide; paraphysoids few, fibrous, late muscular. Asexual: Undetermined	N/A	Petrak (1957)
<i>D. phaeosperma</i>	On dead branches of <i>Lonicera coerulea</i> (Caprifoliaceae)	Switzerland	N/A	Available	Froidevaux (1972)
<i>D. phillyreae</i>	On leaf litter of <i>Phillyrea angustifolia</i> (Oleaceae)	Spain (Balears)	Sexual: Undetermined Asexual: <i>Conidiomata</i> solitary or aggregated in a stroma, brown, to 300 µm diam, with central ostiole; <i>Conidiogenous cells</i> hyaline, smooth, aseptate, ampulliform to broadly ellipsoid or doliiform, 5–10 × 5–7 µm, holoblastic with apical locus, inconspicuously phialidic; <i>Conidia</i> hyaline, smooth, subcylindrical to oblong, guttulate, apex obtuse, tapering to a truncate hilum, 1 µm diam (8–)10–11(–12) × (2.5–)3(–3.5) µm; <i>Hormonema-like asexual morph</i> with hyphae becoming brown, verruculose, constricted at septa; <i>Chlamydospore-like cells</i> up to 8 µm diam, older conidia become brown and verruculose, up to 15 µm long, 5 µm diam.	Available	Crous and Groenewald (2016)
<i>D. pinacea</i>	On fallen branch of <i>Pinus sylvestris</i> (Pinaceae)	Czech Republic	N/A	N/A	Mycobank (2024)
<i>D. platyasca</i> ²	In bark	USA (Alabama)	Sexual: <i>Ascomata</i> aggregated, first with a white margin, then flat and black-margined; <i>Asci</i> numerous, ovate or subspheroid; <i>Ascospores</i> oblong-ovate, muriform, colored, 20 × 12 µm. Asexual: Undetermined	N/A	Saccardo (1889)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
<i>D. polyspora</i>	On branch of <i>Populus tremuloides</i> var. <i>aurea</i> (Salicaceae), and <i>Salix</i> spp. (Salicaceae)	USA (Colorado)	Sexual: <i>Ascomata</i> innate-erumpent, depressed-pulvinate, circular or irregular in outline, densely gregarious, smooth, black; <i>Locules</i> single or occasionally several, thick lenticular, astromous; <i>Asci</i> polysporous (24 or more spores), cylindric-clavate, short stipitate, 90–115 × 12–15 µm, paraphysate; <i>Ascospores</i> when mature muriform with 3 transverse septa and frequently 1 or rarely 2 longitudinal septa in the upper cells, clavate, constricted in the middle, upper half broader, hyaline, 15–18 × 5–6 µm. Asexual: In culture; <i>Conidia</i> hyaline, 1-celled, 8–15 × 4–6 µm.	N/A	Shear and Davidson (1940)
<i>D. pruni</i> ²	<i>Prunus emarginata</i> (Rosaceae), on branches of <i>virginiana</i> var. <i>demissa</i> , and <i>P. virginiana</i> var. <i>melanocarpa</i>	Canada and USA	Sexual: <i>Ascomata</i> 385–660 µm diam., 275–440 µm high; <i>Wall</i> 78–104(–195) µm thick; <i>Asci</i> 104–140 × 15–23.5 µm, more than 32-spored (up to 42 counted); <i>Ascospores</i> 13–18 × 5–6 µm, hyaline, obovate, straight, inequilateral or somewhat bent, broadest above and tapered to the pointed base, 3–(4–5)-septate, constricted at primary septum, with vertical septum in one or both mid cells and often in upper end cell. Asexual: Undetermined	N/A	Barr (1972)
<i>D. pruni-padi</i>	On dead branches of <i>Prunus padus</i> (Rosaceae)	Switzerland	N/A	N/A	Index Fungorum (2024)
<i>D. prunorum</i>	On fruit of <i>Prunus domestica</i> (Rosaceae)	UK	Sexual: Undetermined. Asexual: <i>Young hyphae</i> were 3.5–8 µm diam, while older hyphae, having shorter, fatter, thicker-walled cells, were up to 13.5 µm diam. More or less cylindrical phialoconidia (8–20 × 3.3–5 µm) were produced on minute phialides. Several phialoconidia were often produced at one point on the hyphal cell (multiple phialoconidia). Secondary buds on the phialoconidia and chlamydospores were rarely seen. Arthroconidia and endospores were not observed. <i>Yeast-like</i> multiplication of the strains occurred by polar budding of single cells (4–12.5 × 1.5–4 µm) or by phialoconidial formation on centrally constricted septate cells (6.5–14 × 3–6 µm). Polar and lateral phialoconidia were also produced on cells with several septa.	Available	Dennis and Buhagiar (1973); Crous and Groenewald (2016)
<i>D. pyrenophora</i> ¹	On dead branches of <i>Sorbus</i> sp. (Rosaceae) and on twig of <i>Sorbus aucuparia</i> (Rosaceae)	Germany and Sweden	Sexual: <i>Ascostromata</i> solitary to aggregated, black, immersed to erumpent, unilocular, to 400 µm diam, elliptical, pulvinate, opening by an irregular pore, upper layer dissolving with age; <i>Asci</i> bitunicate, hyaline, oblong to subcylindrical, short stipitate, 8-spored, with apiculus, 2–3 diam., 90–120 × 14–17 µm; <i>Ascospores</i> bi- to triseriate in ascus, hyaline, smooth, at times turning yellow-brown with age, fusiform, inequilateral, slightly curved, with prominent mucoid sheath when young (in water), dissolving at maturity, (5–)5(–8) transversely septate, prominently constricted at primary septum, with oblique or vertical septa in central cells, (22–)25–30(–35) × 7–8 µm, ascospores directly giving rise to asexual morph via budding, with ascomata transforming with age into large conidiomata, with apical opening completely dissolving.	Available	Fries (1849); Crous and Groenewald (2017)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			Asexual: <i>Conidiomata</i> immersed to erumpent, pycnidial, black, globose, to 300 µm diam., separate or gregarious, unilocular; <i>Conidiophores</i> lining the inner cavity, reduced to conidiogenous cells; <i>Conidiogenous cells</i> hyaline, smooth, doliform to ampuliform, 4–9 × 4–6 µm, with minute periclinal thickening at apex; <i>Conidia</i> solitary, aseptate, hyaline, smooth, ovate to ellipsoidal, with minute guttules, subobtuse apex, truncate hilum, (5–)7–8(–9) × 3–4 µm.		
<i>D. rhamni</i>	On branches of <i>Rhamni frangulae</i>	Denmark	Sexual: <i>Ascomata</i> usually erupting in longitudinal series, at first orbicular or elongate, flattened, then umbilical, broad-margined, darkly open, with a rugose margin, black, disc concolorous, necleo dirty white, continuous; <i>Asci</i> cylindrical, sessile, usually curved, 8-spored, 112 × 12 µm; <i>Ascospores</i> obliquely monostichous, oblong, attenuated on both sides, strongly constricted in the middle, then equally double, 3-septate and 1–2 longitudinal, pale colored, 15 × 7 µm; <i>Macrostylospores</i> which are sometimes present, broad-oblong, greatly attenuated on both sides, but obtuse, 4–5-septate and muriform, fucus, 20 × 9 µm; paraphyses simple, filiform, multiguttulates. Asexual: Undetermined	N/A	Saccardo (1889)
<i>D. rhamni-alpinae</i>	On dead branches of <i>Rhamnus alpina</i> (Rhamnaceae) and <i>R. purshiana</i>	Canada and Italy	Sexual: <i>Asci</i> octosporous, clavate, up to 24 µm wide; <i>Ascospores</i> 20–30(–32) × 6–9 µm, hyaline, hemispores nearly equal or upper longer than lower. Asexual: Undetermined	Available	Froidevaux (1972); Barr (2001)
<i>D. ribesia</i>	On branches of <i>Ribes</i> sp. (Grossulariaceae)	Canada, England, Morocco, New Zealand, Scotland and USA	Sexual: <i>Ascomata</i> multiloculate, 500–1000 µm diam., rounded pulvinate, the surface plane; upper portion of locules protruding and roughening surface at times; <i>Walls</i> composed of vertically oriented rows of cells forming <i>textura globosa</i> to <i>textura prismatica</i> , blackened externally, often olivaceous to blackish internally, the hyphae penetrating host tissues; locules 60–80 µm diam., 70–100 µm high; <i>Asci</i> 60–72 × 11–12 µm; <i>Ascospores</i> 15–35 × 4.5–8(–14) µm, hyaline, light dull brown in age, narrowly obovate or elliptic (the ends obtusely pointed), straight or slightly curved, (1–)3–5-septate; no vertical septa formed. Asexual: Undetermined	N/A	Barr (1972); MycoBank (2024)
<i>D. rimicola</i> ²	On dead branches of <i>Diervilla lonicera</i> (Caprifoliaceae)	USA	Sexual: <i>Ascomata</i> 500–1500 µm long or longer by confluence, 38–440 µm wide, 220–270 µm high, elongate or elliptic, erumpent in long rows; <i>Wall</i> thick, externally dark brown and 26–39 µm thick, interior layers yellowish or hyaline and 26–35 µm thick, the basal hyaline region forming a raised cushion 52–78 µm deep in mid portion of locule; apex plane or slightly depressed from sides, surface pulverulent dull blackish. <i>Asci</i> 50–70 × 12–15 µm, oblong clavate; <i>Ascospores</i> inequilateral, 17–25 × 4–5 µm, hyaline, obovate, tapered to an obtuse or pointed base, the upper portion broader than the lower, (1–3–)5–7-septate, constricted at supramedian primary septum, with vertical septum in one or more mid cells. Asexual: Undetermined	N/A	Barr (1972)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
<i>D. rufa</i> ³	N/A	N/A	Sexual: Apothecia on barked wood, widely bleached gregariously sessile, orbicular, then oblong, obtuse, with a dark purple disc, surrounded by a very thin black line, not distinctly marginated, usually convex, roughened, 0.3–2 mm long or wide; <i>Asci</i> oval, thickly coated, 60–70 × 30 µm, 8-spored, covered in a gelatinous, yellowish-brown hymen; <i>Ascospores</i> ellipsoidal, transversely 5-, longitudinally 1-septate, initially hyaline, then gray-brown, 25–27 × 9–10 µm Asexual: Undetermined	N/A	Rehm (1912)
<i>D. salicis</i> ⁴	N/A	N/A	N/A	N/A	N/A
<i>D. sambucina</i> ²	<i>Sambucus</i> sp. (Adoxaceae)	USA	Sexual: <i>Ascomata</i> 230–245 µm diam., 180–200 µm high, often erumpent in long rows; wall 26–33(–50) µm thick; apex short and broadly papillate; <i>Asci</i> 52–78 × 15 µm, oblong; <i>Ascospores</i> 22.5–27 × 6–7.5 µm, hyaline, obovate (the upper portion broader than the lower), tapered to an obtusely pointed base, often inequilateral, (1–)3–6-septate, constricted at supramedian primary septum, with vertical septum in one or several of mid cells. Asexual: Undetermined	N/A	Barr (1972)
<i>D. schizospora</i>	On dead stems of <i>Symphoricarpos orbiculatus</i> (Caprifoliaceae)	USA (Missouri)	Sexual: <i>Ascomata</i> solitary or gregarious, innately erupting, orbicular or oblong, flattened, 0.2–1.5 × 0.2–0.7 mm., with parenchymatic context, black, stomatal, rimmed or irregularly dehiscing, dry underclosed, disc dirty white; <i>Asci</i> bitunicate, aparaphysate, subcylindrical or clavate, short pedicellate, octosporous, 70–119 × 11–15 µm; <i>Ascospores</i> hyaline, fusiform, 1–9-septate, phragmospore or submuriform, gently constricted, deeply constricted to the middle of the septum and easily sessile, 20–44 × 4–6 µm, dichotomous or trichotomous. Asexual: Ascomate-like with conidiferous stromata; <i>Conidia</i> (blastospores) ovoid or oblong, hyaline, continuous, 4–9 × 2–4 µm, emerging in pockets made of ovoid or pyriform cells 4.6–6 µm diam.	Available	Luttrell (1960)
<i>D. slippii</i> ¹	On dead branches <i>Pinus albicaulis</i> (Pinaceae)	USA (Idaho)	Sexual: <i>Ascomata</i> 250–300 µm diam., pulvinate, often in rows along a branch; apex plane, opening irregularly; <i>Wall</i> up to 60 µm thick; <i>Asci</i> 48–60 × 11–13 µm, oblong to clavate, parallel from a basal cushion of hyaline cells; <i>Ascospores</i> 17.5–24 × 3.5–4.5 µm, hyaline, narrowly obovate, tapered to pointed ends, straight or slightly curved, 3-septate, slightly constricted at primary septum. Asexual: Undetermined	N/A	Barr (1972)
<i>D. sorbi</i>	On dry branches of <i>Sorbus aria</i> (Rosaceae), <i>S. aucuparia</i> (Rosaceae), and <i>Persea americana</i> (Rosaceae)	France, Germany, Sweden, and Switzerland	Sexual: <i>Asci</i> elongated, 8-spored, 88 µm long, 24 µm thick; <i>Ascospores</i> distichous, oblong, constricted in the middle, thicker in the upper part, 6–7-septate, hyaline, 26 µm long, 5 µm thick, Asexual: <i>Perithecia</i> with spurious, scattered subcaespitosely, under the yellowish epidermis, nestling, globose, striate, of medium size, obtuse to a conical face, prominum, scarious, tapering to the perithecia; <i>Styspores</i> narrowly fusiform, curved, continuous, 14–18 µm long, 2–3 µm thick., guttulates, hyaline	Available	Fuckel (1870); Saccardo (1889)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
<i>D. spartii</i>	On dead aerial branch of <i>Spartium junceum</i> (Fabaceae)	Italy	Sexual: <i>Ascostromata</i> 370–715 µm high, 320–340 µm diameter, immersed in the epidermis, solitary or clustered, globose, brown to black, with single locules; <i>Peridium</i> 30–50 µm wide; <i>Asci</i> 140–175 × 15–22 µm, 8-spored, bitunicate, fissitunicate, cylindro-clavate, short pedicellate, apically rounded, with a small ocular chamber; <i>Ascospores</i> 17–21 × 5–7 µm, bi-seriate to multi-seriate, hyaline, aseptate, fusoid to ovoid, smooth-walled, with granular contents, lacking a mucilaginous sheath. Asexual: Undetermined	Available	Hyde et al. (2017)
<i>D. sphaerioides</i> ²	On dry bark, especially on the scars left behind by fallen branches of <i>Populus tremula</i> (Salicaceae) and <i>Populus</i> spp.	Canada and Germany	Sexual: <i>Asci</i> oblong, sessile, 8-spored, 100 µm long, 12 µm thick; <i>Ascospores</i> distichous, oblong-clavate, constricted in the middle, 5-septate, muriform, hyaline, 19 µm long, 6 µm thick. (in the wider part); <i>Macrostylospores</i> with mixed asci, oblong, obtuse on both sides, shortly pedicellate, 5-septate, muriform, pale yellow, 28–36 µm long, 14 µm thick. Asexual: <i>Perithecia</i> spurious, superficial, gregarious, minute, total 240 µm long, base 128 µm thick, cylindrical-conical, 60 µm wide, 160 µm long, sometimes with a severed beak, fearful; <i>Stylospores</i> cylindrical, curved, obtuse on both sides, simple, 6 µm long, 1.5 µm thick.	N/A	Fuckel (1870); Barr (1972)
<i>D. staphyleae</i>	On dry branches of <i>Staphylea pinnata</i> (Staphyleaceae)	Germany	Sexual: <i>Ascomata</i> with seriate, often surrounding the entire branch densely and tightly, with a globose-closed subepidermis, then a longitudinal crack v. sometimes breaking out triangularly, black, finally breaking off and falling apart; <i>Asci</i> oblong-clavate, octosporous, 100 µm or longer, 12–16 µm wide; <i>Asci</i> distichous, oblong, attenuated on both sides, but obtuse, 4–6-septate, constricted in the middle and unequally bifurcated, with a few muriform-divided pockets, 25–30 × 6–8 µm, hyaline. Asexual: Undetermined	N/A	Saccardo and Sydow (1902)
<i>D. staphylina</i>	<i>Staphylea trifolia</i> (Staphyleaceae)	USA	Sexual: <i>Ascomata</i> 208–440 µm diam, 117–220 µm high, uni- or multi-loculate; Wall 26 µm thick at sides, up to 90 µm at lower sides at times; apex plane, the pore area pallid under dissecting microscope; <i>Asci</i> 65–90 × 12–16 µm, oblong; <i>Ascospores</i> 18–22.5 × (4–)5–6(–7) µm, hyaline, obovate (the upper portion broader than the lower), straight to inequilateral, tapered to an obtuse base, (1–)3–5(–7)-septate, slightly constricted at the primary septum, with vertical septum in one or two of mid cells. Asexual: Undetermined	N/A	Barr (1972)
<i>D. stictoides</i> ²	On branches of <i>Liriodendron</i> sp. (Magnoliaceae)	USA	Sexual: <i>Ascomata</i> scattered, connected by thick, black mycelia under host epidermis, immersed, subcuticular, becoming erumpent, pulvinate, uni- to multi-loculate, flat to broadly rounded at apex, subiculum at base, 200–225 µm high, 370–400 µm wide; <i>Pseudoparaphyses</i> indistinguishable, sometimes of elongated, compressed pseudoparenchymatic cells between asci, sometimes with thickened septa at both ends, within a gelatinous matrix; <i>Asci</i> 110–140 × 17–26 µm, fissitunicate, numerous, arising from hyaline cells or low, disk-shaped hyaline cells, of various numbers in each locule, at irregular	N/A	Ahn and Shearer (1998)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			heights in hymenial layer, oblong with rounded apex, thin walled throughout or somewhat thick at apex, with eight irregularly biseriate ascospores, short stalked, surrounded by layer of thin gelatinous material, lacking an apical chamber; <i>Ascospores</i> 24–34 × 10–13 µm, hyaline to olivaceous, obovate, straight to slightly curved, rounded at apex or sometimes acute, gradually tapered to base, with five to seven transverse septa, septa sometimes slanted, none to six longitudinal septa, sometimes more than one longitudinal septum in a cell, primary septum supramedian, constricted at primary septum, smooth walled with thin gelatinous sheath. Asexual: Undetermined		
<i>D. symloci</i>	On leaves of <i>Symplocos spicata</i> (Symplocaceae)	Sri Lanka	N/A	N/A	Petrak (1937)
<i>D. syringae</i>	On dead branch of <i>Syringa vulgaris</i> (Oleaceae)	Russia	Sexual: <i>Stromata</i> crowded, arranged serially, sometimes subconfluent, bursting through the bark, flattened, various shapes, such as angular-rounded or angular-elongate, smooth, naked, black, very hard when dry, up to 1 mm wide; <i>Spermatia</i> ovoid or oval, simple, hyaline, 4–6 µm long, 3 µm thick. Asexual: Undetermined	N/A	Karsten (1884)
<i>D. tamaricis</i>	On dead leaves and dry branches of <i>Tamarix gallica</i> (Tamaricaceae)	Portugal	Sexual: <i>Ascomates</i> nestling and erupting in the bark, subglobose, 400 µm diam., black; <i>Asci</i> ovate or clavate, apex rounded, thickly coated, 90–110 × 40–55 µm, non-coerulescent iodine, octospores; <i>Ascospores</i> distichous or irregular, oblong-fusoid, 3-septate, constricted at the middle septum, straight, covered with a mucous layer, made of hyaline olivaceous-brown, 30–42 × 12–14 µm. Asexual: Undetermined	N/A	Dennis et al. (1977) ; Index Fungorum (2024)
<i>D. taxicola</i>	On leaves of <i>Taxus</i> spp. (Taxaceae)	Canada, England and USA	Sexual: <i>Ascomata</i> 130–240 µm diam., 145–165 µm high, globose or depressed, immersed with rounded erumpent apex, epiphyllous, thickly scattered; <i>Wall</i> 14–30 µm thick, consisting of several layers of polygonal cells, blackened externally; <i>Apical pore region</i> stuffed with lighter brown or hyaline cells before maturity; <i>Asci</i> 60–96 × 9–14.5 µm clavate, arising from a low dome-shaped cushion of hyaline cells; <i>Ascospores</i> 13–18.5 × 3–5 µm, hyaline or yellowish, narrowly elliptic or obovate, tapered to pointed ends, straight to slightly curved, (1–) 3-septate, not constricted at septa; contents minutely guttulate, smooth-walled. Asexual: <i>Pycnidia</i> immersed, depressed, 418–435 µm diam., 243–352 µm high, multiloculate; <i>Conidiophores</i> short, 7–15 × 1.5–2 µm; <i>Conidia</i> 3–5 × 1 µm, hyaline, one-celled.	N/A	Barr (1972)
<i>D. thujae</i>	On cone scales of <i>Thuja occidentalis</i> (Cupressaceae)	Great Britain and USA	Sexual: <i>Ascomata</i> 130–245 µm diam., nearly globose, single or few grouped together and connected by hyphae; wall 20–50 µm thick, thickest at base; apex rounded papillate; <i>Asci</i> 37.5–63 × 18–27 µm, broadly oblong; <i>Ascospores</i> 20–30 × 6–9 µm, yellowish brown, obovate, with ends obtuse, straight to inequilateral, (3–)5 (–7)-septate, constricted at the primary and less at the secondary septa,	N/A	Barr (1972)

(Continued)

TABLE 2 Continued

<i>Dothiora</i> species	Host	Locality	Morphology	Sequence data	References
			with vertical septum (rarely two) in mid cells, occasionally extending into the apical cell; thin gelatinous coating 1.5–2 µm thick at times surrounding ascospores. Asexual: Undetermined		
<i>D. vaccinii</i>	On dry stems and branches of <i>Vaccinium uliginosum</i> (Ericaceae)	Switzerland	Sexual: <i>Ascomata</i> breaking out, finally very prominent, gregarious, usually covering the entire branches, brownish-black, 1 mm diam., rounded but usually elliptical and irregular, with a raised edge, swollen, concave apex, first carbonaceous, concolorous, finally irregularly fissured and exposed to a dirty disc; <i>Asci</i> clavate, 8-spored, 74 µm long, 12 µm thick (in the clavula); <i>Ascospores</i> filled, oblong-ovate, obtuse on both sides, slightly curved, continuous, hyaline, 12 µm long, 6 µm thick. Asexual: Undetermined	N/A	Fuckel (1875)
<i>D. valdiviana</i>	On branches of <i>Saxegothaea conspicua</i> (Podocarpaceae)	Chili (Biobío)	Sexual: <i>Ascomata</i> solitary or gregarious, intraepidermal, innately erupting, flattened, black, irregularly orbicular, 100–300 µm diam.; <i>Asci</i> bitunicate, broadly clavate or obovoid, 8-spored, 30–40 × 16–20 µm, paraphysate, arranged in pale multiseriate pockets; <i>Ascospores</i> distichous or tristichous, ellipsoid or clavate, hyaline, phragmospore or muriform, gently constricted at the middle of the septum, 12–16 × 6–7 µm. Asexual: Undetermined	N/A	Butin (1973)
<i>D. versiformis</i> ²	On dead branch of <i>Sorbus sitchensis</i> (Rosaceae)	British Columbia	Sexual: <i>Ascomata</i> 440–660 µm diam., 330–440 µm high, elliptic or rounded from above, single locule; <i>Wall</i> 60–80 µm thick at sides and upper surface, up to 180 µm deep at base; <i>Asci</i> polysporous, 120–140 × 20–26 µm, broadly oblong, pararella; <i>Ascospores</i> 8–10 × 3.5–4.5 µm, 1-celled or 1-septate, 17–25 × 6–9 µm, 3–5-septate, hyaline to brown, elliptic or obovate, often broadest above and tapered to the pointed base, constricted at primary septum or often all septa, with vertical septum in one or more mid cells. Asexual: Undetermined	N/A	Barr (1972); Barr, (2001)
<i>D. viburnicola</i>	On dead leaf of <i>Viburnum tinus</i> (Adoxaceae)	Italy	Sexual: Undetermined Asexual: <i>Conidiomata</i> pycnidial, globose with long neck, brown, to 250 µm diam, with central ostiole, exuding a creamy conidial mass; <i>Conidiophores</i> reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, ampulliform to doliiform, 5–7 × 5–6 µm, with central phialidic locus; <i>Conidia</i> hyaline, smooth, guttulate, subcylindrical, apex obtuse, tapering to a truncate hilum (6.5–)8–10(–13) × (2–)2.5(–3) µm	Available	Crous and Groenewald (2016)
<i>D. viticola</i>	Fruit (grapes) of <i>Vitis vinifera</i> cv. Malvasia (Vitaceae)	Spain	Sexual: Undetermined Asexual: <i>Mycelium</i> composed of hyaline, branched, strongly septate hyphae, smooth- and thin-walled, swollen at septa, 3.5–6 µm diam, becoming monilliform and dark brown with age due to the production of solitary to catenate chlamydospores of up to 20 µm diam, with some segments remaining hyaline or nearly so; <i>Conidiogenous cells</i> integrated on hyphae, intercalary or terminal, inconspicuously to conspicuously denticulate; <i>Conidia</i> holoblastic,	Available	Crous et al. (2015); Crous et al., (2022)

(Continued)

TABLE 2 Continued

Dothiora species	Host	Locality	Morphology	Sequence data	References
			at first synchronously produced in small groups on lateral protrusions of the hyphae on short (1 µm long) conic-truncate denticles, and later percurrently produced along the hyphae and on side branches from larger denticles (1.0–1.5 µm long); <i>Conidia</i> hyaline at first, mostly aseptate, sometimes septate at the middle and slightly constricted at septa, smooth and thin-walled, variable in shape but mostly ellipsoidal, clavate at both ends when septate, 5–17 × 4–10 µm, becoming dark brown and thick-walled with the age, smooth-walled to granulose due to deposition of a dark pigment on the cell surface, 12–20 × 4–12 µm. <i>Microcyclic conidia</i> produced by budding of both hyaline and pigmented conidia, produced singly or in chains of up to 4, at one or both ends but sometimes laterally, being smaller than the primary ones. Endoconidia also present in hyaline segments of hyphae, ellipsoidal, hyaline, 4–6 × 3–4 µm.		
<i>D. wolffii</i> ¹	On branches of <i>Oxydendmm arboretum</i> (Ericaceae)	USA (North Carolina)	Sexual: <i>Ascomata</i> 200–250 µm diam, 150–200 µm high, depressed globose, grouped at times in dark reddish-brown areas on a branch, or scattered; <i>Wall</i> 20–55 µm thick; <i>Asci</i> 55–70 × 12–14.5 µm, oblong, parallel from a flattened basal cushion of hyaline cells; <i>Ascospores</i> 17.5–22 × 4.5–6 µm, hyaline, narrowly obovate, tapered to obtuse ends, inequilateral to slightly curved, (2–)3–5-septate, constricted at primary septum. Asexual: Undetermined	N/A	Barr (1972)
<i>D. xylostei</i>	On dry trunks and thinnest branches of <i>Lonicera xylosteum</i> (Caprifoliaceae)	Germany	Sexual: <i>Spermogonia</i> like a fungus, but smaller; <i>Spermatia</i> oblong-ovate, continuous, hyaline, 6–8 µm long, 3–4 µm thick. The cups of the ascigerus are sparse, bursting through the cracks in the bark, ½ µm long., circular or oblong, flat, sometimes with a protruding or irregular edge, black, dirty inside, cornified; <i>Asci</i> oblong, sessile, unequally bisected, 4-septate, constricted below the middle, a slightly thicker part uniseptate, a narrower part biseptate, straight, hyaline, 20 µm long., 4–5 µm thick. Asexual: Undetermined	N/A	Fuckel (1871)

N/A, no information available
¹Based on the Species Fungorum; the taxon was synonymized under other genera.
²Based on the MycoBank database; the taxon was synonymized under other genera.
³The species shows as Nom. inval., Art. 36.1(c) (Melbourne) in Index Fungorum, but this became legitimate in MycoBank.
⁴The species is regarded as an illegitimate name according to MycoBank (2024), but it was shown as valid in Index Fungorum (2024).

from related species in having polysporous asci, while other species have octosporous asci. These species can be distinguished from each other based on their asci and ascospores sizes; moreover, *D. capparis* can be distinguished from *D. coronillae*, *D. coronillicola*, *D. spartii*, and *D. uzbekistanica* by the presence of a mucilaginous sheath surrounding the ascospores. *Dothiora cactacearum* also clustered among these species; however, its morphological characteristics cannot be compared with others, as it is known only by its asexual morph. *Dothiora buxi* is phylogenetically close to *D. cactacearum*, and both species have similar asexual morphs to the generic, as described by Thambugala et al. (2014) and Crous and Groenewald (2017). The morphology of ascospores of *Dothiora buxi*, *D. capparis*, *D. coronillae*, *D. coronillicola*, *D. rhapontici*, *D. spartii*, and *D. uzbekistanica*, was clearly distinct from that of most *Dothiora*; however, only this evidence did not have enough support to accommodate a distinct lineage in Dothideaceae. Furthermore, an asexual morph of these species has not been cultured or reported to verify its morphological features, except for *D. buxi*. Due to the fact that most of the available sequences of *Dothiora* are only LSU and/or ITS, their taxonomic position remains uncertain. The taxonomic classification of *Dothiora* species is still incomplete; further investigations of freshly collected specimens in different regions and sequence data are needed to better understand their natural classification.

Crous et al. (2018b) transferred *Kabatina mahoniae* A.W. Ramaley to *Dothiora* as *D. mahoniae*. According to the multigene analyses herein (Figure 1), *Dothiora mahoniae* (strain CBS 264.92) clustered with *Neodothiora populina* Crous, G.C. Adams & Winton (strain CBS 147087) with no statistical support for this relationship and separated from *Dothiora* species, which is consistent with Crous et al. (2020). Based on a comparison of morphology, *Dothiora mahoniae* fits well with the generic concepts of *Kabatina* rather than *Dothiora* and *Neodothiora* (Ramaley, 1992; Thambugala et al., 2014; Crous and Groenewald, 2017; Crous et al., 2020). In addition, only LSU and ITS are available for *D. mahoniae* in GenBank. Thus, the species is retained until more evidence of fresh collections with DNA sequence data is available to resolve its phylogenetic placement within the family.

In this study, three new species of hyaline-spored *Dothiora* (*D. capparis*, *D. rhapsodici*, and *D. uzbekistanica*) are described and illustrated. It is interesting to note that *Dothiora* have morphological variability in their spores. Thus, it is inadequate to determine *Dothiora* spp. based solely on morphological data. It can be seen that phylogenetic analyses are necessary to confirm morphology-based identifications and detect species new to science. Many *Dothiora* on the list (Table 2) have not been verified yet based on the molecular data; however, these species have characteristics that match the generic description. Additionally, the classification of several species remains unclear due to the variability in some morphological characters, a lack of molecular information regarding protein-coding genes, and no sexual–asexual links. Hence, these species are not excluded from *Dothiora* until increased taxon samplings and sequence data are available. Further sampling is necessary to improve our knowledge of the diversity, ecology, and impacts of hyaline-spored *Dothiora* species on flowering plants in arid and semi-arid habitats.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/genbank/>, ITS: PP086677, PP086678, PP086679, PP086680, PP086681, PP086682, PP086683 and PP086684; LSU: PP086685, PP086686, PP086687, PP086688, PP086689, PP086690 and PP086691; SSU: PP086692, PP086693, PP086694, PP086695, PP086696 and PP086697; TEF1: PP084936, PP084937, PP084938, PP084939, PP084940, PP084941, PP093832 and PP093833.

Author contributions

CS: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. SH: Conceptualization, Formal

analysis, Project administration, Supervision, Visualization, Writing – review & editing. SK: Formal analysis, Investigation, Visualization, Writing – review & editing. JK: Data curation, Investigation, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. MY: Investigation, Resources, Visualization, Writing – review & editing. YG: Conceptualization, Data curation, Investigation, Project administration, Resources, Supervision, Visualization, Writing – review & editing. AA: Investigation, Resources, Visualization, Writing – review & editing. NS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY

Sinang Hongsan,
Shenzhen University, China

REVIEWED BY

Davide Palmieri,
University of Molise, Italy
Jaturong Kumla,
Chiang Mai University, Thailand
Terd Disayathanooat,
Chiang Mai University, Thailand

*CORRESPONDENCE

Hancheng Wang
✉ xiaobaiyang126@hotmail.com
Feng Wang
✉ 584228992@qq.com
Yingqian Kang
✉ kangyingqian@gmc.edu.cn

[†]These authors share first authorship

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Phyllospheric microbial community structure and carbon source metabolism function in tobacco wildfire disease

Xia Xu^{1†}, Liang Zhao^{1†}, Yanfei Chen¹, Hancheng Wang^{2*}, Liuti Cai², Yanyan Wang¹, Nalin N. Wijayawardene³, Weihua Pan⁴, Feng Wang^{2*} and Yingqian Kang^{1*}

¹Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education of Guizhou & Institute of Health Research & Key Laboratory of Medical Microbiology and Parasitology, School of Basic Medical Sciences, Guizhou Medical University, Guiyang, China, ²Guizhou Provincial Academician Workstation of Microbiology and Health, Guizhou Academy of Tobacco Science, Guiyang, China, ³Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan, China, ⁴Department of Dermatology, Changzheng Hospital, Shanghai, China

The phyllospheric microbial composition of tobacco plants is influenced by multiple factors. Disease severity level is one of the main influencing factors. This study was designed to understand the microbial community in tobacco wildfire disease with different disease severity levels. Tobacco leaves at disease severity level of 1, 5, 7, and 9 (L1, L5, L7, and L9) were collected; both healthy and diseased leaf tissues for each level were collected. The community structure and diversity in tobacco leaves with different disease severity levels were compared using high-throughput technique and Biolog Eco. The results showed that in all healthy and diseased tobacco leaves, the most dominant bacterial phylum was Proteobacteria with a high prevalence of genus *Pseudomonas*; the relative abundance of *Pseudomonas* was most found at B9 diseased samples. Ascomycota represents the most prominent fungal phylum, with *Blastobotrys* as the predominant genus. In bacterial communities, the Alpha diversity of healthy samples was higher than that of diseased samples. In fungal community, the difference in Alpha diversity between healthy and diseased was not significant. LEfSe analysis showed that the most enriched bacterial biomarker was unclassified_Gammaproteobacteria in diseased samples; unclassified_Alcaligenaceae were the most enrich bacterial biomarker in healthy samples. FUNGuild analysis showed that saprotroph was the dominated mode in health and lower diseased samples, The abundance of pathotroph–saprotroph and pathotroph–saprotroph–symbiotroph increases at high disease levels. PICRUST analysis showed that the predominant pathway was metabolism function, and most bacterial gene sequences seem to be independent of the disease severity level. The Biolog Eco results showed that the utilization rates of carbon sources decrease with increasing disease severity level. The current study revealed the microbial community's characteristic of tobacco wildfire disease with different disease severity levels, providing scientific references for the control of tobacco wildfire disease.

KEYWORDS

tobacco wildfire disease, microbial community, high-throughput sequencing, biolog-eco, disease severity

1 Introduction

Tobacco (*Nicotiana tabacum* L.) is an extensively cultivated economic crop in China, whose growth and production are affected by a myriad of biotic and abiotic factors (Ma et al., 2023). Tobacco wildfire disease is caused by *Pseudomonas syringae* pv. *tabaci*, which results in heavy economical losses (Tumewu et al., 2022). The typical symptoms of tobacco wildfire disease include small brown or black lesions bordered by chlorotic halos on tobacco leaves (Sun et al., 2021). The characteristic chlorotic halos are produced by a toxin, tabtoxin (Ichinose et al., 2013).

The phyllosphere is an important habitat for many microorganisms, including commensal, beneficial, and pathogenic microbes affecting plant health and productivity (Lindow and Brandl, 2003; Xin et al., 2018; Zhu et al., 2022). It is notable to study the changes in microbial communities (natural inhabitants) when the host plant is stressed under an infection (Perreault and Laforest-Lapointe, 2022). The development of PCR and sequencing technologies (including high-throughput sequencing) has facilitated the exploration of microbial diversity and structure of microbial communities (Ahmed et al., 2022b; Fitzpatrick et al., 2020; Gong and Xin, 2021).

Tobacco bacterial wilt is caused by *Ralstonia solanacearum* and is reported to reduce the leaf fungal community abundance and diversity (Ahmed et al., 2022a), whereas a similar response was observed by Si et al. (2023) during the investigation of tobacco wildfire disease caused by *P. syringae* pv. *tabaci*. Chen et al. (2020a) noticed a much higher abundance of *Rhizopus oryzae* in cured tobacco leaves infected by tobacco pole rot than that of healthy leaves. The phyllospheric microbial community and composition are closely related to plant health (Zhang et al., 2019).

Disease severity level also affects the leaf microbiome, influencing the dynamics of phyllosphere microbial communities. Huang et al. (2021) studied the influence of the disease severity level on the community structure and diversity of the tobacco leaf spot disease caused by *Didymella segeticola*, identifying that the community diversity of phyllosphere fungi decreased with the increase in the disease severity level. Sun et al. (2022) studied the microbial community of tobacco leaves affected by target spot at different disease severity levels. The results indicate that fungal diversity and relative abundance increased with disease severity level, while bacterial diversity indices decreased. Luo et al. (2019) studied the microbial community structure of symptomatic cucumber leaves affected by angular leaf spot at different disease severity levels. The investigation showed that the pathogen caused an increase in the microbial community richness when disease pressure was higher. Biolog Eco can reflect the main types of carbon source utilization and the strength of metabolic functions of all microbial communities (Rutgers et al., 2016). This technique has been used to identify the carbon-source-associated metabolic pathways of phyllosphere microbial communities in both healthy and diseased tobacco plants (Feng et al., 2023; Shen et al., 2023). However, the effect of disease severity level on the structure and diversity of tobacco wildfire disease phyllosphere microbial community is still poor understood.

Tobacco wildfire disease can lead to serious damage. It is important to understand the microbial community changes in tobacco leaves. Therefore, the goals of this study were to identify

the structure and diversity of phyllosphere bacterial and fungal communities and the metabolism of carbon sources of tobacco microorganisms in tobacco leaves with different disease severity levels. These results will provide theoretical reference for the prevention and control of wildfire disease in tobacco.

2 Materials and methods

2.1 Sampling sites and sampling strategy

In Jun 2022, one tobacco field (cultivar Yunyan 87) with symptoms of tobacco wildfire disease in Dafang County (27°12'15" N, 105°57'18" E), Bijie City, Guizhou Province of China, was selected as the sampling site. The tobacco leaves of different disease severity levels (L1, L5, L7, and L9) were randomly selected based on the Chinese National Standard (GB/T 23222-2008). For L1, L5, L7, and L9 groups, diseased lesion area ranged from 0% to 1%, 6% to 10%, 11% to 20%, and 11% to 100% of a leaf, respectively (Figures 1A–E). There were three biological replicates from each group. A total of 24 samples were collected. The four groups of leaves were separated into two parts, with and without visible leaf spots (Figure 1F), and labeled as diseased and healthy samples, respectively, as presented in Table 1. After sampling, the samples were immediately transported to the laboratory and stored at –80°C until further use.

2.2 DNA extraction, PCR amplification, and high-throughput sequencing

Total DNA was extracted from the leaf samples using the PF Mag-Bind Stool DNA Kit (Omega Bio-Tek, GA, USA) according to the manufacturer's instructions. The quality and concentration of DNA were determined by agarose gel electrophoresis. The hypervariable regions V5–V7 of the bacterial 16S rRNA gene were amplified with primer pairs 799F (5'-AACMGGATTAGATACCCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') (Bulgarelli et al., 2015). Furthermore, the primer pair ITS1F (5'-CTTGGTCATTTAGAGG AAGTAA-3') and ITS2R (5'-GCTGCGTTCCTTCATCGATGC-3') (Adams et al., 2013) were used to amplify the fungal ITS1 region. PCR products were checked by 2% agarose gel electrophoresis. The amplification products were amplified using an Illumina MiSeq platform and constructed the library.

2.3 Sequencing data processing

Qualitative filtering and merging of raw sequencing reads were performed using Flash (version 1.2.11) (Magoč and Salzberg, 2011). All sequences were clustered into operational taxonomic units (OTUs) by Uparse (version 11) (Edgar, 2013) with a 97% similarity cutoff. Subsequently, the OTUs were annotated for bacterial and fungal based on the Silva 16S rRNA (v138) and Unite (version 8.0) database, respectively, setting the confidence threshold to 70% (Huang et al., 2021; Sun et al., 2022). The Alpha and Beta diversities were performed by Mothur (version 1.30.2) and Qiime (2020.2.0), and

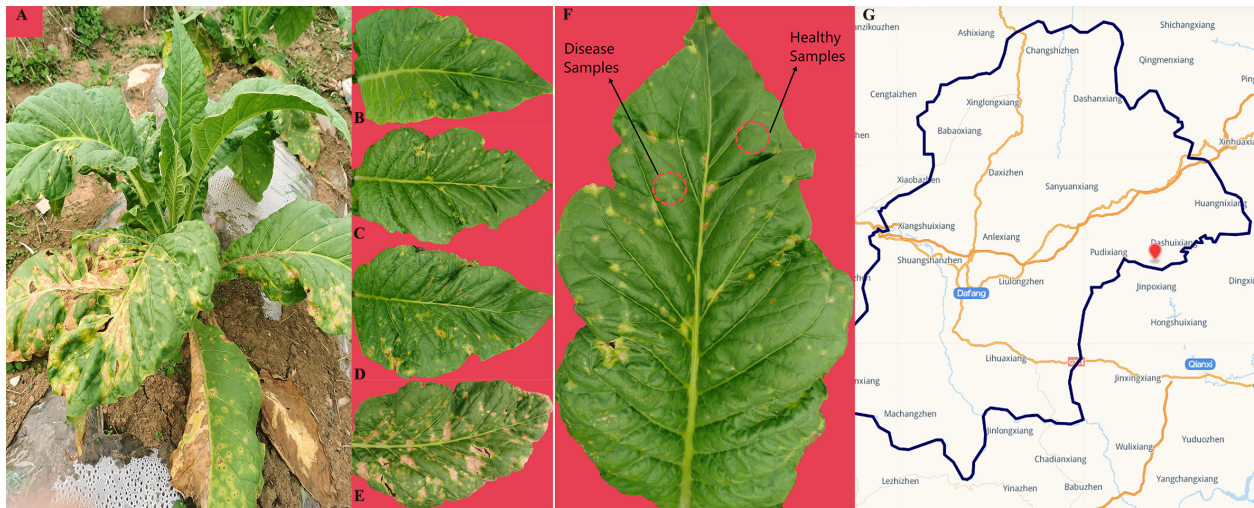


FIGURE 1 Whole plant (A) and leaves displaying disease severity at (B) L1, (C) L5, (D) L7, and (E) L9, respectively. (F) sampling parts display of diseased and healthy samples on tobacco leaves. (G) map of sampling site.

the R statistics package (version 3.3.1) was utilized to draw the principal coordinate analysis (PCoA) diagram. The linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al., 2011) was performed to detect the potential biomarker, using a linear discriminant analysis threshold of 4.0. The PERMANOVA test was used to calculate significant differences. PICRUSt and FUNGuild (Douglas et al., 2020) the databases were used to analyze enzyme functional and fungal trophic mode. Bacterial metabolic functions were analyzed with the bioinformatics software package PICRUSt (Barberán et al., 2012). The correlation of phyllosphere microorganisms was demonstrated using Spearman's rank analysis based on the absolute value of a correlation coefficient >0.6 and a *p*-value <0.05 (Nguyen et al., 2016).

2.4 Biolog inoculation and analysis

Each of the 1-g samples from different disease severity levels was mixed with 50 ml of 0.85% NaCl and shaken (180 rpm) for 2 h at 28°C. The mixture was standing for 30 min, and 100 µL of dilution was added using an electronic pipette and incubated at 28°C in a Biolog OmniLog incubator for 7 days (Dai et al., 2022). The Biolog OmniLog system was equipped with a charge-coupled device

TABLE 1 Sample information for both diseased and healthy tobacco leaves with infected tobacco wildfire disease at different disease severity.

Disease severity	Proportion of lesion area every leaf	Diseased groups (sample)	Healthy groups (sample)
L1	≤1%	B1 (B11, B12, B13)	J1 (J11, J12, J13)
L5	6%–10%	B5 (B51, B52, B53)	J5 (J51, J52, J53)
L7	11%–20%	B7 (B71, B72, B73)	J7 (J71, J72, J73)
L9	≥21%	B9 (B91, B92, B93)	J9 (J91, J92, J93)

camera system and an incubator (Feng et al., 2023). A Heatmap Illustrator (HemI 1.0.3.3) was used to analyze the microbe carbon metabolism of the samples.

2.5 Statistical analysis

The mean value and standard error were used to express the measured results. One-way ANOVA or nonparametric test Kruskal–Wallis was used to compare the differences between multiple groups. *p* ≤ 0.05 was considered to be statistically significant.

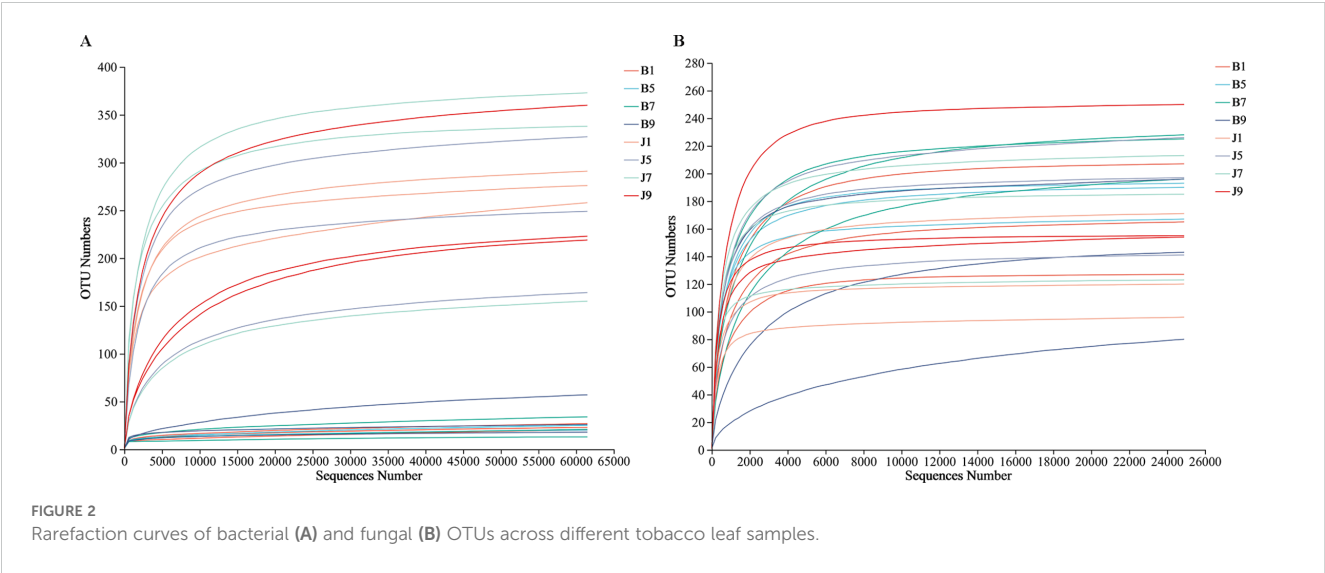
3 Results

3.1 Quality of total bacterial and fungal sequence data

A total of 2,277,441 bacterial sequences were obtained through sequencing of 24 samples. These sequences were classified into 845 OTUs at a 97% similarity level. For fungal sequences, a total of 1,681,293 sequences were classified into 389 OTUs across the 24 samples. Rarefaction curves for each sample reached a plateau, indicating sufficient sequencing depth to capture most microorganisms present (Figure 2). All the bacterial and fungal sequences were deposited in the SRA database under accession numbers PRJNA1096149 and PRJNA1096455, respectively.

3.2 Distribution and diversity of bacterial and fungal operational taxonomic units

Venn diagrams (Figure 3) show the distribution of the OTUs in the four disease severity levels. For bacterial community, a total of 845



OTUs were obtained, including 111 belonging to the diseased group and 842 to the healthy group (Figures 3A, B). The number of shared OTUs among the four diseased groups was 20; there were 14, 15, 9, and 36 unique OTUs found only in B1, B5, B7, and B9, respectively. Meanwhile, the number of shared OTUs among the four health groups was 214, and the groups J1, J5, J7, and J9 only had 82, 46, 104, and 33 unique OTUs, respectively. Notably, the number of bacterial OTUs in the diseased samples was lower than that in the healthy samples. We compared the bacterial Alpha diversity of the tobacco

leaves using the diversity index (Shannon index) and the richness indices (Ace and Chao1 index) in Table 2. The higher the Shannon value, the greater the level of diversity. The higher the Ace and Chao1 value, the greater the level of richness. The Shannon index of diseased and healthy samples ranged from 1.31 ± 0.33 to 1.44 ± 0.17 and 2.11 ± 0.87 to 2.66 ± 1.44 , respectively. The Ace index ranged from 31.34 ± 14.73 to 55.72 ± 37.05 and 332.30 ± 103.30 to 404.90 ± 170.80 for diseased and healthy samples, respectively, whereas the Chao1 index ranged from 26.42 ± 12.45 to 49.11 ± 36.39 for diseased and $334.30 \pm$

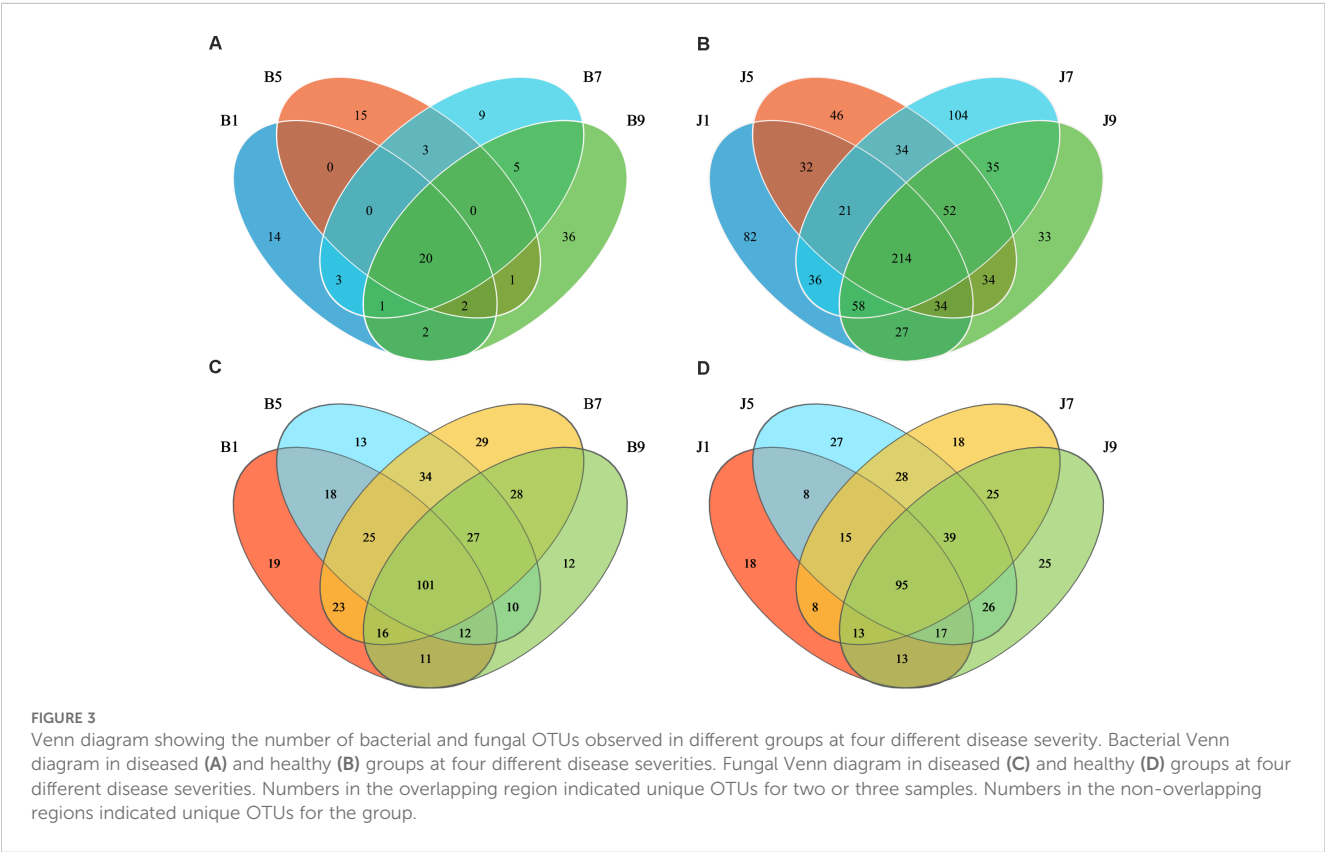


TABLE 2 Alpha-diversity indices of bacterial and fungal communities based on high-throughput sequencing in different samples.

	Disease severity groups	Richness index		Diversity index	Coverage
		Ace	Chao1	Shannon	Coverage
Bacteria	B1	35.91 ± 1.24a	33.33 ± 1.86a	1.39 ± 0.28a	1.000
	B5	33.99 ± 7.97a	31.70 ± 7.43a	1.31 ± 0.33a	1.000
	B7	31.34 ± 14.73a	26.42 ± 12.45a	1.44 ± 0.17a	1.000
	B9	55.72 ± 37.05a	49.11 ± 36.39a	1.42 ± 0.23a	1.000
	J1	380.60 ± 34.04b	371.72 ± 21.74b	2.54 ± 0.28b	0.999
	J5	332.30 ± 103.30b	334.30 ± 106.50b	2.11 ± 0.87ab	0.999
	J7	404.90 ± 170.80b	404.20 ± 166.70b	2.66 ± 1.44ab	1.000
	J9	369.00 ± 111.40b	368.30 ± 108.00b	2.28 ± 0.77ab	0.999
Fungi	B1	125.50 ± 18.91a	127.00 ± 16.56a	2.62 ± 0.34a	1.000
	B5	133.10 ± 8.18a	132.90 ± 8.26a	3.54 ± 0.14a	1.000
	B7	171.60 ± 7.86a	175.60 ± 5.74a	3.11 ± 0.78a	1.000
	B9	121.30 ± 33.89a	121.40 ± 38.26a	2.21 ± 1.45a	0.999
	J1	94.78 ± 17.94a	94.78 ± 17.43a	3.02 ± 0.42a	1.000
	J5	146.00 ± 29.09a	147.30 ± 31.41a	3.55 ± 0.38a	1.000
	J7	132.30 ± 34.55a	133.80 ± 37.27a	3.77 ± 0.25a	1.000
	J9	138.20 ± 37.90a	138.30 ± 38.08a	3.41 ± 0.42a	1.000

Different letters in column direction represent having significant difference between groups (p <0.05).

106.50 to 404.20 ± 166.70 for healthy samples. Correspondingly, the diversity indices Ace, Chao1, and Shannon of healthy samples were greater than those of diseased samples, and the diversity index decreased significantly with increased disease severity level from L1 to L5 and then increased with the increase in the degree of disease from L5 to L9.

In the fungal community, a total of 389 OTUs were obtained, with 378 OTUs in the diseased samples and 375 in the healthy group (Figures 3C, D). The four diseased and four healthy samples contained 101 and 95 shared OTUs, respectively. The unique OTU numbers in the four diseased groups were 19, 13, 29, and 12, respectively, while in the healthy group, the numbers were 18, 27, 18, and 25, respectively. The number of fungal OTUs in the diseased samples was higher than that in the healthy, but there was little difference. Similarly, the Shannon index of diseased and healthy samples ranged from 2.21 ± 1.45 to 3.54 ± 0.14 and 3.02 ± 0.42 to 3.77 ± 0.25, respectively (Table 2). The Ace index ranged from 121.30 ± 33.89 to 171.60 ± 7.86 and 94.78 ± 17.94 to 146.00 ± 29.09 for diseased and healthy samples, respectively, whereas the Chao1 index ranged from 121.40 ± 38.26 to 175.60 ± 5.74 for disease and 94.78 ± 17.43 to 147.30 ± 31.41 for healthy samples. The Ace and Chao1 indices were the highest at B7 and J5 in the disease and health groups, respectively. The Shannon index was the highest at B5 and J7 in the disease and health groups, respectively. Meanwhile, the Shannon index of healthy samples was greater than that of diseased samples, which indicated that the fungal diversity in the healthy group is higher than the diseased group.

3.3 Bacterial and fungal community composition

3.3.1 Bacterial community composition

The 16S dataset showed that the phyllosphere bacteria of diseased and healthy samples contain six phyla of bacteria (Table 3): Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Myxococcota, and Chloroflexi (Figure 4A). Proteobacteria was the predominant phylum, with relative abundances in the diseased groups (B1, B5, B7, and B9) being 99.95%, 99.97%, 99.25%, and 98.97%, respectively, while in healthy groups (J1, J5, J7, and J9), the relative abundance were 66.89%, 84.56%, 79.12%, and 86.06%, respectively. The relative abundances of Actinobacteria in the diseased groups (B1, B5, B7, and B9) were 0.04%, 0.02%, 0.02%, and 0.08%, respectively; the relative abundances in healthy groups (J1, J5, J7, and J9) were 14.17%, 7.49%, 10.72%, and 6.31%, respectively. Notably, the relative abundance of Proteobacteria in diseased samples were higher than those in healthy samples. Conversely, the relative abundance of Actinobacteria and Firmicutes in diseased samples were less than in healthy samples.

At the genus level, the top 10 genera included *Pseudomonas*, *Pantoea*, unclassified_Alcaligenaceae, unclassified_Gammaproteobacteria, *Staphylococcus*, *Brevibacterium*, *Bacillus*, *Kocuria*, *Exiguobacteri*, and *Actinoplanes* (Figure 4B). The abundance of *Pseudomonas*, *Pantoea*, and unclassified_Gammaproteobacteria in diseased samples was higher than that in healthy samples, whereas the abundance of unclassified_

TABLE 3 List of top 10 dominant taxa and their relative abundance in the fungal and bacterial communities of the group.

Community Structure		Relative abundance (%)							
Bacteria		B1	B5	B7	B9	J1	J5	J7	J9
Phylum	Proteobacteria	99.95 ± 0.07a	99.97 ± 0.01a	99.25 ± 0.78a	98.97 ± 1.54a	66.89 ± 10.72b	84.56 ± 8.07ab	79.12 ± 14.41ab	86.06 ± 13.65ab
	Actinobacteria	0.04 ± 0.06a	0.02 ± 0.01a	0.02 ± 0.02a	0.08 ± 0.07a	14.17 ± 4.25b	7.49 ± 5.00ab	10.72 ± 9.28ab	6.31 ± 7.47ab
	Firmicutes	0.01 ± 0.01a	0.01 ± 0.01a	0.71 ± 0.75a	0.94 ± 1.54a	15.57 ± 7.34b	6.92 ± 2.52abc	3.50 ± 1.54ac	6.40 ± 4.82abc
	Bacteroidetes	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.01 ± 0.16a	0.37 ± 0.09a	4.49 ± 5.35a	0.69 ± 0.74a
	Myxococcota	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.71 ± 2.64a	0.06 ± 0.08a	0.09 ± 0.08a	0.07 ± 0.11a
	Chloroflexi	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.31 ± 0.34a	0.21 ± 0.18a	0.68 ± 0.59a	0.28 ± 0.46a
Genus	<i>Pseudomonas</i>	31.59 ± 26.57a	47.24 ± 30.78a	50.53 ± 20.68a	56.10 ± 5.29a	9.58 ± 5.96a	49.98 ± 18.98a	44.15 ± 28.30a	36.18 ± 20.98a
	<i>Pantoea</i>	59.39 ± 30.33a	42.74 ± 30.73ab	38.51 ± 20.71ab	34.28 ± 0.19ab	1.50 ± 0.96b	2.46 ± 1.97b	4.51 ± 3.94b	33.80 ± 7.45ab
	<i>Unclassified Alcaligenaceae</i>	0.02 ± 0.02a	0.04 ± 0.03a	0.01 ± 0.02a	0.01 ± 0.01a	50.77 ± 11.45b	26.83 ± 7.03c	20.15 ± 9.41c	12.78 ± 4.46ac
	<i>Unclassified Gammaproteobacteria</i>	8.74 ± 4.20a	9.40 ± 2.25a	10.12 ± 1.01a	8.21 ± 4.30a	0.01 ± 0.01b	0.05 ± 0.01b	0.28 ± 0.12b	1.16 ± 0.92b
	<i>Staphylococcus</i>	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	5.00 ± 3.49b	1.95 ± 0.24ab	0.48 ± 0.18a	1.70 ± 1.75ab
	<i>Brevibacterium</i>	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	4.46 ± 1.65b	1.55 ± 1.55ac	0.32 ± 0.31ac	0.91 ± 0.75ac
	<i>Bacillus</i>	0.00 ± 0.00a	0.01 ± 0.01a	0.00 ± 0.00a	0.01 ± 0.02a	2.31 ± 1.03a	2.14 ± 2.26a	0.89 ± 0.31a	1.59 ± 0.53a
	<i>Kocuria</i>	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	2.58 ± 1.70b	1.06 ± 0.39ab	0.28 ± 0.07a	0.92 ± 1.03ab
	<i>Exiguobacterium</i>	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	4.26 ± 7.32a	0.01 ± 0.01a	0.02 ± 0.02a	0.03 ± 0.05a
	<i>Actinoplanes</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.09 ± 0.12	1.79 ± 3.07	0.06 ± 0.09
Fungi		B1	B5	B7	B9	J1	J5	J7	J9
Phylum	Ascomycota	89.43 ± 7.14a	82.68 ± 9.21a	90.60 ± 3.61a	79.75 ± 12.56a	91.37 ± 0.45a	87.87 ± 4.54a	83.02 ± 3.78a	89.12 ± 2.94a
	Basidiomycota	10.56 ± 7.13a	17.32 ± 9.20a	9.40 ± 3.61a	20.25 ± 12.56a	8.62 ± 0.45a	12.12 ± 4.54a	16.98 ± 3.78a	10.88 ± 2.94a
	Mucoromycota	0.01 ± 0.01a	0.00 ± 0.01a	0.00 ± 0.00a	0.00 ± 0.00a	0.01 ± 0.01a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Genus	<i>Blastobotrys</i>	25.70 ± 19.68a	12.87 ± 5.19a	2.48 ± 1.67a	4.41 ± 4.36a	24.00 ± 18.00a	10.43 ± 6.41a	4.88 ± 2.74a	22.15 ± 10.54a
	<i>unclassified_Didymellaceae</i>	17.69 ± 23.93a	7.90 ± 4.78a	28.09 ± 21.62a	7.79 ± 9.32a	4.88 ± 1.34a	6.95 ± 2.69a	6.15 ± 1.52a	5.94 ± 2.28a
	<i>Cladosporium</i>	6.45 ± 1.14a	8.75 ± 3.21a	10.22 ± 2.36a	10.20 ± 4.85a	9.01 ± 7.45a	13.55 ± 2.86a	12.04 ± 5.91a	13.93 ± 2.54a
	<i>Alternaria</i>	6.47 ± 8.31a	3.76 ± 0.71a	11.41 ± 13.90a	38.43 ± 35.49a	3.08 ± 0.93a	5.33 ± 3.18a	3.17 ± 1.73a	4.48 ± 1.68a
	<i>Aspergillus</i>	12.19 ± 9.62a	6.74 ± 4.40a	1.59 ± 0.65a	0.65 ± 0.64a	14.34 ± 9.58a	7.46 ± 4.36a	4.87 ± 3.99a	7.66 ± 3.35a
	<i>unclassified_Ascomycota</i>	1.66 ± 0.91a	11.15 ± 11.91a	2.48 ± 0.50a	1.20 ± 1.03a	4.31 ± 1.00a	11.57 ± 6.52a	6.14 ± 2.96a	4.97 ± 3.50a

(Continued)

TABLE 3 Continued

Community Structure		Relative abundance (%)									
Fungi		B1	B5	B7	B9	J1	J5	J7	J9		
	<i>Filobasidium</i>	4.56 ± 3.20a	6.40 ± 7.23a	5.32 ± 2.80a	14.15 ± 15.88a	0.84 ± 0.57a	2.46 ± 3.07a	1.55 ± 2.35a	0.94 ± 1.14a		
	<i>unclassified_Mycosphaerellaceae</i>	1.59 ± 0.70a	3.21 ± 2.63a	6.75 ± 3.30a	2.82 ± 4.40a	2.92 ± 2.72a	3.55 ± 0.97a	6.37 ± 3.20a	5.25 ± 1.42a		
	<i>Epicoccum</i>	1.09 ± 0.74a	2.70 ± 0.39a	3.30 ± 1.01a	3.36 ± 2.59a	8.05 ± 6.97a	3.44 ± 1.15a	2.76 ± 1.00a	2.64 ± 1.42a		
	<i>Leptosphaerulina</i>	0.30 ± 0.41a	0.43 ± 0.31a	3.48 ± 5.22a	0.73 ± 1.11a	0.00 ± 0.00a	3.30 ± 5.12a	1.36 ± 2.12a	2.00 ± 3.00a		

Different letters in column direction represent having significant difference between groups (p <0.05).

Alcaligenaceae in diseased samples was less than that in healthy samples. The abundance of *Pseudomonas* increased with the increase in disease severity level and peaked at L9 sample. On the contrary, the abundance of *Pantoea* decreased with the increase in disease severity level (Table 3). In addition, with the increase in disease severity level, the relative abundance of unclassified_Alcaligenaceae decreased. The result revealed that the bacterial community composition was different for four disease severity levels.

3.3.2 Fungal community composition

The ITS dataset showed that the phyllosphere fungi of diseased and healthy samples contain three phyla of fungi: Ascomycota, Basidiomycota, and Mucoromycota (Figure 4C). The predominant fungal phylum was Ascomycota, with relative abundances in the diseased groups (B1, B5, B7, and B9) were 89.43%, 82.68%, 90.60%, and 79.75%, respectively, while in healthy groups (J1, J5, J7, and J9), the relative abundances were 91.37%, 87.87%, 83.02%, and 89.12%, respectively. The relative abundances of Basidiomycota in the diseased groups (B1, B5, B7, and B9) were 10.56%, 17.32%, 9.40%, and 20.25%, respectively; the relative abundances in healthy groups (J1, J5, J7, and J9) were 8.62%, 12.12%, 16.98%, and 10.88%, respectively (Table 3). The result indicates that the relative abundance of Ascomycota in diseased samples was lower than that in healthy samples, and the relative abundance of Basidiomycota in diseased samples was higher than that in healthy samples, except for L7 samples.

At the genus level, the top 10 genera included *Blastobotrys*, unclassified_Didymellaceae, *Cladosporium*, *Alternaria*, *Aspergillus*, unclassified_Ascomycota, *Filobasidium*, unclassified_Mycosphaerellaceae, *Epicoccum*, and *Leptosphaerulina* (Figure 4D). Similarly, the relative abundance was associated with the four disease severity levels samples, but the difference is not significant. The abundance of unclassified_Didymellaceae, *Alternaria*, and *Filobasidium* in diseased samples was higher than that in healthy samples, whereas the abundance of *Cladosporium* and *Aspergillus* in diseased samples was less than that in healthy samples (Table 3). The abundance of *Aspergillus* decreased with the increase in disease severity level in diseased samples.

3.4 Spatial distribution of bacterial and fungal communities

PCoA plots were used to reveal the spatial distribution of fungal and bacterial communities (Figure 5). In bacterial community, the result showed that diseased groups and healthy groups were obviously separate (Figure 5A). All diseased samples (B1, B5, B7, and B9) from four disease severity levels were irregularly separated from each other. On the contrary, healthy groups (J1, J5, J7, and J9) with four disease severity levels were clustered together. In the fungal community, healthy groups (J1, J5, J7, and J9) with four disease severity levels were clustered together, and diseased samples (B1, B5, B7, and B9) from four disease severity levels were irregularly separated from each other (Figure 5B). Notably, B1 and B5 of the diseased group were clustered together with healthy

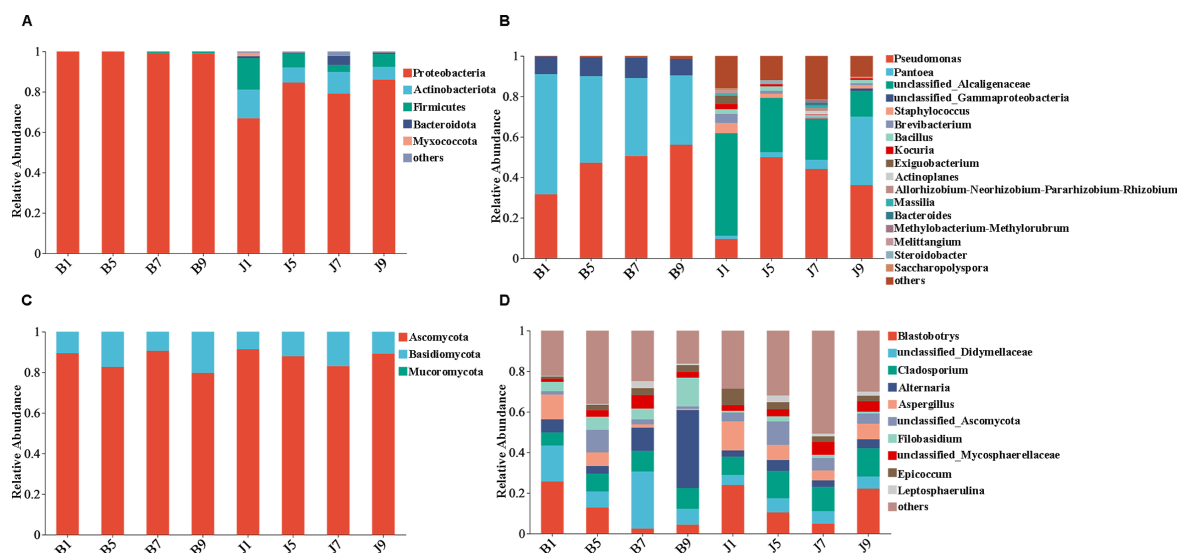


FIGURE 4

Microbial community composition of different groups at the phyla and genus levels. Bacterial community composition of different groups at the phyla (A) and genus (B) levels. Fungal community composition of different groups at the phyla (C) and genus (D) levels.

groups; B7 and B9 were separated from the healthy group. The result indicated that the spatial distributions of bacterial and fungal community composition were significantly different between disease and healthy samples. There were fewer differences in bacterial and fungal community composition of healthy samples compared to diseased samples.

3.5 Significant differences in microbial communities

LEfSe analysis was used to detect the difference in fungal and bacterial communities between the diseased and healthy samples (Figure 6). For bacterial communities, 89 bacteria clades showed statistically significant differences when the LDA threshold was 4.

In L1 leaf samples, 19 and 8 clades showed abundance advantage in healthy and diseased samples, respectively (Figures 6A, B). In L5 leaf samples, 13 and 8 clades present an abundance advantage in healthy and diseased samples, respectively (Figures 6C, D). In L7 leaf samples, 19 clades showed abundance advantage in healthy samples, while eight clades showed abundance advantage in diseased samples (Figures 6E, F). In L9 leaf samples, nine and five clades present abundance advantage in healthy and diseased samples, respectively (Figures 6G, H). At the genus level, *Pantoea* and unclassified_Gammaproteobacteria were enriched in B1, B5, and B7 samples, while the B9 sample only enriched unclassified_Gammaproteobacteria. Unclassified_Alcaligenaceae were enriched in J1, J5, J7, and J9 samples. Meanwhile, J1 healthy samples also were enriched with *Staphylococcus*, *Brevibacterium*, *Bacillus*, and *Kocuria*; J5 healthy samples also were enriched with *Bacillus*; and J7 healthy samples also

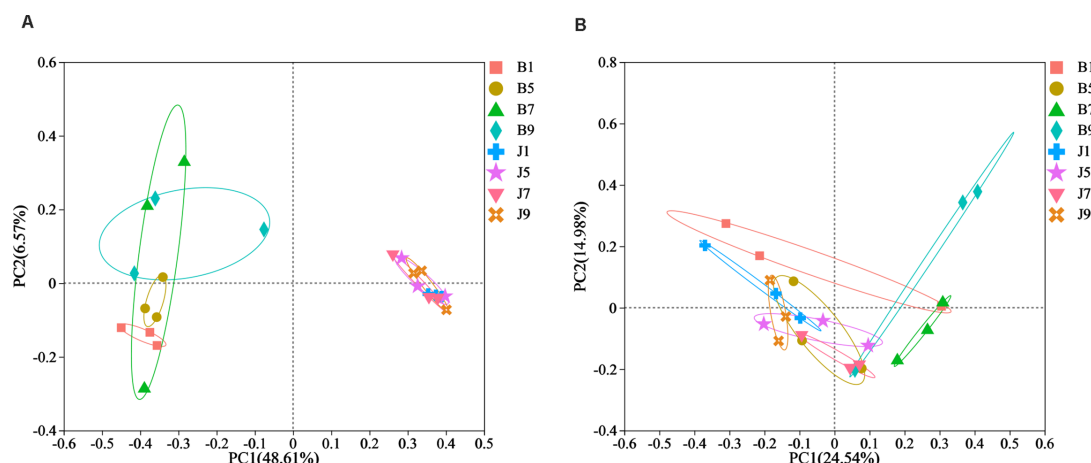


FIGURE 5

Principal Co-ordinate Analysis (PCoA) of the bacterial (A) and fungal (B) communities in the different group sample.

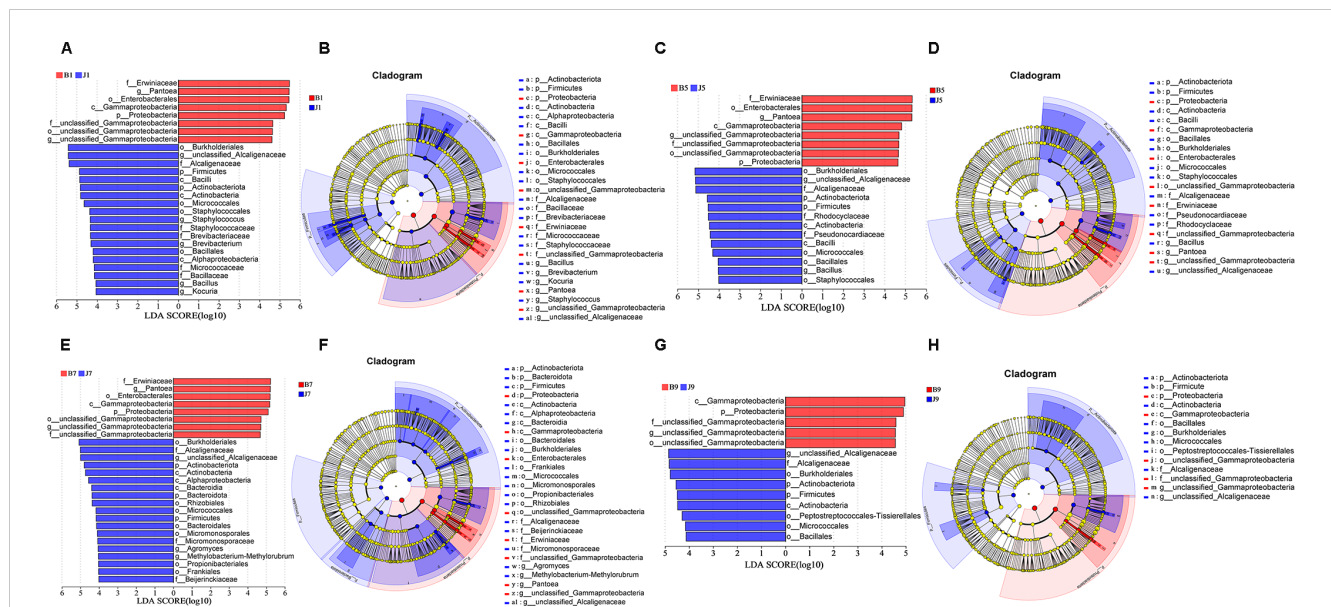


FIGURE 6

LefSe analysis of bacteria abundance between diseased and healthy samples of different disease severities. (A), (C), (E), (G) are LDA score identified the size of differentiation between diseased and healthy groups of 1, 5, 7, 9 disease severity, respectively, with a threshold value of 4. (B), (D), (F), (H) are the cladogram of microbial communities of 1, 5, 7, 9 disease severity, respectively.

were enriched with *Methylobacterium–Methylorubrum*. At the same time, significant differences were found between the different treatment groups using PERMANOVA test. The healthy groups had more characteristic species compared to the diseased groups. However, the B9 did not have characteristic species (Figure 7). Based on the results of LefSe analysis, we found differences between healthy and diseased groups, and differences between samples of different disease severity levels. In addition, it was observed that the diversity of the enriched bacterial species was lower as the disease levels L1–L9 increased, indicating that the disease level has a strong influence on bacterial

diversity. In contrast, there were no significant abundance differences for fungal communities between the diseased and healthy microbial communities of different disease severity levels.

3.6 Microbial community functional characteristics

PICRUSt and FUNGuild were used to predict the trophic modes of fungi in different disease severity levels, respectively (Figure 8).

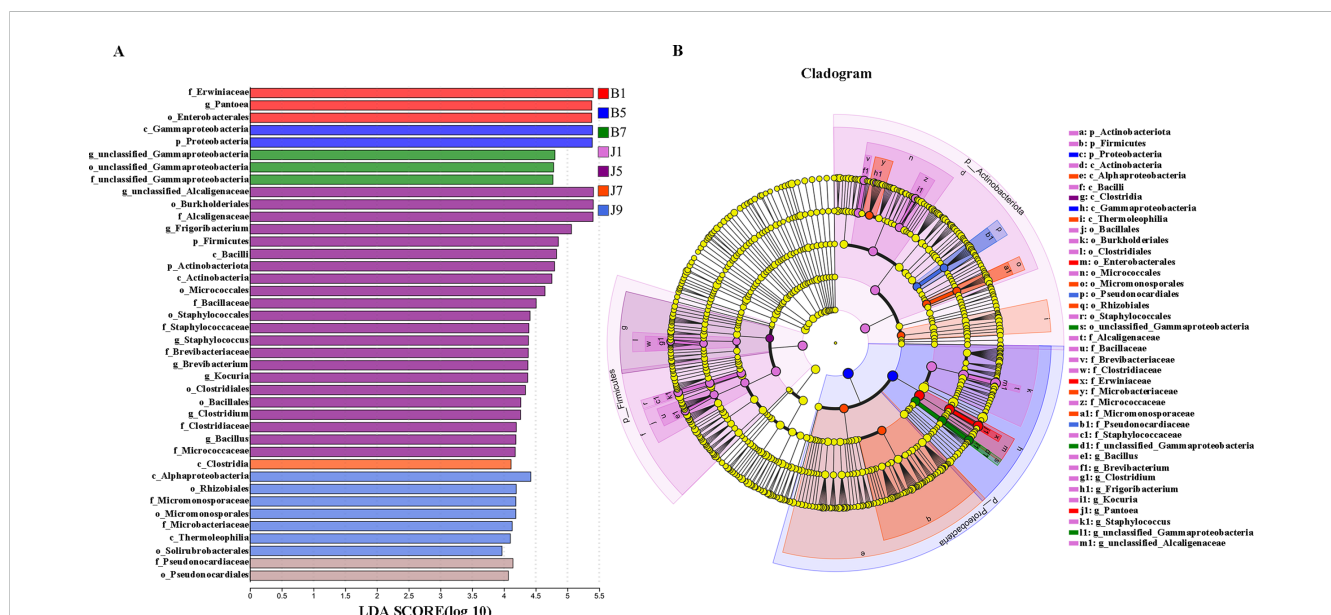


FIGURE 7

LefSe analysis of bacteria abundance in all samples, LDA score (A) and cladogram of microbial communities (B).

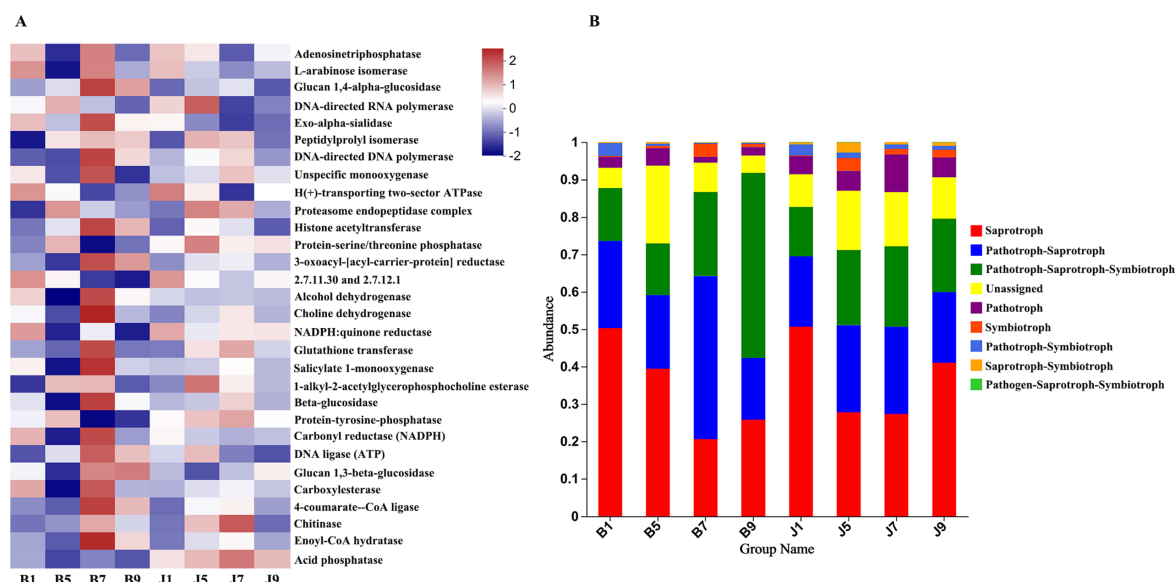


FIGURE 8

PICRUSt analyses of the enzyme functional of fungal in tobacco leaf samples (A). Relative abundance of fungal functional groups (modes) based on OTU annotation table with disturbance frequency level (B).

The PICRUSt analyses result showed that the top 6 enzymes were Adenosinetriphosphatase, L-arabinose isomerase, Glucan 1,4-alpha-glucosidase, DNA-directed RNA polymerase, Exo-alpha-sialidase, and unspecific monooxygenase. Meanwhile, most of the enzymes did not differ significantly between treatment groups. At the FUNGuild result, saprotroph, pathotroph–saprotroph, and pathotroph–saprotroph–symbiotroph were the dominant trophic modes in all samples. The abundance of saprotroph was higher in healthy groups than that in diseased groups and decreased with the increase in disease severity level. In addition, pathotroph–saprotroph–symbiotroph had a higher abundance in diseased groups compared to healthy groups, which increased with increasing disease severity level.

PICRUSt2 was used to predict the bacterial community function. In this study, the most abundant pathway was metabolic (73.67%), followed by environmental information processing (9.14%), cellular processes (6.27%), genetic information processing (5.04%), and human disease (4.17%), with the lowest being the organic system (1.72%). B1 samples were the lowest for all functions, and J9 samples clustered with the diseased samples, which indicates that L9 samples were the most severely diseased, and the bacterial functions in their healthy samples were similar to those of the diseased samples (Figure 9A). There were significant differences between diseased and healthy samples at L1, except for cellular processes. However, most bacterial functions seem to be independent of the disease severity level (Figures 9B–G).

3.7 Microbial co-occurrence network analysis

Network analysis of co-occurrence patterns among the most abundant 50 bacterial and fungal genera presents that most of the

bacterial genera showed positive correlation with each other, and unclassified_Gammaproteobacteria showed negative correlation with 19 bacterial genera such as unclassified_Alcaligenaceae, *Kocuria*, *Bacillus*, and *Brevibacterium* (Figure 10A). For fungal communities, most of the fungal genera were also positively correlated; *Filobasidium* was negatively correlated with six genera including *Phaeosphaeria*, *Leptospora*, and *Exidia*, and *Alternaria* was negatively correlated with *Thermomyces* and *Sclerotiniaceae* (Figure 10B).

3.8 Microbial community metabolic profiles

Phyllosphere microbial metabolic function analysis was performed using Biolog Eco metabolic 96-well plates (Figure 11). The metabolic capacity of carbon sources of different disease level samples decreased with increasing disease level, with B9 samples having the lowest metabolic capacity. The samples with different disease levels had lower capacity to utilize 2-hydroxybenzoic acid, α -cyclodextrin, α -butyronic acid, and phenylethyl-amine, but the B9 sample had a greater capacity to utilize these carbon sources than the other disease level samples.

4 Discussion

Disease severity level is an important factor to affect the structure of microbial communities in tobacco leaves. Understanding of the microbial characteristics of tobacco leaves with different disease severity levels is of great value to disease control. In this study, high-throughput sequencing and Biolog Eco were used to investigate the impact of disease severity level of tobacco wildfire disease on microbial communities and carbon

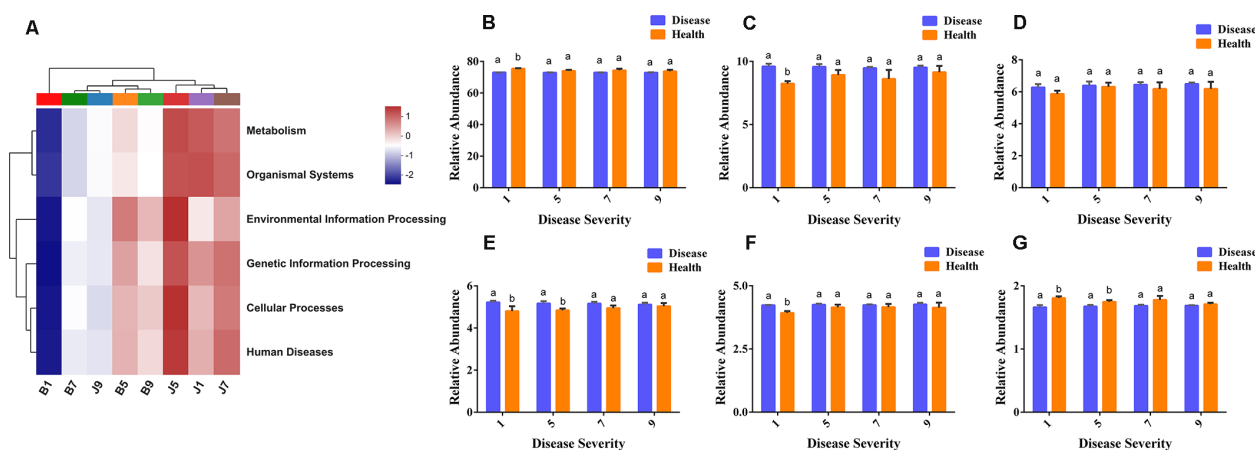


FIGURE 9

PICRUSt analyses of the KEGG level 1 functional categories of bacteria in tobacco leaf samples. (A) Dendrogram of bacterial functional categories, (B) Metabolism, (C) Environmental Information Processing, (D) Cellular Processes, (E) Genetic Information Processing, (F) Human Diseases, (G) Organismal Systems.

source metabolizing capacity. It was shown that healthy plant leaves harbored greater microbial abundances and diversity compared to diseased leaves, with a highly diverse microbial diversity that tends to be conducive to resisting pathogen infection (De Mandal and Jeon, 2023). Similar results have been reported in this study, the Shannon, Ace, and Chao1 indices of bacterial community were higher in healthy groups than the diseased groups, and the Shannon index of the fungal community were higher in healthy groups than that diseased groups. The results suggested that the healthy phyllosphere has greater microbial diversity to resist disease expansion. Moreover, the Shannon index of bacteria decreased and then increased with increasing disease severity level, which was consistent with the conclusion of bacterial community diversity

at different disease levels of powdery mildew in pumpkin reported by Luo et al. (2017). Conversely, Zhang et al. (2019) reported that the fungal diversity index showed increasing and then decreasing trend with the aggravation of the disease, which was consistent with the conclusion in this study about the diversity index of fungi. In general, the results suggested that there may be an interactive relationship between the bacteria and fungi in the leaf of tobacco wildfire disease.

The composition of the microbial community could be affected by the colonization of pathogens. In bacterial communities, Proteobacteria was the dominant phylum in both diseased and healthy samples at four disease severity levels. The abundance of Proteobacteria was higher in diseased samples than in the healthy

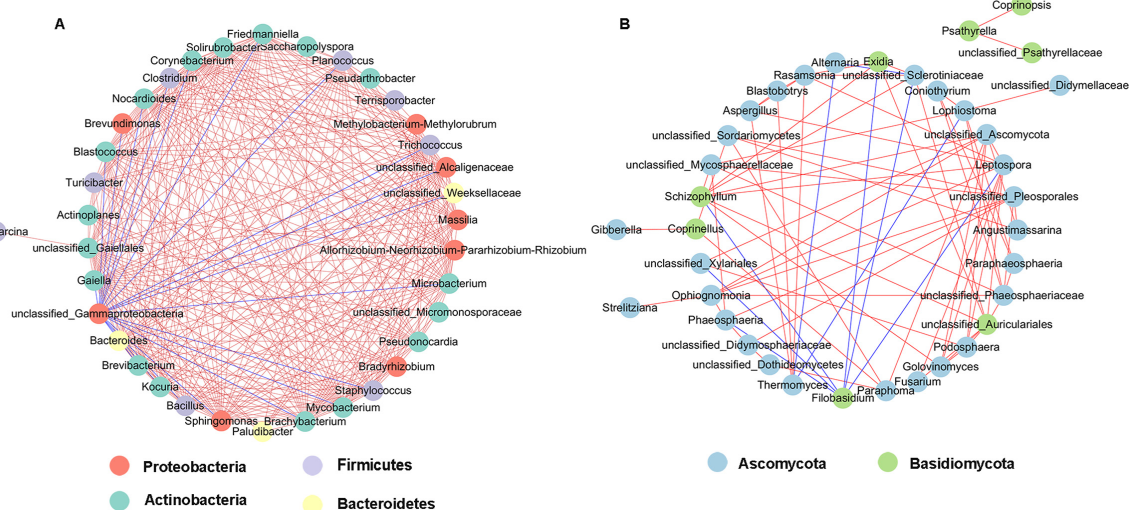


FIGURE 10

Network analysis of bacterial (A) and fungal (B) taxa showing significant positive or negative co-occurrences based on Spearman's rank correlations. Red lines represent positive correlations and blue lines represent negative correlations.

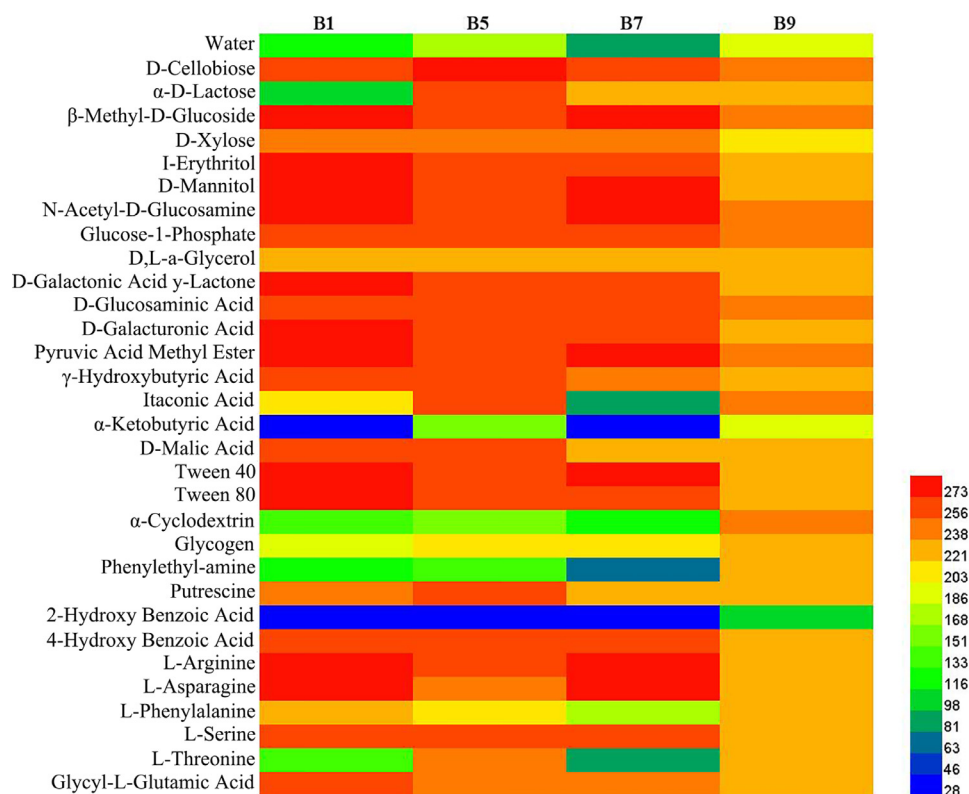


FIGURE 11

Heatmap of carbon sources in BIOLOG ECO-Plate of microbial communities from different samples.

samples (Guo et al., 2023), whereas the abundance of Actinobacteria and Firmicutes was much less in diseased than in healthy samples. The results of a previous study showed that Actinobacteria activates biosynthesis of salicylic acid to induce defense responses in plant leaves (Vergnes et al., 2020). Proteobacteria and Firmicutes are the common bacterial phyla in the phyllosphere; they always show opposite trends, which is thought to be due to the fact that Firmicutes were inhibited by Proteobacteria (Chen et al., 2020b). This may explain why the abundance of Firmicutes in diseased samples was lower than in healthy samples in tobacco wildfire disease. The dominant bacterial genus of both diseased and healthy leaves was *Pseudomonas*, which was the genus to which the pathogen of tobacco wildfire disease belongs. On the other hand, some strains of *Pseudomonas* are thought to produce a wide range of antibiotics that are effective in suppressing agricultural diseases.

The result of this study showed that a high abundance of *Pseudomonas* may be caused by pathogenic colonization or by antimicrobial active strains recruited in the phyllosphere. In addition, the relative abundance of *Pseudomonas* positively correlated with the disease severity level in diseased samples, which is similar to the results reported by Sun et al. (2022) who pointed out that the abundance of *Thanatephorous* (pathogen of tobacco target spot disease) increased with the increase in disease severity level. The abundance of unclassified *Alcaligenaceae* was higher in healthy samples compared to diseased samples, which decreased with increasing disease severity level. *Alcaligenes* has been

heavily reported as a biocontrol agent against tobacco diseases. The result of this study suggests that there are several potentially beneficial genera in healthy samples, and whether plant health can be maintained by maintaining the abundance of *Alcaligenes* is very much worthy of further investigation.

In fungal communities, the dominant phyla were Ascomycota and Basidiomycota in both diseased and healthy leaves, which is similar to Liu et al. (2022). The taxa unclassified *Didymellaceae*, *Alternaria*, and *Filobasidium* were more enriched in diseased samples, whereas the abundance of *Aspergillus* was higher in healthy samples. Si et al. (2023) reported that *Aspergillus* was higher in healthy samples compared to diseased samples, which is consistent with our study. Among them, the relative abundance of *Aspergillus* negatively correlated with the disease severity level in diseased samples. Huang et al. (2021) found that the relative abundance of *Alternaria* increased with a disease severity level and deduced that *Alternaria* could benefit from the disease pressure caused by *Didymella*. The results of this study were consistent with them, and it is possible that the occurrence of wildfire disease is beneficial to *Alternaria* growth, but it remains to be investigated whether this genus accelerates or reduces wildfire disease occurrence. Based on the functional predictions of FUNGuild, most fungal communities were identified as saprotroph and pathotroph-saprotroph. Pathotroph-saprotroph and pathotroph-saprotroph-symbiotroph had a higher abundance in diseased samples. The data signify the presence of a large number of fungi in tobacco leaf, having pathogenic potential.

LEfSe analysis showed that the *Pantoea* and unclassified_Gammaproteobacteria were enriched in the L1, L5, and L7 diseased groups of different disease severity levels, but the L9 diseased groups were only enriched with unclassified_Gammaproteobacteria. The healthy groups were all enriched unclassified_Alcaligenaceae; moreover, the L1 healthy sample was enriched for the genera *Brevibacterium*, *Bacillus*, and *Kocuria*, the L5 healthy sample was also enriched for *Bacillus*, the L7 healthy sample *Methylobacterium-Methylobacterium* was enriched, while the L9 healthy sample did not have any other characterized genera. The results present that the diversity of enriched microbial communities were decreased with increasing disease severity level. In previous studies, *Bacillus*, *Methylobacterium-Methylobacterium*, *Brevibacterium*, and unclassified_Alcaligenaceae were considered to be biocontrol agents, beneficial to plant growth and to protect plants from pathogens (Madhaiyan et al., 2007; Son et al., 2014). Gammaproteobacteria are common bacteria in plants; in this study unclassified_Gammaproteobacteria were found to be in higher abundance in diseased samples compared to healthy samples, which is consistent with previous studies (Wang et al., 2022). Furthermore, the results of correlation network analysis also showed that unclassified_Gammaproteobacteria showed negative correlation with unclassified_Alcaligenaceae, *Kocuria*, *Bacillus*, and *Brevibacterium* in healthy groups. Gammaproteobacteria are eutrophic bacteria (Huang et al., 2017); we speculate that their lower abundance in healthy samples may be due to the higher microbial abundance and diversity in healthy samples, which compete with Gammaproteobacteria for nutrients.

The result of substrate utilization profiling displayed that the metabolizing ability of all samples to most carbon sources decreased with increasing disease severity level, but the metabolizing ability to α -butyronic acid, α -cyclodextrin, glycogen, phenylethylamine, and 2-hydroxybenzoic acid increased with increasing disease severity level, which was similar to the change in *Pseudomonas* abundance, but the relationship between these carbon sources and *Pseudomonas* needs to be further investigated. Meanwhile, we found that all samples had the lowest utilization of 2-hydroxybenzoic acid, which has been reported to play an important role in the regulation of plant growth and development and defense response against pathogenic bacteria (Miura and Tada, 2014), which may explain its low utilization.

5 Conclusion

In this study, we found that the bacterial and fungal abundance and diversity from diseased samples was less than in healthy samples in the leaves affected by tobacco wildfire disease. Moreover, the abundance of bacterial and fungal community composition and diversity was affected by disease severity level. The relative abundance of *Pseudomonas* positively correlated with the disease severity level in diseased samples. The bacterial diversity and relative abundance decrease at the beginning and then increase with the tobacco wildfire disease severity level increases. Nevertheless, the fungal diversity and relative abundance increase

and then decrease with increasing disease severity level. Finally, the metabolizing ability of all samples to most carbon sources decreased with increasing disease severity level. The most significant differences were found in β -methyl-D-glucoside, D-mannitol, N-acetyl-D-glucosamine, D-galacturonic acid, pyruvic acid methyl ester, and Tween 40. The obtained data from this study helps to infer that the composition and diversity of phyllosphere microbiome in tobacco wildfire disease. However, further study is needed to verify the interaction of phyllosphere microbiome. Hence, it is essential to direct attention toward these characteristic microorganisms in further studies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, PRJNA1096149 and PRJNA1096455.

Author contributions

XX: Data curation, Investigation, Writing – original draft. LZ: Data curation, Writing – original draft. YC: Investigation, Writing – original draft. HW: Supervision, Writing – review & editing. LC: Supervision, Writing – review & editing. YW: Supervision, Writing – review & editing. NW: Data curation, Writing – review & editing. WP: Supervision, Writing – review & editing. FW: Supervision, Writing – review & editing. YK: Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JK declared a past collaboration with the authors NW, YK to the handling editor.

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EDITED BY

Sinang Hongsanan,
Shenzhen University, China

REVIEWED BY

Chihiro Kadooka,
Sojo University, Japan
Yanpeng Chen,
Helmholtz Association of German Research
Centers (HZ), Germany

*CORRESPONDENCE

Qiang Li

✉ LQ@mail.qjnu.edu.cn

Dong-Qin Dai

✉ cidaidongqin@gmail.com

[†]These authors have contributed equally to
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Five new species, two new sexual morph reports, and one new geographical record of *Apiospora* (Amphisphaeriales, Sordariomycetes) isolated from bamboo in Yunnan, China

Li-Su Han^{1,2†}, Chao Liu^{1†}, Dong-Qin Dai^{1*},
Itthayakorn Promputtha², Abdallah M. Elgorban³,
Salim Al-Rejaie⁴, Qiang Li^{1*} and Nalin N. Wijayawardene^{1,5}

¹Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, China, ²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ³Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, ⁴Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, ⁵Tropical Microbiology Research Foundation, Pannipitiya, Sri Lanka

Apiospora is an important genus in the Apiosporaceae family with a worldwide distribution. They exhibit different lifestyles including pathogenic, saprophytic, and endophytic. In this study, we aimed to explore the *Apiospora* associated with bamboo and collected 14 apiospora-like taxa from the forests of Yunnan Province, China. Morphological and phylogenetic analyses (combined ITS, LSU, *tef1-α*, and *tub2* sequence data) confirmed that these collections belong to *Apiospora* s. str. and reports five new species (viz., *Ap. dehongensis*, *Ap. jinghongensis*, *Ap. shangrilaensis*, *Ap. zhaotongensis*, and *Ap. zhenxiongensis*). New sexual morphs of asexually typified *Ap. globose* and *Ap. guangdongensis* species, and a new geographical record of *Ap. subglobosa* are also reported. The findings of this study not only enhance the diversity of bambusicolous fungi in the region of Yunnan, but also geographical distribution of some known *Apiospora* species.

KEYWORDS

Apiosporaceae, phylogeny, saprobes, taxonomy, bambusicolous fungi

Introduction

Apiosporaceae (Amphisphaeriales, Sordariomycetes, Ascomycota *vide* Wijayawardene et al., 2022) was introduced by Hyde et al. (1998), with *Apiospora* Sacc. as the type genus (Saccardo, 1875). Currently, Apiosporaceae comprises three genera—*Apiospora*, *Arthrinium* Kunze, and *Nigrospora* Zimm (Pintos and Alvarado, 2021; Jiang et al.,

2022a). The relationship among the genera in Apiosporaceae is confusing as some taxa lack sequence data and show morphological plasticity. For example, *Apiospora* resembles *Arthrinium* s. str. but is phylogenetically well-distinct (Pintos and Alvarado, 2021; Jiang et al., 2022a).

The genus *Apiospora* was introduced by Saccardo (1875), with *Ap. montagnei* Sacc. as the type species (Clements and Shear, 1931). Currently, 175 epithets are listed under the genus of *Apiospora* in Index Fungorum (Index Fungorum 2024; accession date: 16 June 2024). *Apiospora* was reported as a holomorphic genus; the sexual morph is characterized by multi-locular stromata and 1-septate (near the lower cell) ascospores (Dai et al., 2016, 2017; Bhunjun et al., 2022). The asexual morphs usually occur as coelomycetous on natural substrates or hyphomycetous on culture (e.g., MEA, OA, and PDA). The coelomycetous morph is characterized by dark brown conidia, with a longitudinal and transparent slit (Dai et al., 2016; Pintos and Alvarado, 2021; Zhao et al., 2023). The hyphomycetous morph is characterized by hyaline conidiophores, basauxic conidiogenous cells, globose to subglobose, and pale brown to brown conidia (Wang et al., 2018; Pintos and Alvarado, 2021; Tian et al., 2021; Zhao et al., 2023).

Members of *Apiospora* are found in different habitats such as animal tissues (including humans), air, lichens, plants, soil, and seaweeds (Liao et al., 2023). The members of *Apiospora* show a wide distribution and have been reported from tropical, sub-tropical, Mediterranean, and temperate regions (Pintos and Alvarado, 2021; Kwon et al., 2022). Furthermore, the species of *Apiospora* have been reported as endophytes, saprobes, or pathogens, and some particular species have two or three lifestyles (Samuels et al., 1981; Liao et al., 2023; Zeng et al., 2024). For instance, *Ap. arundinis* (Corda) Pintos & P. Alvarado has been reported as pathogens, saprobes, and endophytes (Feng et al., 2021; Liao et al., 2023). Moreover, *Ap. arundinis* has also been reported as a pathogen of plants, animals, and humans (Martínez-Cano et al., 1992; Mavragani et al., 2007; Bagherabadi et al., 2014; Chen et al., 2014; Jiang and Tian, 2021).

According to Monkai et al. (2022); Zhao et al. (2023), and Liu et al. (2024), asexual morphs of *Apiospora* are frequently observable, while sexual morphs are rare. Therefore, this study aims to explore the morphology of the sexual morph of *Apiospora*. Of this, a total of 14 *Apiospora* samples were collected from the Yunnan Province, China. Among these new collections, five new species (e.g., *Ap. dehongensis*, *Ap. jinghongensis*, *Ap. shangrilaensis*, *Ap. zhaotongensis*, and *Ap. zhenxiongensis*), two sexual morphs of asexually typified species (*Ap. globosa* and *Ap. guangdongensis*), and one new country record (*Ap. subglobosa*) are reported along with the morphological descriptions, illustrations, and updated phylogenetic trees.

Materials and methods

Collection and morphological studies

The dead and decaying bamboo culms and branches were collected from several forests in Dehong, Shangri-La, Xishuangbanna, and Zhaotong in Yunnan Province. Collected

samples were kept in envelope bags and transported to the lab for further evaluation. The stromata and micro-morphological characteristics were observed and photographed using Leica S8AP0 and Olympus BX53 stereomicroscopes, respectively, which are equipped with a high-definition digital camera. The sizes of the fungal structures were measured by the Tarosoft (R) Image Frame Work program (IFW). The photo plates were processed with the Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA).

Isolation and preservation

Pure cultures of the new collections were obtained by single spore isolation. The ascospores were picked from stromata and dispersed on sterile water droplets on a cavity slide. Spore suspensions were placed on potato dextrose agar (PDA) and stored at 27°C until germination. The germinated spores were aseptically transferred into new PDA plates and incubated. The characteristics of fungi colonies were recorded and photographed after 20 days.

Dried herbarium samples were preserved at the Mycological Herbarium of Zhongkai University of Agriculture and Engineering (MHZU) and Herbarium of Guizhou Medical University, Guiyang, China (GMB-W). Living cultures were deposited in Zhongkai University of Agriculture and Engineering (ZHKUCC) and Guizhou Medical University Culture Collection (GMBCC) Guiyang.

DNA extraction, PCR, and sequencing

The genomic DNA was extracted from fresh fungal mycelia grown on PDA [using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®)]. However, for two species, single spore isolation was not successful. Thus, we used fruiting bodies to extract DNA using an E.Z.N.A. Forensic DNA Kit (BIO-TEK). The details of the primers used for PCR amplification are presented in Table 1. We followed Tian et al. (2021) for PCR amplification conditions. PCR products were sequenced at Shanghai Mayobio Biomedical Technology Co., China. All newly generated nucleotide sequence data were submitted to GenBank and the accession numbers were obtained (Table 2).

Phylogenetic analyses

All newly obtained forward and reverse sequences were assembled using Geneious 9.1.2. Those assembled sequences were searched using BLASTn (<http://blast.ncbi.nlm.nih.gov/>, accessed on 16 January 2024) to retrieve the sequences of closely related strains. The preliminary identification results showed that our new collections match closest with *Apiospora*, then all available sequences of *Apiospora* were downloaded from the GenBank based on previous literature (Table 2). The matrix of consensus sequences was aligned with MAFFT v. 7 (Katoh and Standley, 2013). The sequence alignments were trimmed by using trimAl.v1.2rev59 [parameters: -gt 0.7 (ITS, *tub2*), -gt 0.8 (LSU),

TABLE 1 LSU, ITS, *tef1-α*, and *tub2* loci primers information.

Loci	Primers and base pairs (5' to 3')	References
LSU (large subunit rDNA)	Forward: LROR 5'-GTACCCGCTGAACCTAAGC-3' Reverse: LR5 5'-ATCCTGAGGGAACTTC-3'	Vilgalys and Hester (1990)
ITS (internal transcribed spacers)	Forward: ITS5 5'-TCTCCGCTTATTGATATGC-3' Reverse: ITS4 5'-GGAAGTAAAGTCGTAACAAGG-3'	White et al. (1990)
<i>tef1-α</i> (elongation factor 1-α)	Forward: EF1-728F 5'-CATCGAGAAGTTTCGAGAAGG-3' Reverse: EF-2 5'-GGARGTACCAAGTATCATGTT-3'	O'Donnell et al. (1998); Carbone and Kohn (1999)
<i>tub2</i> (β-tubulin)	Forward: Bt2a 5'-GGTAACCAATCGGTGCTGCTTTC-3' Reverse: Bt2b 5'-ACCCTCAGTGTAGTGACCTTGGC-3'	Glass and Donaldson (1995)

-gt 0.9 (*tef1-α*); Capella-Gutiérrez et al., 2009] and BioEdit v. 7.0 (Hall, 2004) to remove unclear and uninformative regions. The alignments of four genes (LSU, ITS, *tef1-α*, and *tub2*) were concatenated by Matrix 1.9 (Vaidya et al., 2011). The AliView

1.26 (Larsson, 2014) was used to convert Fasta files to Phylip (for Maximum likelihood) and Nexus (for Bayesian inference) formats. Maximum likelihood analyses (ML) were performed at the CIPRES web portal using RAXML-HPC2 on XSEDE (8.2.12) (Stamatakis et al., 2008; Stamatakis, 2014) with GTRGAMMA model with 1,000 bootstrap pseudoreplicates. Bayesian inference posterior probabilities (BYPP) (Zhaxybayeva and Gogarten, 2002) were evaluated by Markov Chain Monte Carlo (MCMC) in MrBayes on XSEDE (3.2.7a) (Rannala and Yang, 1996) in the CIPRES Science Gateway web (Ronquist et al., 2012). The model of nucleotide evolution was determined by MrMTgui (Ma, 2016); GTR+I+G was the best-fit model for the ITS and *tub2*, SYM+I+G was the best-fit model for the LSU, and GTR+G was the best-fit model for *tef1-α*. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled at every 100th generation. Phylogenetic trees were viewed in FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) (Rambaut, 2012) and formatted by using Adobe Illustrator CS v. 5.

Registration of novel taxa

Newly introduced taxa were registered at the Index Fungorum (2024) and the identifiers were obtained, fulfilling the requirements

TABLE 2 Details of the taxa used in the phylogenetic analyses.

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Apiospora acutiapica</i>	KUMCC 20-0210 ^T	<i>Bambusa bambos</i>	China	Saprobe	MT946339	MT946343	MT947366	MT947360
<i>Ap. adinandrae</i>	SAUCC 1282B-1 ^T	<i>Adinandra glischroloma</i>	China	–	OR739572	OR739431	OR757128	OR753448
<i>Ap. agari</i>	KUC21333 ^T	<i>Agarum cribrosum</i>	Republic of Korea	–	MH498440	MH498520	MH498478	MH544663
<i>Ap. aquatica</i>	S 642 ^T	Submerged wood	China	Saprobe	MK835806	MK828608	NA	NA
<i>Ap. arctoscopi</i>	KUC21331 ^T	Egg of <i>Arctoscopus japonicus</i>	Republic of Korea	–	MH498449	MH498529	MH498487	MN868918
<i>Ap. armeniaca</i>	SAUCC DL1831 ^T	<i>Prunus armeniaca</i>	China	Endophyte	OQ615269	OQ592540	OQ613285	OQ613313
<i>Ap. armeniaca</i>	SAUCC DL1844	<i>Prunus armeniaca</i>	China	Endophyte	OQ615268	OQ592539	OQ613284	OQ613312
<i>Ap. arctoscopi</i>	KUC21331 ^T	Egg of <i>Arctoscopus japonicus</i>	Republic of Korea	–	MH498449	MH498529	MH498487	MN868918
<i>Ap. arundinis</i>	GZCC 20-0116 ^T	<i>Aspergillus flavus sclerotium</i>	USA	Saprobe/ endophyte	MW478899	MW481720	MW522968	MW522952
<i>Ap. aseptata</i>	KUNCC 23-14169 ^T	<i>Dicranopteris pedata</i>	China	Endophyte	OR590335	OR590341	OR634943	OR634949
<i>Ap. aurea</i>	CBS 244.83 ^T	Air	Spain	–	KF144935	AB220251	KF144981	KF145023
<i>Ap. balearica</i>	CBS 145129 ^T	Undetermined Poaceae	Spain	Saprobe	MK014836	MK014869	MK017975	NA
<i>Ap. babylonica</i>	SAUCC DL1841 ^T	<i>Salix babylonica</i>	China	Endophyte	OQ615267	OQ592538	OQ613283	OQ613311

(Continued)

TABLE 2 Continued

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Ap. babylonica</i>	SAUCC DL1864	<i>Salix babylonica</i>	China	Endophyte	OQ615266	OQ592537	OQ613282	OQ613310
<i>Ap. bambusicola</i>	MFLUCC 20-0144 ^T	Culms of <i>Schizostachyum brachycladum</i>	Thailand	Saprobe	MW173087	MW173030	NA	MW183262
<i>Ap. bawanglingensis</i>	SAUCC BW0444 ^T	<i>Indocalamus longiauritus</i>	China	–	OR739570	OR739429	OR757126	OR753446
<i>Ap. biserialis</i>	CGMCC 3.20135 ^T	Bamboo	China	Saprobe	MW478885	MW481708	MW522955	MW522938
<i>Ap. camelliaesinensis</i>	LC 5007 ^T	<i>Camellia sinensis</i>	China	Endophyte	KY494780	KY494704	KY705173	KY705103
<i>Ap. cannae</i>	ZHKUCC 22-0127	<i>Canna</i>	China	Saprobe	OR164948	NA	OR166321	OR166285
<i>Ap. chiangraiense</i>	MFLUCC 21-0053 ^T	Dead culms of bamboo	Thailand	Saprobe	MZ542524	MZ542520	MZ546409	NA
<i>Ap. chromolaenae</i>	MFLUCC 17-1505 ^T	<i>Chromolaena odorata</i>	Thailand	Saprobe	MT214436	MT214342	NA	NA
<i>Ap. cordylinae</i>	GUCC 10027 ^T	<i>Cordyline fruticosa</i>	China	–	NA	MT040106	MT040148	MT040127
<i>Ap. coryli</i>	CFCC 58978 ^T	<i>Corylus yunnanensis</i>	China	Saprobe	OR133586	OR125564	OR139978	OR139974
<i>Ap. cyclobalanopsidis</i>	CGMCC 3.20136 ^T	<i>Cyclobalanopsidis glauca</i>	China	Saprobe	MW478892	MW481713	MW522962	MW522945
<i>Ap. dehongensis</i>	GMBCC1011 ^T	Bamboo	China	Saprobe	PQ111483	PQ111494	PQ463974	PQ464025
<i>Ap. dehongensis</i>	GMBCC1012	Bamboo	China	Saprobe	PQ111484	PQ111495	PQ463975	PQ464026
<i>Ap. dematiacea</i>	KUNCC 23-14202 ^T	<i>Dicranopteris ampla</i>	China	Endophyte	OR590339	OR590346	OR634948	OR634953
<i>Ap. descalsii</i>	CBS 145130 ^T	<i>Ampelodesmos mauritanicus</i>	Spain	Saprobe	MK014837	MK014870	MK017976	NA
<i>Ap. dichotomanthi</i>	LC 4950 ^T	<i>Dichotomanthes tristaniicarpa</i>	China	Saprobe/ endophyte	KY494773	KY494697	KY705167	KY705096
<i>Ap. dicranopteridis</i>	KUNCC 23-14171	<i>Dicranopteris pedata</i>	China	Endophyte	OR590336	OR590342	OR634944	OR634950
<i>Ap. dongyingensis</i>	SAUCC 0302 ^T	On diseased leaves of bamboo	China	–	OP572424	OP563375	OP573270	OP573264
<i>Ap. elliptica</i>	ZHKUCC 22-0131	On dead stem of unidentified plant	China	Saprobe	OR164952	NA	OR166323	OR166284
<i>Ap. endophytica</i>	ZHKUCC 23-0006 ^T	<i>Wurfbainia villosa</i>	China	Endophyte	OQ587984	OQ587996	OQ586075	OQ586062
<i>Ap. esporlensis</i>	CBS 145136 ^T	<i>Phyllostachys aurea</i>	Spain	Saprobe	MK014845	MK014878	MK017983	NA
<i>Ap. euphorbiae</i>	IMI 285638b	<i>Bambusa</i> sp.	Bangladesh	Saprobe	AB220335	AB220241	AB220288	NA
<i>Ap. fermenti</i>	KUC 21289 ^T	Seaweed	Republic of Korea	–	MF615213	MF615226	MF615231	MH544667
<i>Ap. fujianensis</i>	CGMCC 3.25647 ^T	Diseased bamboo leaves	China	–	PP159034	PP159026	PP488470	PP488454
<i>Ap. fujianensis</i>	CGMCC 3.25648	Diseased bamboo leaves	China	–	PP159035	PP159027	PP488471	PP488455
<i>Ap. fuzhouensis</i>	CGMCC 3.25649 ^T	Diseased bamboo leaves	China	–	PP159036	PP159028	PP488472	PP488456
<i>Ap. fuzhouensis</i>	CGMCC 3.25650	Diseased bamboo leaves	China	–	PP159037	PP159028	PP488473	PP488457

(Continued)

TABLE 2 Continued

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	tub2	tef1- α
<i>Ap. gaoyouensis</i>	CFCC 52301 ^T	Phragmites australis	China	Saprobe	NA	MH197124	MH236789	MH236793
<i>Ap. garethjonesii</i>	KUMCC 16-0202 ^T	Dead culms of bamboo	China	Saprobe	KY356091	KY356086	NA	NA
<i>Ap. gelatinosa</i>	HKAS 11962 ^T	Bamboo	China	Saprobe	MW478888	MW481706	MW522958	MW522941
<i>Ap. globosa</i>	KUNCC 23-14210 ^T	<i>Dicranopteris linearis</i>	China	Endophyte	OR590340	OR590347	NA	OR634954
<i>Ap. globosa</i>	GMBCC1021	Bamboo	China	Saprobe	PQ111491	PQ111502	NA	PQ464027
<i>Ap. gongcheniae</i>	YNE00465 ^T	Living stems of <i>Oryza meyeriana</i> subsp. <i>granulata</i>	China	Endophyte	PP033102	PP033259	PP034691	PP034683
<i>Ap. gongcheniae</i>	YNE00565	Living stems of <i>Oryza meyeriana</i> subsp. <i>granulata</i>	China	Endophyte	PP033103	PP0332560	PP034692	PP034684
<i>Ap. guangdongensis</i>	ZHKUCC 23-0004 ^T	<i>Wurfbainia villosa</i>	China	Endophyte	OQ587982	OQ587994	OQ586073	OQ586060
<i>Ap. guangdongensis</i>	GMBCC1022	Bamboo	China	Saprobe	PQ111485	PQ111496	PQ463976	PQ464020
<i>Ap. guangdongensis</i>	GMBCC1023	Bamboo	China	Saprobe	PQ111486	PQ111497	PQ463977	PQ464021
<i>Ap. guiyangensis</i>	HKAS 102403 ^T	Unidentified grass	China	Saprobe	MW240577	MW240647	MW775604	NA
<i>Ap. guizhouensis</i>	LC 5322 ^T	Air in karst cave	China	Endophyte	KY494785	KY494709	KY705178	KY705108
<i>Ap. guizhouensis</i>	GZCC 20-0114	bamboo		–	MW478895	MW481716	MW522964	MW522948
<i>Ap. hainanensis</i>	SAUCC 1681 ^T	On diseased leaves of bamboo	China	Pathogen	OP572422	OP563373	OP573268	OP573262
<i>Ap. hispanica</i>	IMI 326877 ^T	Beach sand	Spain	Saprobe	AB220336	AB220242	AB220289	NA
<i>Ap. hydei</i>	CBS 114990 ^T	Culms of <i>Bambusa tuldooides</i>	China	Saprobe/endophyte	KF144936	KF144890	KF144982	KF145024
<i>Ap. hyphopodii</i>	MFLUCC 15-0003 ^T	<i>Bambusa tuldooides</i>	China	Saprobe	NA	KR069110	NA	NA
<i>Ap. hyphopodii</i>	KUMCC 16-0201	<i>Bambusa tuldooides</i>	China	Saprobe	KY356093	KY356088	NA	NA
<i>Ap. hysterina</i>	ICMP 6889 ^T	Bamboo	New Zealand	Saprobe	MK014841	MK014874	MK017980	MK017951
<i>Ap. iberica</i>	CBS 145137 ^T	<i>Arundo donax</i>	Portugal	Saprobe	MK014846	MK014879	MK017984	NA
<i>Ap. intestini</i>	CBS 135835 ^T	Gut of a grasshopper	India	Saprobe	KR149063	KR011352	KR011350	KR011351
<i>Ap. italica</i>	CBS 145138 ^T	<i>Arundo donax</i>	Italy	Saprobe	MK014847	MK014880	MK017985	MK017956
<i>Ap. jatrophae</i>	AMH 9557 ^T	<i>Jatropha podagrica</i>	Italy	Saprobe	NA	JQ246355	NA	NA
<i>Ap. jiangxiensis</i>	LC 4577 ^T	<i>Maesa</i> sp.	China	Endophyte	KY494769	KY494693	KY705163	KY705092
<i>Ap. jinanensis</i>	SAUCC DL1981 ^T	On diseased leaves of <i>Bambusaceae</i> sp.	China	Endophyte	OQ615273	OQ592544	OQ613289	OQ613317
<i>Ap. jinanensis</i>	SAUCC DL2000	On diseased leaves of <i>Bambusaceae</i> sp.	China	Endophyte	OQ615272	OQ592543	OQ613288	OQ613316
<i>Ap. jinghongensis</i>	GMB-W1013 ^T	Bamboo	China	Saprobe	PQ140163	PQ140160	PQ463971	PQ464022
<i>Ap. jinghongensis</i>	GMB-W1014	Bamboo	China	Saprobe	PQ140164	PQ140161	PQ463972	PQ464023
<i>Ap. kogelbergensis</i>	CBS 113333 K	Dead culms of Restionaceae	South Africa	Saprobe	KF144938	KF144892	KF144984	KF145026

(Continued)

TABLE 2 Continued

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Ap. koreana</i>	KUC21332 ^T	Egg of <i>Arctoscopus japonicus</i>	Republic of Korea	–	MH498444	MH498524	MH498482	MH544664
<i>Ap. koreana</i>	KUNCC23-15553	<i>Bamboo</i> sp.	China	Saprobe	PP584787	PP584690	PP982289	PP933195
<i>Ap. lageniformis</i>	KUC21686 ^T	Branch of <i>Phyllostachys pubescens</i>	Republic of Korea	–	ON787761	ON764022	ON806636	ON806626
<i>Ap. locuta-pollinis</i>	LC 11683 ^T	<i>Brassica campestris</i>	China	Saprobe	NA	MF939595	MF939622	MF939616
<i>Ap. longistroma</i>	MFLUCC 11-0481 ^T	Dead culms of bamboo	Thailand	Saprobe	KU863129	KU940141	NA	NA
<i>Ap. lophatheri</i>	CFCC 58975 ^T	On diseased leaves of <i>Lophatherum gracile</i>	China	–	OR133588	OR125566	OR139980	OR139970
<i>Ap. machili</i>	SAUCC 1175A-4 ^T	<i>Machilus nanmu</i>	China	–	OR739574	OR739433	OR757130	OR753450
<i>Ap. magnispora</i>	ZHKUCC 22-0001 ^T	Bamboo	China	Saprobe	OM486971	OM728647	OM543544	OM543543
<i>Ap. malaysiana</i>	CBS 102053 ^T	<i>Macaranga hullettii</i>	Malaysia	Saprobe	KF144942	KF144896	KF144988	KF145030
<i>Ap. marianiae</i>	AP18219 ^T	<i>Phleum pratense</i>	Spain	Saprobe	ON692422	ON692406	ON677186	NA
<i>Ap. marii</i>	CBS 497.90 ^T	Beach sands	Spain	Saprobe	KF144947	AB220252	KF144993	KF145035
<i>Ap. marina</i>	KUC21328 ^T	Seaweed	Republic of Korea	–	MH498458	MH498538	MH498496	MH544669
<i>Ap. mediterranea</i>	IMI 326875	Air	Spain	Saprobe	AB220337	AB220243	AB220290	NA
<i>Ap. menglaensis</i>	KUNCC 24-17546 ^T	Dead culms of bamboo	China	Saprobe	PP584790	PP584693	PP982292	PP933198
<i>Ap. menglaensis</i>	KUNCC 24-17547	Dead culms of bamboo	China	Saprobe	PP584791	PP584694	PP982293	PP933199
<i>Ap. minutispora</i>	17E-042 ^T	Mountain soil	Republic of Korea	–	NA	LC517882	LC518888	LC518889
<i>Ap. montagnei</i>	AP301120 ^T	Balearic Islands	Spain	Sprobes	ON692424	ON692408	ON677188	ON677182
<i>Ap. mori</i>	MFLU 18-2514 ^T	<i>Morus australis</i>	China (Taiwan)	Saprobe	MW114393	MW114313	NA	NA
<i>Ap. mukdahanensis</i>	MFLUCC 21-0026 ^T	Dead bamboo	Thailand	Saprobe	OP377742	OP377735	NA	OP381089
<i>Ap. multiloculata</i>	MFLUCC 21-0023 ^T	Dead bamboo	Thailand	Saprobe	OL873138	OL873137	OL874718	NA
<i>Ap. mytilomorpha</i>	DAOM 214595 ^T	<i>Andropogon</i> sp.	India	Saprobe	NA	KY494685	NA	NA
<i>Ap. ananasi</i>	MFLUCC 23-0101 ^T	<i>Ananas comosus</i>	Thailand	Saprobe	OR438877	OR438410	OR538085	OR500339
<i>Ap. neobambusae</i>	LC 7106 ^T	Leaves of bamboo	China	Saprobe/endophyte	KY494794	KY494718	KY705186	KY806204
<i>Ap. neochinense</i>	CFCC 53036 ^T	<i>Fargesia qinlingensis</i>	China	Saprobe	NA	MK819291	MK818547	MK818545
<i>Ap. neogarethjonesii</i>	HKAS 102408 ^T	Bamboo	China	Saprobe	MK070898	MK070897	NA	NA
<i>Ap. neogongcheniae</i>	YNE01248 ^T	Living stems of Poaceae plant	China	Endophyte	PP033106	PP033263	PP034695	PP034687
<i>Ap. neogongcheniae</i>	YNE01260	Living stems of Poaceae plant	China	Endophyte	PP033107	PP033264	PP034696	PP034688

(Continued)

TABLE 2 Continued

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	tub2	tef1- α
<i>Ap. neosubglobosa</i>	JHB 006	Bamboo	China	Saprobe	KY356094	KY356089	NA	NA
<i>Ap. neosubglobosa</i>	KUMCC 16-0203 ^T	Bamboo	China	Saprobe	KY356095	KY356090	NA	NA
<i>Ap. obovata</i>	LC 4940 ^T	<i>Lithocarpus</i> sp.	China	Endophyte	KY494772	KY494696	KY705166	KY705095
<i>Ap. oenotherae</i>	CFCC 58972 ^T	On diseased leaves of <i>Oenothera bienni</i>	China	–	OR133590	OR125568	OR139982	OR139972
<i>Ap. olivata</i>	ZY22.052 ^T	Soil	China	–	OR680598	OR680531	OR843234	OR858925
<i>Ap. olivata</i>	ZY22.053	Soil	China	–	OR680599	OR680532	OR843235	OR858926
<i>Ap. ovata</i>	CBS 115042 ^T	<i>Arundinaria hindsii</i>	China	Saprobe	KF144950	KF144903	KF144995	KF145037
<i>Ap. paragongcheniae</i>	YNE00992 ^T	Living stems of Poaceae plant	China	Endophyte	PP033104	PP033261	PP034693	PP034685
<i>Ap. paragongcheniae</i>	YNE01259	Living stems of Poaceae plant	China	Endophyte	PP033105	PP033262	PP034694	PP034686
<i>Ap. paraphaeosperma</i>	MFLUCC 13-0644 ^T	Dead culms of bamboo	Thailand	Saprobe	KX822124	KX822128	NA	NA
<i>Ap. phragmitis</i>	CPC 18900	<i>Phragmites australis</i>	Italy	Saprobe	KF144956	KF144909	KF145001	KF145043
<i>Ap. phyllostachydis</i>	MFLUCC 18-1101 ^T	<i>Phyllostachys heteroclada</i>	China	Saprobe	MH368077	MK351842	MK291949	MK340918
<i>Ap. piptatheri</i>	CBS 145149 ^T	<i>Piptatherum miliaceum</i>	Spain	Saprobe	MK014860	MK014893	NA	NA
<i>Ap. pseudohyphopodii</i>	KUC21680 ^T	Culm of <i>Phyllostachys pubescens</i>	Republic of Korea	–	ON787765	ON764026	ON806640	ON806630
<i>Ap. pseudoparenchymatica</i>	LC7234 ^T	Leaves of bamboo	China	Endophyte	KY494819	KY494743	KY705211	KY705139
<i>Ap. pseudorasikravindrae</i>	KUMCC 20-0208 ^T	<i>Bambusa dolichoclada</i>	China	Saprobe	NA	MT946344	MT947367	MT947361
<i>Ap. pseudosinensis</i>	CPC 21546 ^T	On diseased leaves of bamboo	Netherlands	Saprobe	KF144957	KF144910	NA	KF145044
<i>Ap. pseudospegazzinii</i>	CBS 102052 ^T	<i>Macaranga hullettii</i>	Malaysia	Saprobe	KF144958	KF144911	KF145002	KF145045
<i>Ap. pterosperma</i>	CPC 20193 ^T	<i>Lepidosperma gladiatum</i>	Australia	Saprobe	KF144960	KF144913	KF145004	KF145046
<i>Ap. pusillisperma</i>	KUC21321 ^T	Seaweed	Republic of Korea	–	MH498453	MH498533	MH498491	MN868930
<i>Ap. qinlingensis</i>	CFCC 52303 ^T	<i>Fargesia qinlingensis</i>	China	Saprobe	NA	MH197120	MH236791	MH236795
<i>Ap. rasikravindrae</i>	LC 8179	<i>Brassica rapa</i>	China	Saprobe	KY494835	KY494759	KY705227	KY705155
<i>Ap. rasikravindrae</i>	MFLUCC 21-0051	Dead culms of bamboo	Thailand	Saprobe	MZ542527	MZ542523	MZ546412	MZ546408
<i>Ap. rasikravindrae</i>	MFLUCC 21-0054	Dead culms of maize	Thailand	Saprobe	MZ542526	MZ542522	MZ546411	MZ546407
<i>Ap. sacchari</i>	CBS 372.67	Air		Endophyte	KF144964	KF144918	KF145007	KF145049
<i>Ap. sacchari</i>	CBS 664.74	Soil under <i>Calluna vulgaris</i>	Netherlands	Endophyte	KF144965	KF144919	KF145008	KF145050
<i>Ap. saccharicola</i>	CBS 831.71	Air	Netherlands	Endophyte	KF144969	KF144922	KF145012	KF145054
<i>Ap. sargassi</i>	KUC21228 ^T	<i>Sargassum fulvellum</i>	Republic of Korea	–	KT207696	KT207746	KT207644	MH544677
<i>Ap. sasae</i>	CBS 146808 ^T	Dead culms	China	Saprobe	MW883797	MW883402	MW890120	NA
<i>Ap. septata</i>	CGMCC 3.20134 ^T	Bamboo	China	Saprobe	MW478890	MW481711	MW522960	MW522943

(Continued)

TABLE 2 Continued

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	tub2	tef1- α
<i>Ap. senecionis</i>	KUNCC23-15556 ^T	<i>Senecio scandens</i>	China	Saprobe	PP584794	PP584697	NA	PP993513
<i>Ap. senecionis</i>	KUNCC23-15557	<i>Senecio scandens</i>	China	Saprobe	PP584795	PP584698	NA	PP993514
<i>Ap. serenensis</i>	IMI 326869 ^T	Excipients, atmosphere, and home dust	Spain	Saprobe	AB220344	AB220250	AB220297	NA
<i>Ap. setariae</i>	CFCC 54041	<i>Setaria viridis</i>	China	Saprobe	NA	MT492004	MT497466	MW118456
<i>Ap. setostroma</i>	KUMCC 19-0217	Dead branches of bamboo	China	Saprobe	MN528011	MN528012	NA	MN527357
<i>Ap. shangrilaensis</i>	GMBCC1019 ^T	Bamboo	China	Saprobe	PQ111481	PQ111492	PQ164976	PQ164974
<i>Ap. shangrilaensis</i>	GMBCC1020	Bamboo	China	Saprobe	PQ111482	PQ111493	PQ164977	PQ164975
<i>Ap. sichuanensis</i>	HKAS 107008 ^T	Dead culm of grass	China	Saprobe	MW240578	MW240648	MW775605	NA
<i>Ap. sorghi</i>	URM 93000 ^T	<i>Sorghum bicolor</i>	Brazil	Endophyte	NA	MK371706	MK348526	NA
<i>Ap. sp.</i>	ZHKUCC 23-0010	<i>Wurfbainia villosa</i>	China	Endophyte	OQ587988	OQ588000	OQ586079	OQ586066
<i>Ap. sp.</i>	ZHKUCC 23-0011	<i>Wurfbainia villosa</i>	China	Endophyte	OQ587989	OQ588001	OQ586080	NA
<i>Ap. stipae</i>	CBS 146804 ^T	Dead culm of <i>Stipa gigantea</i>	Spain	Saprobe	MW883798	MW883403	MW890121	MW890082
<i>Ap. subglobosa</i>	MFLUCC 11-0397 ^T	Dead culms of bamboo	Thailand	Saprobe	KR069113	KR069112	NA	NA
<i>Ap. subglobosa</i>	GMB-W1024	Bamboo	China	Saprobe	PQ140165	PQ140162	PQ463973	PQ464024
<i>Ap. subrosea</i>	LC 7292 ^T	Leaves of bamboo	China	Endophyte	KY494828	KY494752	KY705220	KY705148
<i>Ap. taeanensis</i>	KUC21322 ^T	Seaweed	Republic of Korea	–	NA	MH498515	MH498473	MH544662
<i>Ap. thailandica</i>	MFLUCC 15-0202 ^T	Dead culms of bamboo	Thailand	Saprobe	KU863133	KU940145	NA	NA
<i>Ap. trachycarpi</i>	KUNCC23-15558 ^T	<i>Trachycarpus fortune</i>	China	Saprobe	PP584798	PP584701	PP982298	PP933204
<i>Ap. trachycarpi</i>	KUNCC23-15559	<i>Trachycarpus fortune</i>	China	Saprobe	PP584799	PP584702	PP982299	PP933205
<i>Ap. tropica</i>	MFLUCC 21-0056 ^T	Dead culms of bamboo	Thailand	Saprobe	OK491653	OK491657	NA	NA
<i>Ap. vietnamensis</i>	IMI 99670 ^T	<i>Citrus sinensis</i>	Vietnam	Saprobe	KX986111	KX986096	KY019466	
<i>Ap. wurfbainiae</i>	ZHKUCC 23-0008 ^T	<i>Wurfbainia villosa</i>	China	Endophyte	OQ587986	OQ587998	OQ586077	OQ586064
<i>Ap. xenocordella</i>	CBS 478.86 ^T	Soil from roadway	Zimbabwe	–	KF144970	KF144925	KF145013	KF145055
<i>Ap. xenocordella</i>	CBS 595.66	On dead branches	Misiones	Saprobe	KF144971	KF144926	NA	NA
<i>Ap. xishuangbannaensis</i>	KUMCC 21-0695 ^T	The wing of <i>Rhinolophus pusillus</i>	China	–	OP363248	ON426832	OR025930	OR025969
<i>Ap. yunnana</i>	MFLUCC 15-1002 ^T	<i>Phyllostachys nigra</i>	China	Saprobe	KU863135	KU940147	NA	NA
<i>Ap. yunnanensis</i>	ZHKUCC 23-0014 ^T	Grass	China	Saprobe	OQ587992	OQ588004	OQ586083	OQ586070
<i>Ap. zhaotongensis</i>	GMBCC1015 ^T	Bamboo	China	Saprobe	PQ111489	PQ111500	PQ463980	PQ464016

(Continued)

TABLE 2 Continued

Taxa	Strains	Substrate	Country	Lifestyles	GenBank Accession Numbers			
					LSU	ITS	tub2	tef1- α
<i>Ap. zhaotongensis</i>	GMBCC1016	Bamboo	China	Saprobe	PQ111490	PQ111501	PQ463981	PQ464017
<i>Ap. zhenxiongensis</i>	GMBCC1017 ^T	Bamboo	China	Saprobe	PQ111487	PQ111498	PQ463978	PQ464018
<i>Ap. zhenxiongensis</i>	GMBCC1018	Bamboo	China	Saprobe	PQ111488	PQ111499	PQ463979	PQ464019
<i>Neoarthrinium urticae</i>	IMI 326344	Leaf litter	India	–	AB220339	AB220245	AB220292	NA

Newly generated sequences in this study are in bold. “T” indicates type materials; “NA” indicates information not available. AMH, Ajrekar Mycological Herbarium, Pune, Maharashtra, India; CBS, Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CFCC, China Forestry Culture Collection Center, Beijing, China; CGMCC, China General Micro biological Culture Collection; CPC, Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; DAOM, Canadian Collection of Fungal Cultures, Ottawa, Canada; GMBCC, Guizhou Medical University Culture Collection, Guiyang, China; GMB-W, Herbarium of Guizhou Medical University, Guiyang, China; GUCC, Guizhou University Culture Collection, Guizhou, China; GZCC, Guizhou Culture Collection, China; HKAS, Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China; ICMP, International Collection of Microorganisms from Plants, New Zealand; IMI, Culture collection of CABI Europe UK Centre, Egham, UK; JHB, H.B. Jiang; KUC, the Korea University Fungus Collection, Seoul, Korea; SFC the Seoul National University Fungus Collection; KUMCC, Culture collection of Kunming Institute of Botany, Yunnan, China; KUNCC; Kunming Institute of Botany Culture Collection Center, Kunming, China; LC, Personal culture collection of Lei Cai, housed in the Institute of Microbiology, Chinese Academy of Sciences, China; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; SAUCC, Shandong Agricultural University Culture Collection; URM, ZHKUCC, Zhongkai University of Agriculture and Engineering, Guangdong, China.

as mentioned in Art. F.5.1 (International Code of Nomenclature for Algae, Fungi and Plant). (MFLUCC 21-0023, ex-type) with 100% ML and 1.00 posterior probability in BI analysis (Figure 1).

Results

Phylogenetic results

In this study, we selected 145 strains for the phylogenetic analysis and *Neoarthrinium urticae* (IMI 326344) as the outgroup taxon. In the phylogenetic analysis, the final alignment consisted of 2,099 characters in total, including gaps (ITS: 1–503 bp, LSU: 504–1,299 bp, *tef1- α* : 1,300–1,670 bp, *tub2*: 1,671–2,099 bp). The RAXML analysis of the combined dataset yielded the best scoring tree (Figure 1) with a final ML optimization likelihood value of –26,132.916506. The matrix had 1,073 distinct alignment patterns, with 14.23% undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.231558, C = 0.249603, G = 0.260882, and T = 0.257956; substitution rates AC = 1.277568, AG = 3.415629, AT = 1.036259, CG = 0.978503, CT = 5.039154, and GT = 1.000000; and gamma distribution shape parameter α = 0.556683.

Furthermore, according to the phylogenetic results, two isolates (GMBCC1011 and GMBCC1012) have a close affinity to *Ap. garethjonesii* (KUMCC 16-0202, ex-type) with 90% ML and 0.99 BP bootstrap support. Two other isolates, GMBCC1022 and GMBCC1023, had a close affinity to *Ap. guangdongensis* (ZHKUCC 23-0004, ex-type). While GMBCC1019 and GMBCC1020 formed a distinct lineage with 100% ML bootstrap support and 1.00 posterior probability in BI analysis, GMBCC1017 and GMBCC1018 with GMBCC1015 and GMBCC1016 formed a sister branch with 99% ML and 1.00 BP statistical support. The isolate GMBCC1021 clustered with *Ap. globosa* (KUNCC 23-14210, ex-type) with 100% ML and 1.00 BP. The collection GMB-W1024 has a high similarity with *Ap. subglobosa* (MFLUCC 11-0397, ex-type) with 100% ML and 1.00 BP. Two isolates, GMB-W1013 and GMB-W1014, formed a distinct branch with *Ap. multiloculata*

Taxonomic descriptions

Apiospora Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 4 4: 85 (1875); Index Fungorum Registration Identifier: IF264; Classification: Apiosporaceae, Amphisphaeriales, Sordariomycetes. *Apiospora dehongensis* L.S. Han & D.Q. Dai, sp. nov. (Figure 2) Index Fungorum Identifier: IF902463 Etymology: Named after the location “Dehong” where the new taxon was collected.

Description: *Saprobic* on dead branches of bamboo. Sexual morph: *Stromata* 0.4–2.5 mm long, 150–400 μ m wide, 125–140 μ m high, dark brown, fusiform or naviculate, raised on the host surface, with a slit-like opening at the top center, immersed, scattered to gregarious, or forming groups, uniloculate to multi-loculate. *Locules* 100–225 μ m diameter $\times$ 95–130 μ m high ($\bar{x}$ = 145 $\times$ 105 μ m, n = 20), gregarious, clustered, immersed in stromata, arranged in a row, obpyriform to subglobose, ostiolate at center with periphyses, membranous. *Peridium* 5–25 μ m wide, composed of brown to purple to hyaline cells of *textura angularis*. *Hamathecium* 2.5–6.5 μ m wide, filamentous, septate, unbranched, constricted at the septum. *Asci* 85–110 $\times$ 14–20 μ m ($\bar{x}$ = 97.5 $\times$ 17.5 μ m, n = 20), 6-(8)-spored, unitunicate, clavate, apedicellate, apically rounded, straight to slight curved. *Ascospores* 25–30 $\times$ 10–12 μ m ($\bar{x}$ = 27 $\times$ 10.8 μ m, n = 20), 1–3-seriate, ellipsoidal, 2-celled, with a large upper cell and a smaller lower cell, with guttules, hyaline, smooth-walled, with a gelatinous sheath. Asexual morph: Conidiophores and conidiogenous cells were not observed. *Conidia* forming on culture, 13–17.5 μ m ($\bar{x}$ = 16 μ m, n = 20), globose to subglobose, dark brown, unicellular, smooth-walled, with guttules.

Culture characteristics: Ascospores germinate on PDA within 24 h. Colonies reached 60 mm after 20 days at 27 $^{\circ}$ C. The colonies are flat, white to reddish and produce red pigment on agar medium.

Material examined: CHINA, Yunnan Province, Dehong, Ruili, Jiexiang town (23 $^{\circ}$ 97′17″ N, 97 $^{\circ}$ 73′03″ E, 926 m), on dead branches

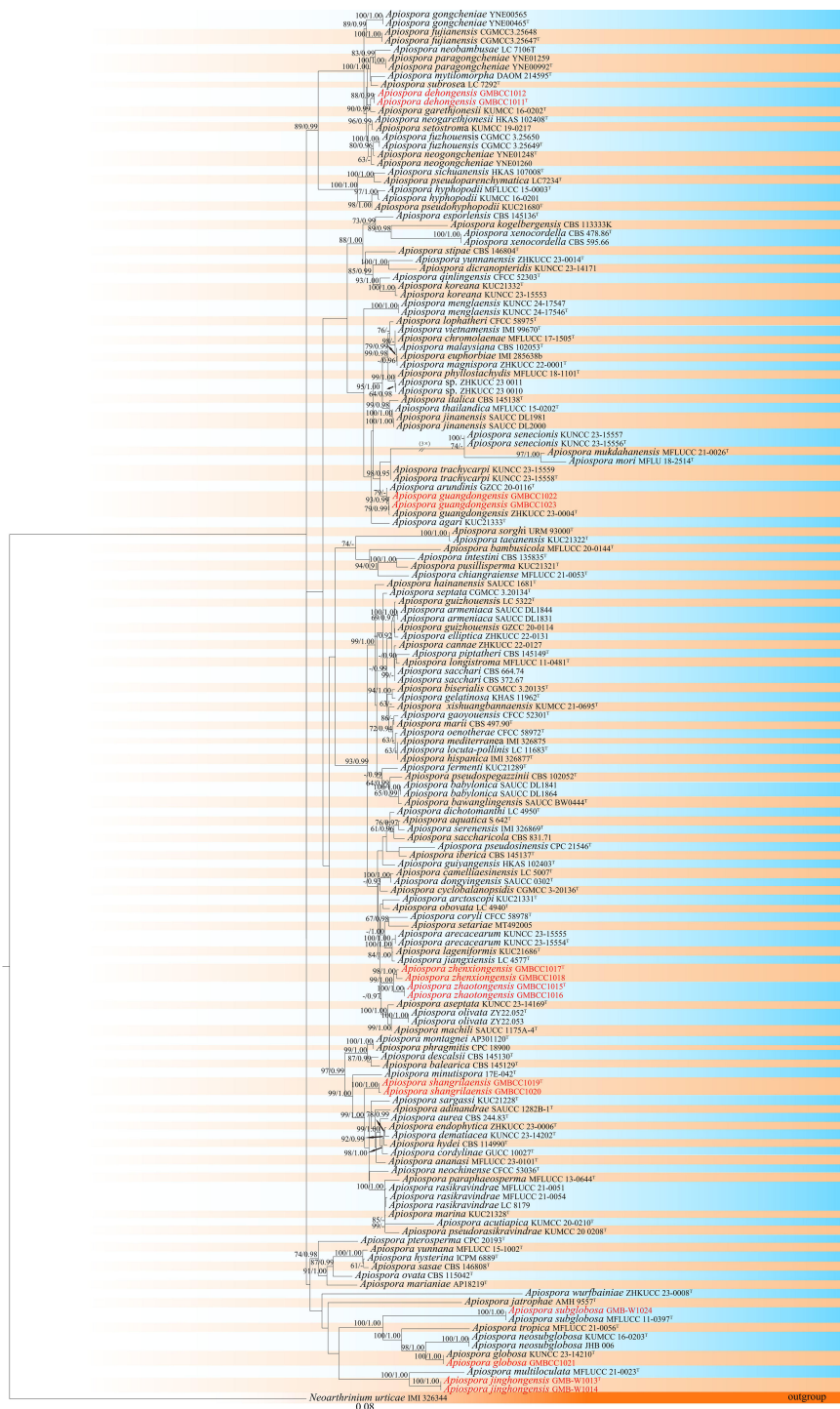


FIGURE 1

Phylogram retrieved from RAxML of *Apiospora* species using the combined dataset of LSU, ITS, *tef1-α*, and *tub2* gene regions. The statistical values are provided at nodes as ML/PP (ML value equal to or above 60% and BI value equal to or above 0.90). The tree is rooted with *Neoeurhynchium urticae* (IMI 326344). Ex-types and new strains are indicated by the superscript “T” and red respectively.

of bamboo, 23 July 2023, L.S. Han & D.Q. Dai, HLS41 (GMB-W1011, holotype), ex-type GMBCC1011; *ibid.* (MHZU 24-0623, isotype), ex-isotype ZHKUCC 24-1160; *ibid.* HLS90 (GMB-W1012, isotype), ex-isotype GMBCC1012.

Notes: Phylogenetic analyses showed that newly generated strains GMBCC1011 and GMBCC1012 formed a sister branch to

Ap. garethjonesii (D.Q. Dai & H.B. Jiang) Pintos & P. Alvarado (KUMCC 16-0202, ex-type) with 90% ML and 0.99 BI support (Figure 1). However, *tef1-α* and *tub2* data of *Ap. garethjonesii* (KUMCC 16-0202, ex-type) are unavailable in GenBank. Morphologically, *Ap. dehongensis* differs from *Ap. garethjonesii* in having smaller asci (85–110 × 14–20 μm vs. 125–154 × 35–42 μm)

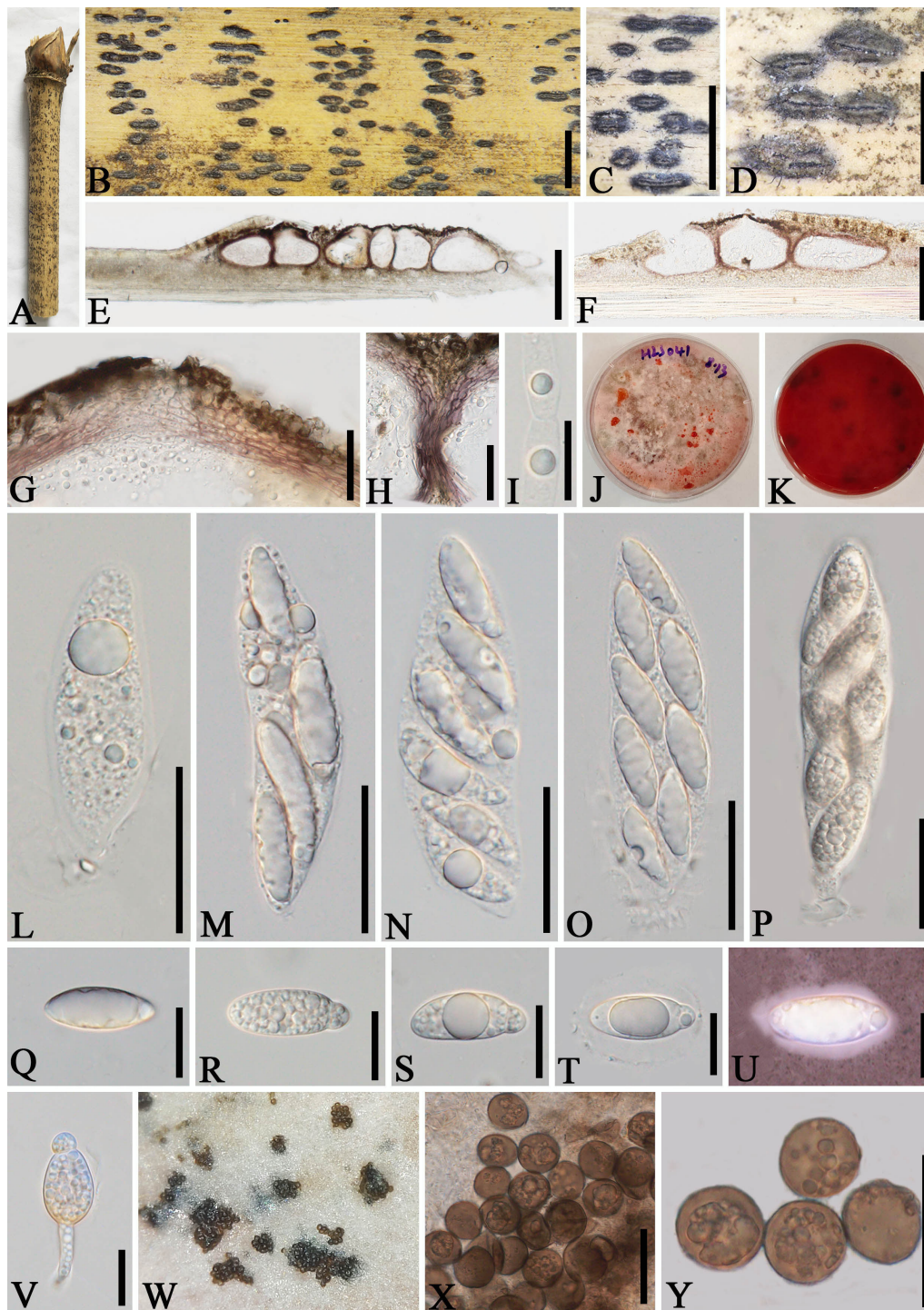


FIGURE 2

Apiospora dehongensis (GMB-W1011, holotype). (A) Bamboo specimen. (B–D) Stromata developing on bamboo branches. (E, F) Vertical sections of stromata. (G, H) Peridium. (I) Paraphyses. (J, K) Cultures on PDA with red pigmentation [upper (J), reverse (K)]. (L–P) Asci. (Q–T) Ascospores. (U) Ascospore stained in Indian ink showing gelatinous sheath. (V) A germinating ascospore. (W) Conidia formed in culture. (X, Y) Conidia. Scale bars: (B–D) 2 mm, (E) 200 μ m, (F) 150 μ m, (G, H, L–P, X, Y) 30 μ m, and (I, Q–V) 15 μ m.

and ascospores ($25\text{--}30 \times 10\text{--}12 \mu\text{m}$ vs. $30\text{--}42 \times 11\text{--}16 \mu\text{m}$) (Dai et al., 2016). Moreover, our cultures produced red pigment on PDA (Figures 2J, K), which was not observed in *Ap. garthjonesii* (Dai et al., 2016). Hence, based on morphological and culture

characteristics and DNA sequence analyses, we introduce our new collection as a new species, viz., *Ap. dehongensis*.

Apiospora jinghongensis L.S. Han & D.Q. Dai, sp. nov. (Figure 3)

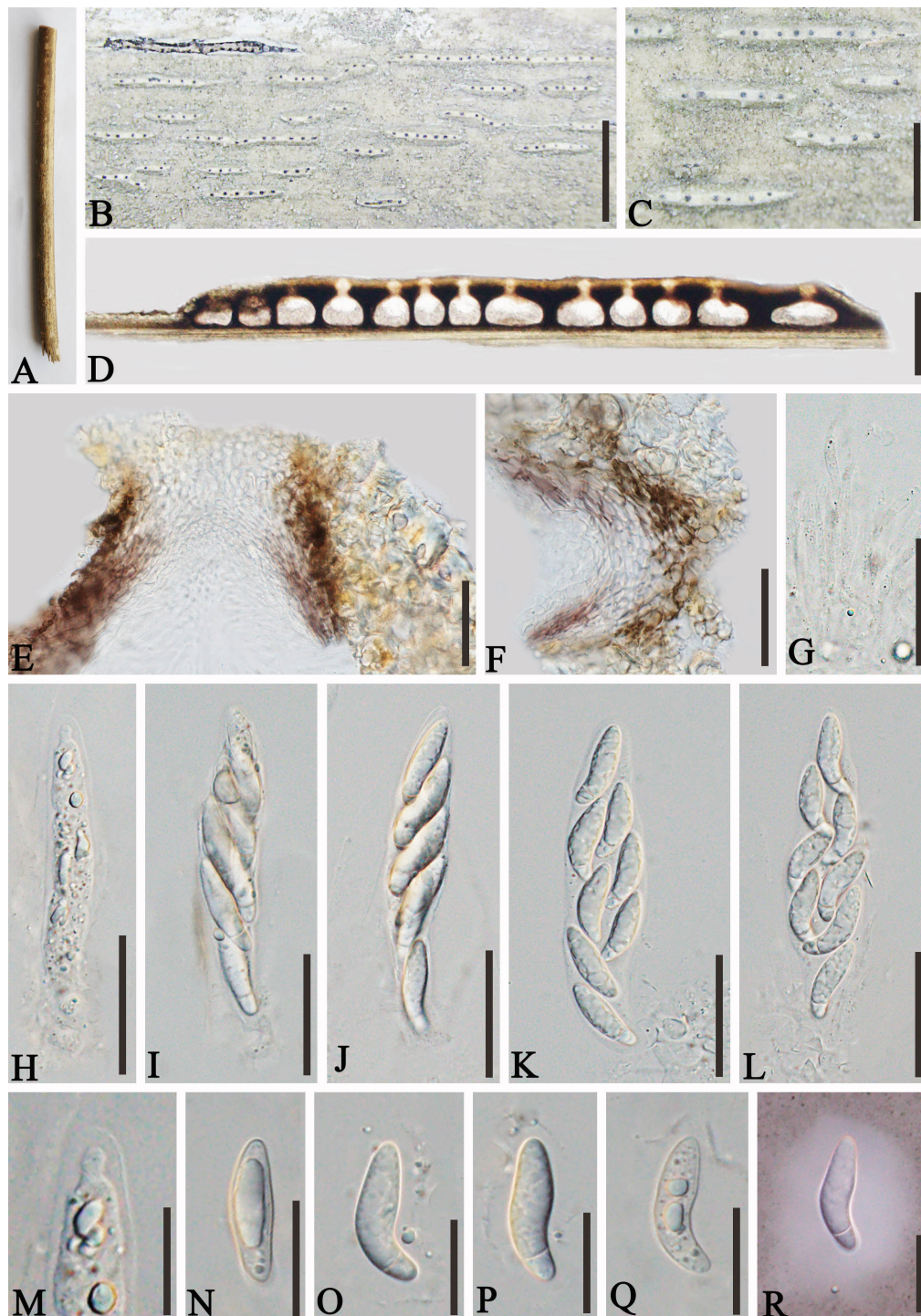


FIGURE 3

Apiospora jinghongensis (GMB-W1013, holotype). (A) Bamboo specimen. (B, C) Stromata developing on bamboo branches. (D) Vertical sections of stromata. (E, F) Peridium. (G) Paraphyses. (H–L) Asci. (M) Ascus with the rounded and smooth apex. (N–R) Ascospores [ascospore stained in Indian ink showing gelatinous sheath (R)]. Scale bars: (B) 2 mm, (C) 1 mm, (D) 300 μ m, (E–L) 30 μ m, and (M–R) 15 μ m.

Index Fungorum Identifier: IF902464

Etymology: Named after the location “Jinghong” where the new taxon was collected.

Description: *Saprobic* on dead branches of bamboo. Sexual morph: *Stromata* 0.6–5.5 mm long, 250–400 μ m wide, 175–200 μ m high, filiform, with parallel black spots, raised when mature, still

under the host surface, scattered to gregarious, 2–20-loculate. *Locules* 110–240 μ m diameter $\times$ 150–185 μ m high ($\bar{x}$ = 160 $\times$ 170 μ m, n = 20), clustered, gregarious, immersed in stromata, forming groups, arranged in a row, ampulliform to obpyriform, usually with flattened base, brown to dark brown, membranous, with a periphysate ostiole in the center. *Peridium* 15–40 μ m thick,

composed of several layers, brown to hyaline cells of *textura angularis*. *Hamathecium* 1.5–4 μm wide, filamentous, hyaline, septate, unbranched paraphyses. *Asci* 85–105 $\times$ 13–20 μm ($\bar{x}$ = 91.5 $\times$ 16 μm , n = 20), 8-spored, unitunicate, clavate to cylindrical, apically rounded, slightly curved, short pedicel. *Ascospores* 22–28 $\times$ 6.5–7.5 μm ($\bar{x}$ = 23 $\times$ 6.7 μm , n = 20), biseriate, ellipsoidal, 1-septate, upper cell larger, and lower cell smaller, hyaline, smooth-walled, rounded at both ends, curved at the bottom, surrounded a gelatinous sheath. Asexual morph: Undetermined.

Material examined: CHINA, Yunnan Province, Xishuangbanna, Jinghong city, Manzhong, Mengla (21°91'97" N, 101°20'42" E, 617 m), on dead branches of bamboo, 16 August 2020, L.S. Han & D.Q. Dai, DDQ1033 (GMB-W1013, holotype); *ibid.* (MHZU 24-0624, isotype); *ibid.* DDQ1033-1 (GMB-W1014, isotype).

Notes: The phylogenetic tree shows that our new collections GMB-W1013 and GMB-W1014 formed a distinct sister branch to *Ap. multiloculata* Zhang et al. (MFLUCC 21-0023) with 100% ML and 1.00 BI support (Figure 1). Additionally, the nucleotide pairwise of new collections and *Ap. multiloculata* in ITS, LSU, and *tub2* have 6.9% (37/535 bp), 0.8% (7/793 bp), and 9.7% (41/421 bp) differences, respectively. Morphologically, our new collection resembles *Ap. multiloculata* in having filiform stromata with central ostiole, 1-septate ascospores curved at the lower cell, but differs by having wider locules (110–240 μm vs. 88–160 μm) (Bhunjun et al., 2022). Although the new taxon morphologically resembles *Ap. multiloculata*, based on multigene phylogenetic analyses, we introduced *Ap. jinghongensis*.

Apiospora shangrilaensis L.S. Han & D.Q. Dai, sp. nov. (Figure 4)

Index Fungorum Identifier: IF902465

Etymology: Named after the location “Shangri-La” where the new taxon was collected.

Description: *Saprobic* on dead culms of bamboo. Sexual morph: *Stromata* 1–3.2 mm long, 200–400 μm wide, 185–210 μm high, elongated fusiform, raised with long, black axis broken at the apex, immersed, multi-loculate. *Locules* 200–250 μm diameter $\times$ 120–190 μm high ($\bar{x}$ = 228 $\times$ 157.5 μm , n = 20), gregarious, clustered, immersed in stromata, arranged in a row, ampulliform to subglobose with poor development base, pseudothecial, brown to dark brown. *Peridium* 10–25 μm wide, composed of three to five layers of hyaline to brown, cells of *textura angularis*. *Hamathecium* 2.5–4.5 μm wide, long, septate, slightly tapering at the top, unbranched paraphyses. *Asci* 130–150 $\times$ 30–40 μm ($\bar{x}$ = 140 $\times$ 36.6 μm , n = 20), 8-spored, unitunicate, clavate, apically rounded, straight to slightly curved, with short pedicel. *Ascospores* 40–55 $\times$ 14–17 μm ($\bar{x}$ = 46.4 $\times$ 15.4 μm , n = 20), 1–2-seriate, ellipsoidal, aseptate when immature, 1-septate when mature, with a larger upper cell, and a smaller lower cell, occasionally with guttules, hyaline, smooth-walled, surrounded by a gelatinous sheath. Asexual morph: Undetermined.

Culture characteristics: Ascospores germinating on PDA within 24 h. Colonies reached 30 mm diameter in 20 days under dark and at 27°C conditions. Colonies flat, circular, cottony, irregular edge, white from above, yellow in the center and outward gradually becoming pale yellow to white from below.

Materials examined: CHINA, Yunnan Province, Shangri-La City, Bigu mountain (27°36'56.9"N, 99°42'6.4"E, 3,460 m), on

dead culms of bamboo, 21 July 2020, L.S. Han & D.Q. Dai, DDQ00801 (GMB-W1019, holotype), ex-type, GMBCC1019, *ibid.* (MHZU 24-0625, isotype), ex-isotype ZHKUCC 24-1161, *ibid.* DDQ00920 (GMB-W1020), living culture GMBCC1020.

Notes: In the phylogenetic analyses, our new isolates, GMBCC1019 and GMBCC1020, formed a distinct branch (Figure 1). Morphologically, the new species is resembling *Ap. hydei* (Crous) Pintos & P. Alvarado (CBS 114990, ex-type) in having immersed, multi-loculate ascostromata, unitunicate asci, 1-septate, smooth-walled ascospores with a gelatinous sheath. However, our new collections can be distinguished from *Ap. hydei* (CBS 114990, ex-type) by having longer and wider asci (130–150 $\times$ 30–40 μm vs. 110–130 $\times$ 17–24 μm) and wider ascospores (40–55 $\times$ 14–17 μm vs. 35–45 $\times$ 8.5–11 μm) (Dai et al., 2016; Zeng et al., 2022). Hence, we introduced *Ap. shangrilaensis* as a new member of *Apiospora* based on morphological characteristics and phylogeny.

Apiospora zhaotongensis L.S. Han & D.Q. Dai, sp. nov. (Figure 5)

Index Fungorum Identifier: IF902466

Etymology: Named after the location “Zhaotong” where the new taxon was discovered.

Description: *Saprobic* on dead branches of bamboo. Sexual morph: *Stromata* 55–270 μm long, 250–450 μm wide, 140–180 μm high, naviculate or filiform, raised but still under on the host tissue with a slit-like opening at the top, scattered to gregarious, uniloculate to multi-loculate, black. *Locules* 150–290 diameter $\times$ 100–170 μm high ($\bar{x}$ = 250 $\times$ 130 μm , n = 20), immersed in stromata, scattered or clustered, dark brown to black, obpyriform to subglobose, with a central ostiole, papillate. *Peridium* 25–45 μm thick, composed of several layers, dark brown to hyaline cells of *textura angularis*. *Hamathecium* 2.5–4.5 μm wide, filamentous distinctly septate, constricted at the septum, unbranched paraphyses, with guttules. *Asci* 85–110 $\times$ 14–20 μm ($\bar{x}$ = 97.5 $\times$ 17.5 μm , n = 20), 8-spored, unitunicate, cylindrical, straight to slightly curved, apically rounded, apedicellate. *Ascospores* 30–40 $\times$ 6–7.5 μm ($\bar{x}$ = 36 $\times$ 6.5 μm , n = 20), overlapping, 2-seriate, 1-septate, conical at both ends, with a larger upper cell and a smaller lower cell, some with guttules, hyaline, smooth-walled, mostly straight, sometimes slightly curved, surrounded by a gelatinous sheath. Asexual morph: Undetermined.

Culture characteristics: Ascospores germinate on PDA within 24 h. Colonies reached 60 mm after 20 days at 27°C. The colonies are white, fluffy, cottony, with irregular edge.

Material examined: CHINA, Yunnan Province, Zhaotong City, Zhenxiong Town (27°62'52"N, 104°81'98"E), on dead branches of bamboo, 4 August 2023, L.S. Han & D.Q. Dai, HLS110 (GMB-W1015, holotype), ex-type GMBCC1015; *ibid.* (MHZU 24-0626, isotype), ex-isotype, ZHKUCC 24-1162; *ibid.* HLS110-1 (GMB-W1016, isotype), GMBCC1016 (ex-isotype).

Notes: Two newly generated strains, GMBCC1015 and GMBCC1016, are phylogenetically close to *Ap. zhenxiongensis* (GMBCC1017 ex-type, GMBCC1018) (Figure 1). Morphologically, the new taxon can be distinguished from *Ap. zhenxiongensis* in having ascospores conical at both ends, whereas *Ap. zhenxiongensis* ascospores are rounded at both ends. Moreover, *Ap.*

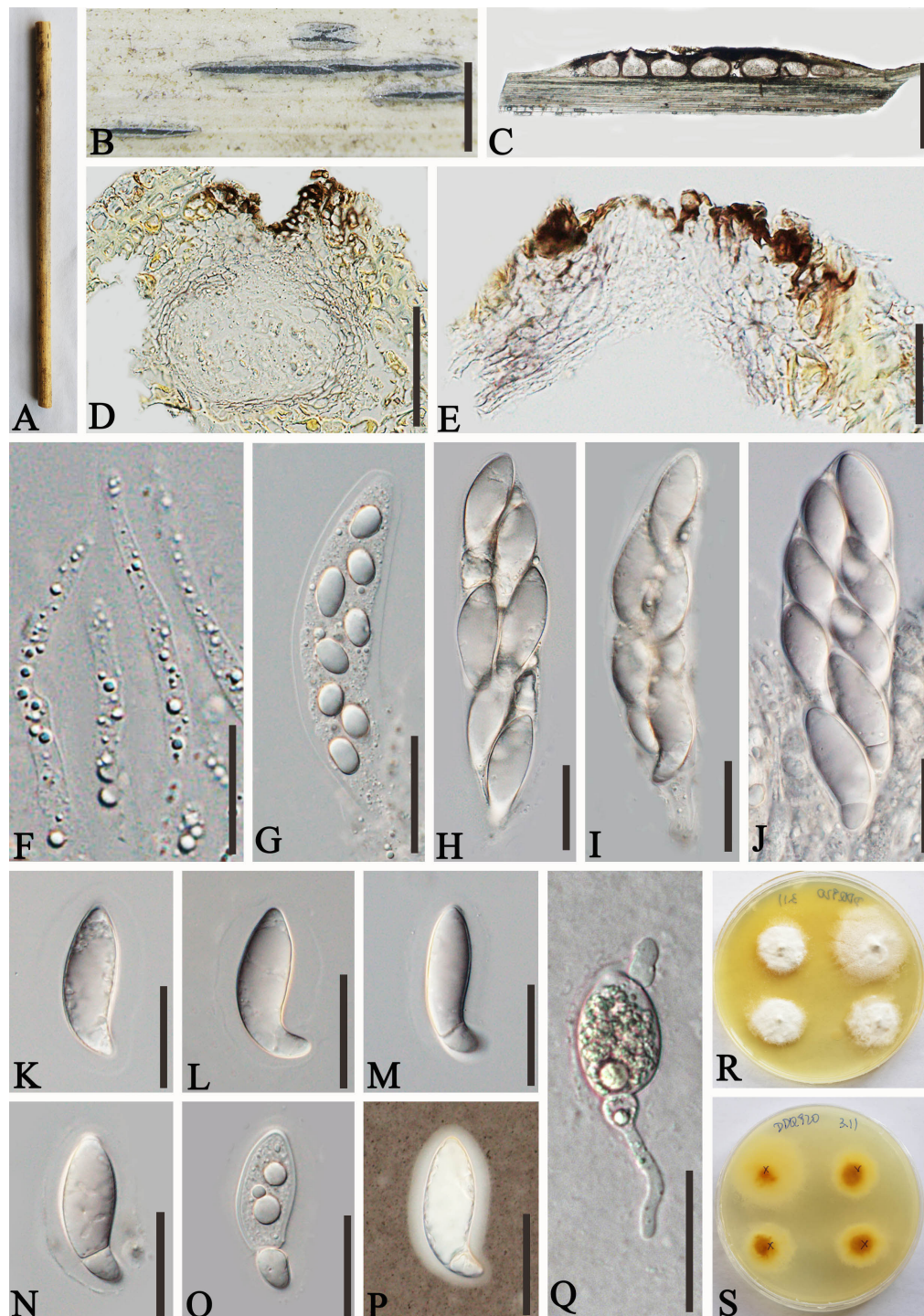


FIGURE 4

Apiospora shangrilaensis (GMB-W1019, holotype). (A) Bamboo specimen. (B) Stromata developing on bamboo branches. (C, D) Vertical sections of stromata. (E) Peridium. (F) Paraphyses. (G–J) Asci. (K–P) Ascospores [a ascospore stained in Indian ink showing gelatinous sheath (P)]. (Q) The germinating ascospore. (R, S) Cultures on PDA [upper (R), reverse (S)]. Scale bars: (C) 500 µm, (D, E, G–Q) 50 µm, (F) 15 µm.

zhaotongensis has straighter ascospores at the bottom than *Ap. zhenxiongensis*. Therefore, we introduce a novel species, *Ap. zhaotongensis*, to accommodate our new collection based on morphology and phylogeny.

Apiospora zhenxiongensis L.S. Han & D.Q. Dai, sp. nov. (Figure 6)

Index Fungorum Identifier: IF902467

Etymology: Named after the location “Zhenxiong” where the new taxon was discovered.

Description: *Saprobic* on dead branches of bamboo. Sexual morph: *Stromata* 0.45–1.6 mm long, 200–450 µm wide, 140–160 µm high, raised, with a slit-like opening at the top, dark brown to

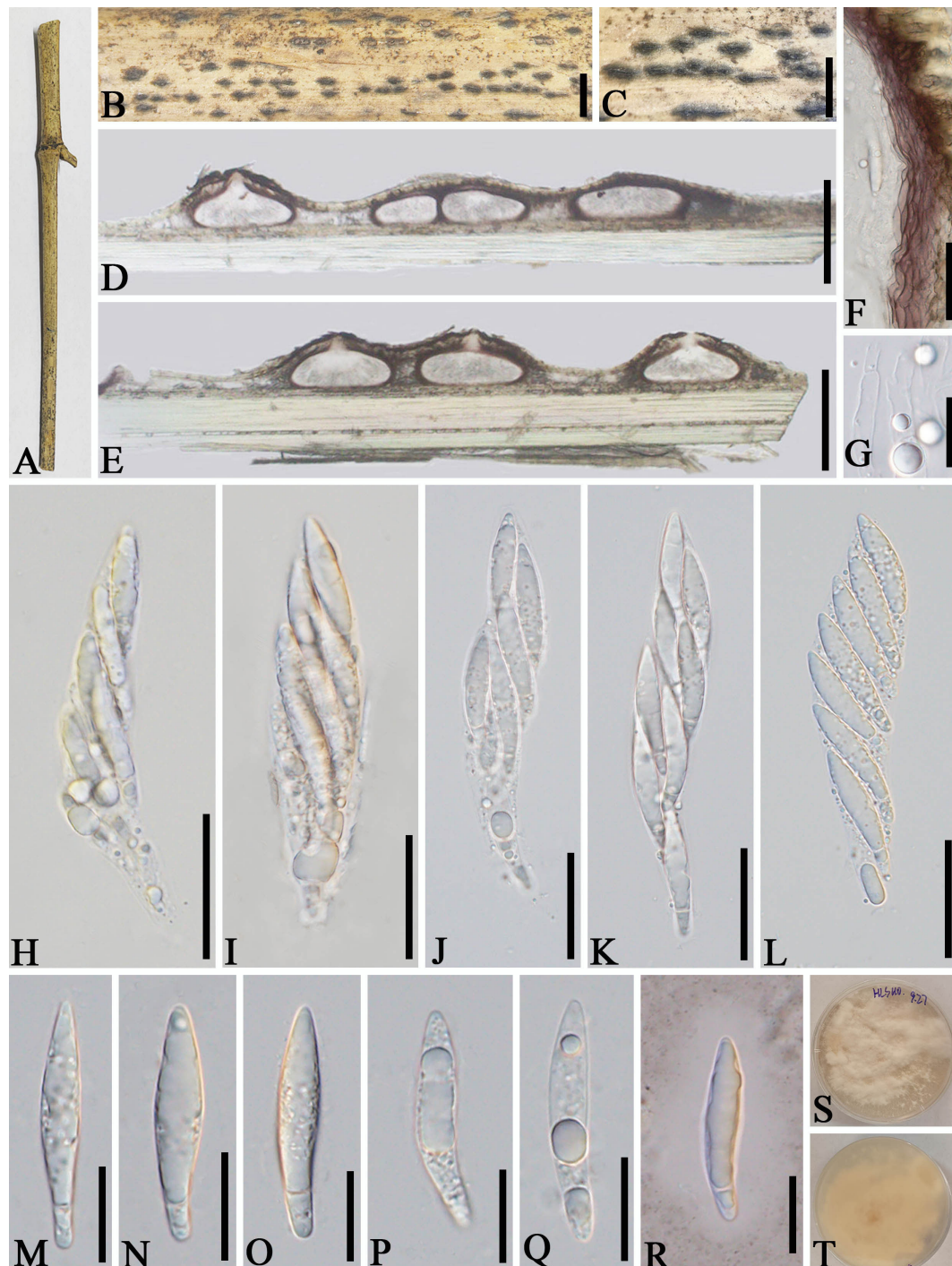


FIGURE 5

Apiospora zhaotongensis (GMB-W1015, holotype). (A) Bamboo specimen. (B, C) Stromata developing on bamboo branches. (D, E) Vertical sections of stromata. (F) Peridium. (G) Paraphyses. (H–L) Asci. (M–R) Ascospores. (R) The ascospore stained in Indian ink showing gelatinous sheath. (S, T) Cultures on PDA [upper (S), reverse (T)]. Scale bars: (B) 1.5 mm, (C) 1 mm, (D, E) 300 μ m, (F, H–L) 30 μ m, and (G, M–R) 15 μ m.

black, scattered to gregarious, naviculate or filiform, multi-loculate. Locules 130–230 μ m diameter $\times$ 90–150 μ m high ($\bar{x}$ = 183 $\times$ 128 μ m, n = 20), immersed in stromata, arranged in a row, obpyriform to ampulliform, dark brown to black. Ostiole 30–60 μ m wide, 35–65 μ m high, with a black papillate. Peridium 5–25 μ m thick, composed of several layers of brown cells of *textura angularis*. Hamathecium 3–8 μ m wide, hyaline, septate, unbranched paraphyses. Asci

80–110 $\times$ 15–25 μ m ($\bar{x}$ = 95 $\times$ 19 μ m, n = 20), 8-spored, unitunicate, cylindrical, apically rounded, with short pedicel. Ascospores 30–40 $\times$ 6–8.5 μ m ($\bar{x}$ = 34 $\times$ 7.5 μ m, n = 20), overlapping, biserial, ellipsoidal, rounded at both ends, 1-septate, cell above septa larger than those below, with guttules, hyaline, smooth-walled, distinctly curved at lower cell when mature, with a gelatinous sheath. Asexual morph: Undetermined.

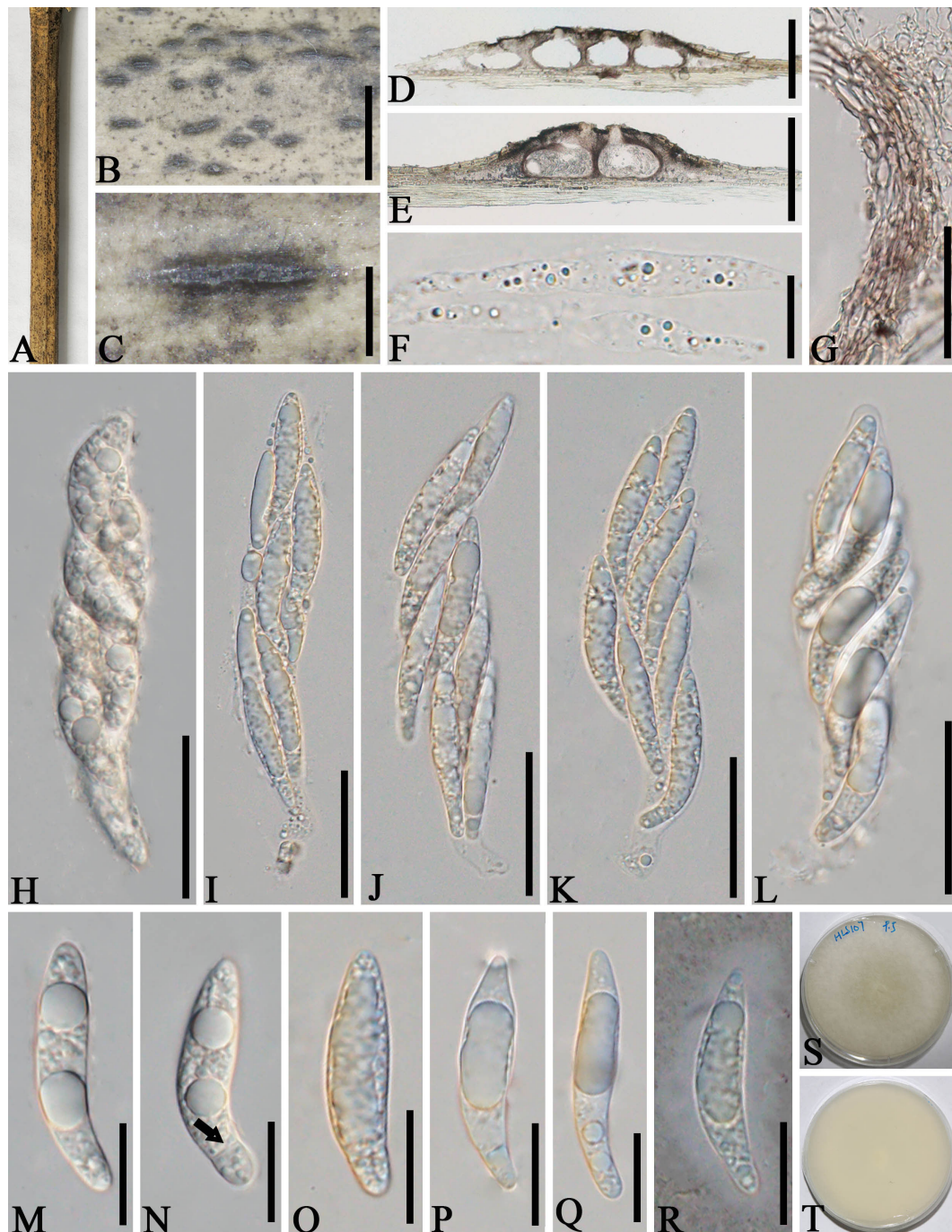


FIGURE 6

Apiospora zhenxiongensis (GMB-W1017, holotype). (A) Bamboo specimen. (B, C) Stromata developing on bamboo branches. (D, E) Vertical sections of stromata. (F) Paraphyses. (G) Peridium. (H–L) Asci. (M–R) Ascospores [a ascospore stained in Indian ink showing gelatinous sheath (R)]. (S, T) Cultures on PDA [upper (S), reverse (T)]. Scale bars: (B) 1.5 mm, (C) 500 μ m, (D, E) 300 μ m, (F, M–R) 15 μ m, and (G, H–L) 30 μ m.

Culture characteristics: Ascospores germinate on PDA within 24 h. Colonies reached 60 mm after 20 days at 27°C. The colonies are flat, white from above and below, dense, circular, cottony, with regular edge.

Material examined: CHINA, Yunnan Province, Zhaotong City, Zhenxiong (27°63'28" N, 104°81'85" E, 1,559 m), on dead branches of bamboo, 4 August 2023, L.S. Han & D.Q. Dai, HLS107 (GMB-W1017, holotype), ex-type GMBCC1017; *ibid.* (MHZU 24-0627,

isotype), ex-isotype, ZHKUCC 24-1163; *ibid.* HLS136 (GMB-W1018), living culture GMBCC1018.

Notes: In our phylogenetic analyses, GMBCC1017 and GMBCC1018 formed a sister branch to *Ap. zhaotongensis* (GMBCC1015, ex-type and GMBCC1016) (99% ML, 1.00 BP, Figure 1). Morphologically, the new collections can be distinguished from *Ap. zhaotongensis* in having ascospores with rounded ends at both ends, while *Ap. zhaotongensis* ascospores have

conical ends. Moreover, the ascospores of the new isolate are more curved at the lower cell than *Ap. zhaotongensis*. Hence, we introduced *Ap. zhenxiongensis* to accommodate our new collections based on morphological comparisons coupled with molecular data.

Apiospora globosa J.Y. Zhang & Y.Z. Lu, *Journal of Fungi* 9 (no. 1,096) (2023) (Figure 7)

Index Fungorum Identifier: IF 901402

Description: *Saprobic* on dead culms of bamboo. Sexual morph: *Stromata* 0.45–3.3 mm long, 200–300 μm wide, 260–300 μm high,

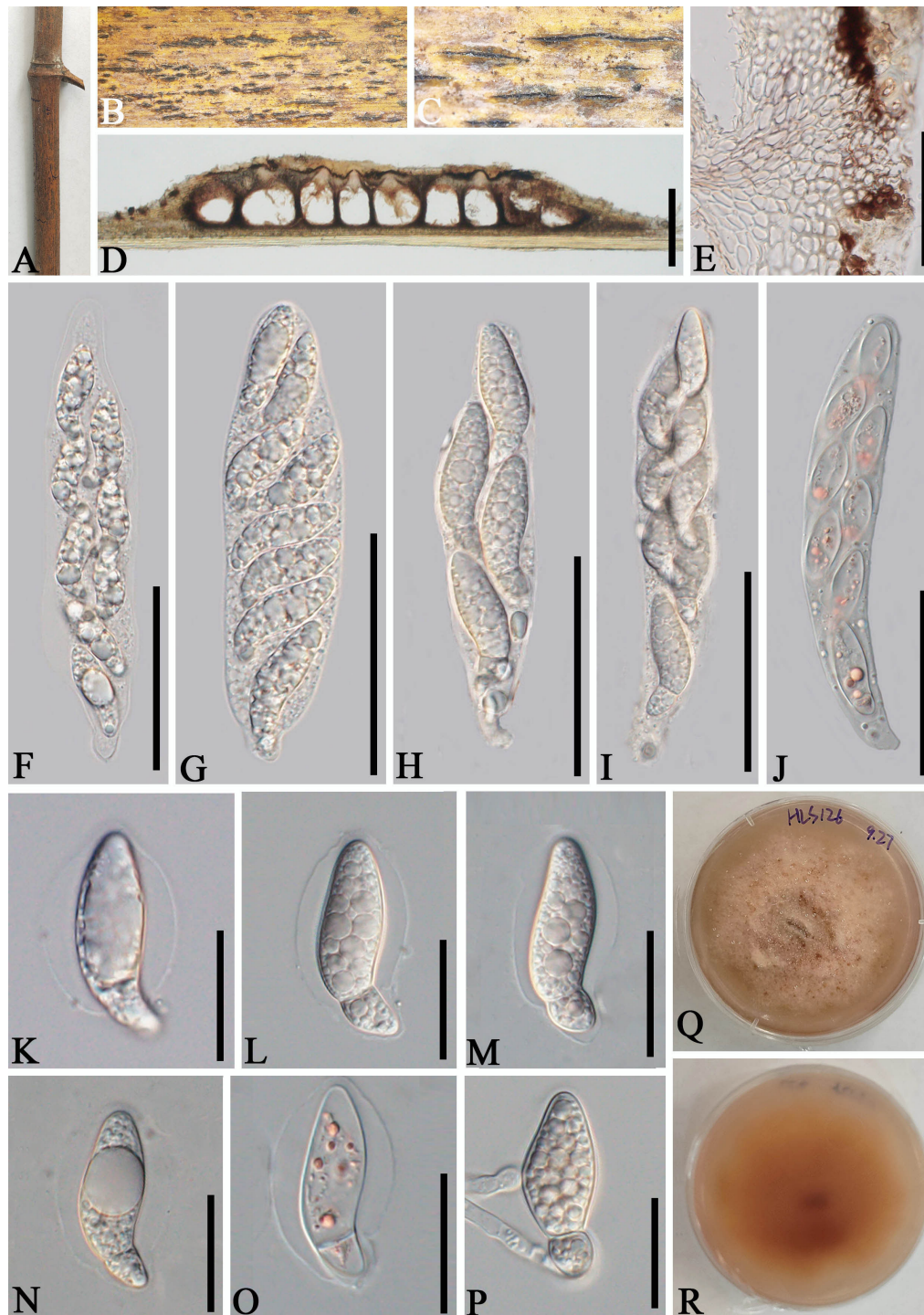


FIGURE 7

Apiospora globosa (GMB-W1021) (A) Bamboo specimen. (B, C) Stromata developing on bamboo branches. (D) Vertical sections of stromata. (E) Peridium. (F–J) Asci. (K–O) Ascospores surrounded by a gelatinous sheath. (P) A germinating ascospore. (Q, R) Cultures on PDA [upper (Q), reverse (R)]. Scale bars: (D) 300 μm , (E–J) 50 μm , and (K–P) 20 μm .

brown to black, fusiform, with stromata breaking through raised cracks at the black center, immersed, gregarious, multi-loculate. *Locules* 180–255 μm diameter $\times$ 100–240 μm high ($\bar{x}$ = 222 $\times$ 164.5 μm , n = 20), gregarious, clustered, immersed in stromata, arranged in a row, obpyriform to ampulliform, ostiole with periphyses, membranous, brown to dark brown. *Peridium* 10–50 μm thick, composed of several layers of brown to hyaline, cells of *textura angularis*. *Asci* 100–135 $\times$ 21–25 μm ($\bar{x}$ = 115.8 $\times$ 22.4 μm , n = 20), 4–(8)-spored, unitunicate, broadly cylindrical to clavate, with a short pedicel, straight to slightly curved, apically rounded. *Ascospores* 32–40 $\times$ 10–12.5 μm ($\bar{x}$ = 34.6 $\times$ 11.3 μm , n = 20), 1–2-seriate, elliptical, 1-septate, with a larger upper cell, and a small lower cell, hyaline, with many guttules, smooth-walled, curved, constricted at the septum, surrounded by an entire gelatinous sheath. Asexual morph: *Endophytic* in the stems of *Dicranopteris linearis*, see Zhang et al. (2023a).

Culture characteristics: Ascospores germinate on PDA within 24 h. Colonies reached 55 mm after 20 days at 27°C. The colonies are white to pale reddish from above, pale reddish from below, circular, cottony, flat, spreading, with irregular edge.

Material examined: CHINA, Yunnan Province, Zhaotong City, Zhenxiong town (27°63'36"N, 104°81'84"E, 1,577 m), on dead culms of bamboo, 4 August 2023, L.S. Han & D.Q. Dai, HLS126 (GMB-W1021, first report of the sexual morph), living culture, GMBCC1021.

Notes: *Apiospora globosa* J.Y. Zhang & Y.Z. Lu was originally described by Zhang et al. (2023a) based on the asexual morph from a healthy stem of *Dicranopteris linearis* (KUNCC 23-14210, ex-type) collected from Guizhou Province, China. Our phylogenetic results (Figure 1) indicated that the strain GMBCC1021 is identical to the ex-type of *Ap. globosa* (KUNCC 23-14210, ex-type) with 100% MLBP and 1.00 BYPP statistic support. Moreover, the base pair arrangement of our collections with KUNCC 23-14210 is identical. Hence, in this study, we report the sexual morph of *Ap. globosa* for the first time.

Apiospora guangdongensis C.F. Liao & Doilom, *Journal of Fungi* 9 (no. 1,087): 12 (2023) (Figure 8)

Index Fungorum Identifier: IF 225951

Description: *Saprobic* on dead culms of bamboo. Sexual morph: *Stromata* 0.4–2.8 mm long, 250–350 mm wide, 130–190 μm high, raised on the host surface, with blackspots on slit-like opening, immersed, scattered to gregarious, 1–5-loculate, fusiform, brown. *Locules* perithecial, 210–380 μm diameter $\times$ 110–180 μm high ($\bar{x}$ = 269 $\times$ 145 μm , n = 20), gregarious, clustered, immersed in stromata, arranged in a row, obpyriform to ampulliform to subglobose. *Ostiole* central, with periphyses. *Peridium* 5–25 μm wide, composed of dark brown to purple to hyaline cells of *textura angularis*. *Hamathecium* 2.5–4.5 μm wide, hyaline, septate, constricted at the septum, unbranched, not anastomosed paraphyses. *Asci* 90–120 $\times$ 16–21 μm ($\bar{x}$ = 102 $\times$ 18 μm , n = 20), 8-spored, unitunicate, cylindrical, apically rounded, with a short pedicel, slightly curved. *Ascospores* 26–35 $\times$ 6.5–10 μm ($\bar{x}$ = 31.5 $\times$ 8 μm , n = 20), biserial, ellipsoidal, 2-celled, with a larger upper cell and a smaller lower cell, with guttules, hyaline, smooth-walled, rounded at both ends, with a gelatinous sheath.

Culture characteristics: Ascospores germinate on PDA within 24 h. Colonies reached 60 mm after 20 days at 27°C. The colonies are floccose, white, circular, cottony, with regular edge, no pigment.

Materials examined: CHINA, Yunnan Province, Zhaotong City, Zhenxiong town (27°63'28" N, 104°81'88" E, 1,557 m), on dead culms of bamboo, 4 August 2023, L.S. Han & D.Q. Dai, HLS51 (GMB-W1022, first report of the sexual morph), living culture GMBCC1022, GMB-W1023; *ibid.* HLS133 (GMB-W1023), living culture GMBCC1023.

Notes: Asexually typified, endophytic species, *Apiospora guangdongensis* C.F. Liao & Doilom (ZHKUCC 23-0004, ex-type) (from the leaves of *Wurfbainia villosa*) was originally described by Liao et al. (2023) from Guangdong Province, China. Our multi-gene phylogenetic tree (Figure 1) showed that our new isolates GMBCC1022 and GMBCC1023 grouped with *Ap. guangdongensis* (ZHKUCC 23-0004, ex-type). Moreover, the base pair arrangement of our collections with ZHKUCC 23-0004 was identical. Therefore, we reported the sexual morph of *Ap. guangdongensis* for the first time in this study.

Apiospora subglobosa (D.Q. Dai & K.D. Hyde) Pintos & P. Alvarado, *Fungal Systematics and Evolution* 7: 207 (2021) (Figure 9)
Index Fungorum Identifier: IF 837715

See Senanayake et al. (2015) for the description.

Material examined: CHINA, Yunnan Province, Dehong, Mang City, Xuangang town (24°45'41"N, 98°43'83"E, 919 m), on dead culms of bamboo, 22 July 2023, L.S. Han & D.Q. Dai, HLS84 (GMB-W1024, new geographical record in China).

Known distributions: Thailand (Senanayake et al., 2015) and China (this study).

Known hosts: Bamboo (Senanayake et al., 2015, this study).

Notes: Senanayake et al. (2015) introduced *Arthrimum subglobosum* D.Q. Dai & K.D. Hyde, but later, Pintos and Alvarado (2021) transferred it to *Apiospora s. str.* as *Apiospora subglobosa* (D.Q. Dai & K.D. Hyde) Pintos & P. Alvarado. In our phylogenetic tree, our new collection GMB-W1024 clustered with *Ap. subglobosa* (MFLUCC 11-0397, ex-type) with 100% ML and 1.00 BI support (Figure 1). Morphologically, our new collection is similar to *Ap. subglobosa* in multi-loculate stromata with black slit-like opening, straight or curved, apical cell large, with smaller basal cell ascospores. However, the asci of GMB-W1024 are narrower than in MFLU 15-0384 (20–25 μm vs. 27–36 μm) (holotype). The ascospores of GMB-W1024 are longer than those of MFLU 15-0384 (25–33 μm vs. 24–28 μm) but narrower (7–10 μm vs. 8.5–12.5 μm), possibly due to the environmental change leading to slight differences in size. Nevertheless, we confirmed that our new collection (GMB-W1024) is *Ap. subglobosa* based on phylogenetic analyses (Figure 1).

4 Discussion

Fungal diversity in southwestern China is very high and a large number of species are introduced annually (Wijayawardene et al., 2021a; Lu et al., 2024; Du et al., 2023; Zhang et al., 2023b, 2024; Chen et al., 2024; Liu et al., 2023a; Tian et al., 2024; Xu et al., 2024; Zhang et al., 2024). Fungi associated with bamboo is one of the popular research topics among the mycologists in this region and several new species have been published in recent studies (e.g., *Bambusicola hongheensis* fide Phookamsak et al., 2024, *Parabambusicola yunnanensis* fide Han et al., 2023, *Paramphibambusa bambusicola*

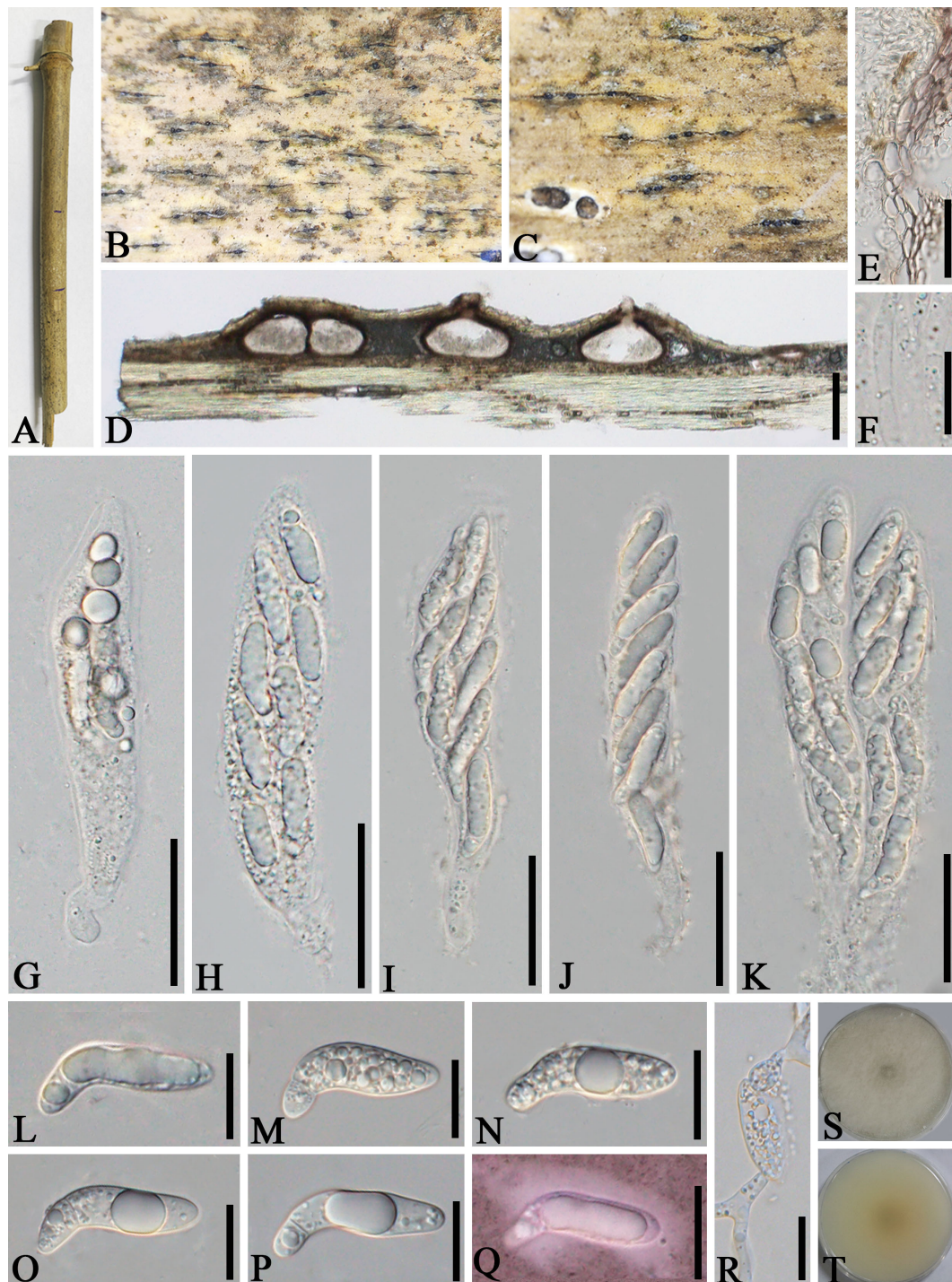


FIGURE 8

Apiospora guangdongensis (GMB-W1022) (A) Bamboo specimen. (B, C) Stromata developing on bamboo branches. (D) Vertical sections of stromata. (E) Peridium. (F) Paraphyses. (G–K) Asci. (L–Q) Ascospore [a ascospore stained in Indian ink showing gelatinous sheath (Q)]. (R) A germinating ascospore. (S, T) Cultures on PDA [upper (S), reverse (T)]. Scale bars: (D) 150 μm , (E, G–K) 30 μm , (F) 10 μm , and (L–R) 15 μm .

fide Han et al., 2024). However, a large number of taxa are yet to be discovered in this region and from bamboo plants, although it has been a well-studied host (Wijayawardene et al., 2021b, 2022).

In this study, we introduced five new species, viz., *Apiospora dehongensis*, *Ap. jinghongensis*, *Ap. shangrilaensis*, *Ap. zhaotongensis*, and *Ap. zhenxiongensis*, and two new sexual morph reports, viz., *Ap.*

globosa and *Ap. guangdongensis*. Furthermore, one new geographical record of *Ap. subglobosa* was also reported based on morphological and multi-locus phylogenetic analyses. All the species were found as saprobic taxa on decaying bamboo branches and culms. Three specimens were collected from western Yunnan Province, China (Dehong), two specimens were obtained from the southwestern part

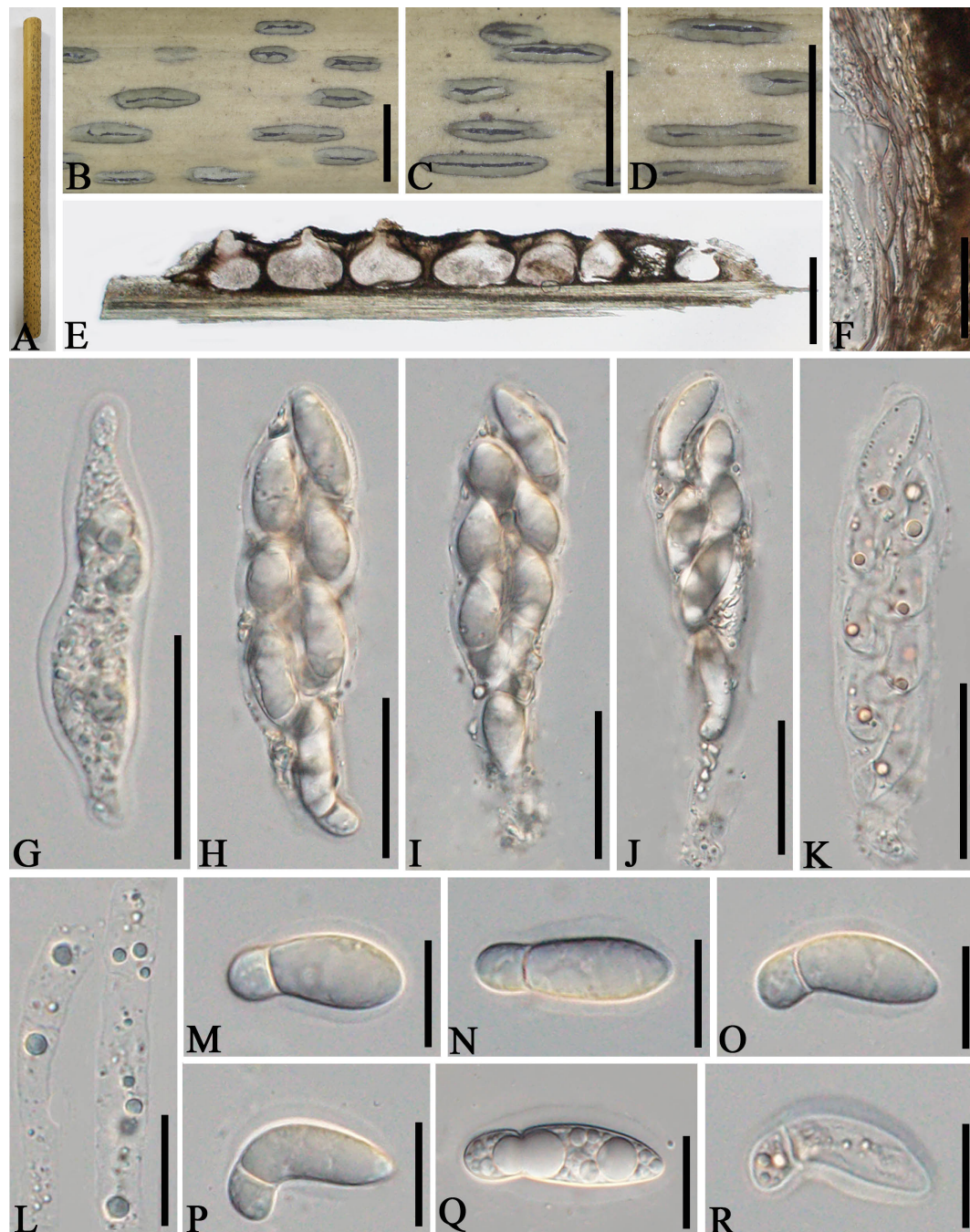


FIGURE 9

Apiospora subglobosa (GMB-W1024) (A) Bamboo specimen. (B–D) Stromata developing on bamboo branches. (E) Vertical sections of stromata. (F) Peridium. (G–K) Asci. (L) Paraphyses. (M–R) Ascospores surrounded by a gelatinous sheath. Scale bars: (B–D) 2 mm, (E) 300 μ m, (F–I, K) 30 μ m, and (L–R) 15 μ m.

(Xishuangbanna), seven specimens were collected from the northeastern section (Zhaotong), and two specimens were gathered from the northwestern part (Shangri-La), which displayed the highly hidden species richness of *Apiospora* in the different regions of Yunnan. Jiang et al. (2022b) and Wang et al. (2018) also emphasized the high species richness of bambusicolous ascomycetes in southwest China, with *Apiospora* as one of the genera with high species diversity. Thus, ongoing research on the genus *Apiospora* is essential.

According to the recently published studies, 90 species of *Apiospora* have been reported to have only an asexual morph, 19 species have been reported to have only a sexual morph, and 22 species have both morphs based on molecular data, including this study (Pintos and Alvarado, 2021; Li et al., 2023; Zhang et al., 2023a; Zhao et al., 2023; Ai et al., 2024; Dissanayake et al., 2024; Liu et al., 2024; Tian et al., 2024; Yan and Zhang, 2024; Zhao et al., 2024). Moreover, regarding the reports of *Apiospora* discovered on bamboo (based on molecular data), 17 species have been

identified solely by their asexual morph, while 15 species have sexual morph only. However, 12 species have been reported with both morphs (Pintos and Alvarado, 2021; Liao et al., 2023; Zhao et al., 2023, 2024; Zhang et al., 2023a; Ai et al., 2024; Dissanayake et al., 2024). Therefore, it is necessary to continue studying bambusicolous *Apiospora* to explore more asexual or sexual morphs of known or unknown species. Furthermore, because some *Apiospora* species were reported to have only asexual or sexual forms, it is crucial to collect more specimens in nature to clarify the status of these species in the genus *Apiospora*.

Note that most of the *Apiospora* species reported from bamboo are saprobes, while only *Ap. dongyingensis* Liu et al. and *Ap. hainanensis* Liu et al. have been reported as pathogens (Liao et al., 2023; Liu et al., 2023b). Jiang et al. (2020) emphasized the importance of researching on bambusicolous pathogenic fungi, because pathogenic fungi have the potential to hinder the advancement of the bamboo industry and even lead to ecological problems. So far, more than 190 bambusicolous pathogenic fungi have been discovered (Kuai, 1996). The continuous study of bamboo pathogenic fungi is related to the conservation and utilization of bamboo resources, which is of great significance to the promotion of sustainable development. Thus, more search works on bambusicolous pathogenic fungi are needed.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/genbank/>, ITS: PQ140160, PQ140161, PQ140162, PQ111492, PQ111493, PQ111494, PQ111495, PQ111496, PQ111497, PQ111498, PQ111499, PQ111500, PQ111501, PQ111502; LSU: PQ140163, PQ140164, PQ140165, PQ111481, PQ111482, PQ111483, PQ111484, PQ111485, PQ111486, PQ111487, PQ111488, PQ111489, PQ111490, PQ111491; *tub2*: PQ463971, PQ463972, PQ463973, PQ463974, PQ463975, PQ463976, PQ463977, PQ463978, PQ463979, PQ463980, PQ463981, PQ164976, PQ164977; *tef1-α*: PQ464016, PQ464017, PQ464018, PQ464019, PQ464020, PQ464021, PQ464022, PQ464023, PQ464024, PQ464025, PQ464026, PQ464027, PQ164974, PQ164975.

Author contributions

L-SH: Writing – original draft, Writing – review & editing, Data curation, Methodology, Software. CL: Writing – review & editing,

Data curation, Writing – original draft. D-QD: Funding acquisition, Methodology, Writing – review & editing, Visualization. IP: Methodology, Writing – review & editing, Software, Visualization. AE: Funding acquisition, Methodology, Writing – review & editing. SA-R: Writing – review & editing, Funding acquisition. QL: Writing – review & editing, Funding acquisition, Methodology. NW: Methodology, Writing – review & editing, Data curation, Software.

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Conflict of interest

The authors declare the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY

Sinang Hongsanan,
Shenzhen University, China

REVIEWED BY

K. W. Thilini Chethana,
Mae Fah Luang University, Thailand
Arun Kumar Dutta,
Gauhati University, India

*CORRESPONDENCE

Thushara C. Bamunuarachchige

✉ tcbamunu@tec.rjt.ac.lk

Dong-Qin Dai

✉ cidaidongqin@gmail.com

†These authors have contributed equally to this work

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Ectophoma salviniae sp. nov., *Neottiosporina mihintaleensis* sp. nov. and four other endophytes associated with aquatic plants from Sri Lanka and their extracellular enzymatic potential

Madhara K. Wimalasena^{1,2,3†}, Nalin N. Wijayawardene^{1,3,4†}, Thushara C. Bamunuarachchige^{3*}, Gui-Qing Zhang¹, R. G. Udeni Jayalal⁵, Darbhe J. Bhat^{6,7}, Turki M. Dawoud⁶, Heethaka K. S. de Zoysa³ and Dong-Qin Dai^{1*}

¹Center for Yunnan Plateau Biological Resources Protection and Utilization, Qujing Normal University, Qujing, Yunnan, China, ²Faculty of Graduate Studies, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka, ³Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, Mihintale, Sri Lanka, ⁴Tropical Microbiology Research Foundation, Colombo, Sri Lanka, ⁵Department of Natural Resources, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka, ⁶Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, ⁷Vishnugupta Vishwavidyapeetam, Gokarna, India

Endophytic fungi associated with selected aquatic plants, *Eichhornia crassipes*, *Nymphaea nouchali*, *Salvinia minima* and *S. molesta* were evaluated. *Ectophoma salviniae* sp. nov. and *Neottiosporina mihintaleensis* sp. nov. are introduced as novel taxa from *Salvinia* spp. from Sri Lanka. *Chaetomella raphigera* is reported as a new geographical record, *Colletotrichum siamense* and *C. truncatum* are reported as novel host records in aquatic plants, while *Phyllosticta capitalensis* has been identified on the same host (*Nymphaea nouchali*) in the North-Central Province of Sri Lanka. Identification of the fungi was based on morphological characteristics and multi-locus phylogenetic analyses using ITS, LSU, SSU, ACT, CHS-1, GAPDH, tub2, rpb2, and tef1- α molecular markers. The identified fungi were analysed for extracellular enzymatic properties. According to the qualitative analysis, *Ectophoma salviniae* sp. nov. exhibited the highest amylase production, *Chaetomella raphigera* exhibited the highest cellulase enzyme production, and *Neottiosporina mihintaleensis* sp. nov. exhibited the highest laccase production. The results demonstrate the aquatic fungal diversity in this region and their extracellular enzymatic potentials, providing valuable insights for future biotechnological approaches.

KEYWORDS

freshwater plants, fungal endophytes, hydrolytic and oxidative enzymes, new species, phylogeny, taxonomy

1 Introduction

The magnitude of the fungal kingdom has been a debatable topic for decades and several studies have revised the prevailing estimations based on the advancements in estimation methods and technologies (e.g., Hawksworth, 1991, 2001; May, 2000; Tedersoo et al., 2015, 2022; Hyde et al., 2023). However, Wu et al. (2019) estimated the global fungal species diversity to be around 12 million, based on culture-independent approaches, while culture-dependent methods yielded a more conservative estimate of 2.2 to 3.8 million species (Hawksworth and Lücking, 2017). Niskanen et al. (2023) revisited the species number estimated by Hawksworth and Lücking (2017) and concluded that it would be 2–3 million species, however, the best estimation is at 2.5 million. Nevertheless, only 160,000 fungal species have been accepted in Species Fungorum (2024; accession date: 06 June 2024, <https://www.speciesfungorum.org/names/names.asp>), thus, a large number of taxa are yet to be described. Additionally, it has reported understudied geographical regions and well-studied hosts but biodiversity regions (temperate and tropical) would harbour more novel taxa (Wijayawardene et al., 2021). It is considered a challenge to reveal the unknown fungal diversity with traditional methods, such as morphological or cultural characteristics. The recent advances in molecular techniques (such as high-throughput sequencing of environmental samples) are accelerating the explorations and further trying to reveal the understudied fungal habitats, life modes and geographical regions (Wijayawardene et al., 2023).

Endophytes are widespread and have been reported in plants from diverse ecosystems such as deserts, temperate zones, arctic tundra, tropical forests, grasslands, and croplands (Arnold, 2007; Arnold and Lutzoni, 2007; Zheng et al., 2015; Rana et al., 2019; Harrison and Griffin, 2020; Dar et al., 2022; Hashem et al., 2023). Based on the ratio of the host (vascular plants) to species current estimates suggest that there are approximately one million species of fungal endophytes (Sun and Guo, 2012; Lugtenberg et al., 2016; Rashmi et al., 2019; Wu et al., 2019; Bhunjun et al., 2024). Endophytic fungi belong to both mitosporic and meiosporic ascomycetes, which reside within plants without causing symptoms and colonize healthy tissue beneath the epidermal cell layer through quiet infections (Lu et al., 2012; Ali et al., 2018; Abdel-Wareth, 2022). Further, it has been reported that comparing endophytic basidiomycetes and basal fungi with endophytic ascomycetes shows that almost 90% of the identified endophytes are *Ascomycota* (Rungjindamai and Gareth Jones, 2024).

Endophytes' ecology, evolution, and applications are interesting topics; however, knowledge about their diversity, geographic and ecological distributions in most plant communities remains limited and unexplored (Gao et al., 2019; Zheng et al., 2021). Previous research has primarily focused on endophytes in terrestrial plants, while endophytic fungal studies related to aquatic plants received little attention (Li et al., 2010; Sandberg et al., 2014; Dissanayake et al., 2016; Myovela et al., 2024). However, the richness of endophytic fungal diversity has been reported in marine ecosystems (Kamat et al., 2020; El-Bondkly et al., 2021), mangrove ecosystems (Deshmukh et al., 2020; Jia et al., 2020; da Silveira Bastos et al., 2024; Myovela

et al., 2024), and freshwater ecosystems (You et al., 2015; Chen et al., 2023; Pramanic et al., 2023). Aquatic plants (including emergent plants, floating-leaved plants, free-floating plants, submerged plants, and wet plants (Ismail et al., 2021; Zhou et al., 2023) serve as hosts for a diverse array of endophytic fungi (Zheng et al., 2021; Wimalasena et al., 2024). Many researchers have studied the diversity and ecological roles of aquatic plants (O'Hare et al., 2018; Kamat et al., 2020; Zheng et al., 2022; Ji et al., 2024). However, most studies have overlooked how endophytes affect these plants and their wider ecological functions. Accordingly, there is a significant gap in the understanding of endophytic fungal communities in aquatic plants (Sandberg et al., 2014; Zheng et al., 2021; Wimalasena et al., 2024).

Sri Lanka is a tropical biodiversity hotspot (Gunawardene et al., 2007; Surasinghe et al., 2019; Sarathchandra et al., 2021; De Zoysa, 2022) and harbours a diverse range of aquatic ecosystems, including both coastal and inland areas (Gunatilleke et al., 2008). Inland freshwater habitats (rivers, streams, marshes, swamp forests, villus, and man-made reservoirs) collectively cover approximately 202,435 hectares (Gunatilleke et al., 2008; Yakandawala, 2012). Sri Lanka is home to over 370 species of aquatic and wetland plants, with 12% being endemic to the country (Yakandawala, 2012). These plants serve as habitats for various fungi, including endophytes (Ratnaweera, 2019; Wimalasena et al., 2024). As highlighted by Wimalasena et al. (2024), Sri Lanka offers significant potential for the identification and study of endophytic fungi. Previous research on endophytic fungi in freshwater plants has been relatively limited in Sri Lanka. For instance, Dissanayake et al. (2016) isolated 20 distinct endophytic fungi from *Nymphaea nouchali*. More recently, Wimalasena et al. (2024) reported the ongoing study on the isolation of endophytic fungi from freshwater plants in Sri Lanka.

In this study, an effort was made to document the endophytic fungi associated with three freshwater plant taxa, viz., *Eichhornia crassipes*, *Nymphaea nouchali*, *Salvinia minima* and *S. molesta*, found in the lentic freshwater habitats of the Mihintale area (in Anuradhapura district, North-Central Province), Sri Lanka. We isolated six fungal species that belong to *Colletotrichum*, *Chaetomella*, *Ectophoma*, *Neottiosporina* and *Phyllosticta*. Among these taxa, two new species, *Ectophoma salviniae* sp. nov. and *Neottiosporina mihintaleensis* sp. nov. are introduced. *Chaetomella raphigera* has been reported as a new geographical record for Sri Lanka. *Colletotrichum siamense* and *C. truncatum* have been identified as new host records on *Eichhornia crassipes*. Isolation of *Phyllosticta capitalensis* on *Nymphaea nouchali* is in confirmative with the findings of Dissanayake et al. (2016) based on multilocus phylogenetic analyses. Furthermore, the study assessed the potential of these endophytic fungi to produce various extracellular enzymes by qualitative assays for amylolytic, cellulolytic, and laccase activities.

2 Materials and methods

2.1 Sampling, isolation and characterization of endophytic fungi

From November to December 2023, healthy aquatic plants were sampled from three lentic freshwater habitats in Mihintale, located in

the Anuradhapura district of Sri Lanka including the Iluppukanniya tank (8.36482° N, 80.50764° E, 118 m), Mahakanadara tank (8.38683° N, 80.38683° E, 117 m), and Mihintale tank (8.36267° N, 80.50591° E, 108 m) (Figure 1; Table 1). Mature plants with undamaged leaves of *Eichhornia crassipes*, *Nymphaea nouchali*, *Nymphaea pubescens*, *Salvinia minima*, and *Salvinia molesta* (Figure 2), were carefully uprooted and brought to the laboratory within one hour in ziplock plastic bags containing fresh water. The samples were maintained separately in freshwater until the isolation process began immediately.

Each plant sample was thoroughly rinsed for 30 seconds under running tap water to remove the debris and adhered mud contaminants. The plants were cut into roots, stems, and leaves and processed in the following sequential surface sterilization steps: an initial immersion in 0.5% sodium hypochlorite for 2 minutes, followed by a rinse in sterile distilled water for 1 minute, immersion in 75% ethanol for 2 minutes, and a final rinse in sterile distilled water for 1 minute. After the final wash, the samples were dried using sterile paper towels under a laminar airflow cabinet (Li et al., 2010; Zheng et al., 2021). However, during surface sterilization of

the plant material, the duration of treatment for each plant species depended on its tissue sensitivity. Specifically, being very spongy, the leaves of *Salvinia molesta* were processed with an initial immersion of 1 minute in 0.5% sodium hypochlorite, followed by a rinse in sterile distilled water for one minute, immersion in 70% ethanol one minute, and a final rinse in sterile distilled water for one minute.

Surface-sterilized plant tissues were cut into 5 × 5 mm segments and placed in Potato Dextrose Agar (PDA; 20% potato, 2% dextrose, 2% agar) media supplemented with tetracycline (50 mg/L). The plates were incubated under aseptic conditions at room temperature (28–30°C) and observed for fungal growth every second day for seven days. Once fungal mycelium emerged from the edges of the plant segments, a portion of the growing colony was aseptically cut and transferred to a freshly prepared PDA plate (Li et al., 2010).

The growth of the subcultures was monitored daily, and colony characteristics, including colour, form, elevation, margin, texture, and dimensions, were recorded for two weeks. The colony colour was identified by a colour guide (Colour guide; ArtyClick Colors,

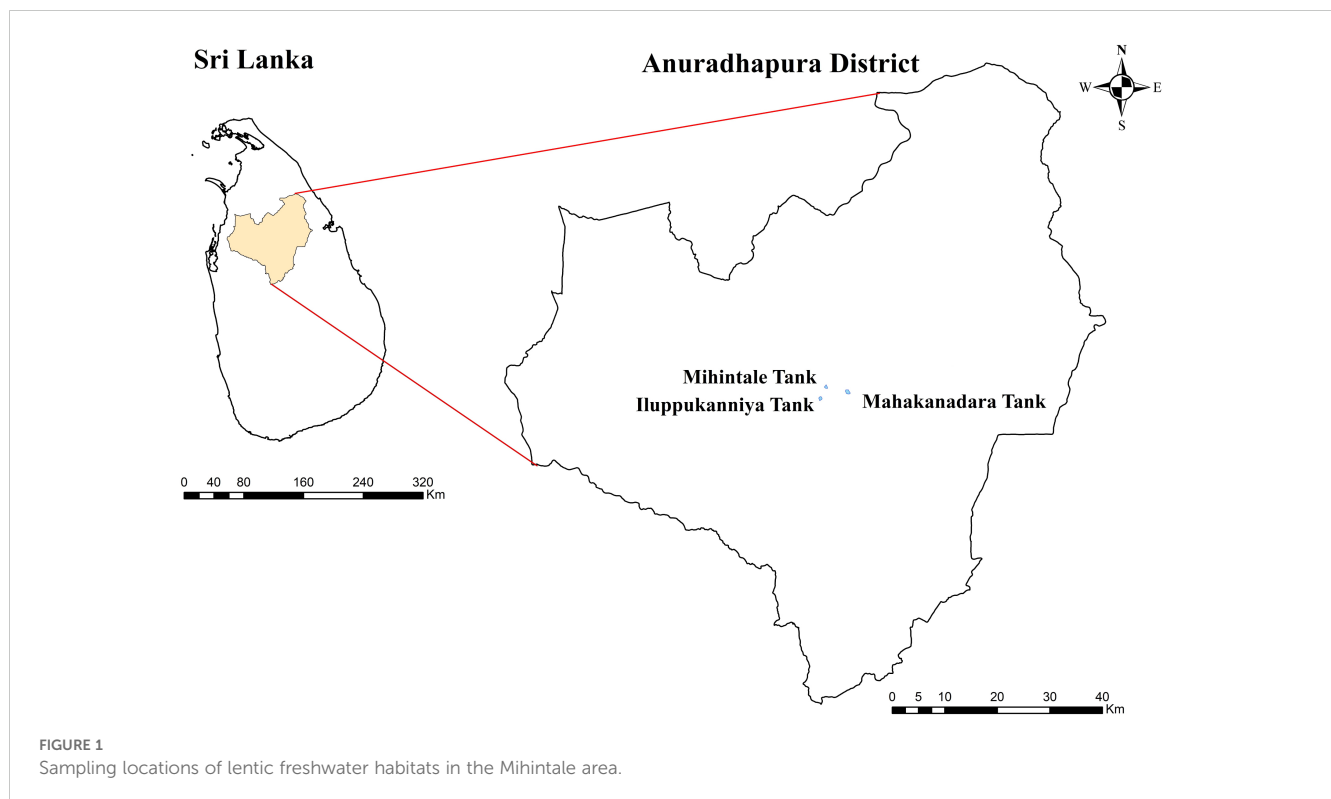


TABLE 1 Lentic freshwater habitats and host substrates for the endophytic fungi.

Locations	Host or substrates
Iluppukanniya tank (8.36482° N, 80.50764° E, 118 m)	Leaf of <i>Eichhornia crassipes</i> (Water hyacinth) Leaf of <i>Salvinia minima</i> (Watermoss) Leaf of <i>Nymphaea nouchali</i> (Blue Water-Lily)
Mahakanadara tank (8.38683° N, 80.38683° E, 117 m)	Leaf of <i>Eichhornia crassipes</i> (Water hyacinth) Leaf of <i>Salvinia molesta</i> (Giant Salvinia)
Mihintale tank (8.36267° N, 80.50591° E, 108 m)	Leaf of <i>Eichhornia crassipes</i> (Water hyacinth)

2024). The stock cultures were stored in sterile water and agar slants and preserved at 4°C at Rajarata University Fungal Culture Collection (RUFCC) in Sri Lanka.

The fungal cultures were induced to sporulate using different stress stimulation techniques. These included reducing the normal

growth temperature (cold treatment), reducing the quantity of PDA volume for each plate to create starvation media (Mattoo and Nonzom, 2022), exposing the plates to UV light for 15 minutes, and placing a double-sterilized pine needle or toothpick on the fungal colony to induce conidiomatal formation (Su et al., 2012).

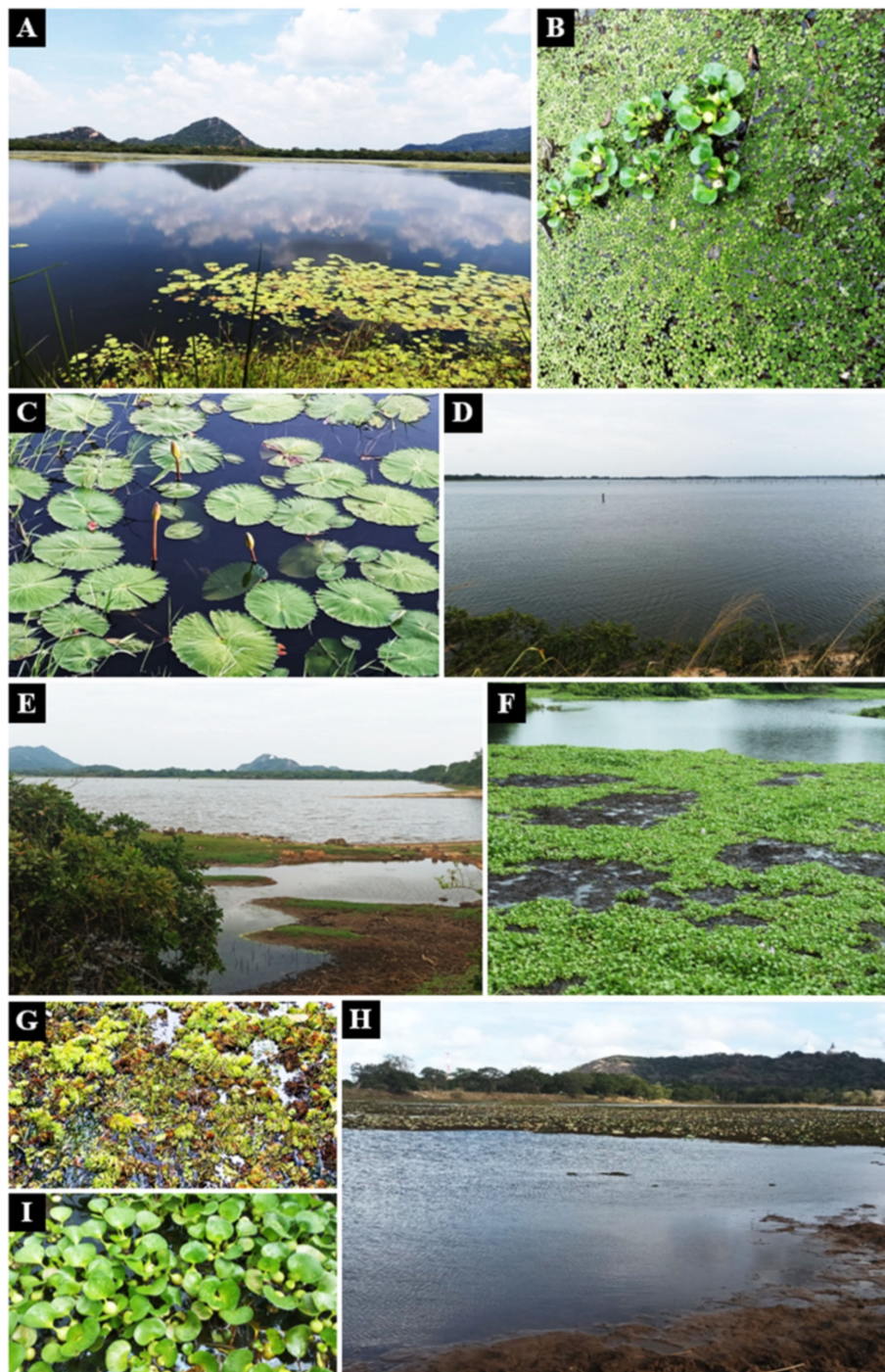


FIGURE 2

Aquatic plants in lentic freshwater habitats in Mihintale area were used to isolate endophytic fungal species. (A) Iluppukanniya tank. (B) *Eichhornia crassipes* (Water hyacinth) and *Salvinia minima* (Watermoss) in the Iluppukanniya tank. (C) *Nymphaea* sp. in the Iluppukanniya tank. (D, E) Mahakanadara tank. (F) *Eichhornia crassipes* in the Mahakanadara tank. (G) *Salvinia molesta* (Giant Salvinia) in the Mahakanadara tank. (H) Mihintale tank. (I) *Eichhornia crassipes* in Mihintale tank.

Post-stressors, the plates were incubated for a week under normal light conditions.

The sporulating cultures were studied using a Nikon SMZ18 stereomicroscope, a Nikon TS2R-FL inverted trinocular microscope and a Nikon ECLIPSE Ci upright microscope. Morphological features were examined and documented (Senanayake et al., 2018).

2.2 Molecular identification of endophytic fungi

2.2.1 Genomic DNA extraction

The genomic DNA was extracted from the freshly cultured fungi using trimethylammonium bromide (CTAB) method, following the protocol described by Gontia-Mishra et al. (2014) and Hatamzadeh et al. (2024).

2.2.2 Polymerase chain reaction

The targeted primers used in the polymerase chain reaction (PCR) included; Actin (*ACT*; ACT-512F/ACT-783R), Chitin (*CHS*-1; CHS-354R/CHS-79F), Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; GDF1/GDR1/Gpd2-LM), Internal transcribed spacers (*ITS*; ITS5/ITS4), Large subunit nuclear ribosomal DNA (*LSU*; LROR/LR5R), RNA polymerase II subunit 2 (*rpb2*; fRPB2-5F2/fRPB2-7cR), Small-subunit ribosomal RNA (*SSU*; NS1/NS4), Translation elongation factor 1 (*tef1-α*; EF1-728F/EF1-986R), and Beta-tubulin (*tub2*; T1/Bt2b), (Table 2), each with specific annealing temperatures. The final volume of the PCR reaction was 25 µl, containing 5 µl of DNA template, 2.5 µl of each forward and reward primer, 12.5 µl of HIMEDIA MBT061-100R 2× PCR TaqMixture (mixture of Taq DNA Polymerase, dNTPs, and optimized buffer) and 2.5 µl of ddH₂O.

The PCR amplification was performed with an initial denaturing step at 95°C for 5 min., followed by 40 amplification cycles consisting of a denaturation step at 95°C for 1 min., an annealing step for 1 min., and a final extension step at 72°C for 10 min. The annealing temperatures were set for the gene loci, with the optimum for each: *ACT*: 58°C, *CHS*-1: 58°C, *GAPDH*: 60°C, *tub2*: 55°C, *ITS*: 54°C, *LSU*: 55°C, *rpb2*: 56°C, *SSU*: 55°C, and *tef1-α*: 54°C. All PCR products were visualized by 1% agarose gel (stained with Diamond TM Nucleic Acid Dye) electrophoresis at 80 V/cm for 30 minutes. The gel was visualized under a UV transilluminator to estimate the fragment size.

2.2.3 DNA sequencing

Amplicons were sequenced using both PCR primers and DNA sequencing results were acquired through Sanger bidirectional sequencing (GeneLabs Medicals Pvt. Ltd., Sri Lanka). The obtained nucleotide sequences were checked for their quality by reviewing the chromatograms using BioEdit version 7.2. After confirming the quality, the sequences were compared with entries in the GenBank database using the Basic Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov>; accessed on 03 April 2024) to identify significant alignments with similarity percentages.

2.3 Phylogenetic analysis

Closely related sequences were downloaded from GenBank based on blast similarity and recent publications. Multiple gene phylogenetic analyses were conducted for endophytic fungi for *Colletotrichum* sp., *ITS*, *GAPDH*, *ACT*, *CHS*-1, *HIS* 3, and *tub2* (Damm et al., 2009). *Chaetomella raphigera* was analyzed using *ITS*, *LSU*, and *SSU* (Suwannarach et al., 2018). *Ectophoma salviniae* sp. nov. underwent analysis with *ITS*, *LSU*, *rpb2* and *tub2* (Hou et al., 2020a). *Phyllosticta capitalensis* was analyzed by using *ITS*, *ACT*, *tef1-α*, and *GAPDH* (Gliénke et al., 2011; Wang et al., 2012). *Neottiosporina mihintaleensis* sp. nov. was analyzed using *ITS*, *LSU*, and *SSU* (de Gruyter et al., 2009) (see Table 2 for the primer details). The phylogenetic trees were constructed via Maximum likelihood (ML) and Bayesian analyses. Maximum likelihood (ML) analysis was constructed by the online portal CIPRES Science Gateway v. 3.3 (Miller et al., 2010), using RAXML-HPC v.8 on XSEDE (8.2.12) tool, with the default settings but adapted: with the GAMMA nucleotide substitution model and 1000 rapid bootstrap replicates. Bayesian analysis was generated from MrBayes v. 3.0b4 (Ronquist and Huelsenbeck, 2003), and the model of evolution was estimated with MrModeltest v. 2.2 (Nylander, 2004). The posterior probabilities (PP) (Rannala and Yang, 1996; Zhaxybayeva and Gogarten, 2002) were determined by the following Markov chain Monte Carlo sampling (MCMC) in MrBayes v.3.0b4 (Huelsenbeck and Ronquist, 2001). Six simultaneous Markov chains were run for 1,000,000 generations, with trees sampled every 100th generation. The preburn was set to 0.25 and the run was automatically stopped when the mean standard deviation of the split frequency reached below 0.01 (Maharachchikumbura et al., 2015). The bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95, are given at the respective branches of each phylogenetic trees (See the Supplementary Tables 1–5). GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis.

2.4 Taxonomic classification

The higher-level taxonomic classification of each freshwater endophytic fungi was based on Wijayawardene et al. (2022a). Index Fungorum identifiers were obtained from Index Fungorum (2024) for the newly introduced taxa following the requirements mentioned in Art. F5.1 of International Code of Nomenclature for Algae, Fungi, and Plant.

2.5 Extracellular enzymatic assay of endophytic fungi

The qualitative analysis of amylolytic, cellulolytic, and laccase enzymatic activities of the endophyte isolates conducted using

TABLE 2 The gene loci utilized in molecular identification techniques for endophytic fungi.

Fungal genera	Gene loci	Primers	Sequence	References
<i>Colletotrichum</i> sp.	ITS	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	(White et al., 1990)
		ITS4	5'-TCCTCCGCTTATTGATATGC-3'	(White et al., 1990)
	GAPDH	GDF1	5'-GCCGTCAACGACCCCTTCATTGA-3'	(Guerber et al., 2003)
		GDR1	5'-GGGTGGAGTCGTACTTGAGCATGT-3'	(Guerber et al., 2003)
	ACT	ACT-512F	5'-ATGTGCAAGGCCGGTTTCGC-3'	(Carbone and Kohn, 1999)
		ACT-783R	5'-TACGAGTCCTTCTGGCCCAT-3'	(Carbone and Kohn, 1999)
	CHS-1	CHS-354R	5'-TGGAAGAACCATCTGTGAGAGTTG-3'	(Carbone and Kohn, 1999)
		CHS-79F	5'-TGCGGCAAGGATGCTTGGAAGAAG-3'	(Carbone and Kohn, 1999)
<i>Chaetomella</i> sp.	ITS	T1	5'-AACATGCGTGAGATTGTAAGT-3'	(O'Donnell and Cigelnik, 1997)
		Bt2b	5'-ACCCTCAGTGTAGTGACCCTTGGC-3'	(Glass and Donaldson, 1995)
	LSU	LROR	5'-ACCCGCTGAACCTTAAGC-3'	(Vilgalys and Hester, 1990)
		LR5	5'-TCCTGAGGGAACTTCG-3'	(Vilgalys and Hester, 1990)
	SSU	NS1	5'-GTAGTCATATGCTTGTCTC-3'	(White et al., 1990)
		NS4	5'-CTTCCGTCAATTCCTTTAAG-3'	(White et al., 1990)
<i>Ectophoma</i> sp.	ITS	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	(White et al., 1990)
		ITS4	5'-TCCTCCGCTTATTGATATGC-3'	(White et al., 1990)
	LSU	LROR	5'-ACCCGCTGAACCTTAAGC-3'	(Vilgalys and Hester, 1990)
		LR5	5'-TCCTGAGGGAACTTCG-3'	(Vilgalys and Hester, 1990)
	rpb2	fRPB2-5F2	5'-GGGGWGAYCAGAAGAAGGC-3'	(Sung et al., 2007)
		fRPB2-7cR	5'-CCCATRGCTTGYYTTRCCCAT-3'	(Liu et al., 1999)
	tub2	T1	5'-AACATGCGTGAGATTGTAAGT-3'	(O'Donnell and Cigelnik, 1997)
		Bt2b	5'-ACCCTCAGTGTAGTGACCCTTGGC-3'	(Glass and Donaldson, 1995)
<i>Phyllosticta</i> sp.	ITS	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	(White et al., 1990)
		ITS4	5'-TCCTCCGCTTATTGATATGC-3'	(White et al., 1990)
	tef1-α	EF1-728F	5'-CATCGAGAAGTTCGAGAAGG-3'	(Carbone and Kohn, 1999)
		EF1-986R	5'-TACTTGAAGGAACCCCTTACC-3'	(Carbone and Kohn, 1999)
	ACT	ACT-512F	5'-ATGTGCAAGGCCGGTTTCGC-3'	(Carbone and Kohn, 1999)
		ACT-783R	5'-TACGAGTCCTTCTGGCCCAT-3'	(Carbone and Kohn, 1999)
	GAPDH	GDF1	5'-GCCGTCAACGACCCCTTCATTGA-3	(Guerber et al., 2003)
		Gpd2-LM	5'- CCCACTCGTTGTCGTACCA-3'	(Myllys et al., 2002)
<i>Neottiosporina</i> sp.	ITS	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	(White et al., 1990)
		ITS4	5'-TCCTCCGCTTATTGATATGC-3'	(White et al., 1990)
	LSU	LROR	5'-ACCCGCTGAACCTTAAGC-3'	(Vilgalys and Hester, 1990)
		LR5	5'-TCCTGAGGGAACTTCG-3'	(Vilgalys and Hester, 1990)
	SSU	NS1	5'-GTAGTCATATGCTTGTCTC-3'	(White et al., 1990)
		NS4	5'-CTTCCGTCAATTCCTTTAAG-3'	(White et al., 1990)

colourimetric changes in the PDA medium. Petri dishes containing PDA supplemented with tetracycline (1600 µg/mL), preventing bacterial contamination (Elshafie et al., 2019). The specific substrates were incorporated into the PDA media for each enzymatic identification, excluding laccase enzymatic activity. Each enzymatic assay included both negative and positive controls. The negative controls consisted of uninoculated fungal PDA plates supplemented with each substrate and treated with the specific chemicals used in each enzymatic assay. The positive controls, involved inoculating fungal PDA plates supplemented with each substrate and treated with the specific chemicals used in each enzymatic assay. All positive and negative controls were incubated at the required incubation temperatures and time periods (see methodology sections 2.5.1, 2.5.2, and 2.5.3).

2.5.1 Qualitative identification of amylase enzymatic activity

Petri dishes containing PDA supplemented with 1% starch were employed for the experiment. The fungal inoculum, comprising small fragments of mycelium (0.5 × 0.5 cm), was carefully placed in the centre of the PDA Petri dishes. Subsequently, the dishes were incubated at 28–30°C for a duration of seven days to facilitate fungal growth and development. Following the incubation period, 1–2 mL of iodine solution was added to each dish. The dishes were then incubated for an additional hour at 28–30°C. Following this incubation, the dishes were thoroughly washed with distilled water to remove any excess iodine solution. The success of the experiment was determined by observing colourimetric changes. A distinct halo appearing around the fungal colony was indicative of a positive result, highlighting the presence of cellulolytic activity (Robledo-Mahón et al., 2020).

2.5.2 Qualitative identification of cellulase enzymatic activity

The 0.5% (w/v) sodium carboxymethyl cellulose (CMC) (Central Drug House Pvt. Ltd., India) was added to the PDA media to evaluate cellulolytic activity. Small pieces of mycelium (0.5 × 0.5 cm) were then positioned on PDA petri dishes. The prepared fungal plates underwent incubation at 28–30°C for a period of three to five days. The qualitative cellulase activity of fungal isolates was assessed based on their ability to proliferate and create cleared zones around colonies on a solid medium. The surface of the media containing the developed fungal colonies was flooded with 0.1% (w/v) Congo red (Himedia Laboratories Pvt. Ltd., Mumbai, India) solution and incubated for 15 minutes at 28–30°C. Afterwards, the dye was removed with sterile distilled water, and the plates underwent an additional 10-minute incubation period at 28–30°C. Subsequently, the plates were further treated by flooding with 1M NaCl (Daejung Chemicals and Metals Co., Ltd., South Korea) for 5 minutes.

2.5.3 Qualitative identification of laccase enzymatic activity

The small pieces of mycelium (0.5 × 0.5 cm) were placed on a PDA medium and incubated for approximately five days at 28–30°C

temperature. The colonized Petri dishes were utilized for adding solutions by droplets at the edge of each colony. Laccase activity was determined using a 0.1 M 1-Naphthol (Research Lab Fine Chem. Industries, Mumbai, India). Following the addition of droplets, Petri dishes were incubated for 24 hours at 28–30°C temperature and changes in the colour of the edge of the colony were considered positive results. The blue-purple appearance was displayed for laccase activity at the edge of each fungal colony (Gramss et al., 1998; Robledo-Mahón et al., 2020).

3 Results

3.1 Phylogenetic analyses

The taxa for each analysis were selected based on blast similarity and related publications and closely related sequences were downloaded from GenBank (See the [Supplementary Tables 1–5](#)).

3.1.1 Multi-gene analyses for *Ectophoma*

The concatenated dataset of LSU, ITS, *rpb2*, and *tub2* regions contained 14 isolates, which comprised 2434 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Didymella exigua* (CBS 183.55) was used as the outgroup taxon. The best-scoring RAXML tree with a final likelihood value of -4415.606556 is presented in [Figure 3](#). The matrix had 127 distinct alignment patterns, with 13.83% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.236991, C = 0.247923, G = 0.273055, T = 0.242031; substitution rates AC = 2.940086, AG = 5.756609, AT = 1.806664, CG = 1.455718, CT = 18.313095, GT = 1.000000; gamma distribution shape parameter alpha = 0.020000. In the phylogenetic analysis, our new strains (RUFCC2458 and RUFCC2462) form the sister clade to *Ectophoma multirostrata* (CBS 274.60 (ex-type) and CBS 380.67) and *E. iranica* (SCUATK1G1 (ex-type) and SCUAK1) with moderate statistical values (95% ML), 96 PP with BP values more than 95%.

3.1.2 Multi-gene analyses for *Phyllosticta*

The concatenated ITS, *tef1-α*, *ACT* and *GADPH* region dataset contained 38 isolates, comprising 1698 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Botryosphaeria obtusa* (CMW 8232) and *B. stevensii* (CBS 112553) were used as the outgroup taxa. The best-scoring RAXML tree with a final likelihood value of -9726.371339 is presented in [Figure 4](#). The matrix had 727 distinct alignment patterns, with 20.00% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.200267, C = 0.311861, G = 0.264840, T = 0.223032; substitution rates AC = 0.893796, AG = 2.729183, AT = 1.229527, CG = 1.044891, CT = 6.112001, GT = 1.000000; gamma distribution shape parameter alpha = 0.344610. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic analysis, our new strain

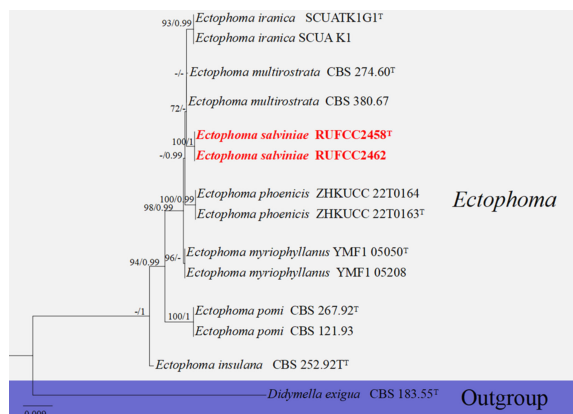


FIGURE 3

Phylogenetic tree from the best scoring of the RAxML analysis based on combined (ITS, LSU, *rpb2* and *tub2*) is rooted to *Didymella exigua* (CBS 183.55). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95, are given at the respective branches. Hyphen (-) means a value lower than 75% (BS) or 0.95 (PP). Ex-types are marked in "T". New isolates are labeled in bold and red.

(RUFCC2452) clustered with *Phyllosticta capitalensis* (CBS 128856) with high statistical support (75% ML, 0.99 PP).

3.1.3 Multi-gene analyses for *Colletotrichum*

The concatenated dataset of ITS, *tub2*, *ACT*, *CHS-1* and *GADPH* regions contained 39 isolates comprising 1818 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Colletotrichum boninense* (CBS 123755) and *C. chamaedoreae* (LC:13868) were used as the outgroup taxa. The best-scoring RAxML tree with a final likelihood value of -8361.798032 is presented in Figure 5. The matrix had 644 distinct alignment patterns, with 3.72% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.232029, C = 0.290209, G = 0.245763, T = 0.231999; substitution rates AC = 1.179354, AG = 3.249038, AT = 1.464001, CG = 0.806083, CT = 5.743800, GT = 1.000000; gamma distribution shape parameter alpha = 0.281325. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic analysis, our new strains (RUFCC2457 and RUFCC2455) clustered in the clade that comprises *Colletotrichum siamense* (CBS 125378 (ex-type), *C.*

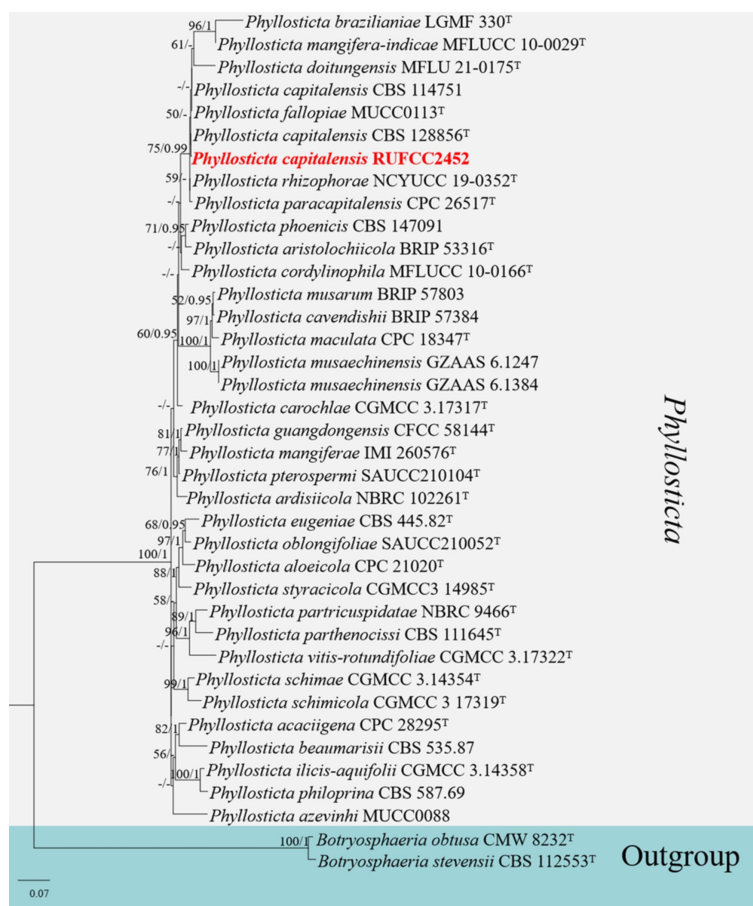


FIGURE 4

Phylogenetic tree from the best scoring of the RAxML analysis based on combined (ITS, *tef1-α*, *ACT* and *GADPH*) is rooted to *Botryosphaeria obtusa* (CMW 8232) and *B. stevensii* (CBS 112553). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 75% and 0.95, are given at the respective branches. Hyphen (-) means a value lower than 50% (BS) or 0.95 (PP). Ex-types are marked in "T". New isolates are labelled in bold and red.

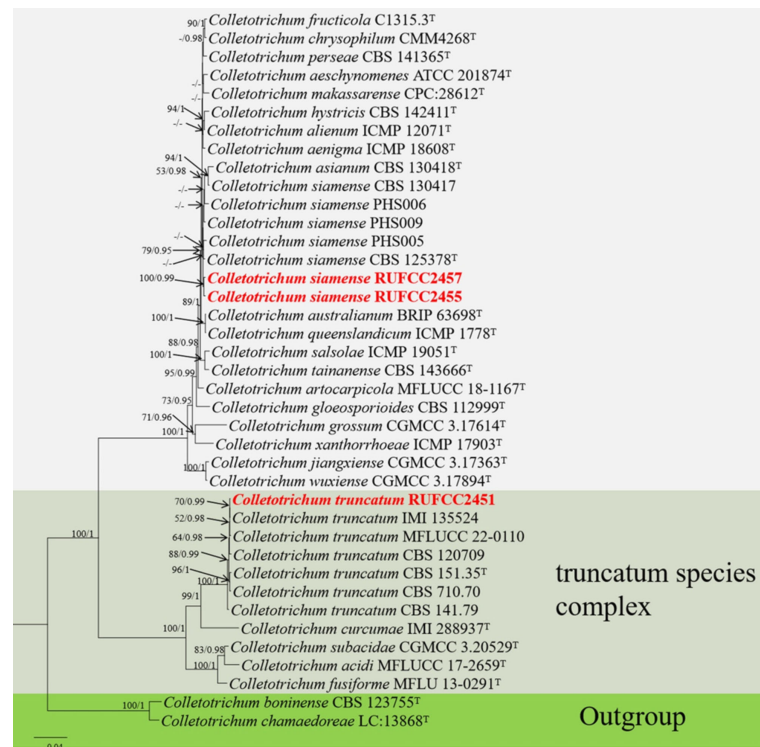


FIGURE 5

Phylogenetic tree from the best scoring of the RAxML analysis based on combined (ITS, *tub2*, *ACT*, *CHS-1* and *GADP*) is rooted to *Colletotrichum boninense* (CBS 123755) and *C. chamaedoreae* (LC:13868). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95, are given at the respective branches. Hyphen (-) means a value lower than 75% (BS) or 0.95 (PP). Ex-typotypes are marked in "T". New isolates are labelled in bold and red.

australianum (BRIP 63698), and *C. queenslandicum* (ICMP 1778) with moderate statistical values (79% ML, 0.95 PP). We compared the conidial morphologies of the new collection against the three species and confirmed that our collections belong to *Colletotrichum siamense* (See the taxonomy section). While another new collection (RUFCC2451) clustered in the clade *Colletotrichum truncatum* with high statistical values (100% ML, 1 PP).

3.1.4 Multi-gene analyses for *Chaetomella*

The concatenated dataset of LSU, ITS and SSU regions contained 24 isolates, which comprised 2977 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Hymenoscyphus scutula* (CBS 101.66) and *H. fructigenus* (CBS 186.47) were used as the outgroup taxa. The best-scoring RAxML tree with a final likelihood value of -7269.420511 is presented in Figure 6. The matrix had 353 distinct alignment patterns, with 37.79% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.263303, C = 0.209012, G = 0.275987, T = 0.251698; substitution rates AC = 1.805316, AG = 2.136673, AT = 0.949986, CG = 0.780819, CT = 4.656160, GT = 1.000000; gamma distribution shape parameter alpha = 0.020000. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic analysis, our new strain (RUFCC2453) clustered in the clade *Chaetomella raphigera* with high statistical values (100% ML, 1 PP).

3.1.5 Multi-gene analyses for *Neottiosporina*

The concatenated dataset of ITS regions contained eleven isolates, which comprised 539 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Suttonomyces rosae* (MFLUCC 15-0051) was used as the outgroup taxon. The best-scoring RAxML tree with a final likelihood value of -1790.869339 is presented in Figure 7. The matrix had 138 distinct alignment patterns, with 3.39% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.220321, C = 0.266236, G = 0.234288, T = 0.279155; substitution rates AC = 4.232102, AG = 5.947883, AT = 5.637501, CG = 0.698220, CT = 13.019587, GT = 1.000000; gamma distribution shape parameter alpha = 0.164930. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic analysis, our new strain (RUFCC2454 (ex-type), and RUFCC2461) form a sister clustered with *Neottiosporina cylindrica* (BRIP 14187 (ex-type) and BRIP (16231) with high statistical values (84% ML, 0.95 PP). Based on the phylogenetic analyses and morphological characters, we confirm our strains differ from *Neottiosporina cylindrica*. Herein, we report our strains as a novel species viz. *Neottiosporina mihintaleensis* sp. nov.

3.2 Taxonomy

In this section, we listed all the collected taxa according to the higher-level classification referenced by Wijayawardene et al. (2022a).

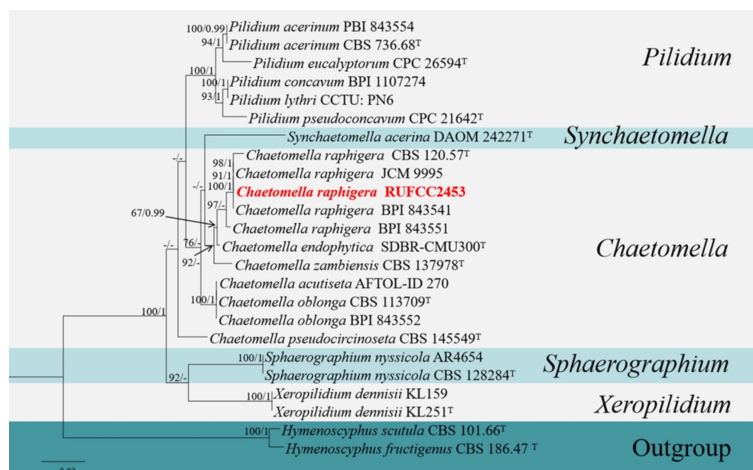


FIGURE 6

Phylogenetic tree from the best scoring of the RAxML analysis based on combined (LSU, ITS and SSU) is rooted to *Hymenoscyphus scutula* (CBS 101.66) and *H. fructigenus* (CBS 186.47). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95, are given at the respective branches. Hyphen (-) means a value lower than 50% (BS) or 0.95 (PP). Ex-types are marked in "T". New isolates are labelled in bold and red.

Ascomycota Caval.-Sm.

Dothideomycetes O.E. Erikss. & Winka

Pleosporales Luttr. ex M.E. Barr

Didymellaceae Gruyter, Aveskamp & Verkley

Ectophoma Valenz.-Lopez, Cano, Crous, Guarro and Stchigel,

Stud. Mycol. 90: 34 (2017)

Index Fungorum Registration Identifier: 819952

Notes: The genus *Ectophoma* was introduced by Valenzuela-Lopez et al. (2018) with *E. multirostrata* (basonym: *Sphaeronaema multirostratum* P.N. Mathur et al.) as the type species. *Ectophoma* comprises six species in diverse habitats (Valenzuela-Lopez et al., 2018) and a well-defined genus in Didymellaceae (Hou et al., 2020a,

b). In this study, we introduce *Ectophoma salviniae* sp. nov. from a healthy leaf of *Salvinia minima* (Watermoss).

Ectophoma salviniae Wimalasena, Wijayaw. & Bamunuarachchige sp. nov.

Index Fungorum Registration Identifier: IF902503 (Figure 8).

Etymology: The name is derived from the host genus *Salvinia*, from which the fungus was isolated.

Holotype: RUSLH/240

Description: Endophytic of healthy leaf of *Salvinia minima*. Sexual morph: Undetermined. Asexual morph on the culture: Colonies on

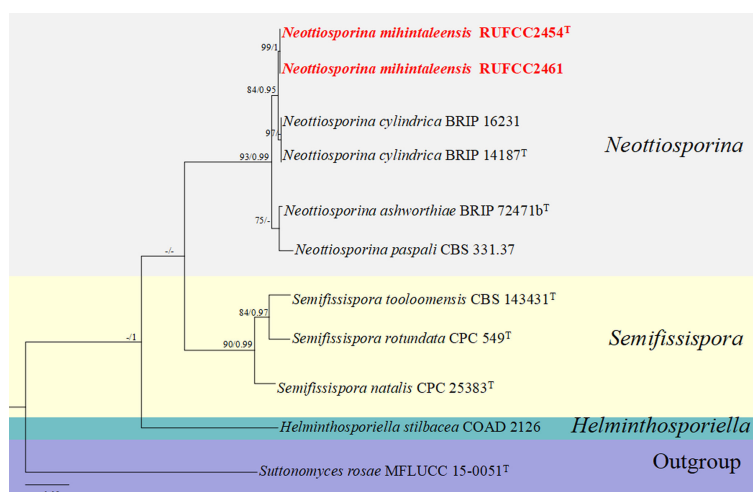


FIGURE 7

Phylogenetic tree from the best scoring of the RAxML analysis based on combined (ITS) is rooted to *Suttonomyces rosae* (MFLUCC 15-0051). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95, are given at the respective branches. Hyphen (-) means a value lower than 50% (BS) or 0.95 (PP). Cultures from holotype and specimens are marked in "T". New isolates are labelled in bold and red.

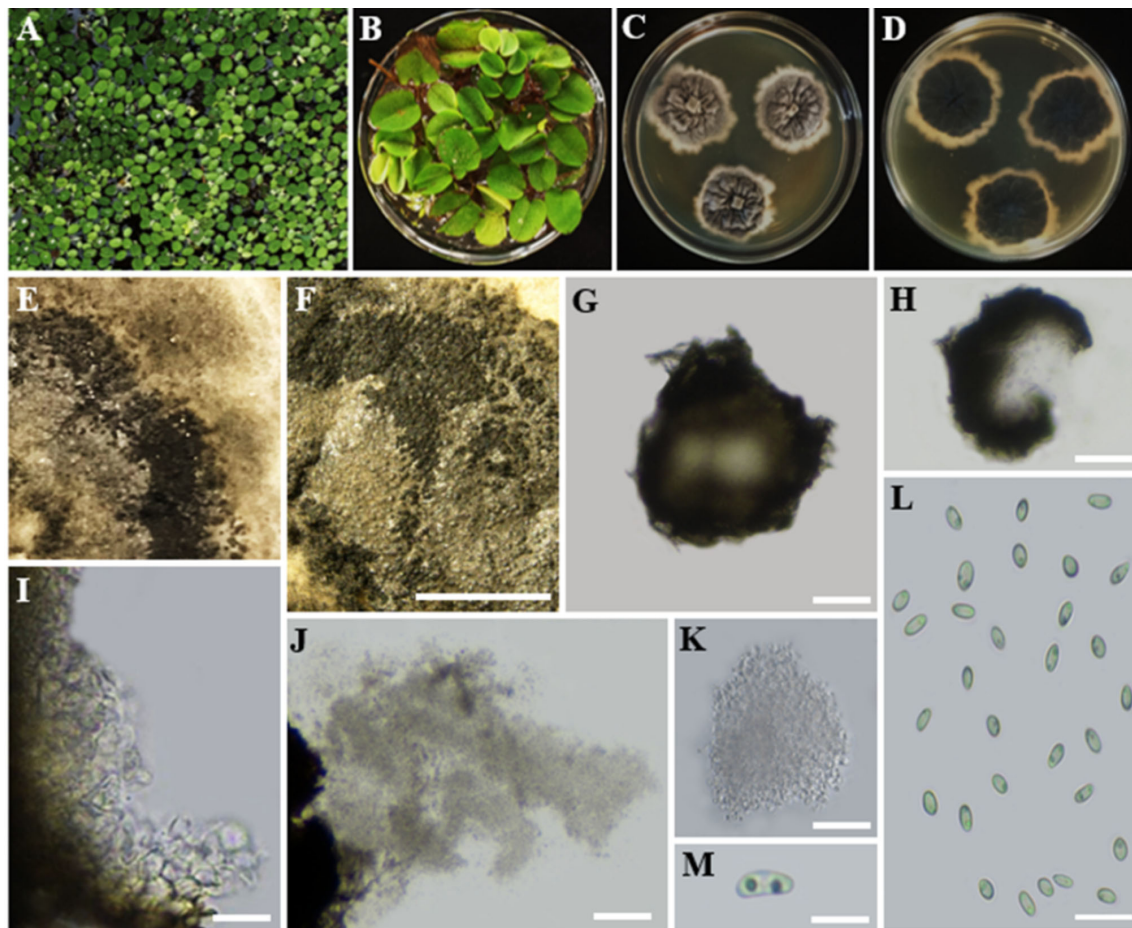


FIGURE 8

Ectophoma salviniae sp. nov. (A, B). *Salvinia minima* in the lake. (A) and in the lab (B) (Watermoss). (C) Top view of the PDA culture plate (diameter; 9.5 cm). (D) Downside of the PDA culture plate (diameter; 9.5 cm) after five days. (E) Sporulated culture after seven days. (F) Stereo microscopic view of sporulation in culture. (G, H) Squashed pycnidium. (I) Inner wall of pycnidium. (J) Conidial production. (K) Conidiogenous cells and conidia. (L, M) Conidia. Scale bars: (F) = 1000 µm, (G–M) = 100 µm.

PDA slow growing, effuse, with a regular margin, flat, white to grey olivaceous reaching a diam of 1.5–2 cm after 7 days at 28°C. *Mycelium* regular, composed of filamentous, septate, branched, smooth, pale olivaceous hyphae 0.5 µm wide. *Conidiomata* pycnidial, 140–300 µm long × 50–80 µm wide, brown to dark brown, solitary or confluent, abundant, ostiolate, with one or more short necks. *Pycnidial wall*, glabrous, with globose to subglobose or irregular cells of *textura angularis*. *Conidiogenous cells* holoblastic to phialidic, minute. *Conidia* 6.1–9.6 × 3.6–6.7 ($\bar{x}$ = 8.0 × 5.0 µm; n = 30) µm, aseptate, hyaline, smooth-walled, oblong to ellipsoidal, end of conidia is acute, guttulate: two guttules are inside the conidial cytoplasm.

Culture characteristics: Colonies on PDA reached a diameter of 1.5–2 cm after 7 days at 28°C, with a regular margin and flat, colourless to weak olivaceous, poorly developed, white to grey olivaceous aerial mycelium. The centre of the colony is olivaceous, followed by a black circle formed by abundant pycnidia. The reverse of the colony was dark black and featured some radially furrowed zones and concentric circles of greyish-black colours.

Material examined: SRI LANKA, North Central Province, Mihintale, Iluppukanniya Tank (8.36482° N, 80.50764° E, 118 m), on healthy leaf of *Salvinia minima* (Watermoss), 28 November 2023, Madhara K. Wimalasena, RUSLH/240 (dried culture as the holotype), RUFCC2462 (ex-type).

Notes: The multi-locus analyses of combined data set of ITS, LSU, *rpb2*, and *tub2* sequence data revealed that our isolates (RUFCC2458, RUFCC2462) are clustered within the *Ectophoma* s. str., forming a sister clade to *E. iranica* (SCUATK1G1 (ex-type) and SCUAK1) and *E. multirostrata* (CBS 274.60 (ex-type) and CBS 380.67). The conidiomata of *E. salviniae* show a slight similarity in shape and the dimensions to those of *E. iranica* and *E. multirostrata*. However, *E. salviniae* has larger conidia than in both *E. iranica* and *E. multirostrata* (Table 3). Furthermore, its cultural characteristics, including dark greyish to black colonies, are also distinct from *E. iranica* and *E. multirostrata* (Table 3). Moreover, phylogenetic analysis further confirms that *E. salviniae* is distinct from *E. iranica* and *E. multirostrata* (Figure 3). Herein, the taxon, represented by RUFCC2458 (ex-type) and RUFCC2462 is introduced as a novel species viz., *Ectophoma salviniae* sp. nov.

Botryosphaeriales C.L. Schoch, Crous & Shoemaker
Phyllostictaceae Fr.

Phyllosticta Pers., *Traité champ. Comest.* (Paris): 55, 147 (1818)
Index Fungorum Registration Identifier: 9384
= *Guignardia* Viala & Ravaz, *Bull. Soc. mycol. Fr.* 8(2): 63 (1892)

Notes: *Phyllosticta* is a well-established genus in *Phyllostictaceae*, *Botryosphaeriales* with over 3000 species epithets in the Index Fungorum 2024 (accession date: 14 of May 2024). This genus currently includes 1,499 recognized species (Sui et al., 2023). Recently, Gong et al. (2024) introduced two novel species (*P. savannaensis* and *P. ovalina*), and Jiang et al. (2024) added three new species (*P. fujianensis*, *P. saprophytica*, and *P. turpiniae*) to this genus. The members of *Phyllosticta* have been mostly reported as pathogens, saprobes, and endophytes of different hosts worldwide (Wikee et al., 2013; Rodrigues et al., 2019; Sui et al., 2023; Jiang et al., 2024). In this study, we isolated *Phyllosticta capitalensis* as an endophytic taxon from *Nymphaea nouchali*.

Phyllosticta capitalensis Henn., *Hedwigia* 48: 13 (1908) [1909]
Index Fungorum Registration Identifier: 168326 (Figure 9).

Description: Endophytic of healthy leaf of *Nymphaea nouchali*. Sexual morph: Undetermined; Asexual morph: *Pycnidia* up to 300 µm diam, 250 µm tall, black, aggregated, erumpent, globose to ampulliform, ostiolate, exuding a colourless, glossy, slimy conidial mass. *Pycnidial wall* consisting of 6–8 layers, up to 40 µm thick, with cells of *textura angularis*. *Ostiole* single, central, 5–15 µm diam. *Conidiophores* subcylindrical to ampulliform, frequently reduced to conidiogenous cells, or branching from a basal supporting cell, coated in mucoid layer, 7–20 × 3–7 µm. *Conidiogenous cells* subcylindrical to ampulliform to doliiform, holoblastic, polyblastic, hyaline, smooth, 7–10 × 3–5 µm; percurrently proliferating 1–2 times near apex. *Conidia* 10–11 × 6–7 µm ($\bar{x}$ = 9.5 × 6.5 µm; n = 30), solitary, hyaline, aseptate, thin- and smooth-walled, coarsely guttulate, ellipsoid to obovoid, tapering toward a narrowly truncate base, enclosed in a mucilaginous 2–4 µm thick

sheath, bearing a hyaline, mucoid, straight to curved, unbranched, 6–8 × 1–1.5 µm, apical appendage on a bluntly rounded apex.

Culture characteristics: Colonies incubated for 14 days at 28–30°C in darkness on PDA initially appear woolly and white with abundant mycelium. Over the next 2–3 days, they transform from greenish to dark green, with white hyphae visible along the undulating margins, eventually becoming black. Over two weeks of incubation in darkness at 28°C, the mycelium extends to the edge of the Petri dish.

Material examined: SRI LANKA, North Central Province, Mihintale, Iluppukanniya tank (8.36482° N, 80.50764° E, 118 m), on healthy leaf of *Nymphaea nouchali* (Blue Water-Lily), 02 December 2023, Madhara K. Wimalasena, RUFCC2452 (living culture), RUSLH/242 (dried culture as the herbarium specimen).

Notes: *Phyllosticta capitalensis* is often found as an endophyte on a wide range of hosts and exhibits a broad geographic distribution (Silva and Pereira, 2007; Silva et al., 2008; Glienke et al., 2011; Wang et al., 2023). It was reported from 70 plant families and is considered a weak plant pathogen (Wikee et al., 2013). *Phyllosticta capitalensis* has been previously reported in Sri Lanka as a rubber foliar pathogen (Herath et al., 2019) and as an endophytic fungus in the leaves of *Camellia sinensis* (Thambugala et al., 2018). Dissanayake et al. (2016) reported *Phyllosticta capitalensis* on healthy specimens of *Nymphaea nouchali* collected from an unpolluted natural freshwater pond in the Western Province of Sri Lanka, based on a single gene locus (ITS) study. In this study, we reconfirmed the occurrence of *Phyllosticta capitalensis* on healthy leaves of *Nymphaea nouchali* in lentic freshwater habitats in the North-Central Province of Sri Lanka, based on four gene loci (ITS, *tefl-α*, *ACT*, and *GADPH*) study, a polyphasic approach. Previous reports of *Phyllosticta* species in freshwater plants worldwide include *Phyllosticta aquatica* (on *Lemna minor* fide (Spegazzini, 1881), *P. fatiscens* (on *Nymphaea odorata* fide (Anonymous, 1960), and *P. nymphaeacea* (on *Nymphaea* sp. fide (Ellis and Everhart, 1900). According to (Farr et al., 2012), *P. capitalensis* has not been reported from *Nymphaea nouchali* so far,

TABLE 3 Diagnostic characters of *Ectophoma iranica*, *E. multirostrata* and *E. salviniae*.

Morphological and colony characters	Species name and references		
	<i>E. iranica</i> (Kularathnage et al., 2023; Lathi et al., 2019)	<i>E. multirostrata</i> (Khanapour et al., 2021; Boerema, 2004)	<i>E. salviniae</i> sp. nov. (This study)
Conidiomata	Pycnidia 145.9–382.7 µm long, hyaline to pale brown to brown, with age becoming blackish brown, variable in shape, mostly globose to subglobose but also ovoid, lemon-shaped	Pycnidia more than 550 µm in diameter, globose to subglobose or irregular. Conidial matrix whitish to cream or buff-coloured	Pycnidia 140–300 µm long × 50–80 µm wide, brown to dark brown, glabrous, globose to subglobose or irregular
Conidiogenous cells	Conidiogenous cells discrete, hyaline, smooth-walled, globose, phialidic. Dimensions of the conidiogenous cell were not reported	Conidiogenous cell were not reported	Conidiogenous cells were not observed.
Conidia	2.2–5.8 µm long aseptate, oblong to ellipsoidal	Variable in dimensions, mostly 3.8–4.2 × 1.8–2.4 µm, oblong to ellipsoidal, sometimes eguttulate	Conidia 6.1–9.6 × 3.6–6.7 µm ($\bar{x}$ = 8.04 × 5.0 µm; n = 30) µm aseptate, hyaline, smooth and thin walled, oblong to ellipsoidal, guttulate
Colony characters	Pale brown to greyish brown colonies	Colourless to weakly olivaceous, white to grey olivaceous to olivaceous buff; reverse olivaceous	Drak greyish to black colour colonies; reverse dark black

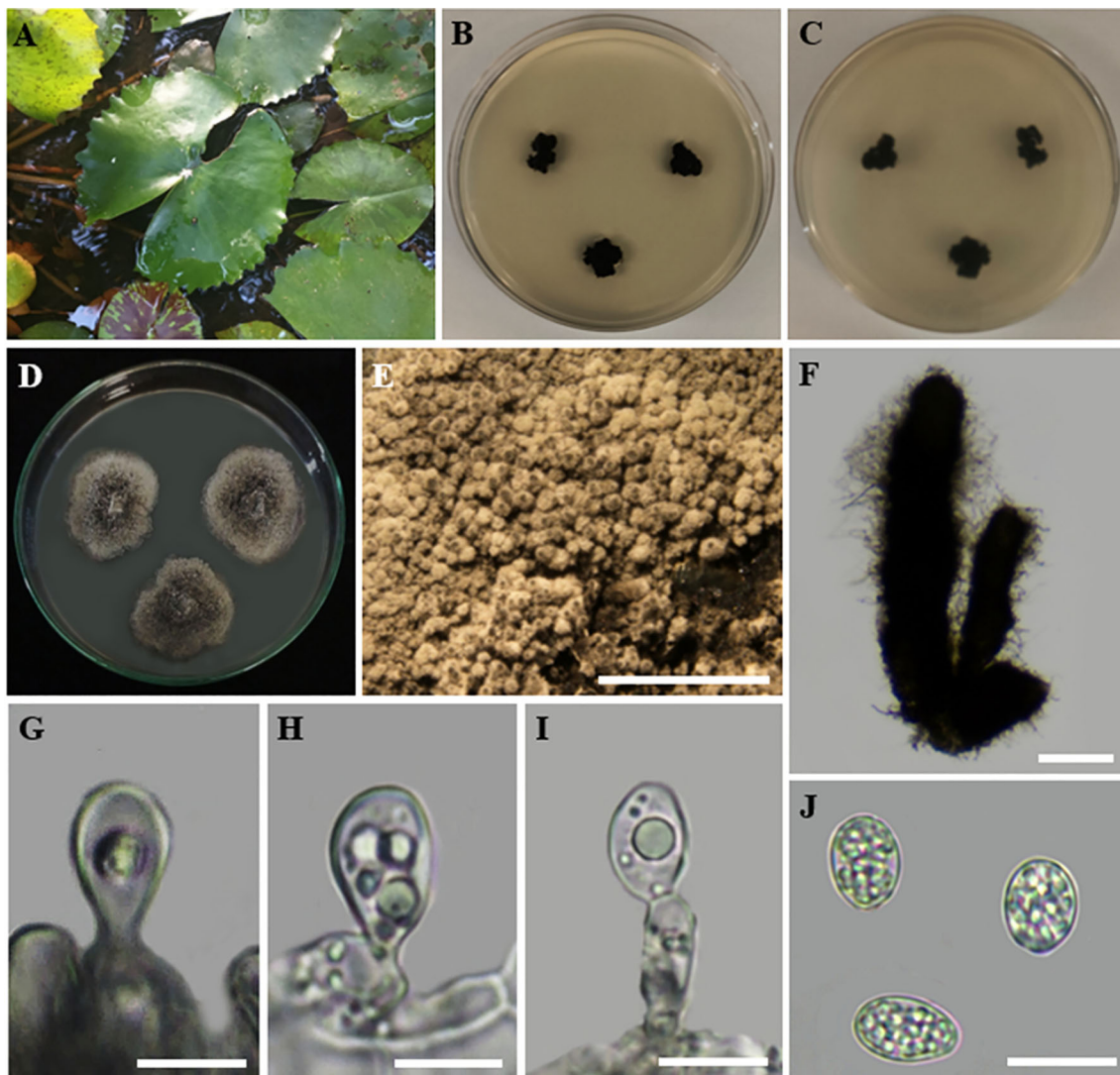


FIGURE 9

Phyllosticta capitalensis (A) Host freshwater plant leaf of *Nymphaea nouchali* (Blue Water-Lily). (B) Upside of the PDA culture plate (diameter; 9.5 cm). (C) Downside of the PDA culture plate (diameter; 9.5 cm) after five days. (D) Sporulated culture after seven days. (E) Stereo microscopic view of sporulation in culture. (F) Conidiomata. (G–I) Conidiogenous cells. (J) Conidia. Scale bars: (E) = 1000 µm, (F) = 50 µm (G–J) = 100 µm.

and thus, this is the first confirmative report of *P. capitalensis* on *Nymphaea nouchali*. When comparing the recently isolated *P. capitalensis* (SDBR-CMU497 and SDBR-CMU498) (Chaiwong et al., 2024) isolates with *P. capitalensis* RUFCC2452, their morphological features, such as pycnidia, conidiophores, and conidiogenous cells, are similar. However, the asexual conidia of *P. capitalensis* RUFCC2452 are slightly larger than those of the SDBR-CMU497 and SDBR-CMU498 (5.2 to 9.4×3.6 to 7.5 µm ($n = 50$) isolates.

Leotiomyces O.E. Erikss. & Winka

Chaetomellales Crous & Denman

Chaetomellaceae Baral, P.R. Johnst. & Rossman

Chaetomella Fuckel, Jb. nassau. Ver. Naturk. 23–24: 401 (1870) [1869–70]

Index Fungorum Registration Identifier: 7575

Notes: Fuckel (1869), established the genus *Chaetomella*, including two species; *C. oblonga*, characterized by hyaline spores, and *C. atra*, characterized by olivaceous spores. Among these, the type species was *C. oblonga* (Rossman et al., 2004). The members of the genus were reported as plant pathogens (Gajbhiye et al., 2016; Pärtel et al., 2017; Nguyen et al., 2018; Suwannarach et al., 2018; Cao et al., 2021), saprophytes and as endophyte (Suwannarach et al., 2018). As of June 2024, the Index Fungorum lists 61 records for the genus *Chaetomella* (<https://www.indexfungorum.org/names/Names.asp>). In this study, we report *C. raphigera* from the healthy leaf of *Eichhornia crassipes* as an endophytic species.

Chaetomella raphigera Swift, Mycologia 22(4): 165 (1930)

= *Volutellospora raphigera* (Swift) Thirum. & P.N. Mathur, Sydowia 18 (1–6):38 (1965)

= *Chaetomella terricola* P.Rama Rao, Mycopathologia et Mycologia Applicata 19 (3):255 (1963)

Index Fungorum Registration Identifier: 163400 (Figure 10).

Description: Endophytic of healthy leaf of *Eichhornia crassipes*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* pycnidial, 200–350×100–250 µm, solitary, short-stipitate, globose to ovate, ostiolate, widely opening lengthwise, dark brown to black, thick-walled, setiferous. Basal stipe short, composed of hyaline,

pseudoparenchymatous cells. *Setae* 50–100×2.5–5 µm, brown, smooth, thick-walled, septate, unbranched, with hooked apices. *Conidiophores* hyaline, short, branched, filiform, septate, and smooth. *Conidiogenous cells* enteroblastic, phialidic, determinate, integrated, filiform, hyaline, and smooth. *Conidia* 3.5–6 × 1–2.3 µm ($\bar{x}$ = 4.8 × 1.6 µm; n = 30), hyaline, aseptate, cymbiform to allantoid,

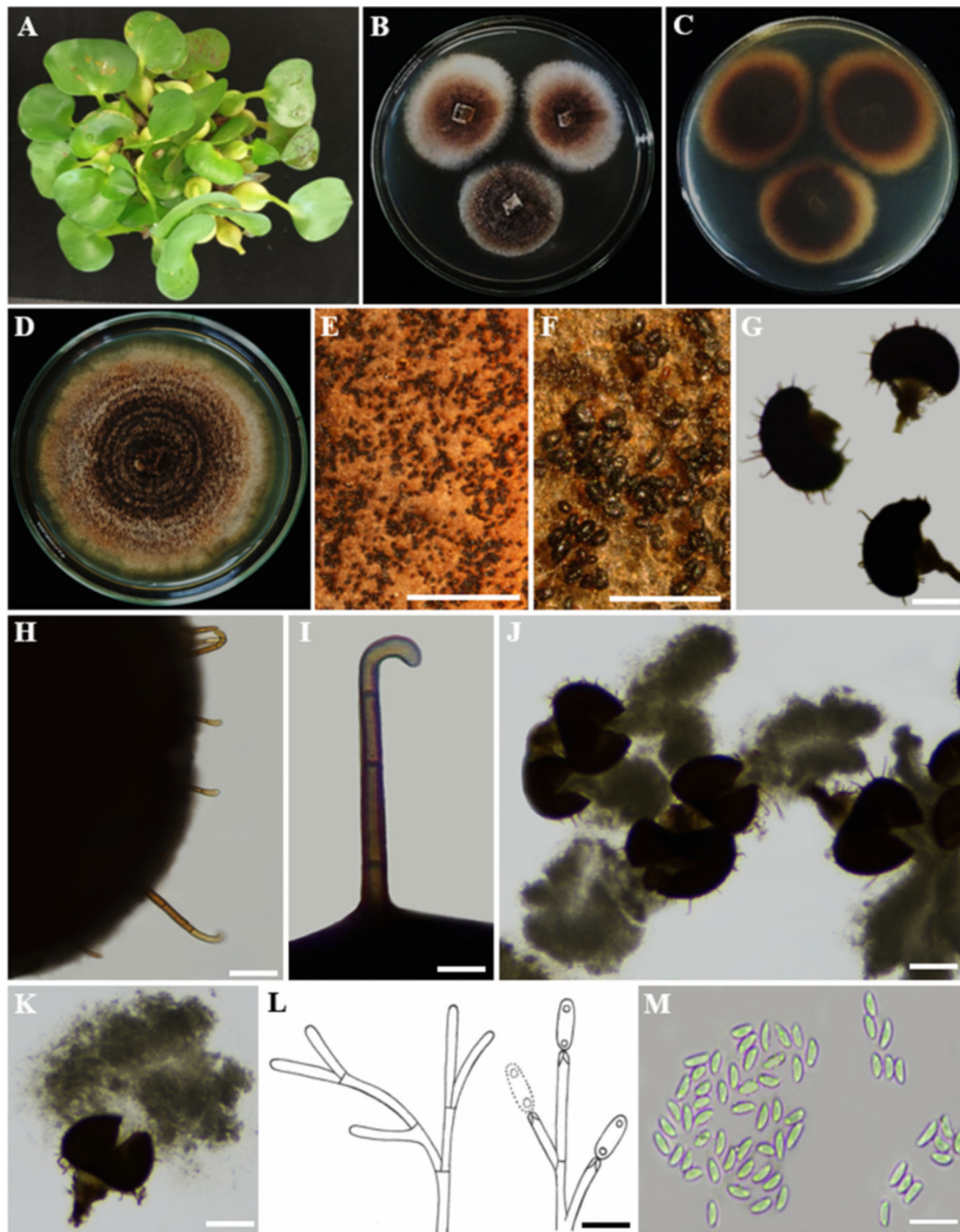


FIGURE 10

Chaetomella raphigera (A) Host freshwater plant leaf of *Eichhornia crassipes* (Water hyacinth). (B) Upside of the PDA culture plate (diameter; 9.5 cm). (C) Downside of the PDA culture plate (diameter; 9.5 cm) after five days. (D) Sporulated culture after seven days. (E, F) Stereo microscopic view of sporulation in culture. (G) Pycnidia. (H, I) Types of setae on pycnidium. (J, K) Pycnidia release the conidia. (L) Conidiogenous cells. (M) Conidia. Scale bars: (E, F) = 1000 µm (G–M) = 100 µm.

on maturity released by splitting the pycnidial wall along the thin-walled cells of the raphe, in mass becoming amber-coloured on aging.

Culture characteristics: Colonies on incubation for 14 days at 28–30°C in darkness on PDA media, attaining 5.5 cm diam., cinnamon brown with yellowish-white margins and with septate, branched mycelium. The reverse of the colony is dark brown in the center with yellowish-white edges. Sporulation is visible in a circular pattern on the surface of the colony.

Material examined: SRI LANKA, North Central Province, Mihintale, Iluppukanniya tank (8.36482° N, 80.50764° E, 118 m), on healthy leaf of *Eichhornia crassipes* (Water hyacinth), 5 December 2023, Madhara K. Wimalasena, RUFCC2453 (living culture), RUSLH/243 (dried culture as the herbarium specimen).

Notes: *Chaetomella raphigera* has been reported as a plant pathogen from India (Gajbhiye et al., 2016). However, this is the first report of *C. raphigera* as a new geographical record in Sri Lanka, found in the freshwater plant *Eichhornia crassipes*. Besides, *Chaetomella* species have not been reported in Sri Lanka thus this is the first genus

report from the country. Morphological similarities of *C. raphigera* (RUFCC2453) with previous studies are mentioned in Table 4.

Sordariomycetes O.E. Erikss. & Winka

Glomerellales Chadeff. ex Rêblová, W. Gams & Seifert

Glomerellaceae Locq. ex Seifert & W. Gams

Colletotrichum Corda

Index Fungorum Registration Identifier: 7737

Note: *Colletotrichum* represents a diverse and complex genus, with currently 344 recognized species grouped into 20 species complexes (Talhinhas and Baroncelli, 2021). These species often lead to considerable economic losses, mostly infecting economically important crops (Peng et al., 2023; Peralta-Ruiz et al., 2023; Zhang et al., 2023). The members of *Colletotrichum* exhibit different lifestyles that are found in varied environments and host species (Jayawardena et al., 2016a, b; Samarakoon et al., 2018; Talhinhas and Baroncelli, 2023). These include necrotrophic (Vargas et al., 2012; De Silva et al., 2017; Talhinhas and Baroncelli, 2021; Páez Redondo et al., 2022), biotrophic and

TABLE 4 Morphological similarities of *Chaetomella raphigera* (RUFCC2453) with previous studies.

<i>C. raphigera</i> strains	Morphological and colony characters			
	Conidiomata	Conidiogenous cells	Conidia	Colony characters
<i>C. raphigera</i> (Rossman et al., 2004)	Pycnidia 150–450 × 100–200 µm on natural substratum, 200–320 × 140–200 µm in culture, elongated, reniform, pale to dark reddish brown, with a short stalk of hyaline textura angularis	Conidiogenous cells enteroblastic, collar and channel minute	Conidia non-septate, hyaline, ellipsoid with broadly rounded ends, straight or slightly curved, smooth, guttulate, 5.2–7.5 × 2.0–3.0 µm ($\bar{x}$ = 6.41 × 2.47 µm, n = 87)	Colonies 4.3–5.0 cm diam., no aerial mycelium, submerged mycelium cinnamon to dark brick, sporulating profusely
<i>C. raphigera</i> (TAC-15/ MUBL No. 665), (Gangadevi and Muthumary, 2009)	Conidiomata are pycnidial, separate, globose but opening widely, very shortly stipitate, dark brown to black, thick-walled, 200–350 × 100–250 µm	Conidiogenous cells enteroblastic, phialidic, determinate, integrated, filiform, hyaline, smooth	Conidia hyaline, aseptate, cymbiform to allantoid, 3.75–6.25 × 1.25–2.5 µm	Colonies are brown, septate, branched mycelium
<i>C. raphigera</i> (BF79/ JX863671 and BF99/ KF308287), (Gajbhiye et al., 2016)	Pycnidia were dark reddish brown, oval, approximately 200 µm × 300 µm	Conidiogenous cells not reported	Conidia were produced apically on conidiophores, aseptate, ellipsoidal with rounded ends, smooth, straight or curved, 10–12 µm × 2–3 µm	Colony characters not reported
<i>C. raphigera</i> (CNUFC-GHD05-1), (Nguyen et al., 2018)	Elongated, reniform, pale to dark reddish brown, and measured 72.5–148.5 µm × 46.5–88.5 µm	Conidiogenous cells not reported	Ellipsoid, and measured 4.8–7.2 µm × 1.8–2.6 µm	Slowly-growing, white at first, becoming cinnamon brown in age
<i>C. raphigera</i> (Cao et al., 2021)	Pycnidia were pale to dark brown, globose or oblate (245.98–491.33 µm × 123.14–274.11 µm), covered with setae	Conidiogenous cells not reported	Conidia were hyaline, oval or boat-shaped (5.19–6.52 µm × 1.87– 2.66 µm)	Colonies were pale brown with rare aerial mycelium and abundant pycnidia production.
<i>C. raphigera</i> RUFCC2453 (This study, 2024)	Conidiomata pycnidial, 200–350 µm × 100–250 µm, solitary, short-stipitate, globose to ovate, ostiolate, widely opening lengthwise, dark brown to black, thick-walled, setiferous	Conidiogenous cells enteroblastic, phialidic, determinate, integrated, filiform, hyaline, and smooth	Conidia 3.5–6 µm × 1.–2.3 µm ($\bar{x}$ = 4.8 × 1.6 µm; n = 30), hyaline, aseptate, cymbiform to allantoid, on maturity released by splitting the pycnidial wall	Colonies 5.5 cm diam., cinnamon brown with yellowish-white margins and with septate, branched mycelium. The reverse of the colony is dark brown in the center with yellowish-white edges. Sporulation is visible in a circular pattern on the surface of the colony

hemibiotrophic (De Silva et al., 2017; Páez Redondo et al., 2022; Jia et al., 2023), quiescent (De Silva et al., 2017; Fu et al., 2022), and endophytic (De Silva et al., 2017; Páez Redondo et al., 2022; Lin et al., 2023; Liu et al., 2023; Zhang et al., 2023; Barreto Ramos et al., 2024) lifestyles. Among these lifestyles, endophytic *Colletotrichum* spp. have been documented in marine environments such as mangroves (Grano-Maldonado et al., 2021; Norphanphoun and Hyde, 2023; Aumentado et al., 2024) and freshwater habitats (Zheng et al., 2022). In this study, we isolated *C. siamense* and *C. truncatum* as two endophytic taxa of *Eichhornia crassipes* in freshwater environments.

Colletotrichum siamense Prihast., L. Cai & K.D. Hyde, *Fungal Diversity* 39: 98 (2009)

Index Fungorum Registration Identifier: 515410 (Figure 11).

Description: Endophytic of healthy leaf of *Eichhornia crassipes*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* acervular, pulvinate, with erect conidiophores formed on a cushion of roundish and medium brown cells. *Setae* not observed. *Conidiophores* maconematous, hyaline, septate, branched. *Conidiogenous* cells hyaline, cylindrical to ampulliform, phialidic, smooth, guttulate, 7–15.5 µm long, 1–2.5 µm wide at apex. *Conidia* 9.89–15.95 × 3.73–5.67 µm ($\bar{x}$ = 13.75 × 4.75 µm; n = 30), hyaline, aseptate, smooth-walled, cylindrical, bluntly rounded at both ends, guttulate.

Culture characteristics: Colonies on incubating for ten days at 28–30°C in dark on PDA media, reaching a diameter of 1–1.5 cm *Eichhornia crassipes*. The aerial mycelium is white, cottony, and sparse. The colony surface features numerous, small acervuli with orange conidial ooze, and the reverse side remain pale yellowish.

Material examined: SRI LANKA, North Central Province, Mihintale tank (8.36267° N, 80.50591° E, 108 m), Mihintale, on healthy leaf of *Eichhornia crassipes* (Water hyacinth), 30 November 2023, Madhara K. Wimalasena, RUFCC2455 and RUFCC2457 (living cultures), RUSLH/244 (dried culture as the herbarium specimen).

Notes: *Colletotrichum siamense* and *C. truncatum* are important plant pathogens causing a wide range of diseases worldwide (Talhinhas and Baroncelli, 2023). Several studies reported *C. siamense* and *C. truncatum* from different hosts and habitats in Sri Lanka and these include *Allium cepa* (Herath et al., 2021), *Hevea brasiliensis* (Herath et al., 2019), *Musa* sp. (Kurera et al., 2023), and *Persea americana* (Dissanayake et al., 2021) as hosts for *Colletotrichum siamense*, while *Begonia* sp. (Wickramasinghe et al., 2019), *Capsicum annuum* (Welideniya et al., 2019), and *Hevea brasiliensis* (Herath et al., 2019) have been identified as hosts for *Colletotrichum truncatum*. De Silva et al. (2019) reported isolates of *Colletotrichum siamense* from different countries showed noticeable differences in growth rates and culture morphology. However, conidial measurements from isolates in distinct subclades of the phylogenetic tree were consistent, and the morphological traits within each subclade were highly uniform within each country (De Silva et al., 2019) (see Table 5 for the morphological comparison).

Huang et al. (2021) reported *C. fructicola* (which causes irregular necrotic lesions on leaves, stems, and crown and petiole rot symptoms) from *Eichhornia crassipes* in China. However, as far as we know, there are no hitherto reports of *C. siamense* or *C. truncatum* on *Eichhornia crassipes* in Sri Lanka or elsewhere (2024;

accession date: 06 June 2024, <https://fungi.ars.usda.gov/>). Hence, this is the first host report of *C. siamense* and *C. truncatum* from *Eichhornia crassipes* from Sri Lanka.

Colletotrichum truncatum (Schwein.) Andrus & W.D. Moore, *Phytopathology* 25: 121 (1935)

Index Fungorum Registration Identifier: 280780 (Figure 12).

Description: Endophytic of healthy leaf of *Eichhornia crassipes*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* acervular, with conidiophores and setae formed directly on hyphae. *Setae* subhyaline to moderately brown, smooth to verruculose, 2 to 5-septate, cylindrical to conical at base, tapering towards the slightly acute to roundish tip, 4–6 µm diam. *Conidiophores* up to 90 µm long, hyaline to pale brown, septate, densely branched, clustered, *Conidiogenous* cells enteroblastic, phialidic, hyaline to pale brown, cylindrical, 6–20 × 2.5–4 µm, with invisible collarette, periclinal thickening not observed. *Conidia* 20.35–28.39 × 2.23–4 µm ($\bar{x}$ = 25 × 3 µm; n = 30), hyaline, cylindrical-fusiform, elongated, smooth-walled, aseptate, curved at tapering apex, truncate at base, guttulate with granular content.

Culture characteristics: Colonies on incubating for seven days at 28–30°C in dark on PDA, exhibit a diameter of 1.5–2 cm. The colonies are flat with an entire margin, devoid of aerial mycelium, bluff at surface and covered by olivaceous-grey to iron-grey acervuli. The reverse of the colony is buff to pale olivaceous-grey. Conidia in mass are whitish, buff to pale saffron.

Material examined: SRI LANKA, North Central Province, Mihintale, Mahakanadara tank (8.38683° N, 80.38683° E, 117 m), on healthy leaf of *Eichhornia crassipes* (Water hyacinth), 8 December 2023, Madhara K. Wimalasena, RUFCC2451 (living culture), RUSLH/245 (dried culture as the herbarium specimen).

Notes: De Silva et al. (2019) found that *Colletotrichum* isolates with curved conidia and ITS sequences matching the ex-type of *C. truncatum* were the most common, making up 44% of all isolates. These isolates came from Indonesia, Malaysia, Sri Lanka, and Thailand, while species with straight conidia were identified separately. The remaining 56% were species with straight conidia, mostly from other complexes within the *Colletotrichum* genus. Liu et al. (2022) reported that the *C. truncatum* species complex produces curved conidia. Interestingly, species with curved conidia appear throughout the phylogenetic tree, suggesting this trait evolved multiple times. While ITS is useful for identifying *Colletotrichum* species complexes (Cannon et al., 2012), other loci like *GAPDH*, *ACT*, *CHS-1*, *HIS 3*, and *tub2* are increasingly used to better define species boundaries, including in the *C. truncatum* complex (Damm et al., 2009, 2014; Liu et al., 2022). See the notes under *Colletotrichum siamense*.

Dothideomycetes genera *incertae sedis*

Neottiosporina Subram., *Proc. Natl. Inst. Sci. India*, B 27: 238 (1961)

Index Fungorum Registration Identifier: 9117

Notes: Sutton and Alcorn (1974) revisited the genus *Neottiosporina*, typified by *N. apoda* (Speg.) Subram. (1961), which is characterized by *pycnidia* that are solitary, dark brown, globose to subglobose, thin-walled, and ostiolate; *conidiogenous* cells are holoblastic, solitary, hyaline, determinate, and originate from the inner wall of the *pycnidium*. The *conidia* are acrogenous, solitary, hyaline,

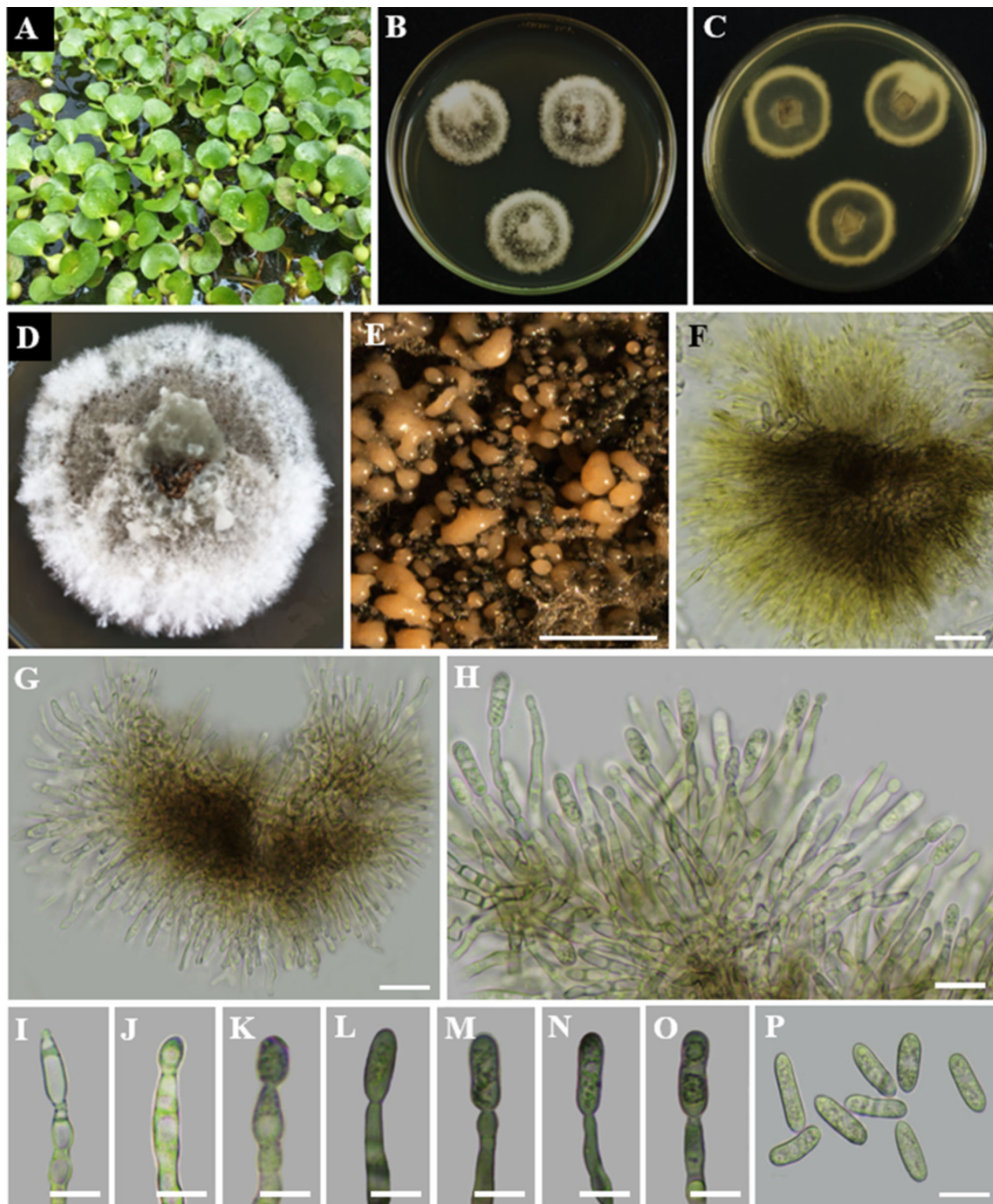


FIGURE 11

Colletotrichum siamense (A) Host freshwater plant leaf of *Eichhornia crassipes* (Water hyacinth). (B) Upside of the PDA culture plate (diameter; 9.5 cm). (C) Downside of the PDA culture plate (diameter; 9.5 cm) after seven days. (D) Sporulated colony. (E) Acervuli with orange conidial ooze. (F, G) Conidiomata and conidiogenous cells. (H) Conidiogenous cells. (I–P), Maturity levels of conidiogenous cells. (P) Conidia. Scale bars: (E) = 500 µm, (F–P) = 100 µm.

multiseptate, smooth-walled, cylindrical to cymbiform, obtuse at apex, and truncate at base. The genus comprises ten species viz., *N. apoda* (Speg.) Subram (Sutton and Alcorn, 1974), *N. ashworthiae* (From Scleria: Queensland fide Tan and Shivas, 2022), *N. asymmetrica* (on *Themeda australis* fide Sutton and Alcorn, 1974), *N. australiensis* (on *Phragmites australis* fide Sutton and

Alcorn, 1974), *N. clavata* (on *Phragmites australis* fide Sutton, 1981), *N. masonii* (on *Pinus caribaea* fide Sutton and Sarbhoy, 1976), *N. cylindrica* (on *Cyperus brevifolius* fide Sutton and Alcorn, 1985), *N. paspali* (Sutton and Alcorn, 1974), *N. phragmiticola* (in Ethiopia, Sudan, and Uganda fide (Nag Raj, 1993), and *N. sorghicola* in China (Sutton and Wu, 1995). In our study of endophytic fungi

TABLE 5 *Colletotrichum siamense* strains reported in different geographical locations and their conidial measurements (CPC-Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute, RUFC-C Rajarata University Fungal Culture Collection, UOM-University of Melbourne culture collection, Victoria, Australia).

<i>Colletotrichum siamense</i> strains reported in different geographical locations	Host and distribution	Conidial measurements
<i>C. siamense</i> (UOM 1116), (De Silva et al., 2019)	Fruit lesion of <i>Capsicum</i> sp., Kandy, Sri Lanka	10.5–16.5 × 3.5–5.5 μm
<i>C. siamense</i> (CPC 30233), (De Silva et al., 2019)	Fruit lesion of <i>Capsicum annuum</i> , Gowa, Indonesia	12.5–17 × 2.5–5.5 μm
<i>C. siamense</i> (UOM 1132), (De Silva et al., 2019)	Fruit lesion of <i>Capsicum</i> sp., Ratchaburi, Thailand	9.5–14.5 × 3.5–5 μm
<i>C. siamense</i> (UOM 1126/F4-1C), (De Silva et al., 2019)	Fruit lesion of <i>Capsicum</i> sp., Kanchana Buri, Thailand	12–15 × 5–7 μm
<i>C. siamense</i> (RUFC2457), This study	On healthy leaf of <i>Eichhornia crassipes</i> , Mihintale, Sri Lanka	9.89–15.95 × 3.73–5.67 μm

inhabiting aquatic plant species, we isolated a novel taxon of *Neottiosporina*.

Neottiosporina mihintaleensis Wimalasena, Wijayaw. & Bamunuarachchige sp. nov.

Index Fungorum Registration Identifier: IF902502 (Figure 13).

Etymology: The name is derived from the locality from where the fungus was collected.

Holotype: RUSLH/241

Description: Endophytic of healthy leaf of *Salvinia molesta*. Sexual and asexual morphs undetermined. Despite efforts to induce the asexual morph using sporulation techniques described in the materials and methods section (2.1), the fungal cultures did not sporulate. Thus, we conclude it is sterile mycelia.

Culture characteristics: Colonies grown on PDA at 28–30°C in a 2 cm Petri dish over a two-week period show that the superficial mycelium is abundant and dark grey at the centre, with white patches towards the periphery. The immersed mycelium appears pale to medium brown with an irregular margin. On the reverse side, the colony displays a centre ranging from yellowish-brown to dark brown, transitioning to a yellowish-white colour at the margin.

Material examined: SRI LANKA, North Central Province, Mihintale, Mahakanadara tank (8.38683° N, 80.38683° E, 117 m), on healthy leaf of *Salvinia molesta* (Giant *Salvinia*), 10 December 2023, Madhara K. Wimalasena, RUSLH/241 (holotype as the dry culture), RUFC2454 (ex-type); *ibid* RUFC2461 (living culture).

Notes: *Neottiosporina mihintaleensis* sp. nov. is a newly identified species within the genus *Neottiosporina*, discovered in freshwater environments in Sri Lanka. Phylogenetic analysis indicates that it is closely related to *N. cylindrica* and *N. ashworthiae* (Figure 7). *Neottiosporina cylindrica* produces cylindrical to slightly clavate conidia (Sutton and Alcorn, 1985; Li et al., 2020) whereas *N. ashworthiae* has not reported its micromorphological characters. However, *Neottiosporina mihintaleensis* did not produce asexual morph in culture, despite the use of sporulation techniques (see sporulation techniques in materials and methods 2.1), making it impossible to compare its morphological features along with phylogenetically related species. Hence, we introduce *Neottiosporina mihintaleensis* as sterile mycelia.

3.3 Qualitative enzymatic assay for extracellular enzymes production by endophytic fungi

This study shows that fungal isolates can produce amylase, cellulase, and laccase enzymes. This enhances our understanding of their ecological roles and opens avenues for future biotechnological applications in diverse industries, thereby addressing the ongoing demand for enzymatic solutions in global markets.

3.3.1 Production of amylase enzyme by fungal isolates

The amylase activity shown by these endophytes can help break down starch when plants start to age (Mahfooz et al., 2017). As biotechnology advances, the significance of amylases in the production of various commodities, such as food and starch-based products, continues to grow. Given the widespread utilization of these enzymes across numerous industries, there exists a persistently high demand for amylases (Khokhar et al., 2011; Bilal and Iqbal, 2019; Patil et al., 2021). As a result, there is an ongoing search for new microbial strains that can produce these enzymes (Khokhar et al., 2011; Patil et al., 2021). The emergence of these newly identified fungal isolates suggests promising prospects for large-scale amylase production.

To assess amylase production, the positive control contained fungal endophytes cultured on PDA media supplemented with 1% soluble starch. Following a seven-day incubation period at 28–30°C (range of the room temperature), 1–2 mL of iodine solution was applied to flood the culture plates, resulting in a blue-black coloration. Observations were recorded at 15 minute and 30 minute intervals. A change from blue-black to a colorless medium indicated the presence of amylase activity, as the enzyme catalyzed the hydrolysis of starch. Among the isolates, *Ectophoma salviniae* sp. nov. exhibited the highest amylase production, forming a clear zone with a diameter of 2.5 cm around the fungal colony and decolorizing the medium completely within 10 minutes, while *Phyllosticta capitalensis* produced a clear zone of 1.5 cm in diameter around its colony after 15 minutes. Other fungal isolates

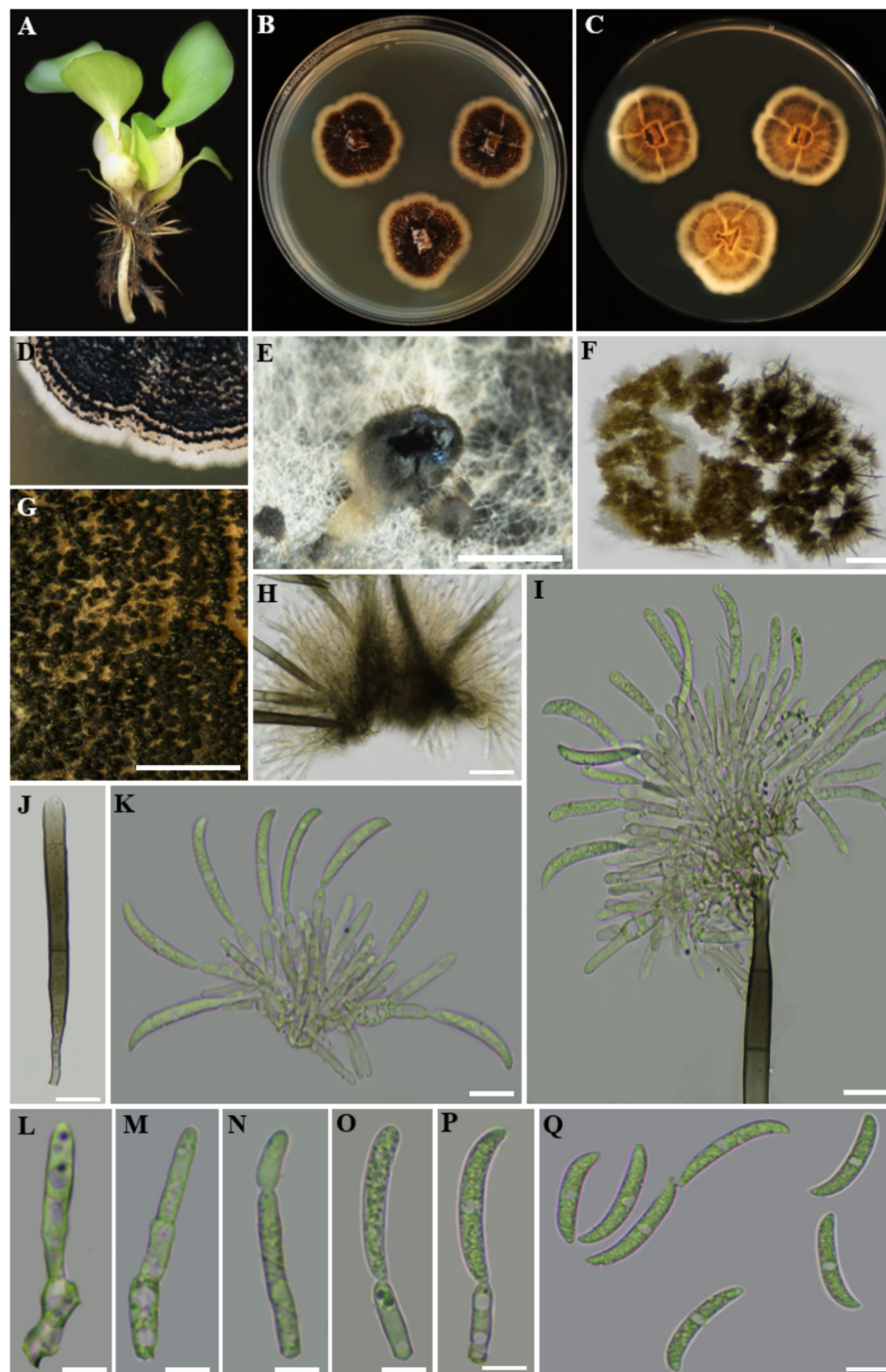


FIGURE 12

Colletotrichum truncatum (A) Host freshwater plant leaf of *Eichhornia crassipes* (Water hyacinth). (B) Upside of the PDA culture plate (diameter; 9.5 cm). (C) Downside of the PDA culture plate (diameter; 9.5 cm) after five days. (D) Sporulated culture after ten days. (E) Stereo microscopic view of sporulation in culture. (F) Stereo microscopic observation of acervuli. (G, H) Acervuli. (J) Aseta. (I, K) Conidiogenesis. (L–P) Maturity levels of conidiogenous cells. (Q) Conidia. Scale bars: (E, F) = 1000 μ m, (G–Q) = 100 μ m.

also displayed amylase production, with discernible effects after 15 minutes. All endophyte isolates, except *Neottiosporina mihintaleensis* sp. nov. and *C. truncatum*, have exhibited the ability to produce extracellular amylase, underscoring their notable enzymatic capabilities (Table 6; Figure 14). Prior research has documented amylase activity in species like *Phyllosticta* spp.

(Wikee et al., 2017; Reyes et al., 2021). and *Colletotrichum* spp. (Prajapati et al., 2013; Armesto et al., 2020; da Silva et al., 2021). The comparison with the negative control, which consisted of PDA media supplemented with 1% soluble starch and without the inoculation of endophytic fungi, involved a seven-day incubation period at 28–30°C. Following this incubation, 1–2 mL of iodine

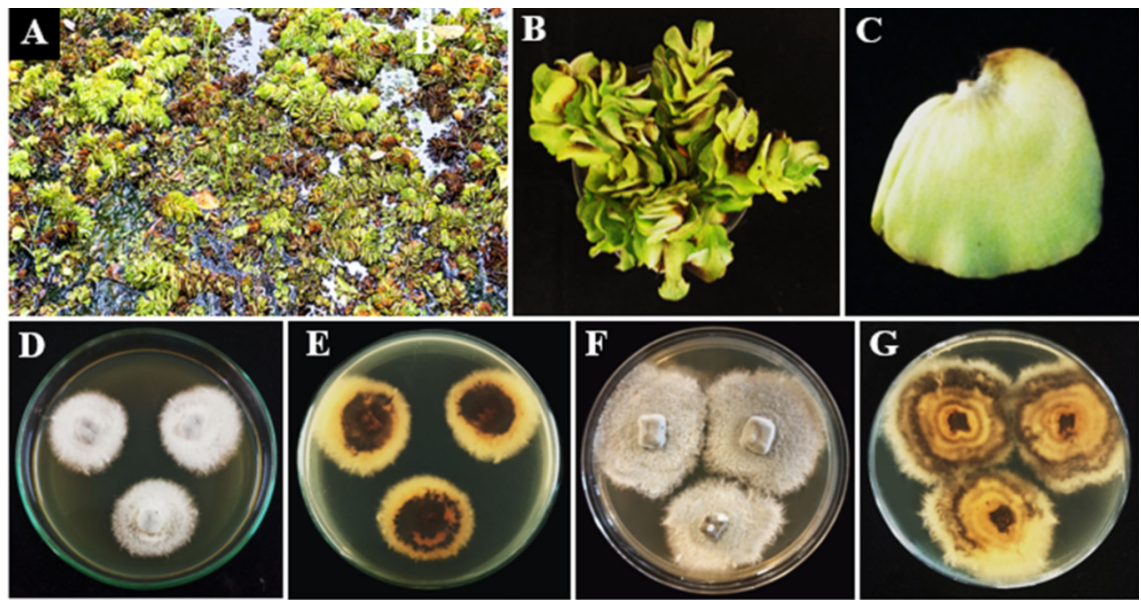


FIGURE 13
Neottiosporina mihintaleensis sp. nov. (A–C) Host freshwater plant leaf of *Salvinia molesta* (Giant Salvinia). (D) Upside of the PDA culture plate (diameter; 9.5 cm). (E) Downside of the PDA culture plate (diameter; 9.5 cm) after five days. (F) Upside of the PDA culture plate (diameter; 9.5 cm). (G) Downside of the PDA culture plate (diameter; 9.5 cm) after ten days.

solution was applied to flood the culture plates, resulting in a blue-black coloration. Observations were recorded at 15 minute and 30minute intervals; however, no color change occurred, and the blue-black coloration remained (Figure 14).

3.3.2 Production of cellulase enzyme by fungal isolates

Cellulase has significant applications across various industries, making it a highly researched enzyme in academic and industrial settings. It is particularly valuable in the pulp and paper, textile industry, bio-ethanol production, wine and brewery sectors, food industry, extraction of pigments and bioactive compounds, pharmaceutical industries, and waste management (Srivastava

et al., 2018; Dhevagi et al., 2021; Ejaz et al., 2021; Maravi and Kumar, 2021; Singh et al., 2021; Lùbek-Nguyen et al., 2022). Due to its broad utility, cellulase is in high demand, accounting for approximately 20% of the global enzyme market (Srivastava et al., 2015; Singh et al., 2021). Fungal cellulase enzymes are particularly effective in breaking down the cellulose component of lignocellulosic materials into hexose sugars, making fungi good producers of cellulase enzymes among microorganisms (Singh et al., 2021).

In this study, the positive control consisted of endophytic fungi inoculated into PDA media supplemented with 0.5% (w/v) sodium carboxymethyl cellulose and incubated for five days at 28–30°C. Following incubation, 0.1% (w/v) Congo red was applied, followed by 1M NaCl for 5 minutes to visualize the enzymatic activity (clear halo around the colonies). *Chaetomella raphigera* exhibited the highest cellulase enzyme production, as evidenced by the red media turning colorless with a clear halo. *Phyllosticta capitalensis* formed clear halos around each colony, indicating significant cellulase enzyme production, second only to *Chaetomella raphigera*. The other isolates (*Colletotrichum truncatum*, *C. siamense*, and *Ectophoma salviniae* sp. nov) exhibited only minimal cellulase enzyme production on solid media, whereas *Neottiosporina mihintaleensis* sp. nov. displayed no cellulase enzyme activity (Table 6; Figure 15). In the comparison between the positive and negative controls, the negative control consisted of PDA media supplemented with 0.5% (w/v) sodium carboxymethyl cellulose, without the inoculation of endophytic fungi, and incubated for five days at 28–30°C. After incubation, 0.1% (w/v) Congo red was applied, followed by a 5-minute treatment with 1M NaCl. No enzymatic activity (clear halo) was observed, leaving only the Congo red stain visible on the plates (Figure 15).

TABLE 6 The ability for extracellular enzymes production by endophytic fungi isolated from freshwater plants.

Endophytic fungal strains	Extracellular enzymes production		
	Amylase	Cellulase	Laccase
<i>Chaetomella raphigera</i>	+	+	–
<i>Colletotrichum siamense</i>	+	+	–
<i>C. truncatum</i>	–	+	+
<i>Ectophoma salviniae</i> sp. nov.	+	+	+
<i>Phyllosticta capitalensis</i>	+	+	+
<i>Neottiosporina mihintaleensis</i> sp. nov.	–	–	+

“+” denotes the ability of fungi to produce extracellular enzymes, while “–” represents the inability of fungi to produce extracellular enzymes.

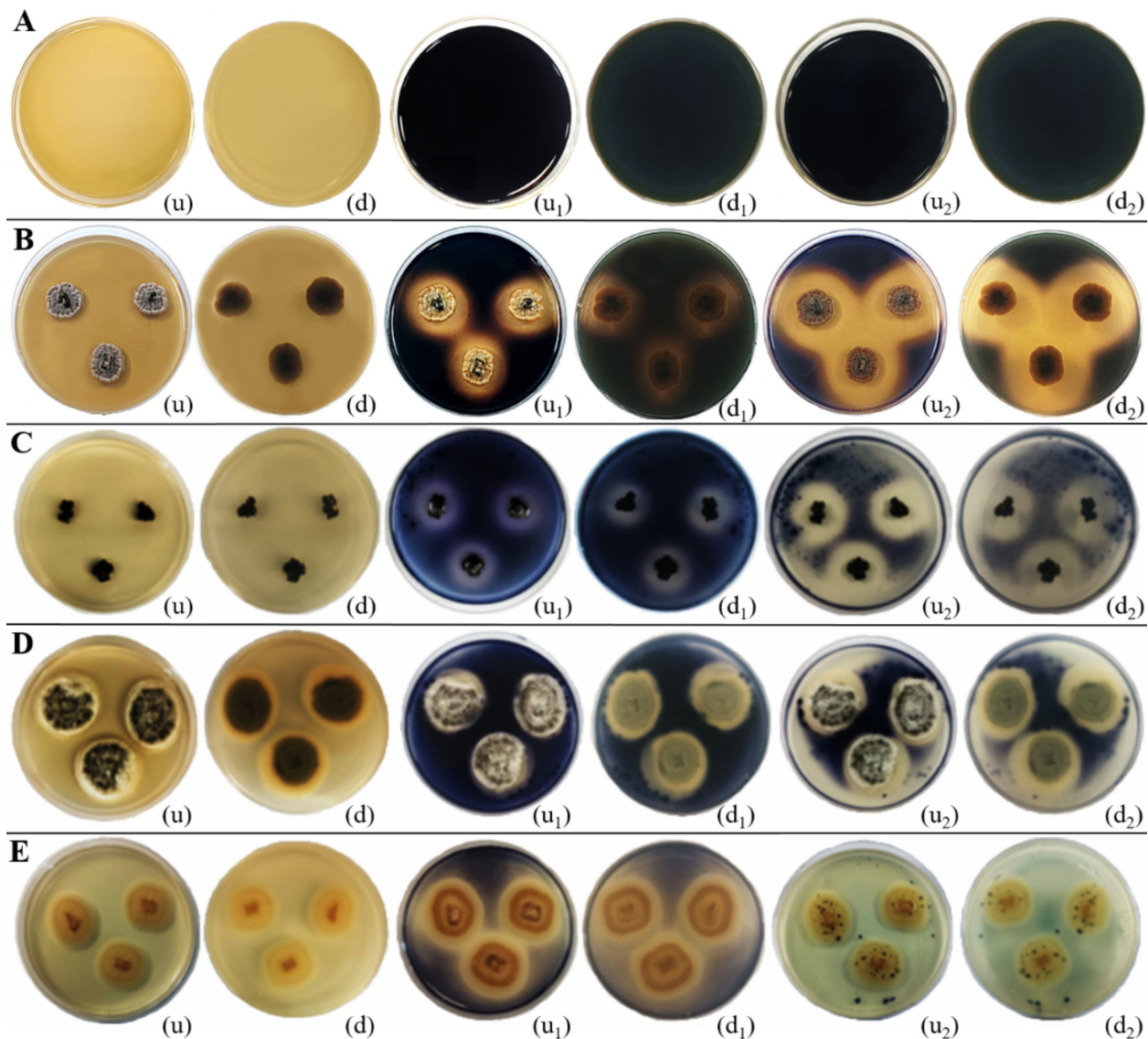


FIGURE 14

Amylase enzymatic activity of endophytic fungi isolated from freshwater plants. (A) Negative control. (B) *Ectophoma salviniae* sp. nov. (C) *Phyllosticta capitalensis*. (D) *Colletotrichum siamense*. (E) *Chaetomella raphigera*. (u, d) Upside and downside of the plate before adding 1–2 mL of iodine solution. (u₁, d₁) Upside and downside of the plate after adding 1–2 mL of iodine solution for 15 minutes (u₂, d₂) Upside and downside of the plate after adding 1–2 mL of iodine solution for 30 minutes, respectively. Amylase enzymatic activity was indicated by the clear zone appearance of the fungal colony on PDA media supplemented with 1% starch. (B–E) Positive control.

Previously, Kao et al. (2019) and Singh et al. (2023) have found that *Chaetomella* sp. exhibits a high capacity for producing glucose-tolerant cellulase enzymes. Similarly, Amirita et al. (2012); Yopi et al. (2017), and Wikee et al. (2017) have highlighted the significant potential of *Phyllosticta* sp. for cellulase enzyme production.

3.3.3 Production of laccase enzyme by fungal isolates

Laccases, classified as blue multicopper oxidases, catalyze the one-electron oxidation of a wide range of substrates and play a crucial role in lignin degradation (Abdel-Hamid et al., 2013; Viswanath et al., 2014; Singh and Gupta, 2020; Kyomuhimbo and Brink, 2023; Sharma et al., 2024). These enzymes are extensively

used in various industries, including industrial effluent decolorization and detoxification, wastewater treatment, paper and pulp production, textiles, xenobiotic degradation, bioremediation, and as biosensors, owing to their key role in the breakdown of lignin and phenolic compounds (Shraddha et al., 2011; Viswanath et al., 2014; Singh and Gupta, 2020; Khatami et al., 2022). Laccase have been identified in approximately 60 fungal strains from the genera *Ascomycetes*, *Deuteromycetes*, and *Basidiomycetes* (Leonowicz et al., 2001; Albu et al., 2019; Abo Nahas et al., 2021; Mahuri et al., 2023). Fungal laccases are categorized into two types: true laccase and false laccase (De Jesus et al., 2009; Mahuri et al., 2023). True laccases can oxidize phenols and aminophenols but cannot oxidize the amino acid residue tyrosine. On the other hand, false laccases can oxidize tyrosine

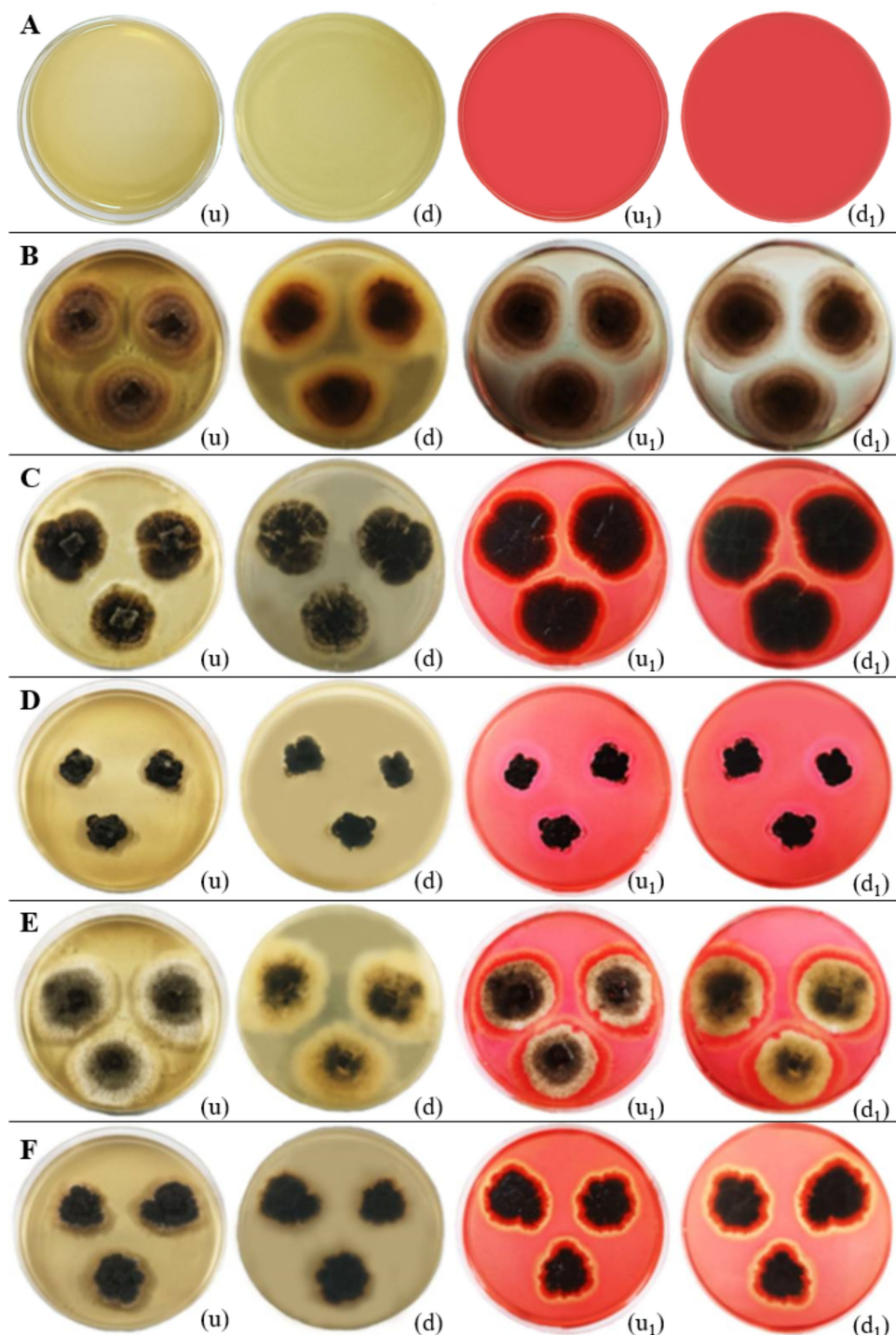


FIGURE 15

Cellulase enzymatic activity of endophytic fungi isolated from freshwater plants. (A) Negative control. (B) *Chaetomella raphigera*. (C) *Colletotrichum truncatum*. (D) *Phyllosticta capitalensis*. (E) *Colletotrichum siamense*. (F) *Ectophoma salviniae* sp. nov. (u, d) Upside and downside of the plate before adding 0.1% (w/v) Congo red solution respectively. (u₁, d₁) Upside and downside of the plate after adding 0.1% (w/v) Congo red solution respectively. Cellulase enzymatic activity was indicated by the clear zone appearance of the fungal colony on PDA media. (B–F) Positive control.

(De Jesus et al., 2009; Chauhan et al., 2017; Jayaram et al., 2023; Mahuri et al., 2023). Jayaram et al. (2023) highlighted that laccase production by fungal endophytes is a promising area of research due to its potential industrial applications, such as bioremediation and detoxification of pollutants.

As per the findings of this study, upon comparing the negative and positive controls in the laccase assay, no color change (blue-purple coloration) was observed in the negative control after the addition of 1-Naphthol solution droplets and 24 hours of incubation at 28–30°C (Figure 16). In the positive control, the

addition of 1-Naphthol solution droplets to each colony of endophyte isolates, followed by subsequent incubation for 24 hours at 28–30°C, resulted in a blue-purple coloration at the edges of the colonies, indicating the presence of laccase enzymes. This reaction was observed in *Colletotrichum truncatum*, *Ectophoma salviniae*, *Neottiosporina mihintaleensis*, and *Phyllosticta capitalensis*. Among these, *Neottiosporina mihintaleensis* exhibited the highest laccase production, with a 3.5 cm diameter blue-purple circle around the colony. *Phyllosticta capitalensis* showed the second-highest laccase production, with a 1 cm diameter blue-purple circle around the colony, compared to its appearance before applying 1-Naphthol droplets. In contrast, *Chaetomella raphigera*, and *C. siamense* did not exhibit laccase enzyme production in this qualitative assay (Table 6; Figure 16).

This research has shown the novel discovery of laccase production in *N. mihintaleensis* and *E. salviniae*. These findings contribute to an expanding understanding of fungal laccases. Notably, prior studies have extensively documented laccase production capabilities in *Phyllosticta* spp. (Wikee et al., 2017; Shankar Naik et al., 2019), *C. truncatum* (Levin et al., 2007; Núñez et al., 2023).

4 Discussion

4.1 Exploration of endophytic fungi in Sri Lanka's freshwater environments

4.1.1 Current status and research gaps on endophytic fungal study in Sri Lanka

Currently, only around 3,000 fungal species are known in Sri Lanka, with an estimated 31,000 plant-associated species still to be described (Adikaram and Yakandawala, 2020; Wijayawardene et al., 2022b). Several of the known fungi in the island nation remain unpublished and have not been sufficiently studied or documented (Adikaram and Yakandawala, 2020; Karunaratna et al., 2022; Wijayawardene et al., 2022b, 2023; Wimalasena et al., 2024). In the Sri Lankan context, particularly regarding endophytic fungi, many studies have focused on terrestrial plants (e.g. Alwis et al., 2021; Pathmanathan et al., 2022; Koshila et al., 2023; Undugoda et al., 2023). Studies on endophytic fungi associated with aquatic plants in Sri Lanka are relatively scarce (Rajagopal et al., 2018; Ravimannan and Sepali, 2020; Ekanayake et al., 2021). Hitherto, the identification of endophytic fungi in freshwater plants was based largely on morphological characters. For instance, Hettiarachchi et al. (1983) reported 15 fungi (*Alternaria* sp., *Cephalosporium* sp., *Cercospora piaropi*, *Curvularia tuberculata*, *Fusarium* sp., *Idriella lunata*, *Mucor* sp., *Myrothecium roridum*, *Neurospora* sp., *Penicillium oxalicum*, *Phaeotrichoconis crotalariae*, and *Septofusidium elegantulum*) isolated from *Eichhornia crassipes*, with identification based only on morphological characteristics. In some of the Sri Lankan studies, endophytic fungal identification was based on a single gene locus, which is inadequate for accurate identification. For example, Dissanayake et al. (2014, 2016) identified *Chaetomium globosum* from healthy *Nymphaea nouchali* using only the ITS locus.

4.1.2 Identification of endophytic fungi in Sri Lankan freshwater habitats: potential for novel species discovery

Sri Lanka harbors over 370 aquatic and wetland plant species, with 12% being unique to the country (Yakandawala, 2012; Bambaranda et al., 2024). These endemic plants serve as essential habitats for fungi, including freshwater fungi and endophytic species that have adapted to unique environmental conditions (Ratnaweera, 2019; Wimalasena et al., 2024). Wimalasena et al. (2024), highlighted that these habitats offer substantial potential for the collection, identification, and utilization of endophytic fungi found in freshwater plants.

This study focused on isolation of freshwater endophytic fungi in three lentic freshwater habitats (Iluppukanniya tank, Mahakanadara tank, and Mihintale tank) located in Mihintale area within the Anuradhapura district. Using polyphasic approaches, six endophytic fungi were isolated including two novel taxa, *Ectophoma salviniae* sp. nov. and *Neottiosporina mihintaleensis* sp. nov. These fungal species were identified in their endophytic life modes, occurring within healthy freshwater plant tissues, particularly in healthy leaves, isolated by a culture-dependent method. In this study, the invasive plant species *Eichhornia crassipes* (Ayanda et al., 2020; Maulidyna et al., 2021; Bayu et al., 2024) provided a wider range of host substrates for fungi compared to other freshwater plants such as *Salvinia* and *Nymphaea*, highlighting its value for biodiversity. Hence, expanding such studies to cover more freshwater habitats could lead to the identification of additional novel species, contributing significantly to the field of mycology and biotechnology worldwide.

4.2 Reference cultures of pathologically important taxa, *Colletotrichum siamense*, *C. truncatum* and *Ectophoma* sp. in Sri Lanka

Precise identification of fungi is an important step in taxonomy. DNA sequence analyses and morphological characters play an important role in modern taxonomy which aids in identifying species and providing their classification (Wijayawardene et al., 2023). A large number of species originally described from Sri Lanka lack sequence data and were identified based on only morphological characteristics (Wijayawardene et al., 2022b). Nevertheless, delineating species boundaries of species complexes of specious genera would depend only on DNA sequence data analyses.

Adikaram and Yakandawala (2020) listed pathologically important *Colletotrichum* species in Sri Lanka, including *C. siamense* and *C. truncatum*. However, either *C. siamense* or *C. truncatum* have not been reported as a pathogenic species from aquatic plants. Table 7 lists the studies that provided phylogenetic identifications of *C. siamense* and *C. truncatum*.

In a previous study, Dissanayake et al. (2016) reported *C. siamense* from *Nymphaea nouchali* but they used only the ITS region to identify the taxon. Use of one locus is not recommended

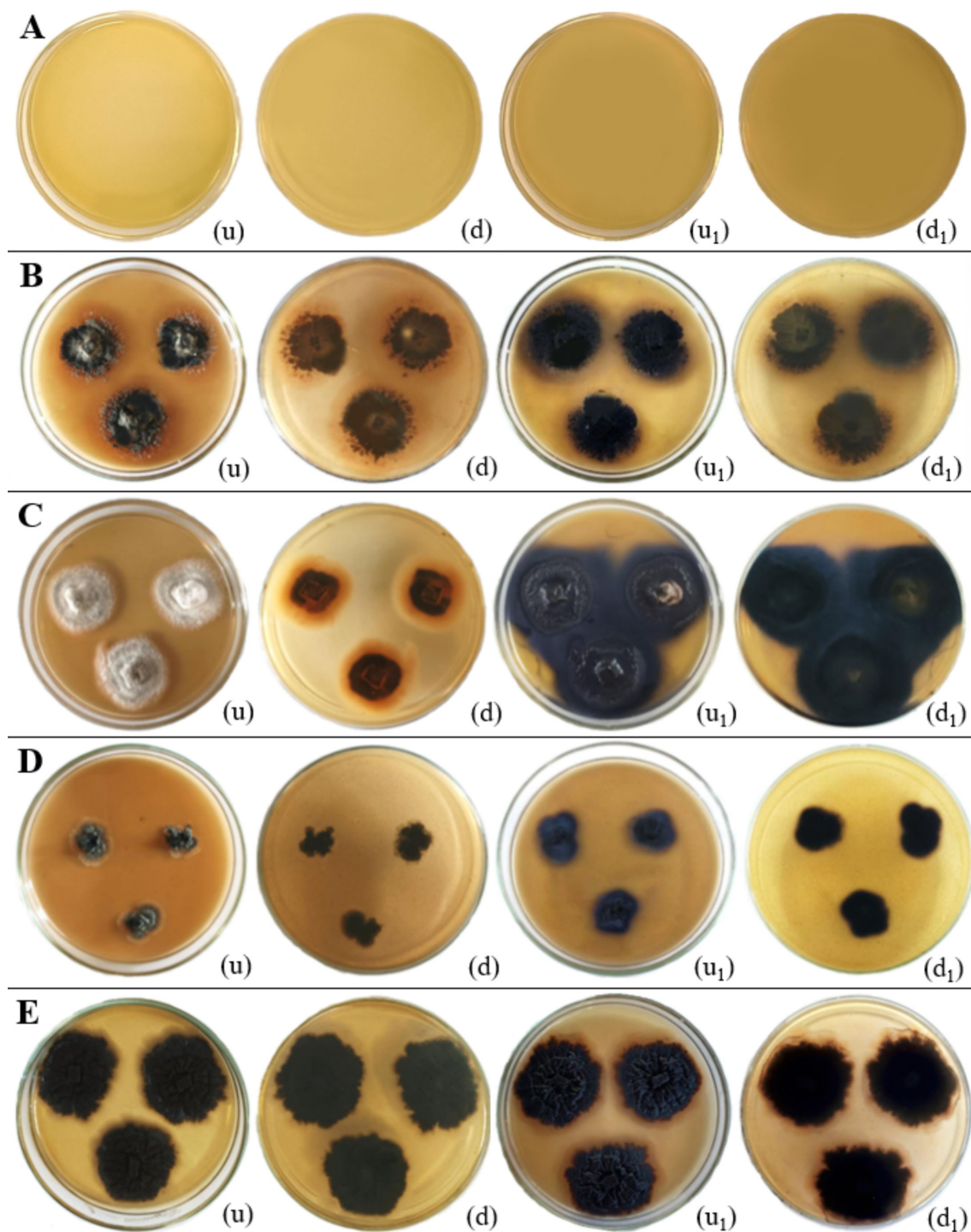


FIGURE 16

Laccase enzymatic activity of endophytic fungi isolated from freshwater plants. (A) Negative control. (B) *Colletotrichum truncatum*.

(C) *Neottiosporina mihintaleensis* sp. nov. (D) *Phyllosticta capitalensis*. (E) *Ectophoma salviniae* sp. nov. (u, d) Upside and downside of the plate before adding 0.1M 1-Naphthol respectively. (u₁, d₁) Upside and downside of the plate after adding after adding 0.1M 1-Naphthol respectively. Laccase enzymatic activity was indicated by the blue purple colour appearance of the fungal colony on PDA media. (B–E) Positive control.

for *Colletotrichum* thus, we used ITS, *tub2*, *ACT*, *CHS-1* and *GADPH* regions in our phylogenetic analyses following Armand et al. (2023) and Armand and Jayawardena (2024). We have not observed any disease symptoms in the leaves of *Eichhornia crassipes*. Hence, it is concluded that both *C. siamense* and *C.*

truncatum are endophytic species of *Eichhornia crassipes*. This is the first study that provided multi-locus phylogenetic evidence to identify two pathologically important (but endophytic in this study) *Colletotrichum* species (e.g., *C. siamense* and *C. truncatum*) in aquatic habitats. It is important to maintain the reference living

cultures of both species; thus, it has been deposited at the Rajarata University Culture Collection.

Ectophoma species have been reported as important plant pathogens. *Ectophoma multirostrata*, the type species of *Ectophoma* (Valenzuela-Lopez et al., 2018), has been originally reported as a soil-inhabiting fungi in India (as *Sphaeronaema multirostratum fide* (Mathur and Thirumalachar, 1959). Later, this species was reported as a pathogen of different plants worldwide (e.g. (Aveskamp et al., 2010; Valenzuela-Lopez et al., 2018; Chobe et al., 2020; Ahmadpour et al., 2021; Kularathnage et al., 2023). Lee et al. (2022) reported *Ectophoma multirostrata* as a pathogenic agent infecting the aquatic plant water spinach (*Ipomoea aquatica*) in Korea. *Ectophoma myriophyllana* Huang Y. and Yu Z. F. was recently introduced as an epiphyte of leaves of *Myriophyllum spicatum* (Chen et al., 2023). Our novel species, *Ectophoma salviniae* did not cause any diseased symptoms on the leaves of *Salvinia minima* and thus, we conclude it is an endophytic taxon inhabiting the host. As far as we know, this is the first report of *Ectophoma* species from *Salvinia* species in Sri Lanka (Farr and Rossman, 2024). We have not observed *Ipomoea aquatica* (which was affected by *Ectophoma multirostrata*) in the same aquatic environment, and the distribution of *Ectophoma salviniae* sp. nov. is unknown. Future studies would be essential to recognise the potential host jumping and life mode switching of *Ectophoma salviniae* sp. nov. and its impact on *Ipomoea aquatica* since it is a widely-used leafy vegetable in Sri Lanka.

4.3 The possibility of endophytic fungi being used as mycoherbicides against invasive weed management in wetland environments

4.3.1 Threat of invasive aquatic plants

Invasive plant species pose a major threat to natural ecosystems by reducing biological diversity (Rodríguez-Merino, 2023; Xiong et al., 2023). Over the past few decades, the spread of aquatic alien

TABLE 8 The potential application of *Colletotrichum* species in weed management.

<i>Colletotrichum</i> spp.	Target weeds	References
<i>C. gloeosporioides</i> BWH-1	<i>Alopecurus aequalis</i> , <i>Amaranthus retroflexus</i> , <i>Ageratum conyzoides</i> , <i>Bidens pilosa</i> , <i>Capsella bursa-pastoris</i> , <i>Celosia argentea</i> , <i>Echinochloa crusgalli</i> , and <i>Mikania micrantha</i>	(Xu et al., 2019)
<i>C. dematium</i>	<i>Parthenium hysterophorus</i>	(Singh et al., 2010)
<i>C. gloeosporioides</i>	Russian thistle, Tumbleweed	(Berner et al., 2009)
<i>C. graminicola</i>	<i>Echinochloa</i> sp.	(Yang et al., 2007)
<i>C. lini</i>	<i>Convolvulus arvensis</i>	(Damm et al., 2014; Tunali et al., 2008, 2009)

plant species in the lentic water bodies of Sri Lanka has created significant ecological, environmental, and economic problems (Dissanayake, 2020; Kariyawasam et al., 2021). Thus, fungi can be used as mycoherbicides for a more effective solution to eradicate invasive aquatic plant species due to their pathogenic activity and host-specific targeting.

4.3.2 Application of fungi as mycoherbicides

Bioherbicides are biological products used to control weed species and are typically formulated using microbiological agents, especially fungi, and are often referred to as mycoherbicides (Golijan et al., 2023; Ravlić and Balićević, 2014). The concept of mycoherbicides emerged during the 1980s and 1990s, as documented by TeBeest and Templeton (1985); Templeton (1987); Templeton (1992) and Wall et al. (1992). Mycoherbicides are considered environmentally friendly alternatives to chemical herbicides because they are harmless to the environment, eco-

TABLE 7 Studies provided phylogenetic analyses for *Colletotrichum siamense* and *C. truncatum* species in Sri Lanka (RUFCC-Rajarata University Fungal Culture Collection, UOM-University of Melbourne culture collection, Victoria, Australia, UPBT-University of Peradeniya, Department of Biotechnology, USJCC-University of Sri Jayewardenepura Culture Collection, Department of Botany, University of Sri Jayewardenepura, Nugegoda, Sri Lanka).

Species name	Host	Life mode	Gene regions	Culture collections	References
<i>C. truncatum</i>	<i>Capsicum annuum</i>	Pathogen	ITS, <i>tub2</i> , <i>GADPH</i> , <i>CHS-1</i> , <i>HIS3</i> and <i>ACT</i>	UOM	(De Silva et al., 2019)
	<i>Eichhornia crassipes</i>	Endophyte	ITS, <i>tub2</i> , <i>ACT</i> , <i>CHS-1</i> and <i>GADPH</i>	RUFCC	This study
<i>C. siamense</i>	<i>Capsicum annuum</i>	Pathogen	ITS, <i>tub2</i> , <i>GADPH</i> , <i>CHS-1</i> , <i>HIS3</i> and <i>ACT</i>	UOM	(De Silva et al., 2019)
	<i>Persea Americana</i>	Pathogen	ITS, <i>tub2</i> , and <i>GADPH</i>	UPBT	(Dissanayake et al., 2021)
	<i>Allium cepa</i>	Pathogen	ITS, <i>GADPH</i> and <i>tub2</i>	USJCC	(Herath et al., 2021)
	<i>Eichhornia crassipes</i>	Endophyte	ITS, <i>tub2</i> , <i>ACT</i> , <i>CHS-1</i> and <i>GADPH</i>	RUCC	This study

friendly, and specifically target certain types of plants (Chakraborty and Ray, 2021; Hasan et al., 2021; Keshamma, 2022). The efficiency of fungi in weed management is exemplified by the reported potential of *Colletotrichum* species (Table 8). *Colletotrichum siamense* and *C. truncatum* have previously been identified as pathogenic fungi across various plant species. According to this study, these *Colletotrichum* spp. may demonstrate potential for managing the invasive weed *Eichhornia crassipes* in Sri Lanka.

5 Conclusion

This study identified the culturable mycobiota in three lentic freshwater habitats located in Mihintale, within the Anuradhapura District of Sri Lanka, revealing a rich fungal diversity. Through identification, six endophytic fungal species were found, including two novel endophytic fungal species: *Ectophoma salviniae* sp. nov. and *Neottiosporina mihintaleensis* sp. nov., recorded on the freshwater plant *Salvinia*. The identification was confirmed using a polyphasic approach. The next step involved qualitatively assessing the extracellular enzymatic potentials of these endophytic isolates. *Ectophoma salviniae* sp. nov. exhibited the highest amylase production, *Chaetomella raphigera* showed the highest cellulase enzyme production, and *Neottiosporina mihintaleensis* sp. nov. demonstrated the highest laccase production, offering novel insights for future biotechnological applications. Besides, this study discussed the potential of fungi as mycoherbicides for managing invasive freshwater weeds.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/nuccore/genbank/>, ITS: PP989214, PP989215, PP989216, PP989217, PP989218, PP989219, PP989220, PP989221, PP989222; LSU: PP989223, PP989224, PP989225, PP989226, PP989227; SSU: PP989228, PP989229, PP989230; *GAPDH*: PQ014240, PQ014241, PQ014242, PQ014243; *CHS-1*: PQ014237, PQ014238, PQ014239; *ACT*: PQ014233, PQ014234, PQ014235, PQ014236; *tub2*: PQ014246, PQ014247, PQ014248; *rpb2*: PQ014244, PQ014245; *tefl-α*: PQ014249.

Author contributions

MW: Writing – original draft, Writing – review & editing. NW: Writing – original draft, Writing – review & editing. TB: Writing – original draft, Writing – review & editing. G-QZ: Writing – original draft, Writing – review & editing. RJ: Writing – original draft, Writing – review & editing. DB: Writing – original draft, Writing – review & editing. TD: Writing – original draft, Writing – review & editing. HZ: Writing – original draft, Writing – review & editing. D-QD: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2024.1475114/full#supplementary-material>

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EDITED BY

Sinang Hongsanan,
Shenzhen University, China

REVIEWED BY

Huang Zhang,
Kunming University of Science and
Technology, China
Nattawut Boonyuen,
National Center for Genetic Engineering and
Biotechnology (BIOTEC), Thailand
Dian-Ming Hu,
Jiangxi Agricultural University, China

*CORRESPONDENCE

Ratchadawan Cheewangkoon

✉ ratchadawan.c@cmu.ac.th

Rungtiwa Phookamsak

✉ phookamsak@mail.kib.ac.cn

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New insights into freshwater ascomycetes: discovery of novel species in diverse aquatic habitats

Lu Li^{1,2,3}, Darbhe Jayarama Bhat^{4,5}, Hong-Bo Jiang^{6,7,8,9},
Jun-Fu Li^{6,7,8,9}, Turki M. Dawoud⁴, Fangqi Sun^{6,7,8,9},
Sukanya Haituk^{1,10}, Ratchadawan Cheewangkoon^{1,2*}
and Rungtiwa Phookamsak^{6,7,8,9*}

¹Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, ²Agrobiodiversity in Highland Agriculture and Sustainable Utilization Research Group, Chiang Mai University, Chiang Mai, Thailand, ³Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China, ⁴Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, ⁵Vishnugupta Vishwavidyapeetam, Gokarna, India, ⁶Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China, ⁷Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China, ⁸Centre for Mountain Futures (CMF), Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China, ⁹CIFOR-ICRAF China Program, World Agroforestry (ICRAF), Kunming, China, ¹⁰Office of the Research Administration, Chiang Mai University, Chiang Mai, Thailand

During investigations of freshwater fungi in Hunan and Yunnan provinces, China, *Chaetopsina yunnanensis* sp. nov. (Nectriaceae), *Parafuscosporella hunanensis* sp. nov. (Fuscosporellaceae), and *Pleurotheciella yunnanensis* sp. nov. (Pleurotheciaceae) were discovered on submerged decaying wood and branches. Based on phylogenetic analyses, *C. yunnanensis* formed a separate branch with *Chaetopsina pinicola* and nested among other *Chaetopsina* species in Nectriaceae (Hypocreales). Furthermore, hitherto known *Chaetopsina beijingensis* shared the same branch with *Chaetopsina fulva*, a type species of the genus, demonstrating their conspecific status. Therefore, *C. beijingensis* is formally synonymized under *C. fulva*, with an amended species circumscription. *Pa. hunanensis* formed a well-separated subclade with the ex-type strain of *Parafuscosporella mucosa* and clustered with other *Parafuscosporella* within Fuscosporellaceae (Fuscosporellales). In addition, the genus *Parafuscosporella* is treated as distinct from *Vanakripa* due to a lack of phylogenetic evidence in clarifying their congeneric status with the latter. *Pl. yunnanensis* is found to be sister to *Pleurotheciella saprophytica*, forming a subclade with *Pleurotheciella dimorphospora* within the Pleurotheciaceae (Pleurotheciales). Morphologically, *C. yunnanensis* fits well with the generic concept of *Chaetopsina* in forming a holomorphic state with hyphomycetous asexual morph producing pigmented, setiform conidiophores, phialidic conidiogenous cells, hyaline conidia, and nectria-like sexual morph. *Pa. hunanensis* fits well with *Parafuscosporella* in having acrogenous, apiosporous, versicolored, obovoid to obpyriform conidia. In contrast, *Pl. yunnanensis* resembles *Pl. dimorphospora* in forming asexual dimorphism with two types of conidia (Type I, brown, muriform/

phragmosporous conidia; Type II, hyaline, amerosporous/didymosporous conidia). The novelty of taxa is explained with detailed descriptions, photo-micrographic illustrations, polymorphism, and multigene phylogenetic analyses of Bayesian inference and maximum likelihood criteria.

KEYWORDS

Fuscosporellaceae, hyphomycetes, morpho-molecular-based taxonomy, Nectriaceae, novel taxa, Pleurotheciaceae

1 Introduction

Freshwater fungi are a diverse and heterogeneous taxonomic group occurring saprobially on partially or fully submerged organic substrates in aquatic habitats. Their life cycle, in whole or part, relies on free freshwater and submerged substrates (Calabon et al., 2020, 2023a). Freshwater environments are categorized into three types: 1) lentic, any natural aquatic environment lacking continuous flow but exhibiting static, low, or slow movement of water as in lakes, ponds, swamps, and pools; 2) lotic, any natural aquatic environment with a continuous flow of water such as rivers, streams, creeks, and brooks; and 3) other habitats, which include artificial water bodies such as in cooling towers and tree holes (Luo et al., 2004). Fungi found in freshwater habitats were grouped into several morphological and ecological entities, viz., freshwater ascomycetes, freshwater hyphomycetes (i.e., Ingoldian fungi, aero-aquatic hyphomycetes or asexual ascomycetes, terrestrial-aquatic hyphomycetes, and submerged aquatic hyphomycetes), freshwater basidiomycetes, coelomycetes, microsporidia, zoosporic fungi, and zygomycetes (Tsui et al., 2016; Schuster et al., 2022). Furthermore, freshwater fungi subsist as saprobes, mutualists, or parasites and have also been isolated as endophytes (Tsui et al., 2016). However, freshwater fungi have an important ecological function in decomposing wood by breaking down complex organic compounds into simpler inorganic materials and facilitating the passage of energy and nutrients across all trophic levels in the food chain (Bärlocher et al., 2011; Sridhar and Sudheep, 2011; Tsui et al., 2016). Two crucial biological characteristics of these fungi include the ability to sporulate underwater and thrive on decaying deciduous leaves and twigs in streams and rivers (Krauss et al., 2011; Wurzbacher et al., 2014; Tsui et al., 2016). In addition, the biotechnological potential of freshwater fungi as producers of bioactive metabolites, with promising values in drug discovery, is becoming evident (Duarte et al., 2013; Shen et al., 2022; El-Elimat et al., 2021; Calabon et al., 2023a). Lignicolous freshwater fungi can degrade indigestible lignocellulose in submerged wood, releasing nutrients into the water. However, their precise ecological role and economic value remain less understood (Bucher et al., 2004; Shen et al., 2022).

Freshwater fungi have been documented since the mid-19th century, and a wealth of information is now available

(de Wildeman, 1895; Ingold, 1942, 1955; Calabon et al., 2021). Over the recent past decades, these fungi have been relatively well-studied in Asia, particularly in China, India, and Thailand (Sridhar and Sudheep, 2011; Mehboob et al., 2021; Luo et al., 2019; Dong et al., 2020; Shen et al., 2022; Calabon et al., 2023a, b). In the early stages of studies on freshwater fungi, identification has primarily relied on morphology (Ingold, 1975; Krauss et al., 2011; Calabon et al., 2023a). In the case of yeasts, additional methods such as biochemical, fermentation, and assimilation tests were included. Presently, in addition to morphology, molecular data analyses were applied to taxonomic studies of freshwater fungi (Ranghoo et al., 1999; Nikolcheva and Bärlocher, 2002; Luo et al., 2019; Dong et al., 2020; Calabon et al., 2023a, b). Recent studies revealed that lignicolous freshwater fungi constitute a highly diverse taxonomic group with a substantial population. To date, more than 3,870 species of freshwater fungi have been documented from various substrates and geographical locations (Luo et al., 2019; Dong et al., 2020; Shen et al., 2022; Calabon et al., 2023a, b). Lignicolous freshwater fungi are complex assemblages of mostly filamentous fungi, single-celled chytrids, and yeasts (Tsui et al., 2016). Freshwater fungi belong to Ascomycota, Basidiomycota, Chytridiomycota, and Rozellomycota; in class levels within the Ascomycota, most species are accommodated in Dothideomycetes and Sordariomycetes (Calabon et al., 2022).

Chaetopsina (family Nectriaceae) was established by Rambelli (1956), with the type species *Chaetopsina fulva*, which was isolated from decaying leaves in northern Italy. *Chaetopsina* is a dematiaceous hyphomycete genus, the members of which occur on decaying wood, leaves, pine needle litter, and bark, as well as on ascomycetous stromata and soil (Seifert et al., 2011). Most *Chaetopsina* species are found in tropical and subtropical areas (Lechat and Fournier, 2019). *Chaetopsina* is initially characterized by reddish-brown setose conidiophores turning yellow in lactic acid, and hyaline, smooth, fusiform ameroconidia, and fertile regions situated terminally or along the axes of the setiform conidiophores (Samuels, 1985; Kirk and Sutton, 1985). Later, some species with dark brown conidiophores or lateral branches on the conidiophores also have been accommodated (Bakhit and Abdel-Aziz, 2021). According to Luo and Zhuang (2010), *Chaetopsinectria chaetopsinae* was distinct as the sexual morph of *C. fulva*. Lombard et al. (2015) and Rossman et al. (2016)

subsequently recommended using *Chaetopsina* despite *Chaetopsinectria* in respect to the 1F = 1N policy, and hitherto, *Chaetopsinectria* was treated as a synonym of *Chaetopsina*. The sexual morph of *Chaetopsina* is seen featuring superficial, non-stromatic, reddish brown to bright red, oval ascomata with an acute ostiolar apex, with walls composed of cells of *textura epidermoidea*, with paler papilla; unitunicate, clavate, short-stipitate asci containing 8-spored, fusiform, 0–1-septate, hyaline ascospores, and growing in association with upright conidiophores (Lechat and Fournier, 2020). So far, a total of 33 species epithets are listed under *Chaetopsina*, and four epithets are listed under *Chaetopsinectria* in Index Fungorum (<https://indexfungorum.org/Names/Names.asp>; accessed on 21 June 2024). However, eight previously described *Chaetopsina* epithets have been synonymized under other genera in Nectriaceae or *incertae sedis* (Index Fungorum, 2024), and the genus now embodies only 25 accepted species wherein molecular data of only 13 species are available (Lechat and Fournier, 2019; Bakhit and Abdel-Aziz, 2021). There are four species in *Chaetopsina* that have so far been recorded from freshwater habitats, including *Chaetopsina aquatica*, *C. fulva*, *Chaetopsina hongkongensis*, and *Chaetopsina polyblastia* (Sivichai et al., 2000; Ho et al., 2002; Luo et al., 2019; Bakhit and Abdel-Aziz, 2021). *C. fulva* and *C. polyblastia* were found on woody test blocks of *Xylia dolabriformis* in a freshwater stream of Khao Yai National Park, Thailand (Sivichai et al., 2000). *C. hongkongensis* was found on decaying submerged wood in Tai Po Kau Forest Stream, Hong Kong, China (Ho et al., 2002). *Chaetopsina beijingensis* (treated herein as a synonym of *C. fulva*) was isolated from decaying wood submerged in a freshwater stream in Yunnan, China (Luo et al., 2019). *C. aquatica* was collected on decaying submerged stems of *Phragmites australis* (Poaceae) in the River Nile, Sohag, Egypt (Bakhit and Abdel-Aziz, 2021).

Parafuscosporella (family Fuscosporellaceae), typified by *Parafuscosporella moniliformis*, was introduced by Yang et al. (2016). Yang et al. (2016) also introduced the new order Fuscosporellales to accommodate a new single family Fuscosporellaceae and to which six genera, viz., *Bactrodesmiastrum*, *Fuscosporella*, *Mucispora*, *Parafuscosporella*, *Plagiascoma*, and *Pseudoascotaiwania* were initially accommodated. *Parafuscosporella* is one of four new genera established by Yang et al. (2016) when the new order Fuscosporellales and the new family Fuscosporellaceae were established. The genus is characterized by spherical to cushion-shaped, black, gelatinous sporodochia, with a jelly-like cover, semi-macronematous, mononematous, compact, flexuous, simple or branched, mostly moniliform, globose to subglobose, ellipsoidal or clavate celled conidiophores, monoblastic, integrated, sometimes discrete, terminal, globose or subglobose, ellipsoidal or clavate conidiogenous cells and acrogenous, ellipsoidal to broadly obpyriform, smooth, dark brown to black conidia with a septum near the base, sometimes with a small protuberance and a pale brown basal cell (Boonyuen et al., 2016; Yang et al., 2016). Ten species are listed in *Parafuscosporella*, comprising *Parafuscosporella aquatica*, *Parafuscosporella ellipsoconidiogena*, *Parafuscosporella garethii*, *Parafuscosporella lignicola*, *Pa. moniliformis*, *Parafuscosporella mucosa*, *Parafuscosporella nilotica*, *Parafuscosporella obovata*, *Parafuscosporella pyriformis*, and *Parafuscosporella xishuangbannaensis* (Yang et al., 2016, 2020;

Boonyuen et al., 2021; Boonmee et al., 2021; Wang et al., 2023; Li et al., 2023). Species of the genus have been reported from freshwater habitats in China and Thailand (Yang et al., 2016, 2020; Boonmee et al., 2021; Boonyuen et al., 2021). Goh et al. (2023) introduced two novel freshwater fungi from Taiwan, namely, *Vanakripa oblonga* and *Vanakripa taiwanensis*. Based on the morphological resemblance between *Parafuscosporella* and *Vanakripa*, Goh et al. (2023) treated *Parafuscosporella* as a synonym of *Vanakripa* and transferred all *Parafuscosporella* species to *Vanakripa*. In contrast, *Vanakripa chiangmaiensis* and *Vanakripa minutiellipsoidea* clustered with *Conioscypha* species were excluded from *Vanakripa* (Goh et al., 2023). Unfortunately, the type species of *Vanakripa*, *Vanakripa gigaspora*, lacks molecular data to clarify the phylogenetic placement. Hence, the congeneric status of *Parafuscosporella* and *Vanakripa* is questionable.

Pleurotheciella (family Pleurotheciaceae), typified by *Pleurotheciella rivularia*, was introduced by Réblová et al. (2012). To date, within the genus *Pleurotheciella*, only three species, viz., *Pleurotheciella erumpens*, *Pleurotheciella fusiformis*, and *Pl. rivularia*, are known for their sexual morphs, with non-stromatic perithecia, unitunicate asci, and hyaline to subhyaline, 1- or 3–5-septate ascospores, lacking a mucilaginous sheath. The asexual morph of the genus is a dactylaria-like hyphomycete, which is characterized by polyblastic, denticulate conidiogenesis, subhyaline conidiophores, and hyaline, ellipsoidal to ellipsoidal-fusiform, aseptate to multi-septate conidia (Luo et al., 2018). At present, 18 species are accommodated in *Pleurotheciella*, viz., *Pleurotheciella aquatica*, *Pleurotheciella centenaria*, *Pleurotheciella dimorphospora*, *Pl. erumpens*, *Pl. fusiformis*, *Pleurotheciella ganzhouensis*, *Pleurotheciella guttulata*, *Pleurotheciella irregularis*, *Pleurotheciella krabiensis*, *Pleurotheciella lunata*, *Pleurotheciella nilotica*, *Pl. rivularia*, *Pleurotheciella saprophytica*, *Pleurotheciella submersa*, *Pleurotheciella sympodia*, *Pleurotheciella tropica*, *Pleurotheciella uniseptata*, and *Pleurotheciella verrucosa*, and all accepted species of the genus have been reported for their asexual morphs (Hyde et al., 2018; Luo et al., 2018; Abdel-Aziz et al., 2020; Réblová et al., 2020; Shi et al., 2021; Boonmee et al., 2021; He et al., 2024). The genus has been so far represented from freshwater habitats in China and Thailand, except for *Pl. dimorphospora*, which is the only species described from terrestrial habitats in China (Boonmee et al., 2021).

In this study, we introduce three new species, *Chaetopsina yunnanensis*, *Parafuscosporella hunanensis*, and *Pleurotheciella yunnanensis*, based on morphology and phylogenetic studies, from freshwater habitats in China. In addition, *C. beijingensis* is formally synonymized under *C. fulva*, and the congeneric status of *Parafuscosporella* and *Vanakripa* is discussed.

2 Materials and methods

2.1 Sample collection, morphological studies, and isolation

Submerged decaying wood and branches were collected from a freshwater stream and lakes in Hunan and Yunnan provinces, China, from July to September 2022 (wet season). Detailed

environmental parameters (e.g., pH, temperature, and dissolved oxygen) were recorded for each sample collected from lakes. Fresh specimens were brought to the laboratory in Ziploc bags and studied following the methods described by Luo et al. (2018). The samples were incubated in high-density plastic boxes lined with moisturized absorbent paper at room temperature for 1 week. Macro-morphological characters of the fungi on the host surface were observed using an Optec SZ760 compound stereomicroscope. Temporarily prepared microscope slides were placed under a Nikon ECLIPSE 80i compound microscope fitted with a Nikon DS-Ri2 digital camera for observation and micro-morphological photography. The morphologies of colonies on the substrates were photographed using a Nikon SMZ1000 stereo zoom microscope. Microscopic structures were measured using the Tarosoft® Image Frame Work program, and the photographic plates were processed using Adobe Photoshop CS6 version 10.0 software (Adobe Systems, San Jose, CA, USA).

Single spore isolation was performed following the method described by Luo et al. (2018). The germinated conidia were aseptically transferred to fresh potato dextrose agar (PDA) plates and incubated at room temperature. The specimens were dried under natural light, wrapped in absorbent paper, and placed in a Ziploc bag with mothballs. Herbarium specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS), Kunming, China. The cultures were deposited in Kunming Institute of Botany, Chinese Academy of Sciences (KUNCC), Kunming, Yunnan, China. The novel species were registered in the Index Fungorum repository (<https://indexfungorum.org/Names/IndexFungorumRegisterName.asp>; accessed on 27 September 2024).

2.2 DNA extraction, PCR amplification, and sequencing

Fresh mycelia were scraped from colonies grown on PDA medium. DNA extraction was carried out using a DNA extraction kit (TOLOBIO Plant Genomic DNA Extraction Kit, Tsingke Company, Beijing, China) following the manufacturer's instructions. PCR amplification was performed using primer pairs LR0R/LR5 (Vilgalys and Hester, 1990) for the nuclear ribosomal large subunit 28S rDNA gene (LSU), NS1/NS4 (White et al., 1990) for the nuclear ribosomal small subunit 18S rDNA gene (SSU), ITS5/ITS4 (White et al., 1990) for the internal transcribed spacer rDNA region (ITS), and fRPB2-5F/fRPB2-7cR (Liu et al., 1999; Rehner and Buckley, 2005) for the RNA polymerase second largest subunit (*RPB2*). The PCR amplification was carried out in a 25- μ L reaction volume containing 12.5 μ L of 2 $\times$ Power Taq PCR Master Mix, 1 μ L of each forward and reward primer (10 μ M), 1 μ L of genomic DNA template (30–50 ng/ μ L), and 9.5 μ L sterilized double-distilled water. Amplifications were carried out using the BioTeke GT9612 thermocycler (Tsingke Company, Beijing, China). The PCR amplification conditions for ITS, LSU, and SSU consisted of initial denaturation at 98°C for 3 minutes, followed by 35 cycles of denaturation at 98°C for 20 seconds, annealing at 53°C for 10 seconds, an extension at 72°C for 20 seconds, and a final extension

at 72°C for 5 minutes. The PCR amplification conditions for *RPB2* consisted of initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 52°C for 2 minutes, an extension at 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. The quality of PCR products was checked using 1% agarose gel electrophoresis, and distinct bands were visualized in the gel documentation system (Compact Desktop UV Transilluminator analyzer GL-3120). The PCR products were purified, and Sanger sequences were obtained by Tsingke Company, Beijing, China.

2.3 Sequence assembly, alignment, and phylogenetic analyses

The newly generated sequences were subjected to the nucleotide BLAST search via the NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; accessed on 1 May 2024) to search the closely related taxa and confirm the correctness of the sequences. The sequence datasets were obtained by compiling the closely related taxa of the novel taxa retrieved from GenBank based on nucleotide BLAST searches and recent publications (Luo et al., 2018; Lechat and Fournier, 2020; Bakhit and Abdel-Aziz, 2021; Boonmee et al., 2021; Shi et al., 2021; Li et al., 2023; He et al., 2024). Outgroups were selected based on recently published data (Bakhit and Abdel-Aziz, 2021; Shi et al., 2021; Li et al., 2023) (Tables 1–3). Multiple sequence alignments were aligned with MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>; accessed on 10 May 2024) (Katoh et al., 2019) and automatically trimmed using TrimAl (<http://phylemon.bioinfo.cipf.es/utilities.html>; accessed on 10 May 2024) (Capella-Gutiérrez et al., 2009). A combined sequence dataset was obtained using SquenceMatrix v.1.7.8 (Vaidya et al., 2011). Phylogenetic relationships of the new taxa were performed based on maximum likelihood (ML) and Bayesian inference (BI) analyses.

ML analysis was performed by RAxML-HPC2 v.8.2.12 on the XSEDE (8.2.12) tool via the CIPRES Science Gateway (<http://www.phylo.org/portal2>; accessed on 25 May 2024) (Stamatakis, 2006; Miller et al., 2015) following the default setting but adjusted by setting 1,000 bootstrap replications and GTRGAMMA model of nucleotide substitution. The evolutionary model of nucleotide substitution for the BI analyses was performed independently for each locus using MrModeltest v 2.3 (Nylander, 2008). GTR+I+G was selected as the best-fit model for ITS, LSU, SSU, and *RPB2* datasets in all analyses under the Akaike information criterion (AIC). Markov Chain Monte Carlo (MCMC) sampling was computed to estimate Bayesian posterior probabilities (BYPP) in MrBayes v.3.2.7 (Ronquist et al., 2012). Two parallel runs with six simultaneous Markov chains were run for 1,000,000 generations but stopped automatically when the critical value for the topological convergence diagnostic reached 0.01. Trees were sampled every 200th generation. The first 10% of the total trees were set as burn-in and were discarded. The remaining trees were used to calculate posterior probabilities in the majority rule consensus tree.

Phylograms were visualized using FigTree v1.4.4 (Rambaut, 2018) and rearranged in Adobe Photoshop CS6 software (Adobe

Systems, USA). The new sequences were deposited in GenBank (Tables 1–3), and the final alignment and phylogenetic tree were registered in TreeBASE under the submission IDs: 31916 (*C. yunnanensis*), 31910 (*Pa. hunanensis*) and 31135 (*Pl. yunnanensis*) (<http://www.treebase.org/> accessed on 25 December 2024).

TABLE 1 Taxon names, strain numbers, and GenBank accession numbers of the ITS and LSU sequences used in the phylogenetic analyses of *Chaetopsina*.

Taxon name	Voucher/culture	GenBank accession numbers	
		ITS	LSU
<i>Calonectria parvispora</i>	CBS 111465	MT359775	MT359535
<i>C. parvispora</i>	CMW 30981	MT359774	MT359534
<i>Chaetopsina acutispora</i>	CBS 667.92	MH862382	MH874045
<i>Chaetopsina aquatica</i>	SUMCC H-18001	MW633072	MW633073
<i>Chaetopsina aurantisalinicola</i>	MFLU 18-0566	NR_168213	NG_068297
<i>C. aurantisalinicola</i>	MFLUCC 17-0414	MN047103	MN017868
<i>Chaetopsina eucalypti</i>	CPC 32857	MH327799	MH327835
<i>Chaetopsina fulva</i>	CBS 138004	KJ869159	KJ869216
<i>C. fulva</i>	MFLU 18-2327	MK828667	MK828234
<i>C. fulva</i>	CBS 142.56	KM231772	NG_070573
<i>C. fulva</i>	FMR 13129	KY853432	KY853492
<i>C. fulva</i>	HMAS 188462	GU075861	GU075867
<i>Chaetopsina gautengina</i>	CPC 34896	NR_170049	NG_073870
<i>Chaetopsina penicillata</i>	CBS 608.92	NR_154780	NG_058781
<i>C. penicillata</i>	KUNCC22-12664	OP985128	OP985134
<i>Chaetopsina pini</i>	CPC 21622	KF777144	KF777200
<i>Chaetopsina pinicola</i>	CPC 21819	NR_137823	KF777201
<i>Chaetopsina pnagiana</i>	BRFM 3055	NR_175643	NG_088111
<i>Chaetopsina saulensis</i>	CLLG 18029	MN017104	MN017106
<i>Chaetopsina yunnanensis</i>	KUNCC23-12940	OQ860234	PP151255
<i>C. yunnanensis</i>	KUNCC23-13014	OQ860233	PP151256
<i>Graphium carbonarium</i>	CBS 123610	MH863310	MH874834
<i>Graphium jumulu</i>	CBS 139898	NR_137980	NG_069278
<i>Volutella gilva</i>	CBS 128258	MH864864	MH876309
<i>Volutella leucaenae</i>	MFLUCC 17-2620	NR_189395	NG_241997
<i>Volutella salvadorae</i>	CBS 147070	NR_173060	NG_076746
<i>Volutella thailandensis</i>	MFLUCC 16-0366	NR_169676	MH376742

The newly generated sequences are indicated in red, while the type strains are in black bold font. “–” indicates unavailable sequences. ITS, internal transcribed spacer; LSU, large subunit.

3 Results

3.1 Phylogenetic analyses

Analysis 1

The concatenated LSU and ITS sequence dataset comprises 27 representative taxa in *Calonectria*, *Chaetopsina*, and *Volutella* with *Graphium carbonarium* (CBS 123610) and *Graphium jumulu* (CBS 139898) as the outgroup taxa (Table 1). The concatenated sequence matrix comprises 1,420 characters, including gaps (LSU, 828 bp; ITS, 588 bp). BI and ML analyses of the combined dataset were performed to determine the placement of our new taxon and infer relationships at the intrageneric level as well as resolve the phylogenetic relationships of the core genera in Nectriaceae. The phylogenetic trees obtained from BI and ML analyses resulted in trees with largely similar topologies. A phylogenetic investigation based on ML analysis was carried out with the best RAXML tree with a final likelihood value of –5,889.450811. The matrix had 385 distinct alignment patterns, with 6.19% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.238371, C = 0.250160, G = 0.286306, T = 0.225163, with substitution rates AC = 1.768750, AG = 2.223229, AT = 2.277765, CG = 1.698772, CT = 7.040906, GT = 1.000000; gamma distribution shape parameter α = 0.526092. The final average standard deviation of split frequencies at the end of total MCMC generations was calculated as 0.009709 in BI analysis.

Molecular analysis of a concatenated LSU and ITS sequence dataset (Figure 1) demonstrated that the phylogenetic relationship of the representative genera, *Calonectria*, *Chaetopsina*, and *Volutella*, is not well-resolved. The genera *Calonectria* and *Volutella* formed distinct clades with well-supported value in ML analyses but low-supported value in BI analysis. *Chaetopsina* formed a distinct clade with *Calonectria* and *Volutella* with low support. The interspecific status of many *Chaetopsina* species is not well-resolved in the present study, including *Chaetopsina acutispora*, *Chaetopsina pinicola*, *Chaetopsina pnagiana*, and *Chaetopsina saulensis*. Two new strains (KUNCC23-12940 and KUNCC23-13014) formed a robust subclade [100% maximum likelihood bootstrap support (MLBS) and 1.00 Bayesian posterior probabilities (BYPP)] and clustered with *C. pinicola* (CPC 21819) with low-supported values. *C. beijingensis* (CBS 138004) shared the same branch with the type (CBS 142.56) and representative strains of *C. fulva* (FMR 13129 and MFLU 18-2327). In contrast, *C. fulva* (HMAS 188462) remained distinct from other strains.

Analysis 2

The phylogenetic analyses of concatenated ITS, LSU, and SSU sequence data were conducted to demonstrate the phylogenetic relationships of the new isolates with other species in *Parafuscosporella*. Twenty strains were included in the combined dataset (Table 2), which comprised 2,340 characters (ITS, 535 bp; LSU, 829 bp; SSU, 976 bp) after alignment (including gaps). The

TABLE 2 Taxon names, strain numbers, and GenBank accession numbers of the ITS, LSU, and SSU and sequences used in the phylogenetic analyses of *Parafuscosporella*.

Taxon name	Voucher/culture	GenBank accession number		
		ITS	LSU	SSU
<i>Fuscosporella aquatica</i>	MFLUCC 16–0859	NR_156398	NG_059853	NG_062433
<i>Fuscosporella pyriformis</i>	MFLUCC 16–0570	NR_152555	KX550896	NG_061248
<i>Parafuscosporella aquatica</i>	KUMCC 19–0211	NR_173178	MN512343	–
<i>Parafuscosporella ellipsoconidiogena</i>	TBRC 15503	OK044749	OK044741	OK054346
<i>Pa. ellipsoconidiogena</i>	TBRC 15504	OK044750	OK044742	OK054347
<i>Parafuscosporella garethii</i>	BCC79986	OK135602	KX958430	KX958428
<i>Pa. garethii</i>	BCC79987	OK135603	KX958431	KX958429
<i>Parafuscosporella lignicola</i>	MFLUCC 23–0047	OQ917244	OQ875867	OQ917245
<i>Pa. lignicola</i>	MFLUCC 23–0048	OQ925403	OQ875868	OQ925402
<i>Parafuscosporella moniliformis</i>	MFLUCC 15–0626	NR_152557	KX550895	NG_063614
<i>Parafuscosporella mucosa</i>	MFLUCC 16–0571	MG388214	NG_059855	NG_063663
<i>Parafuscosporella hunanensis</i>	KUNCC23–13574	OR230704	PP744555	PP744557
<i>Pa. hunanensis</i>	KUNCC24–17774	PP744554	PP744556	PP744558
<i>Parafuscosporella nilotica</i>	CBS H22128	MN921198	–	MN921199
<i>Parafuscosporella obovata</i>	TBRC 15505	OK044751	OK044743	OK054348
<i>Parafuscosporella pyriformis</i>	MFLUCC 18–1400	MN513030	MN512339	–
<i>Pa. pyriformis</i>	KUMCC 19–0008	MN513031	MN512340	–
<i>Parafuscosporella xishuangbannaensis</i>	IFRDCC 3133	ON540716	ON540747	–
<i>Vanakripa oblonga</i>	BCRC FU31423	MT452512	OQ079570	OQ079568
<i>Vanakripa taiwanensis</i>	BCRC FU31428	MT452513	OQ079569	OQ079567

The newly generated sequences are indicated in red, while the type strains are in black bold font. “–” indicates unavailable sequences. ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

TABLE 3 Taxon names, strain numbers, and GenBank accession numbers of the ITS, LSU, SSU, and *RPB2* sequences used in the phylogenetic analyses of *Pleurotheciella*.

Taxon name	Voucher/culture	GenBank accession numbers			
		ITS	LSU	SSU	<i>RPB2</i>
<i>Pleurotheciella aquatica</i>	MFLU 17-0911	NR_160591	NG_066193	–	–
<i>Pl. aquatica</i>	MFLUCC 17-0464	MF399236	MF399253	MF399220	MF401405
<i>Pleurotheciella centenaria</i>	DAOM 229631	NR_111709	NG_060098	NG_064996	JQ429265
<i>Pleurotheciella dimorphospora</i>	KUMCC 20-0185	NR_175737	NG_081519	NG_078760	–
<i>Pl. dimorphospora</i>	KIB049/MFLU 20-0138	MW981446	MW981444	MW981454	MZ509665
<i>Pleurotheciella erumpens</i>	CBS 142447	NR_170010	MN699435	NG_070323	MN704311
<i>Pleurotheciella fusiformis</i>	IFRD500 014	MT555417	MT559121	MT555733	–
<i>Pleurotheciella ganzhouensis</i>	JAUCC6079	OR853417	OR853422	OR853426	PP078759
<i>Pl. ganzhouensis</i>	JAUCC6678	PP800192	PP800214	PP801261	PP816289

(Continued)

TABLE 3 Continued

Taxon name	Voucher/culture	GenBank accession numbers			
		ITS	LSU	SSU	RPB2
<i>Pleurotheciella guttulata</i>	KUMCC 15-0442	MF399239	MF399256	MF399222	MF401408
<i>Pl. guttulata</i>	KUMCC 15-0296	MF399240	MF399257	MF399223	MF401409
<i>Pleurotheciella irregularis</i>	JAUCC6080	OR853418	OR853423	PP801258	PP816286
<i>Pl. irregularis</i>	JAUCC6679	PP800193	–	PP801262	–
<i>Pleurotheciella krabiensis</i>	MFLUCC 16-0852	MG837018	MG837013	MG837023	–
<i>Pl. krabiensis</i>	MFLUCC 18-0856	MG837019	MG837014	MG837024	–
<i>Pleurotheciella lunata</i>	S-426	MK878378	MK835847	MK834782	–
<i>Pl. lunata</i>	MFLUCC 17-0111	MF399238	MF399255	MF399221	MF401407
<i>Pleurotheciella rivularia</i>	CBS 125238	NR_111711	NG_057950	NG_061124	JQ429263
<i>Pleurotheciella saprophytica</i>	MFLUCC 16-1251	MF399241	MF399258	MF399224	MF401410
<i>Pleurotheciella submersa</i>	MFLUCC 17-1709	MF399243	MF399260	MF399226	MF401412
<i>Pl. submersa</i>	DLUCC 0739	MF399242	MF399259	MF399225	MF401411
<i>Pleurotheciella sympodia</i>	MFLUCC 18-0983	MT555419	MT555425	MT555734	–
<i>Pl. sympodia</i>	MFLUCC 18-0658	MT555418	MT559086	MT559094	–
<i>Pl. sympodia</i>	KUMCC 19-0213	MT555420	MT555426	–	–
<i>Pleurotheciella tropica</i>	MFLU 18-0141	MG837020	MG837015	MG837025	–
<i>Pleurotheciella uniseptata</i>	S-936	MK878377	MK835846	MK834781	MN194025
<i>Pleurotheciella verrucosa</i>	JAUCC6076	OR853414	OR853419	OR853424	PP078756
<i>Pl. verrucosa</i>	JAUCC6675	PP800189	PP800211	PP801259	PP816287
<i>Pl. verrucosa</i>	JAUCC6078	OR853416	OR853421	PP801257	PP078758
<i>Pl. verrucosa</i>	JAUCC6677	PP800191	PP800213	–	–
<i>Pleurotheciella yunnanensis</i>	KUNCC23-13328	OR234682	PP095383	PP095382	PP131261
<i>Pl. yunnanensis</i>	KUNCC23-13682	PP095384	PP095381	PP095385	PP131262
<i>Rhexoacrodictys erecta</i>	HSAUPmyr4622	KU999964	KX033556	KX033526	–
<i>Rhexoacrodictys fimicola</i>	HMAS 47737	KU999960	KX033553	KX033522	–

The newly generated sequences are indicated in red, while the type strains are in black bold font. “–” indicates unavailable sequences. BCC, BIOTEC Culture Collection, Pathum Thani, Thailand; BCRC, Bioresource Collection and Research Centre, Food Industry Research and Development Institute, Hsinchu, Taiwan; BRFM, Biological Resource Center CIRM-CF (International Center of Microbial Resources, Marseille, France; CBS, Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CMW, Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CLLG, Collection of Christian Lechat at French Guiana deposited in LIP herbarium (university of Lille); CPC, Culture Collection of Pedro Crous, Netherlands; FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain; DAOM, Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; DLUCC/HMAS, Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; HSAUP, Herbarium of Department of Plant Pathology, Shandong Agricultural University, Taian, Shandong, China; IFRD/IFRDCC, Research Institute of Resources Insects, China Academy of Forestry; JAUCC, Jiangxi Agricultural University Culture Collection; KIB, Collection of Rungtiwa Phookamsak at Kunming Institute of botany, Chinese Academy of Sciences; KUMCC/KUNCC, Kunming Institute of Botany, Chinese Academy of Sciences Culture Collection, Kunming, Yunnan, China; MFLU, the herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; S, Collection of Hongyan Su; SUMCC, Sohag University microbial culture collection, Egypt; TBRC, Thailand Bioresource Research Center; ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

best RAxML tree with a final likelihood value of –6,818.339932 is presented (Figure 2). RAxML analysis yielded 415 distinct alignment patterns and 16.25% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.231018, C = 0.253572, G = 0.287376, T = 0.228033, with substitution rates AC = 1.005383, AG = 1.978020, AT = 1.365847, CG = 0.802886, CT = 4.380984, GT = 1.000000; gamma distribution shape parameter alpha = 0.765977. The final average standard deviation of split

frequencies at the end of total MCMC generations for BI analysis was 0.009867.

Phylogenetic analyses retrieved from ML and BI analyses were not significantly different and showed similar topologies. Phylogenetic analyses demonstrated that *Parafuscospora* formed the well-resolved clade and separated into two subclades: clade A comprises *Pa. garethii*, *Pa. hunanensis*, *Pa. mucosa*, *Pa. nilotica*, *Pa. obovata*, *Pa. pyriformis*, and *Pa. xishuangbannaensis*, whereas clade B comprises *Pa.*

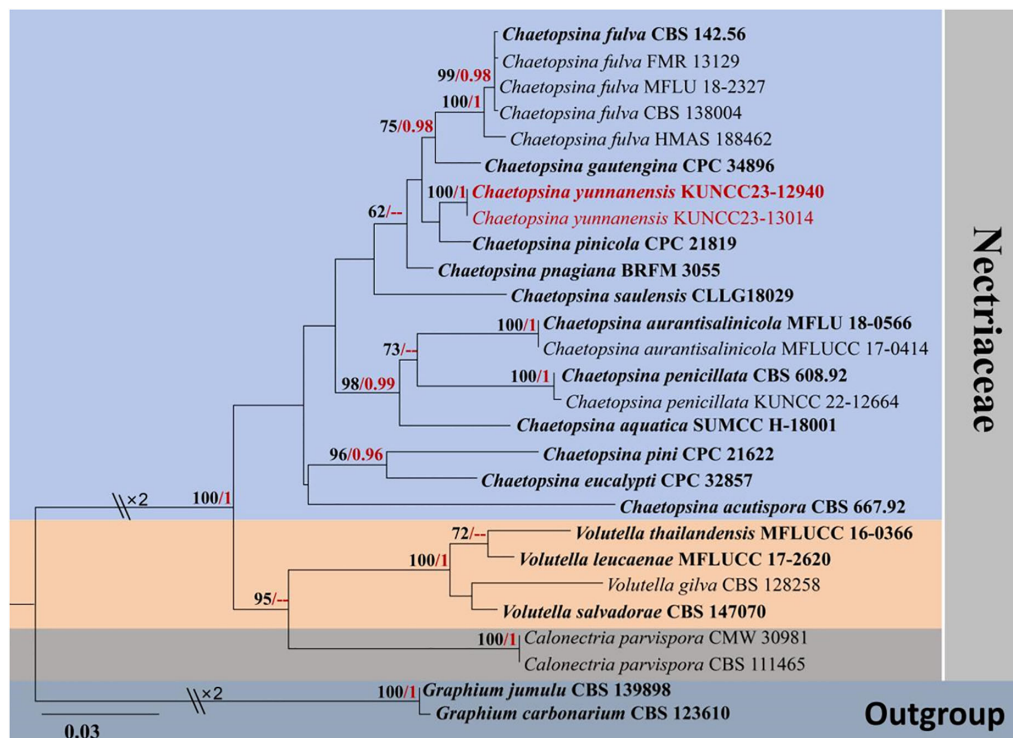


FIGURE 1

RAXML phylogenetic tree of a concatenated LSU and ITS sequence dataset. The BI and ML support values equal to or greater than 0.90 BYPP and 60% MLBS are shown as “MLBS/BYPP” at the nodes. The tree is rooted to *Graphium carbonarium* (CBS 123610) and *Graphium jumulu* (CBS 139898). Type strains are in bold, and newly generated strains are in red. LSU, large subunit; ITS, internal transcribed spacer; BI, Bayesian inference; ML, maximum likelihood.

aquatica, *Pa. ellipsoconidiogena*, *Pa. lignicola*, and *Pa. moniliformis*. Two *Vanakripta* species, *V. oblonga* and *V. taiwanensis*, also formed a high-support subclade closely related to *Pa. ellipsoconidiogena* and *Pa. lignicola*. The interspecific status of most *Parafuscospora* is well-clarified, except for *Pa. xishuangbannaensis*, in the present study. The two strains (KUNCC23-13574 and KUNCC24-17774) of *Pa. hunanensis* sp. nov. clustered with *Pa. mucosa* in subclade A with 100% MLBS and 1.00 BYPP support values.

Analysis 3

The phylogenetic analyses of concatenated ITS, LSU, SSU, and *RPB2* sequence data were conducted to demonstrate the relationship between the new species and other known species in *Pleurotheciella*. Thirty-four strains were included in the combined dataset (Table 3), which comprised 2,919 characters (ITS, 564 bp; LSU, 825 bp; SSU, 682 bp; *RRB2*, 848 bp) after alignment (including gaps). *Rhexoacrodactylus erecta* (HSAUPmyr4622) and *Rhexoacrodactylus fimicola* (HMAS 47737) were selected as the outgroup taxa. The best RAXML tree with a final likelihood value of -10,754.565198 was selected to represent the phylogenetic affinity of the novel species with closely related species (Figure 3). RAXML analysis yielded 677 distinct alignment patterns and 18.84% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.234132, C = 0.258625, G = 0.287873, T = 0.219371, with substitution rates AC = 1.285686, AG = 3.496823, AT = 1.635783, CG = 0.882608, CT = 9.634626, GT =

1.000000; gamma distribution shape parameter alpha = 0.461774. The final average standard deviation of split frequencies at the end of total MCMC generations for BI analysis was 0.009778.

Phylogenetic analyses retrieved from ML and BI analyses were not significantly different and showed similar topologies. Phylogenetic affinities of most *Pleurotheciella* species are well-resolved (up to 70% MLBS and 0.90 BYPP support values) in the present study, except for *Pl. uniseptata*. However, the species always formed a stable subclade with *Pl. aquatica*. Two strains (KUNCC23-13328 and KUNCC23-13682) of the new species, *Pl. yunnanensis*, formed a distinct subclade (100% MLBS/1.00 BYPP), sister to *Pl. saprophytica* (MFLUCC 16-1251) with significant support (96% MLBS/1.00 BYPP) and clustered with *Pl. dimorphospora* (KUMCC 20-0185) with significant support (96% MLBS/1.00 BYPP).

3.2 Taxonomy

Class Sordariomycetes O.E. Erikss. & Winka

Subclass Hypocreomycetidae O.E. Erikss. & Winka

Order Hypocreales Lindau

Family Nectriaceae Tul. & C. Tul.

Genus *Chaetopsina* Rambelli

Chaetopsina fulva Atti Accad. Sci. Ist. Bologna, Cl. Sci. Fis., Rendiconti 11: 5 (1956). **Amend.** L. Li, Phookamsak & Bhat

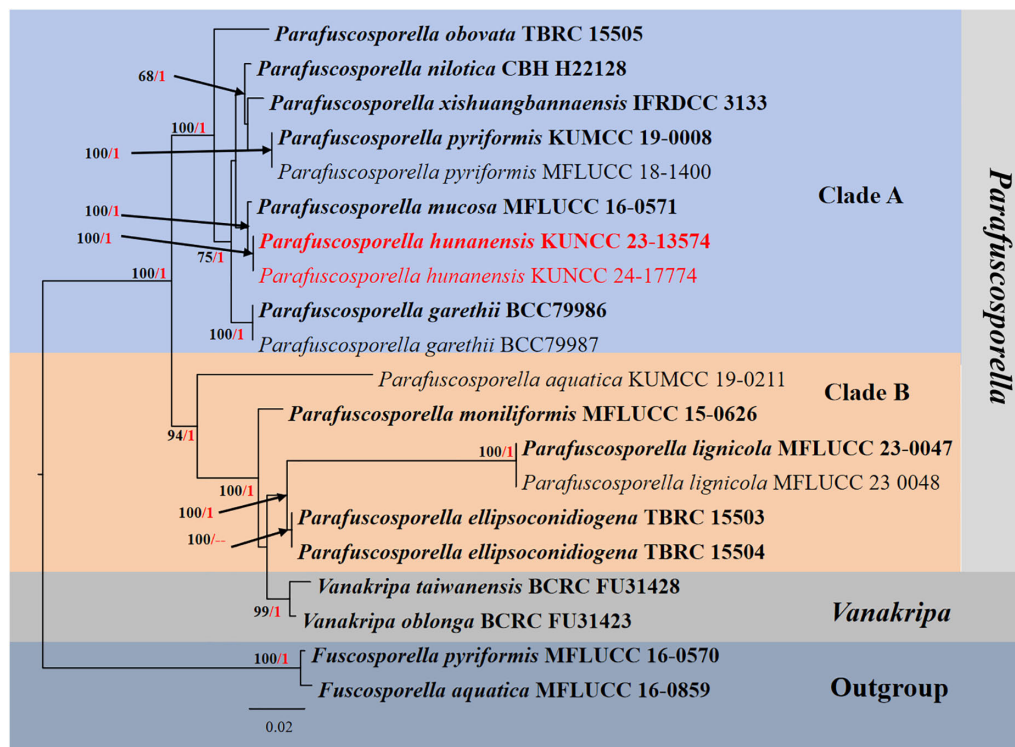


FIGURE 2

RAxML phylogenetic tree of a concatenated ITS, LSU, and SSU sequence datasets. The BI and ML support values equal to or greater than 0.90 BYPP and 60% MLBS are shown as "MLBS/BYPP" at the nodes. The tree is rooted to *Fuscosporella aquatica* (MFLUCC 16-0859) and *Fuscosporella pyriformis* (MFLUCC 16-0570). Type strains are in red, and newly generated strains are in red. ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit; BI, Bayesian inference; ML, maximum likelihood.

Index Fungorum number: IF 294735

= *C. beijingensis* Crous & Y. Zhang ter, in Crous et al., Persoonia 32: 267 (2014)

Typification: Italy, on fallen leaves (needle) of *Cedrus deodara* (Pinaceae), Feb 1956, A. Rambelli, IMI 62199 (type of *C. fulva*), ex-type culture, CBS 142.56; China, Beijing, Fragrant Hill, N39°59' 18.4" E116°11'25", on needles of *Pinus tabulaeformis* (Pinaceae), 1 Sep 2013, P.W. Crous & Y. Zhang, CBS H-21718 (holotype of *C. beijingensis*), CPC 23629 = CBS 138004.

Saprobic on Pinaceae and various plant hosts as well as soil. Sexual morph: Referred to *Chaetopsinectria chaetopsinae* ($\equiv$ *Nectria chaetopsinae* Samuels; Lombard et al., 2015; Rossman et al., 2016). Asexual morph: *Conidiophores* 180–300 $\times$ 7.5–10 μ m, macronematous, simple, sparse, erect, setiform, often swollen, bulbous at the base (up to 15–20 μ m), tapering toward acutely rounded apex, straight or slightly curved, mostly flexuous, yellow-brown to red-brown, less pigmented at the base, turning red-brown in 3% KOH and yellow in lactic acid, unbranched, smooth to verruculose, up to 13-septate (12–16-septate as in *C. beijingensis*), thick-walled (2 μ m diam.), sterile or occasionally fertile; fertile region situated below the middle of the main axis or higher, occasionally terminal, comprising an irregularly branched, densely aggregated, hyaline, conidiogenous apparatus. *Conidiogenous cells* 6–12(–20) $\times$ 3.5–5 μ m monophialidic, discrete, hyaline, ampulliform to lageniform, smooth, irregularly branched, periclinal thickening visible, with minute collarettes. *Conidia* 8–12

$\times$ 2 μ m, solitary, hyaline, subcylindrical to cylindrical, with rounded ends, aseptate, smooth, with guttules, rarely with flattened hilum (adopted from Ellis, 1971; Kirk and Sutton, 1985; Samuels, 1985; Luo and Zhuang, 2010; Crous et al., 2014; Perera et al., 2023).

Known habitats and host: On needle of *C. deodara* and fallen leaves of *Laurus nobilis*, *Quercus*, and *Carpinus*, needles of *P. tabulaeformis* (as *C. beijingensis*), woody test blocks of *X. dolabriformis*, dead leaves, decaying wood and soil (Rambelli, 1956; Ellis, 1971; Sivichai et al., 2000; Luo and Zhuang, 2010; Crous et al., 2014; Arias et al., 2015; Hernández-Restrepo et al., 2017; Luo et al., 2019; Vu et al., 2019; Perera et al., 2023).

Known distribution: Canada, China, Italy, Spain, and Thailand (Rambelli, 1956; Ellis, 1971; Sivichai et al., 2000; Luo and Zhuang, 2010; Crous et al., 2014; Arias et al., 2015; Hernández-Restrepo et al., 2017; Luo et al., 2019; Vu et al., 2019; Perera et al., 2023).

Notes: *C. fulva* was designated as the type species of *Chaetopsina* when Rambelli (1956) introduced the genus. The species was poorly studied to begin with, and molecular data of the type specimen were obtained by Vu et al. (2019), who also confirmed its phylogenetic affinity in the Nectriaceae. The species, originally isolated from a needle of *C. deodara* and fallen leaves of *L. nobilis*, *Quercus*, and *Carpinus* in northern Italy, produces pigmented, erect, septate, setiform conidiophores that are apically sterile (Rambelli, 1956; Kirk and Sutton, 1985; Bakhit and Abdel-Aziz, 2021; Index Fungorum, 2024). Ellis (1971) provided a detailed morphological description of *C. fulva*, which produces macronematous, up to 280

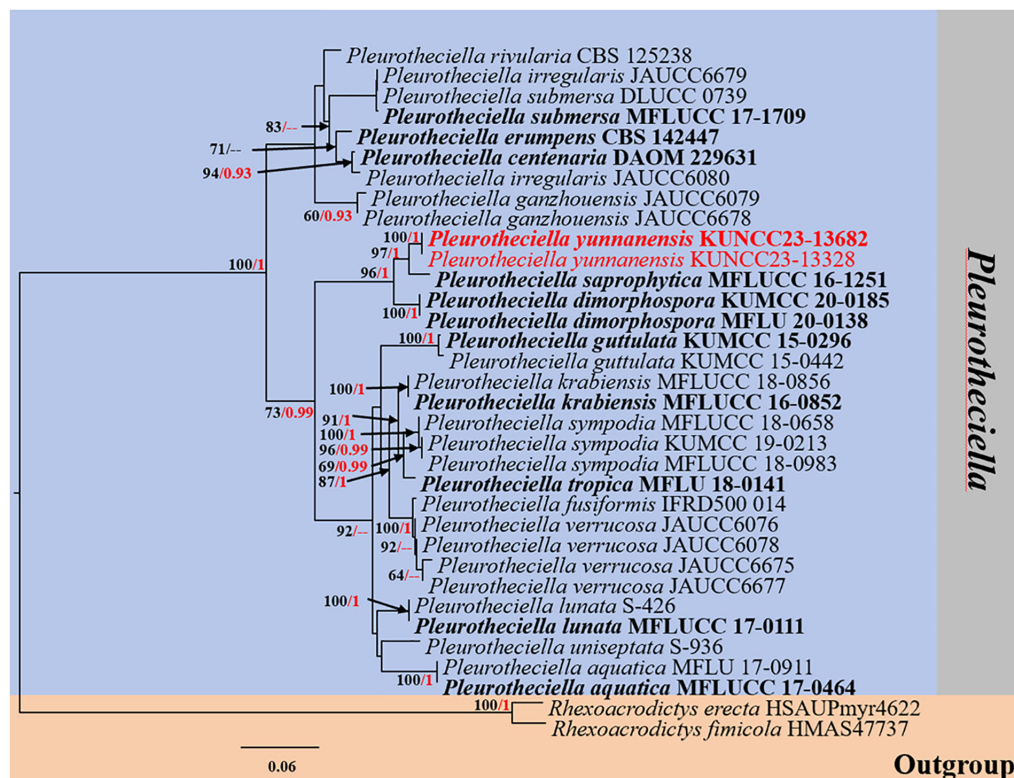


FIGURE 3

RAxML phylogenetic tree of a concatenated ITS, LSU, SSU, and RPB2 sequence dataset. The BI and ML support values equal to or greater than 0.90 BYPP and 60% MLBS are shown as "MLBS/BYPP" at the nodes. The tree is rooted to *Rhexoacrodictys erecta* (HSAUPmyr4622) and *Rhexoacrodictys fimicola* (HMAS 47737). Type strains are in bold, and newly generated strains are in red. ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit; BI, Bayesian inference; ML, maximum likelihood.

$\times 5\text{--}8\text{ }\mu\text{m}$ conidiophores, with bases often swollen to $15\text{--}20\text{ }\mu\text{m}$. The conidiogenous cells (phialides) are $7\text{--}15\text{ }\mu\text{m}$ long, with a swollen base $3\text{--}4\text{ }\mu\text{m}$ wide and a phialidic neck approximately $1\text{ }\mu\text{m}$ thick. Conidia are $7\text{--}11 \times 1\text{ }\mu\text{m}$. The species were reported from dead fallen leaves and soil, with distribution noted in Canada and Italy. Kirk and Sutton (1985) re-circumscribed the species based on type studies and emended the generic circumscription incorporating only five described species, viz., *Chaetopsina catenulata*, *C. fulva*, *Chaetopsina penicillata*, *C. polyblastia*, and *Chaetopsina splendida*. Kirk and Sutton (1985) treated *Chaetopsina romantica* (= *Chaetopsis romantica*) as a synonym of *C. fulva* on the insistence of Rambelli (1987).

Luo and Zhuang (2010) introduced a sexual genus *Chaetopsinectria* to accommodate sexual morphs of *Chaetopsina*. Based on ITS and 28S rDNA sequence analyses, Luo and Zhuang (2010) mentioned that *C. fulva*, *C. chaetopsinae*, and *C. chaetopsinae-penicillatae* have a close relationship. Regarding the generic type, *C. fulva*, the common features include red-brown and setose conidiophores that turn yellow in lactic acid, hyaline phialides, and sienna-colored colonies on PDA; these morphological traits are shared by other *Chaetopsina* asexual morph of nectriaceous fungi and provide phenotypic information in defining this asexual genus (Samuels, 1985; Okada et al., 1997; Luo and Zhuang, 2010). However, Rossman et al. (2016) mentioned that *C. fulva* is the asexual morph of *C. chaetopsinae* ($\equiv N.$

chaetopsinae), and hence, these generic names were synonyms and further recommended to use *Chaetopsina* rather than *Chaetopsinectria* due to its prior establishment, following the 1F = 1N policy (McNeill et al., 2012).

Crous et al. (2014) introduced *C. beijingensis*, collected from decaying wood submerged in a freshwater stream, in Beijing, China, and further demonstrated the similarity of nucleotide pairwise (ITS and LSU) between *C. beijingensis* and *C. fulva* (HMAS 188462) with 98% similarity of ITS and 100% similarity of LSU. Morphologically, *C. beijingensis* resembles *C. fulva* but can be distinguished from the latter in having subcylindrical and slightly larger conidia [$(11\text{--})12\text{--}13\text{--}(14) \times 2\text{--}(2.5)\text{ }\mu\text{m}$; Crous et al., 2014]. In contrast, *C. fulva* (described from the type's slide) has cylindrical, $8\text{--}12 \times 1.5\text{ }\mu\text{m}$ conidia (Kirk and Sutton, 1985). Vu et al. (2019) provided the molecular data from the type of *C. fulva* (CBS 142.56). Subsequently, Lechat and Fournier (2019) informally treated *C. beijingensis* as a synonym of *C. fulva*, in view of their morphological resemblance, phylogenetic evidence, and nucleotide pairwise similarities—99.6% similarity of ITS and 100% similarity of LSU sequences based on the nucleotide pairwise comparison of the type strains (CBS 142.56 vs. CBS 138004)—but did not taxonomically synonymize these taxa. Phylogenetically, *C. beijingensis* (CBS 138004) shares the same branch with the type strain of *C. fulva* and other representative strains with high support (100% MLBS/1.00 BYPP; Figure 1) in the present study. In accordance with

Lechat and Fournier (2019) and our own observations, we formally synonymized *C. beijingensis* under *C. fulva* based on morphological indistinctiveness and phylogenetic support coupled with the conspecific in nucleotide polymorphism. Detailed description is amended to incorporate the morphological features of *C. beijingensis* and *C. fulva*.

Chaetopsina yunnanensis L. Li, Bhat & Phookamsak, **sp. nov.**

Index Fungorum number: IF901629, [Figure 4](#)

Etymology: The name reflects the location of Yunnan where the holotype was collected.

Holotype: KUN-HKAS 126987

Saprobic on an unidentified branch, submerged in a freshwater habitat. **Sexual morph:** *Ascomata* perithecial, raised from pseudostomatic, visible as orangish to reddish brown, solitary or in groups (2–3 *ascomata*), superficial, scattered, associated with *Chaetopsina*-like asexual morph, ovoid to obpyriform, with an acutely conical apex, ostiolate with pore-like opening, soft and freshly, shiny, collapsing when dry. *Peridium* 20–50 μm ($\bar{x}$ = 38 μm , n = 20) thick in vertical section, composed of thick-walled, orangish cells, of *textura angularis* to *textura prismatica* or *epidermoidea*, becoming paler and slightly elongated toward interior. *Asci* and *ascospores* were not observed. **Asexual morph:** *Conidiophores* 200–310 μm long, 5–11 μm wide, broader at bulbous base with 8–10 μm wide, erect, macronematous, mononematous, straight to slightly curved, 5–8-septate, thick-walled, smooth, orangish brown to reddish brown, paler toward apex, fertile at above half. *Fertile region* yellow to orangish brown comprising loosely and regularly arranged penicillate, smooth-walled, 2-septate branches bearing densely aggregated conidiogenous cells, arranged in several whorls. *Conidiogenous cells* 6–8 $\times$ 4–6 μm ($\bar{x}$ = 7 $\times$ 4.5 μm , n = 20), discrete, ampulliform, phialidic, hyaline. *Conidia* 8–11 $\times$ 5–7 μm ($\bar{x}$ = 9.5 $\times$ 6 μm , n = 20), cylindrical to cylindric-fusoid or ellipsoidal, hyaline, solitary, unicellular, guttulate, smooth.

Culture characteristics: *Conidia* germinating on PDA within 48 h. Colonies on PDA reaching 20 mm diam. at room temperature in natural light after 1 month. Colonies from above medium dense, circular, dull, flat to slightly raised, effuse to fairly fluffy, white to cream, edge entire, with a rough surface, slightly radiating with pale yellowish concentric ring outward colonies, sometimes with raised floccose mycelial turfs; reverse: dark reddish brown, paler at margins, with black reddish pigments produced in PDA. Sporulation not observed.

Material examined: China, Yunnan Province, Xishuangbanna (99°56′–101°50′E, 21°08′–22°36′N, 470–2,429.5 msl), on a branch submerged in a freshwater stream, 9 September 2022, L. Li, LILU-133 (KUN-HKAS 126987, holotype), ex-type living culture KUNCC23-12940; *ibid.*, Kunming, Yang Zonghai Lake (102°5′–103°02′E, 24°51′–24°58′N, 1,770 msl; pH of water = 8.92, temperature = 26°C, dissolved oxygen = 7.6 mg/L, purification level 3), on submerged wood, 12 July 2022, L. Li, LILU-133-1 (KUN-HKAS 126988), living culture KUNCC23-13014.

Notes: The nucleotide BLAST searches of ITS and LSU sequences indicated that *C. yunnanensis* sp. nov. (KUNCC23-12940) is close to *Chaetopsina* species, with LSU of 99% similarity to *C. pinicola* (CBS 136444), *Chaetopsina gautengina* (CPC 34896), and *C. pnagiana* (BRFM 3055); ITS of 97% similarity to *C. pinicola*

(CBS 136444); and 96% similarity to *C. fulva* (CBS 138004) and *C. gautengina* (CPC 34896). In addition, the morphological characteristics of *C. yunnanensis* well match the concept of *Chaetopsina*, with red-pigmented upright conidiophores bearing discrete, phialidic conidiogenous cells and unicellular, cylindrical to cylindric-fusoid or ellipsoidal conidia (Kirk and Sutton, 1985; Samuels, 1985; Lechat and Fournier, 2020). The concatenated ITS and LSU phylogenetic analyses also demonstrated that the new strains KUNCC23-12940 and KUNCC23-13014 clustered within the species group of *Chaetopsina* but constituted an independent branch and sister to *C. pinicola* (CPC 21819) ([Figure 1](#)). The nucleotide base pair comparison between the type strain of the new species, KUNCC23-12940, and *C. pinicola* (CPC 21819) revealed 18/586 bp (3.07%) of ITS and 4/800 bp (0.5%) of LSU differences. Morphologically, *C. yunnanensis* differs from *C. pinicola* in having darker reddish-pigmented, 5–8-septate conidiophores, with penicillate fertile region at the apex and cylindric-fusoid or ellipsoidal conidia. In contrast, *C. pinicola* has medium brown, turning red-brown in 3% KOH, unbranched, verruculose, 11–15-septate conidiophores, with fertile mid region and subcylindrical, larger conidia [(11–)13–15(–17) $\times$ 2(–2.5) μm ; Crous et al., 2013].

Class Sordariomycetes O.E. Erikss. & Winka

Subclass Savoryellomycetidae O.E. Erikss. & Winka

Order Fuscosporellales Jing Yang, Bhat & K.D. Hyde

Family Fuscosporellaceae Jing Yang, Bhat & K.D. Hyde

Genus *Parafuscosporella* Jing Yang & K.D. Hyde

Parafuscosporella hunanensis L. Li, Bhat & Phookamsak, **sp. nov.**

Index Fungorum number: IF 902698, [Figure 5](#)

Etymology: The name reflects the location Hunan Province of China where the holotype was collected.

Holotype: KUN-HKAS 136260

Saprobic on decaying submerged wood in a freshwater stream.

Sexual morph: Undetermined. **Asexual morph** *Colonies* on natural substrate sporodochial, scattered, sparse, visible as black, dull, soft, granular on the host surface. *Mycelium* semi-immersed to superficial, composed of septate, hyaline, and smooth hyphae. *Conidiophores* 10–35 $\times$ 3–10 μm ($\bar{x}$ = 23 $\times$ 7 μm , n = 20), micronematous to semi-macronematous, mononematous, inconspicuous, hyaline, cylindrical, dense, erect or flexuous, branched, septate, smooth-walled. *Conidiogenous cells* 7–15 $\times$ 4–12 μm ($\bar{x}$ = 11 $\times$ 8 μm , n = 20), holoblastic, monoblastic, integrated, terminal, determinate, hyaline to pale brown, ampulliform to doliiform, smooth-walled. *Conidia* 20–29 $\times$ 15–22 μm ($\bar{x}$ = 26 $\times$ 19 μm , n = 20), acrogenous, obovoid to pyriform, with base truncate, dark brown to black, versicolored, paler brown at basal cell, 1-septate near the base, sometimes with pale brown to hyaline protuberance, or with a distinct basal frill derived from the distal end of the conidiogenous cell on cessation, smooth-walled.

Culture characteristics: *Conidia* germinating on PDA within 24 h. Germ tubes produced from both ends. Colonies on PDA reaching 1 mm diam. at room temperature in natural light after 3 months. Colonies from above, dense, irregular in shape, with undulate margin, dull, flat to slightly raised, velvety to felted, dark greenish gray at the margin, pale greenish gray toward the center, slightly radiated with

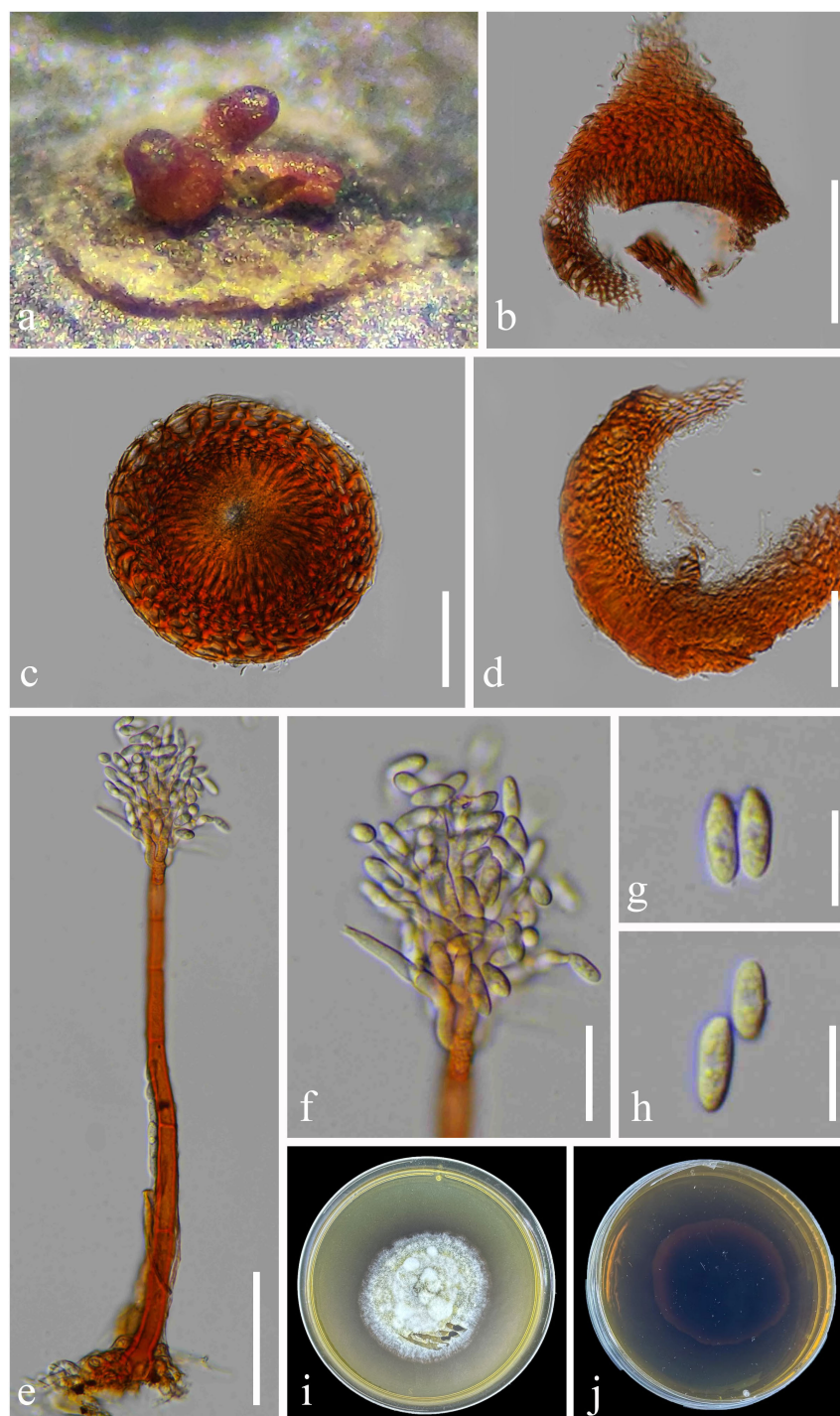


FIGURE 4

Chaetopsina yunnanensis (KUN-HKAS 126987, holotype). (A) Hyphomycetous colonies associated with ascomata (in group) on pseudostomata on submerged branch. (B) Pyriform ascoma with a conical apex. (C) Upper view of ascoma with pore-like opening ostiole. (D) Vertical section of peridium. (E, F) Conidiophores and conidiogenous cells bearing conidia. (G, H) Conidia. (I, J) Culture characteristics on PDA (I = from up-front, J = down-reverse). Scale bars: (B) = 100 μm , (C–E) = 50 μm , (F) = 20 μm , and (G, H) = 10 μm . PDA, potato dextrose agar.

convex ring, surface rough, with wrinkled folded aspect; reverse, dark greenish at the margin, pale greenish gray toward the center, radiated with black and white ring, radially furrowed at the edge; not produced pigmentation. Sporulation not observed.

Material examined: China, Hunan Province (108°47'–114°15'E, 24°38'–30°08'N, 500–1,500 msl), saprobic on decaying wood submerged in a freshwater stream, 26 August 2022, L. Li, LILU-203 (KUN-HKAS 136260, holotype), ex-type living culture,

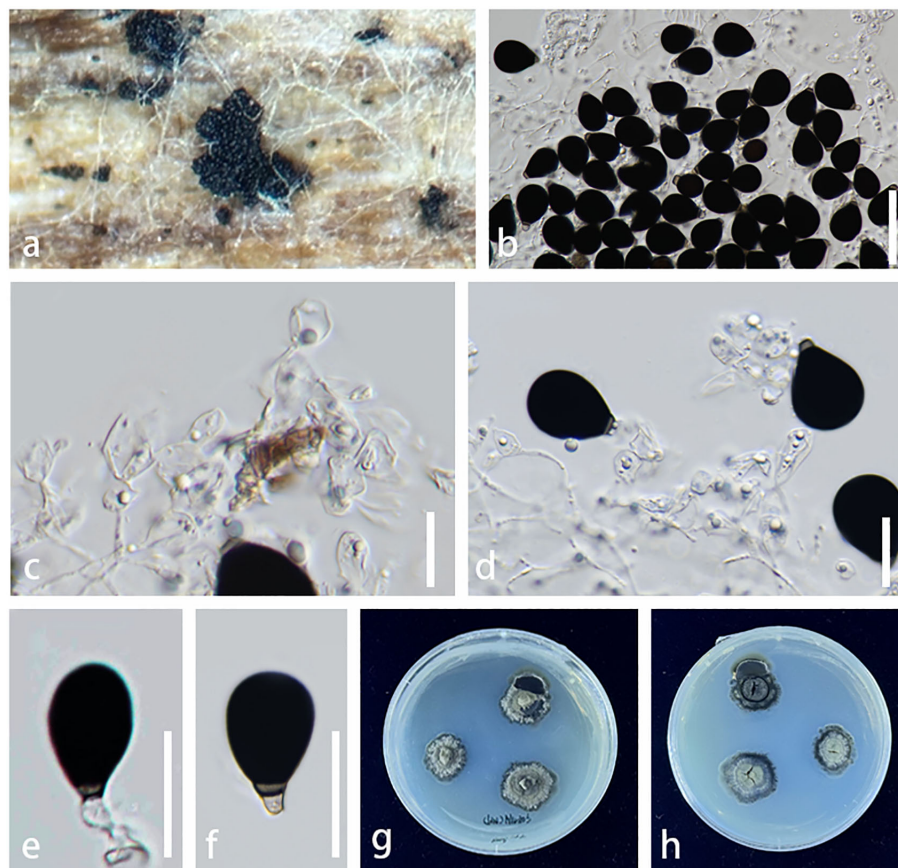


FIGURE 5

Parafuscospora hunanensis (KUNCC23-13574, holotype). (A) Sporodochia on host surface. (B) Conidial mass with dense, inconspicuous conidiophores, embedded in gelatinous matrix. (C) Micronematous, branched septate conidiophores with ampulliform to doliform conidiogenous cells. (D) Conidiophores and conidiogenous cells bearing conidia. (E, F) Conidia with a distinct basal frill derived from the distal end of the conidiogenous cell or with pale brown to hyaline protuberance. (G, H) Colony on PDA (G = from above, H = from below). Scale bars: (B) = 50 μ m, (C–F) = 20 μ m. PDA, potato dextrose agar.

KUNCC23-13574; *ibid.*, LILU-203-2 (KUN-HKAS 136261), living culture KUNCC24-17774.

Notes: The nucleotide BLAST searches of ITS, LSU, and SSU sequences indicated that *Pa. hunanensis* sp. nov. (KUNCC23-13574) is similar to *Pa. mucosa* (MFLUCC 16-0571, type strain) with 97.73% similarity of ITS, 99.88% similarity of LSU, and 100% similarity of SSU. Multigene phylogenetic analyses demonstrated that *Pa. hunanensis* (KUNCC23-13574 and KUNCC24-17774) clustered with the type strain of *Pa. mucosa* (MFLUCC 16-0571) with high support (100% MLBS/1.00 BYPP; Figure 2). *Pa. hunanensis* morphologically resembles *Pa. mucosa* (MFLU 16-1980) in having obovoid to pyriform, versicolored conidia, with brown to dark brown distal cell, paler basal cell, truncate base, and septate near the basal cell. However, *Pa. hunanensis* can be distinguished from *Pa. mucosa* by the absence of jelly-like cover on sporodochia and with inconspicuous, branched, septate, cylindrical conidiophores, hyaline to pale brown, doliform conidiogenous cells and obovoid to pyriform conidia. In contrast, *Pa. mucosa* produced sporodochia with distinct jelly-like covering, macronematous, conspicuous, cylindrical conidiophores with globose, subglobose, ellipsoidal, or clavate conidiogenous cells, and obovoid to pyriform, or ellipsoidal conidia

(Yang et al., 2016). The nucleotide pairwise difference comparison between *Pa. hunanensis* (KUNCC23-13574) and *Pa. mucosa* (MFLUCC 16-0571) revealed 14/602 bp (2.32%) for ITS, 1/839 bp (0.11%) for LSU, and 0/777 bp (0%) for SSU sequences. Based on the morphological distinctions, and phylogenetic evidence, coupled with the significant nucleotide pairwise differences of ITS region, the new species *Pa. hunanensis* is introduced herein.

Class Sordariomycetes O.E. Erikss. & Winka

Subclass Savoryellomycetidae O.E. Erikss. & Winka

Order Pleurotheciales R  blov   & Seifert

Family Pleurotheciaceae R  blov   & Seifert

Genus *Pleurotheciella* R  blov  

Pleurotheciella yunnanensis L. Li, Bhat & Phookamsak, sp. nov.

Index Fungorum number: IF901610, Figure 6

Etymology: In reference to Yunnan Province of China, where the holotype was collected.

Holotype: KUN-HKAS 132016

Saprobic on decaying, submerged wood from freshwater habitats. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on natural substrates visible as small, black, scattered, dots on the

host surface. *Mycelium* immersed to partially superficial, brown to dark brown, composed of branched, septate, smooth, 2–3 μm wide, thin-walled hyphae. *Conidiophores* difficult to distinguish on the host, semi-macronematous, mononematous, sub-hyaline to brown, erect, or bent on the host surface. *Conidiogenous cells* 3–5 $\times$ 3–6 μm ($\bar{x}$ = 4 $\times$ 4.5 μm , n = 20), holoblastic, hyaline, raised from hyphae, terminal or intermediate. *Conidia* 18–25 $\times$ 22–30 μm ($\bar{x}$ = 21.5 $\times$ 26 μm , n = 20), varied in shape, ellipsoidal to subglobose, dark brown to black, initially forming phragmoconidia, later becoming muriform, chairoid at maturity. *In vitro*: Dimorphic, with two conidial types. Type I: *Conidiophores* 30–50 $\times$ 3–5 μm ($\bar{x}$ = 36 $\times$ 4.5 μm , n = 20), semi-macronematous or macronematous, mononematous, hyaline to dark brown, cylindrical, septate, unbranched. *Conidiogenous cells* holo- to polyblastic, terminal,

lateral, or intercalary, brown. *Conidia* 15–22 $\times$ 12–15 μm ($\bar{x}$ = 19 $\times$ 13.5 μm , n = 20), phragmosporous to muriform, variedly shaped, brown to dark brown, subglobose to cordiform, or irregular in shape, with a protuberant hilum, initially 2–3-phragmoseptate, at maturity becoming 1–2 transverse and longitudinally dictyoseptate, sectoried, leaf clover-like, brown to dark brown. Type II: *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–8 $\times$ 2–6 μm ($\bar{x}$ = 5.5 $\times$ 5 μm , n = 20), phialidic, terminal, integrated, with minute denticles, subhyaline to pale brown, 1–2-septate, unbranched, arising in pseudo-chains. *Conidia* 7–12 $\times$ 3–5 μm ($\bar{x}$ = 9.5 $\times$ 4 μm , n = 20), hyaline, ellipsoidal, 0–1-septate, guttulate, smooth-walled.

Culture characteristics: Conidia germinating on PDA within 48 h. Germ tubes produced from the basal cell. Colonies reaching

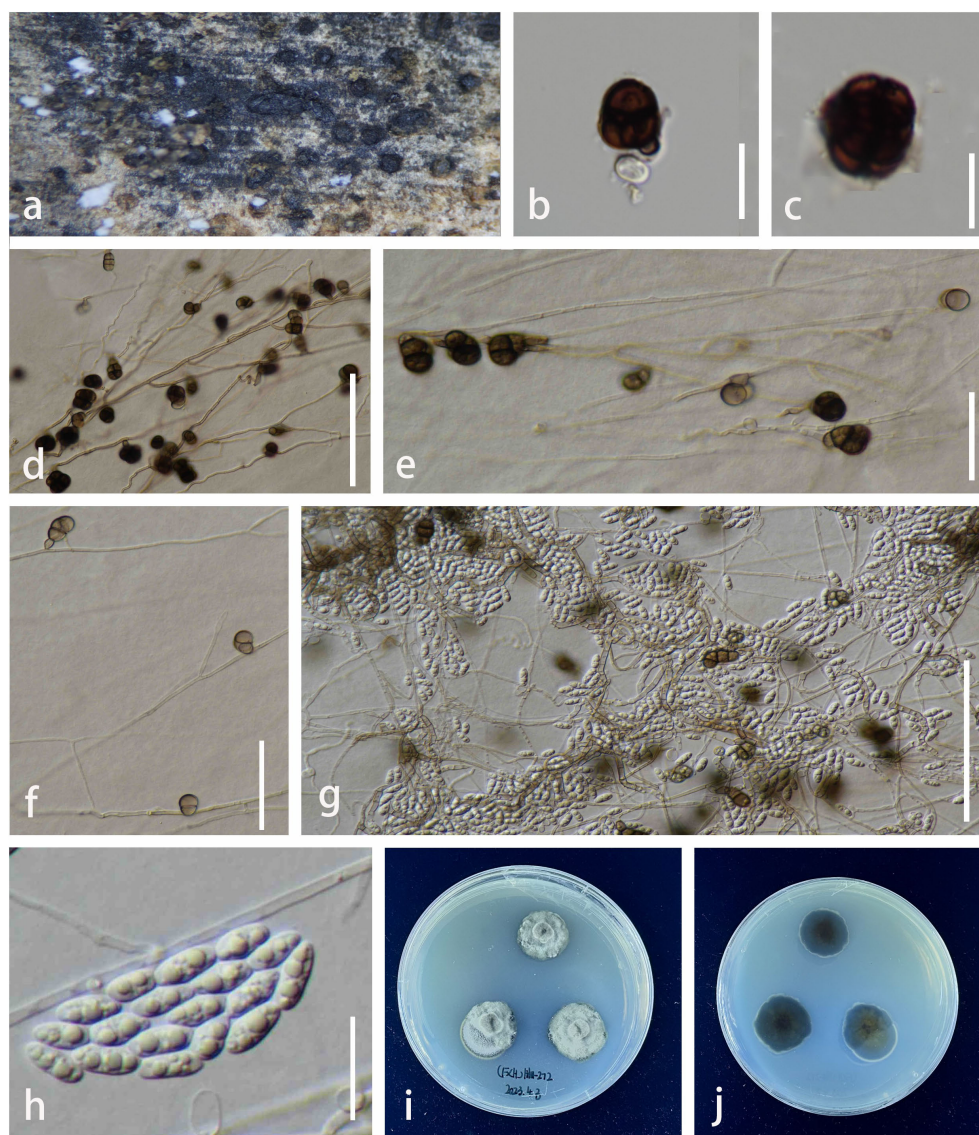


FIGURE 6

Pleurotheciella yunnanensis (KUN-HKAS 132016, holotype). (A) Colonies on surface of submerged wood. (B, C) Dark brown, muriform conidia on natural substrate. (D–F) Conidiogenous cells bearing muriform conidia *in vitro*. (G) Conidiogenous cells bearing brown, muriform conidia (Type I) and hyaline, ellipsoidal conidia (Type II) on PDA. (H) Hyaline conidia (Type II). (I, J) Colonies on PDA (I = from above, J = from below). Scale bars: (B–E, G, H) = 20 μm ; (F) = 10 μm . PDA, potato dextrose agar.

2.3 mm diam at room temperature in normal day and night light after 1 month. Colonies from above, white to pale gray, dense, slightly circular to irregular in shape, slightly raised to umbonate, rough at the surface, with wrinkled folded aspect, undulate at the edge, slightly radiating, velvety to felted; reverse, white at the margin, dark green to black in the middle, olive in the center, wrinkled folded; not producing pigmentation. Mycelium superficial to immersed in media, brown, composed of septate, branched, smooth hyphae. Sporulation on PDA after 2 weeks.

Material examined: China, Yunnan Province, Fuxian Lake (102°43′–102°59′E, 24°31′–24°51′N, 1,720 msl; pH of water = 8.593333, temperature = 23°C, dissolved oxygen = 7.39 mg/L, purification level 1), on decaying wood submerged in a freshwater lake, 14 August 2022, L. Li, LILU-272 (KUN-HKAS 132016, holotype), ex-type living culture KUNCC23-13328; *ibid.*, Yang Zonghai Lake (102°5′–103°02′E, 24°51′–24°58′N, 1,770 msl; pH of water = 8.92, temperature = 26°C, dissolved oxygen = 7.6 mg/L, purification level 3), 12 July 2022, L. Li, LILU-136 (KUN-HKAS 132017), living culture KUNCC23-13682.

Notes: Based on the nucleotide BLAST search, *Pl. yunnanensis* (KUNCC23-13328) is similar to *Pl. dimorphospora* (KUMCC 20-0185) with 96.21% similarity of the ITS, 98.88% similarity of LSU, 99.44% similarity of SSU, and 92.29% similarity of RPB2. The multigene phylogenetic analyses confirmed the phylogenetic affinity of the new species (KUNCC23-13328 and KUNCC23-13682) as a member of *Pleurotheciella* by nested with *Pl. dimorphospora* and *Pl. saprophytica* forming a well-resolved subclade among other species within the *Pleurotheciella* (Figure 3). The nucleotide pairwise comparison between *Pl. yunnanensis* (KUNCC23-13328) and *Pl. saprophytica* (MFLU 17-0915) revealed 16/464 bp (3.4%) of ITS, 7/804 bp of LSU (0.9%), 0/813 bp of SSU (0%), and 40/781 bp of RPB2 (5%) differences. *Pl. yunnanensis* shares similar morphology with *Pl. dimorphospora* in having two types of conidial morphology *in vitro*. However, they differ by phragmoconidia comprising 2–3-septate and leaf clover-like, brown to dark brown dictyoconidia. In contrast, *Pl. dimorphospora* mainly produced multi-septate, sectored, ellipsoidal to subglobose dictyoconidia (Boonmee et al., 2021). Furthermore, *Pl. dimorphospora* was found in terrestrial habitats, whereas *Pl. yunnanensis* has been found in aquatic habitats. *Pl. yunnanensis* differs from *Pl. saprophytica* by the lack of cylindrical or apically tapering sympodial, conspicuously denticulate conidiogenous cells and subcylindrical to obovoidal, obtuse conidia with a rounded apex and tapering base (Luo et al., 2018). However, both species were isolated from submerged wood in a freshwater environment in Yunnan Province, China. Interestingly, most *Pleurotheciella* have been found in aquatic environments, except for *Pl. dimorphospora*. Details on habitats, hosts, and distributions of *Pleurotheciella* are provided in Table 4. Furthermore, *Pl. yunnanensis* fits well with the generic concept of *Pleurotheciella* by sharing similar morphological characteristics as polyblastic, denticulate conidiogenesis, subhyaline conidiophores, and hyaline, ellipsoidal to ellipsoidal-fusiform, and aseptate to multi-septate conidia. Detailed morphological characteristics of all known *Pleurotheciella* species are also provided in Table 5.

4 Discussion

Freshwater fungi exhibit remarkable ecological diversity, playing essential roles in aquatic ecosystems. They act as decomposers, breaking down organic matter like wood and leaf litter, which contributes to nutrient cycling and energy flow. These fungi also form symbiotic relationships with aquatic plants and algae, aiding in nutrient uptake and survival. Some freshwater fungi are pathogens, impacting aquatic plants and animals, while others help control populations within ecosystems. Adapted to both flowing (lotic) and still (lentic) water, they show a variety of morphological and physiological adaptations that allow them to thrive in diverse aquatic environments. Freshwater fungi are also important as bioindicators, providing insights into water quality and ecosystem health. Overall, they are key contributors to ecosystem function, biodiversity, and the balance of freshwater habitats (Gessner et al., 2007; Krauss et al., 2011; Jones et al., 2014; Kuehn, 2016).

Sordariomycetes is the largest class of lignicolous freshwater fungi in Ascomycota, containing approximately 823 species and 298 genera (Luo et al., 2019; Calabon et al., 2022; Shen et al., 2022). In this study, three new freshwater species belonging to the subclasses Hypocreomycetidae and Soryellomycetidae (Sordariomycetes) are introduced from Hunan and Yunnan provinces, China. The three new species, *C. yunnanensis*, *Pa. hunanensis*, and *Pl. yunnanensis*, are found inhabiting the freshwater habitats, both lotic and lentic. *C. yunnanensis* was collected in a lake (lentic) and a freshwater stream (lotic), *Pa. hunanensis* in a freshwater stream (lotic), and *Pl. yunnanensis* from a lake (lentic). These three species were isolated from submerged decaying branch and wood, indicating their roles as decomposers degrading organic matter in nutrient cycling. It is notable that species of *Chaetopsina*, *Parafuscosporella*, and *Pleurotheciella* are commonly known from aquatic habitats. However, many species such as *Chaetopsina guyanensis*, *C. saulensis*, and *Pl. dimorphospora* have also been found in terrestrial habitats (Lechat and Fournier, 2019; Boonmee et al., 2021). Hence, we speculate the species in genera *Chaetopsina* and *Pleurotheciella* tend to inhabit a wide range of environments, including submerged freshwater or wet terrestrial environments. However, all species of *Parafuscosporella* have so far been found in aquatic environments.

Similar to Tsui et al. (2016), the three new species—*C. yunnanensis*, *Pa. hunanensis*, and *Pl. yunnanensis*—may belong to the group of aquatic–terrestrial hyphomycetes (mitosporic ascomycetes) that are initially found growing on decaying plant material and capable of sporulation underwater. The freshwater fungi in this group such as *Canalisporium*, *Dactylaria*, *Dictyochaeta*, and *Sporoschisma*, were typically distinguished based on the features of their conidia, conidiophores, and the process of conidiogenesis (Tsui et al., 2016). Since *C. yunnanensis*, *Pa. hunanensis*, and *Pl. yunnanensis* exhibit characteristics similar to those described in aquatic–terrestrial hyphomycetes, they should fit well into this group. Their ability to grow in aquatic environments, along with their possible role in decaying plant material (e.g., branches, leaves, and wood), aligns with the ecological

TABLE 4 Distribution, habitats, and hosts of *Pleurotheciella* species.

Species name	Host	Habitats	Distribution	Reference
<i>Pleurotheciella aquatica</i>	Submerged wood	Freshwater	Dulong River, Yunnan, China	Luo et al., 2018
<i>Pleurotheciella centenaria</i>	Submerged wood	Freshwater	Ontario, Canada	Réblová et al., 2012
<i>Pleurotheciella dimorphospora</i>	Dead wood	Terrestrial	Kunming, Yunnan, China	Boonmee et al., 2021
<i>Pleurotheciella erumpens</i>	Submerged wood	Freshwater	Ariège, France Asturias, Spain	Réblová et al., 2020
<i>Pleurotheciella fusiformis</i>	Submerged wood	Freshwater	Dulong River, Yunnan, China	Luo et al., 2018
<i>Pleurotheciella ganzhouensis</i>	Submerged wood	Freshwater	Ganzhou, Jiangxi, China	He et al., 2024
<i>Pleurotheciella guttulata</i>	Submerged wood	Freshwater	Qiubei, Yunnan, China	Luo et al., 2018
<i>Pleurotheciella irregularis</i>	Submerged wood	Freshwater	Nanchang, Jiangxi, China	He et al., 2024
<i>Pleurotheciella krabiensis</i>	Submerged wood	Freshwater	Krabi, Thailand	Hyde et al., 2018
<i>Pleurotheciella lunata</i>	Submerged wood	Freshwater	Dulong River, Yunnan, China	Luo et al., 2018
<i>Pleurotheciella nilotica</i>	Submerged wood	Freshwater	Sohag, Egypt. Nakhon Phanom, Thailand Qiubei, Yunnan, China	Abdel-Aziz et al., 2020 Shi et al., 2021
<i>Pleurotheciella rivularia</i>	Submerged wood	Freshwater	Ariège, France	Réblová et al., 2012
<i>Pleurotheciella saprophytica</i>	Submerged wood	Freshwater	Dulong River, Yunnan, China	Luo et al., 2018
<i>Pleurotheciella submersa</i>	Submerged wood	Freshwater	Dulong River, Yunnan, China	Luo et al., 2018
<i>Pleurotheciella sympodia</i>	Submerged wood	Freshwater	Nakhon Phanom, Thailand Chiang Mai, Thailand Phayao, Thailand	Shi et al., 2021
<i>Pleurotheciella tropica</i>	Submerged wood	Freshwater	Phang Nga, Thailand	Hyde et al., 2018
<i>Pleurotheciella uniseptata</i>	Submerged wood	Freshwater	Ontario, Canada Dulong River, Yunnan, China	Réblová et al., 2016
<i>Pleurotheciella verrucosa</i>	Submerged wood	Freshwater	Jian, Jiangxi, China	He et al., 2024
<i>Pleurotheciella yunnanensis</i>	Submerged wood	Freshwater	Fuxian Lake and Yangzonghai Lake, Yunnan, China	This study

The new species is indicated by black bold.

characteristics described for this group of fungi. However, further studies would be required to confirm their precise classification within the group, especially considering conidial morphology and conidiogeneses, as noted by Tsui et al. (2016).

Some freshwater fungi exhibit morphological and ecological specificity (e.g., aquatic spore morphology, attachment structures, growth form adaptation, and spore release strategies), which may display different morphological traits depending on their growth environment, and these traits are often linked to factors such as habitat type, nutrient sources, and interactions with other organisms (Suberkropp, 2011; Naranjo-Ortiz and Gabaldón, 2019). In this study, the three new species, *C. yunnanensis*, *Pa. hunanensis*, and *Pl. yunnanensis*, do not exhibit specific morphological and ecological adaptations to lotic and lentic environments, although detailed studies on their precise adaptations in these habitats are limited. The special morphological characteristics may inconspicuously occur in these three new species, which allow them to adapt to an aquatic environment. However, these three new three species were explored from decaying plant material submerged in freshwater

lakes and streams. For instance, most species in *Parafuscosporella* possess a jelly-like cover on sporodochia or hyaline appendages at the base of conidia. *Pa. hunanensis* may produce a jelly-like cover; however, this characteristic may disappear during the shifting from the collection site to the laboratory, or this species may generally occur as saprobe in terrestrial and produce some ecological functions that allow its adaptation during its submerged in aquatic environments.

Nonetheless, we can infer potential adaptations based on their general ecological characteristics and roles in aquatic ecosystems. *C. yunnanensis*, *Pa. hunanensis*, and *Pl. yunnanensis* may exhibit morphological traits suited to their respective aquatic environments. In lotic systems, where water flow is continuous, these species may develop stronger attachment structures on their spores or hyphae, which help them remain anchored to substrates such as decaying wood or aquatic plants. In contrast, species in lentic environments, where the water is still, may not require such robust attachment features but may have adaptations for better spore dispersal in stagnant water. Additionally, species in flowing water (lotic systems) may possess conidia with characteristics that

TABLE 5 Morphological characteristics of *Pleurotheciella* species (asexual morphs).

Species name	Conidiophores	Conidiogenous cells	Conidia	References
<i>Pleurotheciella aquatica</i>	28–46 × 4–5 μm, cylindrical, dark brown at below half, pale brown to hyaline at above half, straight or slightly sinuous, with a terminal node of denticles	Polyblastic, integrated, terminal, cylindrical or tapering toward tip, hyaline to subhyaline	15.5–17.5 × 3–4 μm, hyaline, broadly lunate to suballantoid, obtuse and tapering at both ends, 0–3-septate, mostly 1-septate with an inconspicuous central septum	Luo et al., 2018
<i>Pleurotheciella dimorphospora</i>	On substrate: 9–15(–21) × 2–4 μm, macronematous, mononematous, subhyaline to brown, septate, unbranched to branched <i>In vitro</i> : Type I: 4.5–6 × 2.5–4 μm, semi-macronematous or macronematous, mononematous, cylindrical, hyaline to brown, straight or flexuous, septate, unbranched Type II: reduced to conidiogenous cells	Holoblastic, terminal, integrated Holoblastic, terminal or intercalary, integrated, brown 6–8 × 2–3 mm, holoblastic, terminal, hyaline, ellipsoidal, aseptate, unbranched	(16.5–)20–27(–32) × (15–)20–25(–28) mm, dark brown to black, muriform, variedly shaped, ellipsoidal, subglobose, or irregular in shape, sectoried, inconspicuously septate 20–29 × 14–19 mm, brown, muriform, varied in shape, subglobose to cordiform, or irregular in shape, with a protuberant hilum 8.5–10.8 × 4–5 mm, hyaline, ellipsoidal, 0–1-septate, 2-guttulate, smooth-walled	Boonmee et al., 2021
<i>Pleurotheciella fusiformis</i>	34–40 × 2.5–3.5 μm, hyaline, erect, arising directly on substrate, usually with a terminal node of denticles, or sometimes extending through the original node with a new extension of the conidiophore	Polyblastic, integrated, terminal, cylindrical or tapering toward tip, sympodial extended, denticulate, with conspicuous denticles, hyaline to subhyaline near base, hyaline toward apex	16–18 × 3–4 μm, hyaline, broadly lunate to suballantoid, obtuse and tapering at both ends, 0–1-septate with an inconspicuous central septum, often with 2 large guttules in each cell	Luo et al., 2018
<i>Pleurotheciella ganzhouensis</i>	9.9–41.9 × 2.2–3.7 μm, macronematous, mononematous, cylindrical, erect or slightly curved, the top slightly swollen with denticulate conidiogenous loci	Polyblastic, integrated, terminal, hyaline, cylindrical, or verrucose, forming conidia sympodially on cylindrical denticles	14.4–19.4 × 2.5–3.3 μm, hyaline, capsule-shaped, fusiform, cylindrical or subclavate, guttulate, round and tapering at both ends, one end is usually sharper, 1-septate	He et al., 2024
<i>Pleurotheciella guttulate</i>	188–310 × 3–4 μm, cylindrical, hyaline, straight or slightly sinuous, arising directly on substrate, with a terminal node of denticles	Polyblastic, integrated, terminal, hyaline, cylindrical or tapering toward tip, sympodial	17–19 × 4–5 μm, hyaline to grayish, subcylindrical, fusiform to slightly obovoid, rounded at both ends, aseptate, smooth-walled	Luo et al., 2018
<i>Pleurotheciella irregularis</i>	50–110 × 2.6–3.5 μm, macronematous, mononematous, hyaline, cylindrical, mostly tapering toward the apex, mostly curved, verrucous, irregular, some with a terminal node of denticles, with verruca in the middle and upper parts	Polyblastic, integrated, terminal, hyaline, cylindrical, or verrucose, forming conidia sympodially on cylindrical denticles	24.2–33.9 × 4.2–6.4 μm, hyaline, narrowly fusiform, subclavate, guttulate, straight or slightly arcuate, pointed at one end the other round and wider in the middle, 1–3-septate, slightly constricted at the septum	He et al., 2024
<i>Pleurotheciella krabiensis</i>	240–390 × 3.3–4.8 μm, brown, septate, becoming paler toward the apex, straight or slightly curved, splaying out at the apex	Polyblastic, sympodial, pale brown or hyaline, cylindrical or tapering apex, denticulate, denticles conspicuously cylindrical	19–25 × 4.5–6 μm, hyaline, fusiform, subcylindrical to obovoid, or subclavate, 1-septate, often guttulate, straight or slightly curved, obtuse at the apex, pointed at the base	Hyde et al., 2018
<i>Pleurotheciella lunata</i>	26–44 × 3.5–4.5 μm, cylindrical, dark brown at the base, pale brown to grayish toward the apex, straight or slightly sinuous, 2–7-septate, arising directly on substrate, with a terminal node of denticles	Polyblastic, integrated, terminal, subhyaline to hyaline, cylindrical or tapering toward tip, sympodial extended, denticulate, with conspicuous denticles	13–23 × 3–4 μm, hyaline to whitish gray, Broad lunate, rounded at the apex, obtuse and tapering at the base, 1-septate with an inconspicuous central septum	Luo et al., 2018
<i>Pleurotheciella nilotica</i>	23–110 × 3–4 μm, cylindrical, dark brown at the base, pale brown to grayish toward the apex, straight or slightly curved, unbranched, 1–8-septate, with a limited, terminal node of denticles, thick and smooth-walled, arising from thick, brown to dark brown hyphae (3–4 μm wide), similar to conidiophores and running parallel to the substrate surface	2–5 × 2.5–3.5 μm, polyblastic, integrated, terminal, cylindrical or tapering toward tip, pale-brown to subhyaline near base, hyaline toward apex, smooth-walled, forming conidia sympodially on conspicuous denticles	8–13 × 2–4 μm, clavate, lunate, hyaline, 1-septate with inconspicuous central septum, rounded at the apex, obtuse and tapering toward the base, smooth-walled, often with one or two guttules in each cell	Abdel-Aziz et al., 2020
<i>Pleurotheciella rivularia</i>	9.5–20 × 2.5–3(–3.5) μm, sub-hyaline, hyphae, often reduced to the conidiogenous cells, 1–2-septate, unbranched to sparingly branched	1.5 × 1 μm, integrated, cylindrical to ampulliform, subhyaline to hyaline,	12.5–16.5(–17.5) × 4.5–5 μm, ellipsoidal to obovoidal, 0–1–2-septate, not or slightly constricted at the septa, individually hyaline to subhyaline, subhyaline in mass, smooth,	Rěblová et al., 2012

(Continued)

TABLE 5 Continued

Species name	Conidiophores	Conidiogenous cells	Conidia	References
		elongating sympodially, with 1–2 denticles	thin-walled, solitary, rounded at the apical end, tapering toward the truncate base; subglobose conidia (8–12 × 6.5–8 μm), sometimes occur	
<i>Pleurotheciella saprophytica</i>	44–52 × 3–4 μm, cylindrical, dark brown at the base, pale brown to grayish toward the apex, straight or sinuous, arising directly on substrate	Polyblastic, integrated, terminal or intercalary, hyaline, cylindrical or tapering toward tip, sympodial extended, denticulate, with conspicuous denticles	10–14 × 2.5–3.5 μm, hyaline, subcylindrical to obovoid, rounded at the apex, obtuse and tapering toward base, 1-septate with an inconspicuous central septum	Luo et al., 2018
<i>Pleurotheciella submersa</i>	113–146 × 4.5–5.5 μm, erect, cylindrical, dark brown at the base, pale brown to grayish toward the apex unbranched, mostly 7-septate, arising directly on substrate	Polyblastic, integrated, terminal, cylindrical, sympodial extended, denticulate, with conspicuous denticles	25–28 × 5.5–6.5 μm, hyaline, subcylindrical, slightly curved, rounded at the apex, obtuse and tapering toward base, aseptate, often with 4 large guttules	Luo et al., 2018
<i>Pleurotheciella sympodia</i>	135–355 × 1.5–3.5 μm, synnemata erect, mid brown to dark brown, brown rigid, velvety, smooth, conidiophores splaying out or divergent at the apical part	Polyblastic, terminal, integrated, subhyaline to pale brown, cylindrical or subulate, smooth, denticulate, with several tiny sympodial denticles	22.5–29 × 4.5–6.5 μm, hyaline, clavate, straight or slightly curved, rounded at apex, tapering toward base, 1-septate, guttulate	Shi et al., 2021
<i>Pleurotheciella tropica</i>	100–250 × 4–4.8 μm, erect, dark brown at the base, becoming paler toward the apex, cylindrical, straight or slightly curved	Polyblastic, integrated, terminal, pale brown to hyaline, cylindrical, forming conidia sympodially on cylindrical denticles	16–21 × 5.5–7 μm, hyaline, narrowly obovoid or subclavate, guttulate, straight, obtuse at the apex, pointed at the base, 1-septate	Hyde et al., 2018
<i>Pleurotheciella uniseptata</i>	126–174 × 3.5–5.5 μm, straight or sinuous, cylindrical, dark brown at the base, becoming paler upward, smooth-walled or slightly granular or roughened, arising directly on substrate, usually with a terminal node of denticles, but rarely extending through the original node with a new extension of the conidiophore	Polyblastic, integrated, terminal, pale brown to subhyaline near base, hyaline toward apex, cylindrical or tapering toward tip, smooth-walled or slightly granular, forming conidia sympodially on conspicuous denticles	12.5–15.5 × 3.5–4.5 μm, hyaline to grayish, fusoid or slightly clavate, straight, rounded at the apex, obtuse and tapering toward base, 1-septate with an inconspicuous central septum, often with 1–2 large guttules in each cell	Luo et al., 2018
<i>Pleurotheciella verrucosa</i>	51.3–131.8 × 1.9–3.4 μm, macronematous, mononematous, dark brown at the base, becoming paler toward the apex, cylindrical, erect or slightly curved, slightly swollen at the base	Polyblastic, integrated, terminal, pale brown to hyaline, cylindrical or verrucous, forming conidia sympodially on cylindrical denticles or wart	10.2–16.9 × 2.3–4.3 μm, hyaline, narrowly fusiform, meniscus or subclavate, guttulate, pointed at one end, the other round and wide in the middle, 1-septate	He et al., 2024
<i>Pleurotheciella yunnanensis</i>	On substrate: Difficult to distinguish on host, semi-macronematous or macronematous, mononematous, sub-hyaline to brown, erect on host surface <i>In vitro</i> : Type I: 30–50 × 3–5 μm, semi-macronematous or macronematous, mononematous, hyaline to dark brown, cylindrical, septate, unbranched Type II: reduced to conidiogenous cells.	3–5 × 3–6 mm, holoblastic, hyaline, raised from hyphae, terminal or intermediate Holo- to polyblastic, terminal, lateral, or intercalary, brown 3–8 × 2–6 mm, phialidic, terminal, integrated, with minute denticles, subhyaline to pale brown, 1–2-septate, unbranched, arising in pseudochains	18–25 × 22–30 mm, varied in shape, ellipsoidal to subglobose, dark brown to black, initially forming phragmoconidia and becoming muriform, chairoid at maturity 15–22 × 12–15 mm, phragmosporous to muriform, variedly shaped, brown to dark brown, subglobose to cordiform, or irregular in shape, with a protuberant hilum, phragmoconidia comprise 2–3-septate, dictyoconidia comprise 1–2 transverse and longitudinal septa, sectoried, leaf clover-like, brown to dark brown 7–12 × 3–5 mm, hyaline, ellipsoidal, 0–1-septate, guttulate, smooth-walled	This study

The new species is indicated by black bold.

allow them to float or travel further, optimizing dispersal in currents. Conversely, in still water (lentic systems), these fungi may produce denser spores or develop morphology that enhances their survival and growth on submerged wood and organic matter, as they would be more reliant on localized nutrient sources (Kuehn, 2016).

The three new species may play crucial roles in nutrient cycling, particularly in wood degradation. In both lotic and lentic environments, they contribute to the breakdown of organic matter such as decomposing wood, which is an important source of nutrients for other organisms in the ecosystem. Their activity in wood degradation releases essential nutrients like nitrogen, carbon, and phosphorus back into the ecosystem, influencing nutrient cycling and supporting a wide range of aquatic life (Gulis et al., 2006, 2008). Moreover, the three new species may interact with other aquatic organisms, including bacteria and invertebrates, through their decomposition activities. The release of small organic molecules during wood degradation can attract microbial communities, facilitating a rich nutrient web. These interactions are crucial for maintaining the health and balance of aquatic ecosystems. The ecological relevance of these fungi in nutrient cycling and wood degradation in lotic and lentic systems is profound. In lotic environments, where constant water movement aids the dispersal of spores, the fungi can contribute to nutrient cycling over large areas, potentially impacting the entire aquatic food web. In lentic environments, they may play a more localized role but still significantly contribute to the recycling of nutrients trapped in stagnant water and organic matter. While studies on these species-specific interactions with aquatic organisms are limited, it is likely that their roles in wood degradation and nutrient cycling are shared across many freshwater fungi.

The species of *Chaetopsina* are distributed in different climate zones in various countries such as China (*C. fulva* as *C. beijingensis* and *C. hongkongensis*) (Goh and Hyde, 1997; Crous et al., 2014) Egypt (*C. aquatica*) (Bakhit and Abdel-Aziz, 2021), France (*C. guyanensis*, *C. pnagiana*, and *C. saulensis*) (Lechat and Fournier, 2019, 2020), Thailand (*C. penicillata*) (Bao et al., 2023), and South Africa (*C. gautengina*) (Crous et al., 2020). This genus has been reported from both freshwater and terrestrial habitats. For instance, *C. penicillata* was introduced by Samuels (1985) from terrestrial habitats in Ecuador, Jamaica, and New Zealand. Bao et al. (2023) reported *C. penicillata* from freshwater habitats in China. Through our study on *C. yunnanensis*, we noticed the *Chaetopsina* species can be segregated by their morphological characteristics, such as ascumata dimensions and perithecial wall anatomy, size, shape, and ornamentation of ascospores and conidia of the asexual morph. Furthermore, the morphological traits of our isolates closely resemble those attributed to *Chaetopsina*. These include red-pigmented erect conidiophores producing unicellular, cylindrical to cylindro-fusoid or ellipsoidal conidia. Phylogenetic analyses of a combined ITS and LSU sequence data indicated that our isolates *C. yunnanensis* (KUNCC23-12940 and KUNCC23-13014) are grouped with the species of *Chaetopsina*, forming a distinct lineage that is basal to *C. pinicola* (CPC 21819). Therefore, considering both the morphological comparisons and the

phylogenetic analyses, we proposed the establishment of a new species, *C. yunnanensis*, in this study.

Meanwhile, we noticed that *C. beijingensis* has not been formally synonymized under *C. fulva* so far. While describing a new aquatic species, *C. aquatica*, from River Nile in Egypt, Bakhit and Abdel-Aziz (2021), quoting Lechat and Fournier (2019), referred that *C. beijingensis* is a synonym of *C. fulva*, the type species of the genus. Lechat and Fournier (2019) described two new species of *Chaetopsina* from Saül (French Guiana) and noted that *C. beijingensis* is similar to *C. fulva*, both morphologically and phylogenetically but did not taxonomically synonymize these taxa. In the present study, the phylogenetic analyses coupled with the comparison of their polymorphism demonstrated that *C. beijingensis* is conspecific with *C. fulva*, concurring with previous studies (Lechat and Fournier, 2019; Bakhit and Abdel-Aziz, 2021). Hence, *C. beijingensis* is formally synonymized under *C. fulva* herein.

Only ITS and LSU sequence data are available for most species of *Chaetopsina*, of which the ITS gene is currently remarkable as the sufficient phylogenetic marker in delineating the interspecific status of *Chaetopsina* (Lechat and Fournier, 2019, 2020; Bakhit and Abdel-Aziz, 2021). However, many sufficient phylogenetic markers derived from the protein-coding genes were recommended for resolving a better phylogenetic resolution of the family Nectriaceae where *Chaetopsina* does belong as well as the class Sordariomycetes [e.g., the ATP citrate lyase (*ACL1*), α -actin (*ACT*), β -tubulin (*TUB2*), calmodulin (*CMDA*), histone H3 (*HIS3*), the RNA polymerase II largest subunit (*RPB1*), the RNA polymerase II second largest subunit (*RPB2*), and translation elongation factor 1 alpha (*TEF1- α*)] (Lombard et al., 2015; Perera et al., 2023). Of these, sequences of the protein-coding genes are available for few *Chaetopsina* species such as *C. acutispora*, *C. fulva* (type species), and *C. penicillata* (Lombard et al., 2015). The limitation on molecular data of *Chaetopsina* can cause taxonomic ambiguities. We, therefore, recommend utilizing multigene phylogeny for resolving taxonomic ambiguities and also providing a better taxonomic resolution on *Chaetopsina*, expanding to Nectriaceae as well as the class Sordariomycetes, of which the sufficient phylogenetic markers based on the protein-coding genes of *Chaetopsina* should be derived.

In our studies on *Pa. hunanensis*, we noticed that the species of *Parafuscosporella* are morphologically indistinguishable. Hence, it was found necessary to understand their taxonomic boundaries using multigene phylogenetic markers. Phylogenetic affinities of species in *Parafuscosporella* have been delineated by ITS, LSU, and SSU phylogenetic markers, of which ITS region is currently recommended as a measurable gene for resolving phylogenetic relationships among species in *Parafuscosporella* (Boonmee et al., 2021; Boonyuen et al., 2021; Li et al., 2023). Nevertheless, the *RPB2* gene has always been utilized for delineating taxa in Fuscosporellaceae (Fuscosporellales) where *Parafuscosporella* accommodated, with other related orders such as Conioscyphales, Pleurotheciales, and Savoryellales in Savoryellomycetidae (Boonyuen et al., 2016; Yang et al., 2016, 2020; Wang et al., 2023). Unfortunately, the *RPB2* gene is only available for *Pa.*

garethii, *Pa. pyriformis*, and *Pa. xishuangbannaensis* in the total of the known species. This may cause the insufficient phylogenetic resolution on *Parafuscospora* correlated with the closely related genera in the family Fuscosporellaceae, extending to the correlation with taxa among the closely related orders in Sordariomycetidae. Thus, the *RPB2* gene is recommended in further clarifying species levels of *Parafuscospora* corresponding with other closely related genera in Fuscosporellaceae.

The confusion between *Parafuscospora* and *Fuscospora* was also discussed in a previous study (Yang et al., 2016). *Fuscospora* is morphologically similar to *Parafuscospora* but differs in the structure of conidia, which are produced in culture. *Fuscospora* produces multi-celled, filamentous to helicoid conidia, while *Parafuscospora* produces globose to obpyriform, uni-septate conidia in culture. The same situation exists for *Conioscypha* in that the species of *Conioscypha* are largely indistinguishable in morphology. Li et al. (2024) proposed to use the potential of phylogenetic markers to clarify their phylogenetic relationships. The single gene trees of *Conioscypha* (ITS, LSU, SSU, and *RPB2*) and combined sequence datasets (LSU-ITS, LSU-ITS-SSU, and LSU-ITS-*RPB2*) were previously obtained to compare the reliable phylogenetic markers. The results of these prior analyses demonstrated that analysis of the *RPB2* gene could provide a better phylogenetic resolution of *Conioscypha*. Therefore, the *RPB2* gene is recommended as a genetic marker for resolving phylogenetic relationships among species in *Conioscypha* (Li et al., 2024). Simultaneously, the protein-coding genes such as *TEF1-α* and *RPB2* have been recommended for clarifying the taxonomic ambiguities of various genera in Sordariomycetes due to their high utility in resolving phylogenetic relationships at multiple taxonomic levels. *TEF1-α* and *RPB2* are relatively conserved within Sordariomycetes but also exhibit enough variability to distinguish closely related species or genera. Their nucleotide sequences evolve at rates suitable for both deep and shallow phylogenetic analyses, making them useful for resolving both inter- and intra-generic relationships (Matheny et al., 2007; Lücking et al., 2020, 2021). Therefore, it is concluded that the protein-coding genes, especially the *RPB2* gene region, are necessary for resolving the taxonomic ambiguities of some genera in Sordariomycetidae as well as Sordariomycetes, and hence, *TEF1-α* and *RPB2* should be derived for novel taxa in further study.

It is worth noting that Goh et al. (2023) synonymized *Parafuscospora* under *Vanakripa* due to the morphological features of *V. gigaspora*, the type of *Vanakripa*, resembling *Parafuscospora*. Based on the principle of nomenclatural priority, all nine *Parafuscospora* species were transferred to *Vanakripa* (Goh et al., 2023). However, *V. gigaspora* as the type species of *Vanakripa* lacks molecular data to confirm their phylogenetic placement. Hence, this leads the taxonomic confusion on the phylogenetic placement of *Vanakripa*. *Vanakripa* was introduced by Bhat and Kendrick (1993), with *V. gigaspora* as the type species. The genus was considered as genus *incertae sedis* in Pezizomycotina (Wijayawardene et al., 2021), while a key to species of the genus was provided by Arias et al. (2008). *Vanakripa* has a special clavate to vermiform, hyaline, separating cells attached to the conidia (Bhat and Kendrick, 1993). The genus

currently accommodated a few species lacking genetic sequences. In Goh et al. (2023), phylogenetic analyses demonstrated that *Vanakripa* formed separated clades relating to *Conioscypha* and *Parafuscospora*. Two new species that were identified as *V. oblonga* and *V. taiwanensis* by Goh et al. (2023) formed a clade with *Parafuscospora* in Fuscosporellaceae, whereas *V. chiangmaiensis* and *V. minutiellipsoidea* are related to *Conioscypha* in Conioscyphaceae. Goh et al. (2023) re-circumscribed morphological features of *Vanakripa* compared to *Parafuscospora*. Based on conidial ontogeny resemblance, Goh et al. (2023) demonstrated that *Parafuscospora* is congeneric with *Vanakripa*. Therefore, Goh et al. (2023) transferred all *Parafuscospora* to *Vanakripa* without providing phylogenetic evidence from type studies.

In accordance with the morphological trait, many genera in Fuscosporellaceae and Conioscyphaceae are morphologically somewhat similar; however, these genera can be distinguished based on multigene phylogeny of sufficient genes. Unfortunately, *Vanakripa* formed polyphyletic clades in Conioscyphales and Fuscosporellales (Goh et al., 2023). Considering the morphology of *Vanakripa* species, Goh et al. (2023) excluded *V. chiangmaiensis* and *V. minutiellipsoidea* (clade in Conioscyphales) and some other described species from *Vanakripa* (viz., *Vanakripa chinensis*, *Vanakripa ellipsoidea*, *Vanakripa fasciata*, *Vanakripa inflata*, *Vanakripa menglaensis*, *Vanakripa parva*, and *Vanakripa rhizophorae*) due to their conidial morphology being different from *V. gigaspora*, the type species of *Vanakripa*, in producing ellipsoidal or broadly obovoid, one-celled conidia. In contrast, *V. oblonga*, *V. taiwanensis* (clade as basal of *Parafuscospora* in Fuscosporellales in the present study), and other synonymized *Parafuscospora* species were treated as *Vanakripa sensu stricto* due to the conidial morphology resemblance in producing septate, apiosporous, versicolored conidia, with or without the presence of a hyaline appendage at the conidial base (Goh et al., 2023). With this point of view, it is reasonable to segregate *Vanakripa* into two morphological groups. However, molecular data of *V. gigaspora*, the type species of *Vanakripa*, have not yet been derived, and/or the epitype has not yet been designated, leading to the phylogenetic uncertainty for *Vanakripa*. Hence, the treatment of *Vanakripa* in different orders may cause taxonomic confusion in *Vanakripa*, corresponding with *Parafuscospora*. In the present study, we, therefore, tentatively place *Parafuscospora* as a distinct genus from *Vanakripa* to avoid taxonomic confusion until the type strain of *V. gigaspora* is derived from molecular data and/or the epitype is designated for clarifying its phylogenetic affinity. We also believe that caution of synonymization should be maintained at this stage. However, if future studies could confirm that *Parafuscospora* and *Vanakripa* are congeneric based on morphological and phylogenetic evidence, this would greatly advance the taxonomic discussion of the Fuscosporellaceae. We recognize that any changes in classification should take into account the importance of nomenclatural stability, ensuring that the naming and classification system of the family remains consistent and carefully considered within the scientific community.

It is notable that 17 of the total 18 species of *Pleurotheciella* were collected from freshwater habitats, and only *Pl. dimorphospora* is

from the terrestrial environment. Furthermore, most species have been found in China and Thailand, as well as in Canada, Egypt, France, and Spain (Table 4). In addition, *Pl. dimorphospora* is dimorphic (with two types of conidial morphology), which can be well distinguished from other species in *Pleurotheciella* (Boonmee et al., 2021). Initially, the original authors felt that the two types of conidial morphology *in vitro* were caused by contamination in the culture. However, through single spore isolation and molecular work on both types of conidia, they confirmed that these two types of conidia are of the same species (Boonmee et al., 2021). It is interesting that our study also meets the same situation with the new species, *Pl. yunnanensis*, showcasing two-type conidial morphology *in vitro*.

Although in recent times freshwater fungi have been continuously discovered, compared to soil ecosystems, these fungi still lacked sufficient spatial and temporal resolution, especially from those environments falling in various latitudinal zones, ecosystems (such as the water column and sediments), snowclad mountains, and extreme environments like deep-sea vents (Grossart et al., 2019). Yunnan has emerged as a hotspot for lignicolous freshwater fungal research since 2015, resulting in the discovery of a number of new species and new records in some extremely varied genera such as *Acrogenospora*, *Dictyosporium*, *Distoseptispora*, *Pleurotheciella*, *Sporidesmium*, and *Sporoschisma* (Bao et al., 2020; Wan et al., 2021; Shen et al., 2022). In contrast, the lignicolous freshwater fungi in Hunan Province still remained understudied. Hyde et al. (2016) discussed the impacts of riparian vegetation, water pollution, sampling methods, and global warming on the diversity of lignicolous freshwater fungi. However, the diversity, quantitative abundance, and ecological functions of freshwater fungi, particularly their interactions with other microorganisms, remain largely speculative, unexplored, and overlooked in the current understanding of aquatic ecology and biogeochemistry (Haraldsson et al., 2018; Grossart et al., 2019). The three new species explored in the present study from freshwater lakes and streams in Hunan and Yunnan provinces will add to the species number of lignicolous freshwater fungi in China and upgrade the global species numbers of freshwater fungi.

Additionally, genomic studies have revealed that freshwater fungi contain a vast diversity of secondary metabolite pathways (El-Elimat et al., 2021). However, the genes and gene clusters involved in these metabolic processes, as well as the secondary metabolite products, remain mostly unknown (Chiang et al., 2009; El-Elimat et al., 2021). Over the last 30 years, several freshwater fungi have been subjected to chemical investigations, resulting in the isolation of 283 secondary metabolites of wide chemical diversity and a broad range of biological activities (Wang et al., 2008; Hernández-Carlos and Gamboa-Angulo, 2011; Canto et al., 2022; El-Elimat et al., 2021). El-Elimat et al. (2021) reviewed the secondary metabolites of freshwater fungi and summarized those compounds as mainly belonging to alkaloids, terpenes, polyketides, phenylpropanoids and peptides, and some unclassified secondary metabolites. Dong et al. (2009) isolated two novel naphthalene-containing compounds, colelomycerones A and B, and three known metabolites from the culture broth of an unidentified freshwater fungus YMF 1.01029, and those metabolites showed noticeable

antifungal and antibacterial activities. Prabhu et al. (2018) found Greensporone C, a secondary metabolite from freshwater fungi inducing mitochondrial-mediated apoptotic cell death in leukemic cell lines. de Souza et al. (2023) investigated communities of 154 culturable freshwater fungi from Antarctic lakes and the capabilities of all cultured fungi to produce various extracellular enzymes at low temperatures and found that the most widely produced enzymes were proteases and pectinases. These active enzymes produced by freshwater fungi have various applications in biotechnological processes in industries including textile, pharmaceutical, food, detergent, and paper, as well as in bioremediation of environmental pollutants (Raghukumar, 2008; Fathima et al., 2022; de Souza et al., 2023). Unfortunately, the study on secondary metabolites of *Chaetopsina*, *Parafuscospora*, and *Pleurotheciella* is unexplored. Therefore, further studies on secondary metabolites of freshwater fungi in *Chaetopsina*, *Parafuscospora*, and *Pleurotheciella* will be most rewarding.

The limitations of the current study are essential for providing a balanced perspective and guiding future research. One of the key challenges encountered in this study was the difficulty in morphological differentiation of some freshwater fungal species, particularly given the high morphological plasticity within certain genera. Additionally, our sampling efforts were constrained by limited geographic coverage and sampling time, which may have affected the comprehensiveness of the data. Furthermore, genetic sequencing efforts were limited by the availability of high-quality DNA samples from all species, which constrained the depth of phylogenetic analyses. These limitations underscore the need for more extensive and targeted genetic studies, such as the use of multigene sequencing or the application of environmental DNA (eDNA) sampling techniques, which could help capture a broader diversity of fungi in various aquatic ecosystems. Future research should prioritize these approaches to better understand the full scope of freshwater fungal biodiversity and address the unresolved taxonomic and ecological questions raised in this study.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/genbank/> ITS: OQ860234, OQ860233, OR230704, PP744554, OR234682, PP095384; LSU: PP151255, PP151256, PP744555, PP744556, PP095383, PP095381; SSU: PP744557, PP744558, PP095382, PP095385; RPB2: PP131261, PP131262. The final alignment and phylogenetic tree were registered in TreeBASE under the submission IDs: 31916 (*C. yunnanensis*), 31910 (*Pa. hunanensis*) and 31135 (*Pl. yunnanensis*) (<http://www.treebase.org/> accessed on 25 December 2024).

Author contributions

LL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources,

Writing – original draft, Writing – review & editing. DB: Investigation, Methodology, Writing – review & editing. H-BJ: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. J-FL: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. TD: Funding acquisition, Writing – review & editing. FS: Project administration, Writing – review & editing. SH: Project administration, Writing – review & editing. RC: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. RP: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY

Sinang Hongsan,an,
Shenzhen University, China

REVIEWED BY

Rajesh Jeewon,
University of Mauritius, Mauritius
Ajay Kumar Gautam,
Abhilashi University Mandi, India

*CORRESPONDENCE

Alan J. L. Phillips

✉ alan.jl.phillips@gmail.com

Chao Liu

✉ Liuchao_80@163.com

†These authors have contributed equally to this work

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Species evolution: cryptic species and phenotypic noise with a particular focus on fungal systematics

Anusha H. Ekanayaka^{1,2†}, Samantha C. Karunarathna^{1,3†}, Saowaluck Tibpromma¹, Arun Kumar Dutta⁴, Danushka Sandaruwan Tennakoon⁵, Anuruddha Karunarathna⁶, Ekachai Chukeytirote⁷, Dong-Qin Dai¹, Steven L. Stephenson⁸, Sajeewa S. N. Maharachchikumbura⁹, Chao Liu^{1*} and Alan J. L. Phillips^{10*}

¹Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biology and Food Engineering, Qujing Normal University, Qujing, China, ²Department of Urban Bioresources, Faculty of Urban and Aquatic Bioresources, University of Sri Jayewardenepura, Nugegoda, Sri Lanka, ³National Institute of Fundamental Studies (NIFS), Kandy, Sri Lanka, ⁴Molecular & Applied Mycology Laboratory, Department of Botany, Gauhati University, Guwahati, Assam, India, ⁵Guangdong Provincial Key Laboratory for Plant Epigenetics, Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen, China, ⁶Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand, ⁷School of Science, Mae Fah Luang University, Chiang Rai, Thailand, ⁸Department of Biological Sciences, University of Arkansas, Fayetteville, AR, United States, ⁹School of Life Science and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu, China, ¹⁰Universidade de Lisboa, Faculdade de Ciências, Biosystems and Integrative Sciences Institute (BioISI), Lisbon, Portugal

The evolution of a species can be understood in the context of two major concepts—the cryptic species concept and the phenotypic noise concept. The former represents morphologically indistinguishable but genetically distinct evolutionary lineages, while the latter represents the phenotypic variations of an isogenic population. Although the concept of cryptic species currently represents a general topic, its effect on other aspects of biology, such as biodiversity, ecology, evolutionary biology, and taxonomy, is still unclear. In particular, cryptic species cause complications and prevent the development of a clear taxonomy. The phenotypic noise concept or phenotypic plasticity generally refers to the various expressions of phenotypes in different environments. Hence, the cryptic species concept refers to genetic variations, while the phenotypic noises concept is about non-genetic variations. Although both concepts are opposites, they each contribute significantly to the evolutionary process of an organism. Despite the extensive research studies and publications discussing those two concepts in separate accounts, a concise account that combines and compares both concepts are generally lacking. Nevertheless, these are essential to understand the evolutionary process clearly. This review addresses the available literature on this topic, intending to provide a general and overall discussion on both the cryptic species concept and

the phenotypic noise concept and their effect on evolution, ecology, biodiversity, and taxonomy with a special focus on fungal systematics. hence, several fungal case studies representing the two concepts are presented, compared, and discussed for a better understanding.

KEYWORDS

biodiversity, ecology, evolution, phylogeny, taxonomy

1 Introduction

Evolution occurs when a group of organisms undergoes gradual changes in their properties over generations. The changes in genome, physiology, or morphology make the particular group of organisms in a population different from their recent ancestors, causing those individuals in question To achieve reproductive isolation from other individuals within the population who share a particular ecological niche, maintaining the ability to interbreed (Mayr, 1982; Aldhebiani, 2018). This process is referred to as speciation. This evolutionary process or speciation event results from a complex series of circumstances that directly connect with the species concept. The species circumscription and delimitation are based on two major concepts—the cryptic species concept and the phenotypic noise concept.

The matter of delineating species has been compounded by a fundamental challenge related to what constitutes a species. This challenge arises from divergent perspectives among biologists regarding the definition of species. Over the past fifty years or so, various factions within the biological community have advocated for distinct and sometimes conflicting species concepts, as documented by (Mayden, 1997; De Queiroz, 1998; Harrison, 1998). Among them, Mayden (1997) enumerated 24 distinct species concepts with numerous additional alternative definitions. A definition here refers to a concise depiction of a concept, meaning that a single species concept may be associated with multiple definitions that vary in minor linguistic nuances. Many of these concepts and their corresponding definitions are incompatible, often leading to disparate conclusions regarding the delineation and enumeration of species. Consequently, the conundrum of species concepts, which is related to the discord of the current theoretical concept of species, is intricately bounded by the challenge of determining species' boundaries and quantities based on empirical evidence (De Queiroz, 2007).

More commonly discussed species concepts include morphological, biological, evolutionary, and phylogenetic concepts (Taylor et al., 2000). Among them, the biological and evolutionary species concepts are widely used. Currently, there are conflicts among these terms. A biological species refers to inter-fertile populations reproductively isolated from other such groups and occupying a distinct ecological niche (Mayr, 1982). An

evolutionary species refers to a single lineage of ancestor-descendant populations with a unique evolutionary history and the ability to maintain its identity from other such lineages. It also fits into its ecological niche (Simpson, 1961; Wiley, 1981; Paris et al., 1989). However, there is a congruence between the theoretical and operational species concepts. The operational species concept surpasses the theoretical one in prevalence, which causes conflicts in other related areas of evolution, ecology, biodiversity, and taxonomy (Mayden, 1997; Taylor et al., 2000). Hence, a better understanding of the causes for the congruence of the theoretical and operational species concepts is essential.

As the evolutionary species concept defines a species as a single lineage of ancestor-descendent populations, the theoretical species concept was primarily considered the evolutionary species concept (Taylor et al., 2000). However, current understandings of evolutionary consequences deviate from this primary idea about the theoretical species concept. With the slow process of evolution, specific individuals of a biological species acquire minor changes in their genotype. At a specific stage of this evolution, the newly changed individuals become non-fertile with the original population, and they thus represent a distinct evolutionary lineage (Paris et al., 1989; Struck and Cerca De Oliveira, 2019). However, both groups (original and newly changed) can still be morphologically indistinguishable (Struck and Cerca De Oliveira, 2019). This is the stage at which a cryptic species arises. It is supposed that as time passes, those two populations diverge gradually in their morphology and physiology to be suited to unique ecological conditions (Wiley, 1981; Paris et al., 1989; Struck and Cerca De Oliveira, 2019). Although most authors adopt the evolutionary species concept, they often recognize and classify species based only on distinctive phenotypic characteristics. Afterward, the classification followed also becomes morphologically based, the basis upon which the taxa can be easily identified. Generally, the evolutionary or phylogenetic species concept is superior to morphology-based and biological species concepts. Therefore, the morphological species concept is neither an evolutionary nor a biological species concept. As a result, a conflict arises between theory and practice (Paris et al., 1989; Struck and Cerca De Oliveira, 2019). Despite everything, the morphological species concept has become a more significant inference to the biological or evolutionary species concepts and

the foundation for the cryptic species concept (Dobzhansky, 1982; Aldhebiani, 2018; Struck and Cerca De Oliveira, 2019). On the other hand, the morphological species concept is strongly influenced by ecological and environmental conditions. Therefore, gene expression can vary with the environment and is reflected in phenotype variations. Most importantly, even if the phenotype changes, the genotype is still the same (West-Eberhard, 2008; Levin, 2013; de Vienne, 2021).

The cryptic species concept also called the sibling species concept, describes two or several species that are indistinguishable in their phenotypes but differ in their genotypes (Zúniga-Reinoso and Benítez, 2015; Korshunova et al., 2019). Hence, in a morphological concept, they have been classified as a single nominal species (Zúniga-Reinoso and Benítez, 2015). Phenotypes of organisms within an isogenic population are not identical since they exhibit some degree of difference/variation from each other (Kaneko and Furusawa, 2008). Phenotypic noise refers to these variations in a phenotype developed by an individual genotype; it is also referred to as phenotypic plasticity (West-Eberhard, 2008). The environment and history of an organism influence the development of the variability in the cellular phenotype while the genotype is still unchanged (Raser and O'shea, 2005). As such, a single species can be given different names simply because of its phenotypic plasticity in different habitats. Both concepts are contradictory to one another and have created confusion in modern taxonomy. Both have significantly influenced other related research areas, including evolutionary biology, ecology, and biodiversity. Since morphology is a contentious marker in taxonomy, it hides cryptic species that share certain phenotypic characteristics but are otherwise distinct. As a result of the presence of cryptic species or the inability to recognize existent species, actual biodiversity levels are underestimated or overestimated (Lefebure et al., 2006; Padial et al., 2010).

Although cryptic species are more or less morphologically indistinguishable, their genotypes are significantly different (Wei et al., 2021). Therefore, molecular phylogenetic tools are the only fast, reliable, and efficient methods to discover species and face the taxonomic problems caused by cryptic species (Jörger and Schrödl, 2013; Korshunova et al., 2019). This is again applicable to the phenotypic noise concept. Molecular phylogenetic tools clearly recognize the different phenotypes influenced by the environment of the same species.

The concept of cryptic species dates back about three centuries, and the first record was provided by Derham in 1718 (*in Ray and Willughby, 1718*). Later authors have defined cryptic species in different ways. The first explicit definition for cryptic species was “population systems which were believed to belong to the same species until genetic evidence shows the existence of isolating mechanisms separating them” (Stebbins, 1950). Korshunova et al. (2019) recently defined cryptic species as “species which manifest low morphological but considerable genetic disparity”. Considering several definitions for cryptic species (Stebbins, 1950; Paris et al., 1989; Zúniga-Reinoso and Benítez, 2015; Korshunova et al., 2019; Struck and Cerca De Oliveira, 2019), they are considered to represent distinct evolutionary lineages and are reproductively isolated; however, morphologically indistinguishable, they have historically been misinterpreted as members of a single species.

The discussions on phenotypic noise began with the origin of the term “phenotype”. The first clear definition of phenotype was “All types of organisms, distinguishable by direct inspection or only by finer methods of measuring or description” (Johannsen, 1909). The general consideration among scientists is that the phenotype refers to the observable characteristics in an individual and reflects the genotype of an organism resulting from the expression of genes. The concept of phenotypic noise describes how the environment exerts an effect on gene expression and then how phenotypes vary based on environmental conditions (West-Eberhard, 2008; Levin, 2013). Phenotypic noise is also defined as the environmental sensitivity of a genotype (West-Eberhard, 2008).

One of the earliest examples of cryptic species is the genus *Drosophila*, which includes fruit flies (Dobzhansky and Epling, 1944; Dobzhansky, 1951). The two species *D. pseudoobscura* and *D. persimilis* were originally treated as one species. Later, in laboratory cultures, researchers observed these two species were reproductively isolated. Further studies identified and confirmed their differences in morphology (in wings and male genitalia), physiology, behavior, and chromosome morphology (Dobzhansky and Epling, 1944; Dobzhansky, 1951). Another example of cryptic species is provided by Adams et al. (2014), who explored the hyper-cryptic species complexes of Australian freshwater fish collections based on allozyme, mtDNA, and morphological data. They found that these species, across their broad geographic range, revealed a 1500% increase in species-level biodiversity, including 15 distinct species of phenotypically similar minnows (*Phoxinus* spp, a small freshwater fish) from different lakes (Adams et al., 2014). Investigations of *Phoxinus* communities of adjacent freshwater ecosystems of the westernmost part of the Po River basin in Italy, based on mitochondrial cytochrome oxidase I (COI) sequences from 239 specimens, revealed a complex of species of *Phoxinus*, consisting of *P. septimaniae*, *P. csikii*, and *P. lmaireul*, which are morphologically indistinct (De Santis et al., 2021). In another example, phylogenetic analyses of 37 samples from 13 taxa of the Asian forked fern genus (*Dicranopteris*) from five chloroplast gene regions (rbcL, atpB, rps4, matK, and trnL-trnF) showed that *D. linearis* is polyphyletic, which suggests that there is cryptic diversity within the species. Further comparisons erected the new species *Dicranopteris austrosinensis* and *D. baliensis* (Wei et al., 2021). Peintner et al. (2019) identified and analyzed *Fomes* strains from different habitats in Italy and Austria using rDNA ITS region phylogenetics. These findings proved the existence of subclades within the *Fomes fomentarius* clade and led to the formal recognition of the new species *Fomes inzengae* (Peintner et al., 2019). Researchers analyzed 96 Japanese specimens of the *Hypholoma fasciculare* complex, a group of common wood-decomposing fungi, using mitochondrial ribosomal RNA (mt-rRNA) sequences, nuclear ITS region, and 24 single-copy genes. The results showed that the *H. fasciculare* complex encompassed two species, *H. fasciculare* and *H. subviride* (Sato et al., 2020).

The phenotypic noise concept has been discussed at length in several research papers (Raser and O'shea, 2005; West-Eberhard, 2008; Levin, 2013; de Vienne, 2021). Some interesting studies on phenotypic noise include the identification of intraspecific phenotypic variation and morphological divergence of *Folsomia*

candida by Tully and Potapov (2015) and phenotype microarray profiling of *Staphylococcus aureus* by von Eiff et al. (2006). Compared with the cryptic species concept, the phenotypic noise concept lacks detailed studies. However, many confusing points remain on these topics. Moreover, the combined effects of the cryptic species concept and the phenotypic noise concept on other fields, such as taxonomy, evolution, ecology, and biodiversity require further studies. Hence, the present study reviews and discusses the influence of the cryptic species concept and the phenotypic noise concept on taxonomy, evolution, ecology, and biodiversity research. Future research trends related to these concepts are summarized and discussed.

Further, fungi are versatile and reflect their diversity in morphology, ecology, physiology, and phylogeny. Fungi rank as the second most diverse group of organisms in terms of species, followed by insects (Purvis and Hector, 2000; Antonelli, 2023). Among fungi, a remarkable range of morphological diversity exists, spanning from single-celled yeasts to substantial 'fruiting bodies' that can generate trillions of spores (Stajich et al., 2009). Numerous fungi's developmental phases occur within or on intricate substrates like soil, wood, plants, or animals, rendering them challenging to observe. Consequently, developmental stages and true ecological diversity of most fungi remain undiscovered (Rodriguez et al., 2004). Both fungi that produce large reproductive structures (e.g., mushrooms and truffles) and those that make no reproductive structures apart from the meiotic cells themselves (e.g., yeasts) show such complex reproductive models (Stajich et al., 2010). The most intricate formations within the fungal realm are the multicellular sexual fruiting bodies, characterized by well-defined fungal tissues and various cell types (Kües et al., 2018). Nevertheless, unicellular yeasts show huge complexities in their life cycles (Yurkov et al., 2015). They also produce numerous bioactive molecules, which are helpful for various research areas such as agriculture, industrial, and pharmaceuticals (Raja et al., 2017). The estimated number of fungal species on Earth was approximately 12 million (ranging from 11.7 to 13.2 million), a significant increase from the previous estimate of 2.2 to 3.8 million species obtained through various estimation techniques. A team of fungal experts recently assessed the fungal diversity in the world using four main academic pathways viz. scaling laws; fungus: plant ratios; actual versus previously known number of species; and DNA-based studies; according to them, there are likely to be 2–3 million species of fungi, with a best estimate of 2.5 million (Antonelli, 2023). On the other hand, molecular phylogeny has revealed the existence of numerous cryptic species within this diverse fungal kingdom (Wu et al., 2019). However, recent studies revealed that fungal species circumscription and delimitation are a considerable challenge (Raja et al., 2017; Baturo-Ciesniewska et al., 2020; Stengel et al., 2022). Specifically, fungal species delimitation is complicated by cryptic species (Crespo and Lumbsch, 2010; Shivas and Cai, 2012; Peintner et al., 2019; Sato et al., 2020; Wang et al., 2022). Phenotypic noises or plasticity also cause controversies incorrect species identification in fungi (Lehner, 2010; Behm and Kiers, 2014). Hence, this review is also expected to improve the identification of fungal species and aid in developing future fungal research applications.

2 Application of the concepts in fungal systems

To review the available literature related to cryptic species and phenotypic noises concepts, various sources such as scholarly papers, digital databases, and personal communications were used. Data on the cryptic species concept and the phenotypic noise concept were collected primarily from "Google Scholar," "ResearchGate," "PubMed," and "Web of Science." The keywords such as species concept, cryptic species, phenotypic noises, biological, phenotype, genotype, ecology, biodiversity, and evolution were used in different combinations to derive references for the review. The derived literature was reviewed and analyzed to understand the fundamentals of cryptic species and phenotypic noise concepts and an in-depth literature review was done to determine their effect on biodiversity, ecology, evolutionary biology, and taxonomy.

Several suitable examples to reflect the occurrence of cryptic species and phenotypic noises within species circumscription and delimitation, were obtained from recently published literature with the approval of the original authors (Thambugala et al., 2015; De Silva et al., 2020). Authors re-examined a few specimens to confirm their morphological characteristics viz. *Marasmius imitarius* specimen was loaned from the Calcutta University herbarium (CUH), *Rhytidhysterion neorufulum* specimens were obtained from the Mae Fah Luang University herbarium (MFLU), and *Misturatosphaeria aurantiacinotata* from the National Museums of Kenya/East African Herbarium (EA). The specimens of *Rhytidhysterion neorufulum* and *Misturatosphaeria aurantiacinotata* were initially examined with a Motic SMZ 168 stereomicroscope (Motic Asia, Kowloon, Hong Kong), and further observations were made with a Nikon ECLIPSE 80i compound microscope (Nikon Instruments Inc., Melville, New York, USA) with photographs recorded with a Canon 550D digital camera (Canon Inc., Ota, Tokyo, Japan). Hand-cut sections were mounted in sterile water for study and photographs. Tarosoft (R) Image Frame Work programme (Version: 1.3) was used to make measurements, and Adobe Photoshop CS3 extended version 10.0 was used to process the images used for the figures (Adobe Systems, USA). The specimen of *Marasmius imitarius* was studied with a Dewinter 'crown' trinocular microscope (Dewinter Optical Inc., New Delhi). Photographs were taken with the dedicated camera attached to the microscope.

The phylogenetic trees were reproduced using published sequence data in the NCBI database related to above mentioned examples. For each gene, sequences were aligned using MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) and manually adjusted in BioEdit v. 7.0.4 (Hall, 2004) where necessary. Datasets were concatenated with FaBox (1.41) (Villesen, 2007). Ambiguously aligned regions were excluded and gaps were treated as missing data.

As a model of evolution, the GTR+G+I substitution model was used. Maximum likelihood (ML) phylogenetic analyses were conducted using the RAxML-HP2 Workflow on XSEDE (8.2.9) available through the CIPRES web portal (Miller et al., 2011; Stamatakis, 2014). Each ML tree bootstrap analysis was run using

1000 in-depth replicates with identical parameters. Markov chain Monte Carlo (MCMC) sampling in the CIPRES web portal (Miller et al., 2011) with MrBayes on XSEDE was used to calculate posterior probabilities (PP). Four Markov chains were run in parallel for two million generations, with tree samples taken every thousand generations. The temperature parameter of the MCMC heated chain was fixed at 0.2. Tracer v.1.5 (Rambaut and Drummond, 2009) was used to analyze the log-likelihood score distribution and determine whether more search iterations were necessary to reach convergence. Topologies below the asymptote (the top 80% of the sample) were rejected as part of the burn-in phase, and the remaining trees were used to determine posterior probabilities in the majority rule consensus tree.

2.1 Cryptic species examples

2.1.1 Example - *Marasmius imitarius*

The genus *Marasmius* is cosmopolitan in distribution and can be recognized as those taxa that possess reviving basidiomata with a sulcate or corrugated pileus, a centrally placed cartilaginous stipe, and white basidiospores (Fries, 1835). *Marasmius imitarius* is morphologically very similar to many other species in the same genus (Wannathes et al., 2009). In fact, the specific epithet suggests how phenotypically this species resembles others in the genus. The taxon can be identified morphologically by its light brown sulcate pileus with a darker brown to reddish brown center, its distant (10–12), cream-colored lamellae with brown edges, its clavate basidiospores with a mean range of $17.8\text{--}19.2 \times 4.1\text{--}4.7 \mu\text{m}$, the absence of pleurocystidia and caulocystidia, and the fact that it grows primarily on woody twigs (Wannathes et al., 2009). According to Wannathes et al. (2009), most of these morphological features of *M. imitarius* overlap with other taxa like *M. bambusiniiformis*, *M. mazatecus*, *M. striaepileus*, and *M. sierraleonis*.

To confirm the observation reported by Wannathes et al. (2009), we examined one of the Indian collections of *Marasmius imitarius* (CUH AM078) and *M. bambusiniiformis* (AKD 382/2014, the author's (Arun Kumar Dutta) personal collection; Figure 1). The examination revealed that the taxon *M. imitarius* (CUH AM078) possesses morphological characteristics such as a small (5–17 mm diam.), obtusely conical to convex pileus that matures to convex to plano-convex with a slight central depression, often with an upturned margin showing the faces of brownish orange to reddish orange lamellae with a smooth to minutely pruinose surface; distant ($L = 9\text{--}11$, $l = 0\text{--}1$) white to cream lamellae often with brown edges; fusoid to clavate basidiospores ($14.5\text{--}21.5 \times 3.5\text{--}6.8 \mu\text{m}$); absence of pleurocystidia; *Siccus*-type cheilocystidia with clavate to pyriform main body ($7.5\text{--}14.5 \times 4\text{--}9 \mu\text{m}$) and cylindrical apical setulae ($3.5\text{--}7.5 \mu\text{m}$); a hymeniform pileipellis consisting of *Siccus*-type broom cells with main body measuring $10\text{--}21.5 \times 6.5\text{--}10 \mu\text{m}$, and apical setulae measuring $3.0\text{--}7.0 \mu\text{m}$; absence of caulocystidia; and the presence of clamp-connections in all tissues.

Further observation of *M. bambusiniiformis* (collection no. AKD 382/2014) revealed that *M. bambusiniiformis* consists of features such as a small (5–10 mm diam.), conic to convex or often

applanate pileus, with smooth to slightly pruinose surface colored light brown to brownish yellow with brownish orange center; distant to subdistant ($L = 18\text{--}21$, $l = 0\text{--}1$) cream lamellae; fusoid to clavate basidiospores ($14\text{--}19.5 \times 3.5\text{--}6.0 \mu\text{m}$); absence of pleurocystidia; *Siccus*-type cheilocystidia with clavate to pyriform main body ($11.5\text{--}16 \times 5\text{--}8 \mu\text{m}$) and cylindrical apical setulae ($3.5\text{--}8 \mu\text{m}$); a hymeniform pileipellis consisting of *Siccus*-type broom cells with a main body measuring $12.5\text{--}20 \times 7\text{--}8.5 \mu\text{m}$, and apical setulae measuring $3.5\text{--}8.0 \mu\text{m}$; absence of caulocystidia; and the presence of clamp-connections in all tissues.

Hence, our study on the detailed morphology of both taxa (like *M. imitarius* and *M. bambusiniiformis*) also suggests that differentiating these two taxa based solely on morphological features is insufficient. However, phylogenetic analyses based on the nrITS DNA data of this study (Figure 2) show *M. imitarius* lies far away from *M. bambusiniiformis*, and previous studies also show similar results with this study (Wannathes et al., 2009). In fact, based on phylogenetic analyses, *M. imitarius* is not closely related to *M. bambusiniiformis* (Figure 2).

2.2 Phenotypic noise concept examples

2.2.1 Example 1- morphology variations of *Rhytidhysterion neorufulum*

The hysteriaceous genus *Rhytidhysterion* belongs to the family Hysteriaceae in the Dothideomycetes (Thambugala et al., 2016). These fungi have a worldwide distribution mostly as saprobes, endophytes or weak pathogens on woody plants and rarely as human pathogens (Thambugala et al., 2016; De Silva et al., 2020; Cobos-Villagrán et al., 2021). Species of *Rhytidhysterion* are characterized by having large, conspicuous, elongated, superficial, carbonaceous to coriaceous, navicular ascomata, mostly opening by a longitudinal slit and with thick-walled ascospores (Thambugala et al., 2016). Since these fungi express varied shapes of ascomata depending on the environmental conditions, their ascomata traits are quite fascinating. For instance, their ascomata are disc-shaped (irregular opening/apothecial-shape) under wet conditions, but when dry, they fold at the margin, forming an elongated slit (hysterothecial-shape) (Thambugala et al., 2016; Cobos-Villagrán et al., 2021). In some cases, the same specimen may also contain disc-shaped and hysterothecial-shaped ascomata. For example, we observed the ascoma characteristics of several isolates of *Rhytidhysterion neorufulum* (MFLU 18-2644, MFLU 21-0248 and MFLU 14-0608) and found that all consist of both disc-shaped and hysterothecial-shaped ascomata (Figure 3). Thus, their apothecial or hysterothecial behavior could not be considered as a valid trait for delimitation of species of *Rhytidhysterion* as those differences can be attributed to environmental changes.

We reexamined the ascospore characteristics of three isolates of *R. neorufulum* (MFLU 18-2644, MFLU 21-0248 and MFLU 14-0608) and found that they are highly diverse in their shape, color, and septation (Figure 4). Ascospores can range from ellipsoidal to fusiform, ellipsoid to oblong or narrowly fusiform, and with pointed or obtuse ends (Thambugala et al., 2016; De Silva et al., 2020). Indeed, a single specimen can possess all these shapes (MFLU 18-



FIGURE 1

Field photographs of basidiomata. (A) *Marasmius imitarius* (CUH AM078), (B) *Marasmius bambusiniformis* (author's (Arun Kumar Dutta) personal collections, AKD 382/2014). Scale bars = 10 mm.

2644). Ascospores of *R. neorufulum* have a variety of colors, including hyaline, pale brown, golden brown, and dark brown. The ascospores can be mono-septate, symmetrical, or asymmetrical and constricted at the central septum, while others are 2–3-septate, symmetrical, or asymmetrical and slightly constricted at the central septa. In addition, some ascospores have enlarged second cells from the apex. *Rhytidhysterion neorufulum* has a high phenotypic plasticity of ascospores and ascomata characteristics, and those characteristics may be considered insufficient for delimitation of species of *Rhytidhysterion*. Therefore, species-level delimitation in

Rhytidhysterion is best accomplished using a multi-gene phylogeny (Figure 5) in combination with morphological characteristics.

2.2.2 Morphology variation in *Misturatosphaeria aurantiacinotata*

Misturatosphaeria is a member of the family *Teichosporaceae* in the Dothideomycetes. This genus was introduced by Mugambi and Huhndorf (2009) based on mixed ascospore morphological characteristics of this group. It is undeniably true that ascospores of this genus exhibit high phenotypic plasticity even within the

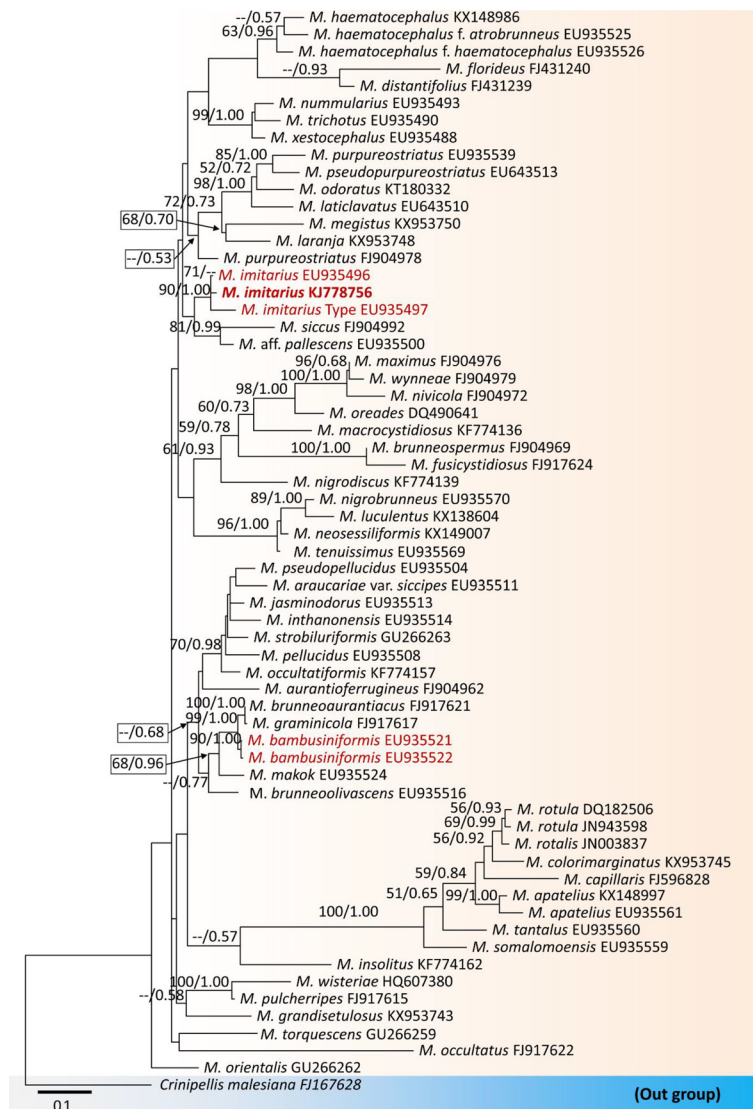


FIGURE 2

Maximum likelihood tree constructed using ITS rDNA sequence data. The tree is rooted with *Crinipellis malesiana*. Morphologically look-a-like taxa, *Marasmius imitarius* and *M. bambusiniiformis* are in red. The collected specimen of *M. imitarius* is in bold. Bootstrap support values $\geq 50\%$ from the maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) values ≥ 0.50 are given above the nodes, respectively. The abbreviation 'M.' represents the genus *Marasmius*.

same species (GKM 1238). For instance, the ascospore characteristics of *Misturatosphaeria aurantiacinctata* vary from hyaline to light brown or dark brown, are 1–3-septate, deeply or slightly constricted at the central septum and some have a mucilaginous sheath while some lack this feature (Figure 6). As such, their ascospores display highly diverse morphological characteristics (Mugambi and Huhndorf, 2009; Thambugala et al., 2015; Tennakoon et al., 2021). Moreover, it is very important to select fruiting bodies of the same level of maturity for morphological examinations, especially with respect to ascospores. Otherwise, there is a possibility of misidentifying a specimen as an entirely different species. In the case of *M. aurantiacinctata*, when only immature ascomata are present on a substrate, it is possible to observe hyaline, 1-septate ascospores that are deeply constricted at the central septum have a distinct

mucilaginous sheath. But in mature ascomata, one can observe light brown to dark brown, 3-septate ascospores that lack a mucilaginous sheath (Figure 6). Thus, multi-gene phylogeny takes on an important role in the classification of species of *Misturatosphaeria aurantiacinctata* (Figure 7).

3 Cryptic species - phenotypic noise and taxonomy

A comprehensive taxonomic framework serves as a cornerstone in many biological research endeavors. It is imperative to thoroughly scrutinize and articulate the specific delineation of species within a taxonomic group, as well as to define its boundaries, to establish a robust taxonomic structure. The

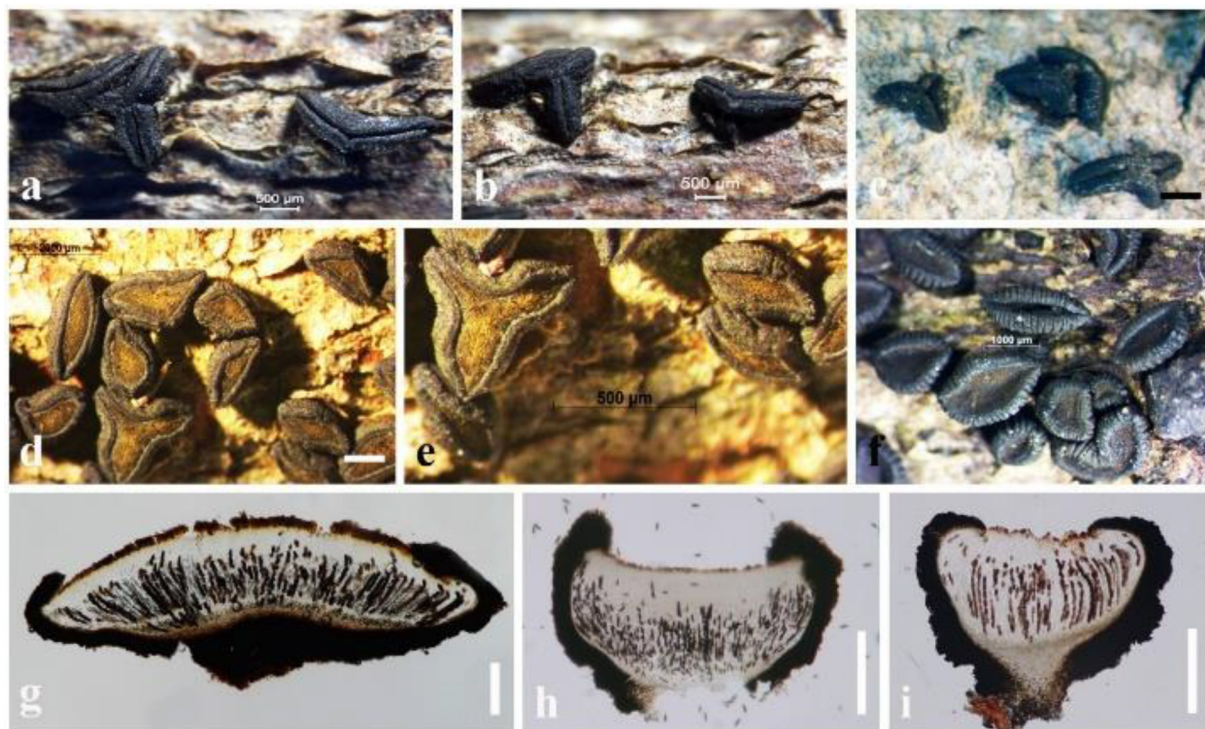


FIGURE 3

Ascomata variation in *Rhytidhysteron neurufulum*. (A, B) Ascomata of MFLU 18-2644. (C) Ascomata of MFLU 21-0248. (D–F) Ascomata of MFLU 14-0608. (G) Vertical section of MFLU 14-0608. (H, I) Vertical section of MFLU 18-2644. Scale bars: (A–C, E) = 500 µm, (D) = 2000 µm, (F) = 1000 µm, (G–I) = 300 µm.

presence of cryptic species and phenotypic noise often contributes to the lack of taxonomic clarity and leads to confusion within the field (Bochner, 2008; Delić et al., 2017). In some cases, the same species has been listed under different taxonomic names; in other cases, different species have been classified under the same name.

Distinct species may be hidden within species complexes because the characteristics expressed by the phenotype are not sufficient or strong enough to classify them as different species even though they are genotypically different and reproductively isolated. Hence, it causes difficulties in setting up species circumscriptions and delimitations. Furthermore, the same species having several taxonomic names results in an inaccurate taxonomic system, leading to errors in taxonomic applications in other research areas.

There are practical difficulties in detailed observations of microorganisms and even microstructures of both macro- and microorganisms; thus, detecting cryptic species by their micromorphology could be more difficult. However, in the case of pseudo-cryptic species, a detailed re-examination of phenotypic data provides better solutions for correctly identifying cryptic species (Lajus et al., 2015).

In some cases, detailed anatomical characteristics are not supportive enough to get a clear taxonomy. Instead, molecular tools provide better comparisons and more effectively reveal cryptic species (Karanovic and Cooper, 2012; Karanovic et al., 2016). The detailed microanatomical re-description for *Pontohedyle verrucosa* was insufficient to reveal reliable characteristics for diagnosing the two major clades identified within the genus. Thus, the molecular

analyses based on four genetic markers (mitochondrial cytochrome c oxidase subunit I, 16S rRNA, nuclear 28S, and 18S rRNA) provided sufficient characterization to discover and formally describe nine cryptic new species (Jörger and Schrödl, 2013).

Moreover, fast-evolving molecular markers are even more effective in revealing cryptic species (Karanovic et al., 2016). For example, the 5'-end of the mitochondrial COI gene has been recommended as the barcoding gene for all species of animals (Hebert et al., 2004). The main benefit of this mtCOI gene is that it has little genetic variation within a species but considerable variation between species (Karanovic et al., 2016). Furthermore, metabarcoding has recently emerged as a prominent tool for species identification. This approach proves particularly valuable when conventional morphological identification of species encounters difficulties, as it hinges on the distinctive genetic information inherent to individual species (Semmouri et al., 2021). Metabarcoding can help distinguish cryptic species by targeting specific barcoding DNA regions (e.g., COI for animals, rbcL for plants, and ITS for fungi). In some cases, phenotypic noise, which includes variation in appearance or phenotype within a single species, can make traditional identification methods less reliable. Metabarcoding can overcome this challenge by focusing on the underlying genetic information, which tends to be more consistent. This helps reduce the impact of phenotypic noise and cryptic species on species identification (De Luca et al., 2021; McInnes et al., 2021; Semmouri et al., 2021). However, when molecular markers are inadequate in clarifying taxonomic uncertainties,

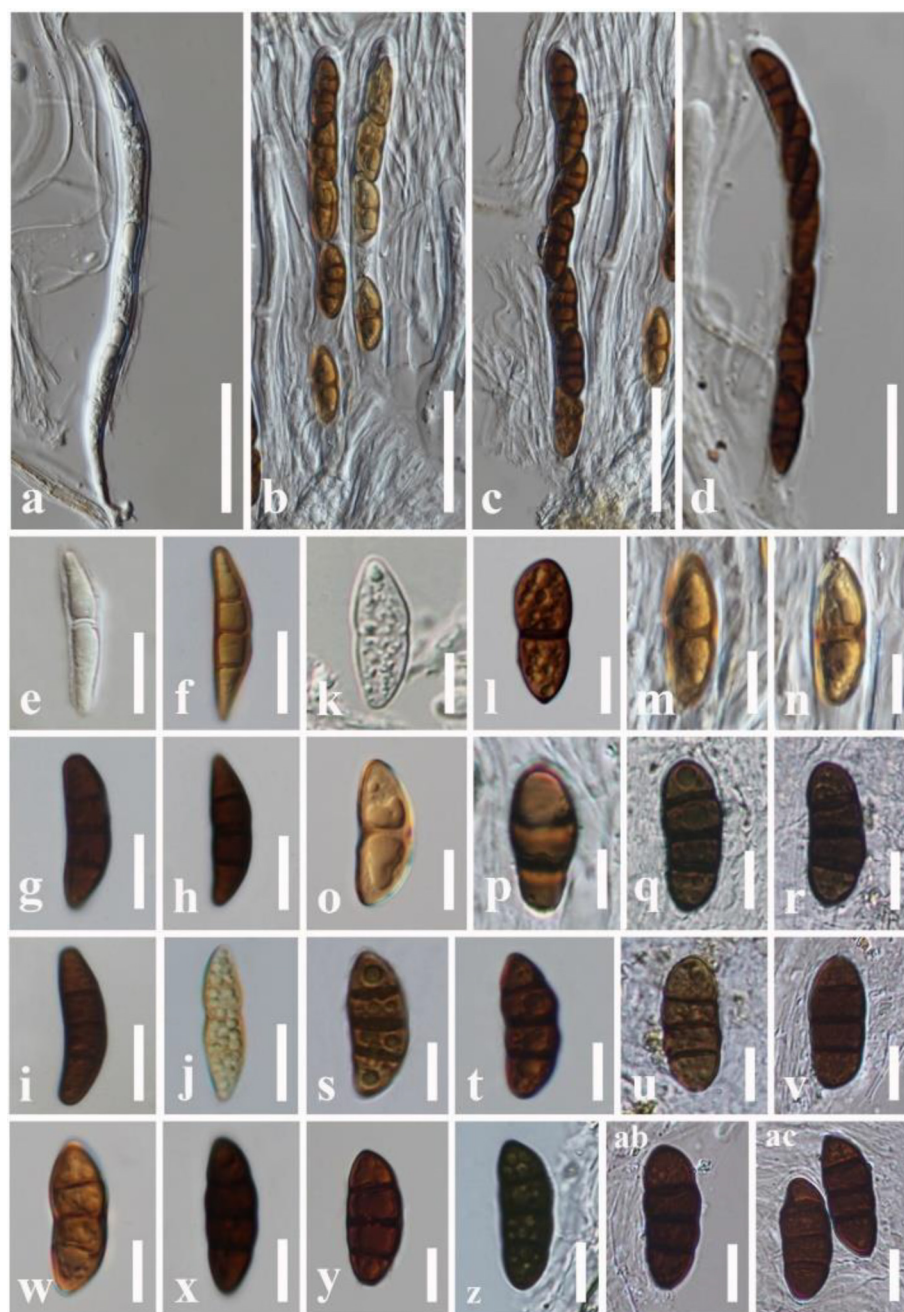


FIGURE 4

Variations in *Rhytidhysterium neurufulum* ascospores. (A, B) Asci of MFLU 18-2644. (C, D) Asci of MFLU 14-0608. (E–J) Ascospores of MFLU 14-0608. (K–R) Ascospores of MFLU 18-2644. (S–AC) Ascospores of MFLU 21-0248. Scale bars: (A–D) = 50 μ m, (E–J) = 15 μ m, (K–AC) = 12 μ m.

whole-genome sequencing emerges as a promising approach to establishing a consistent taxonomy (Thompson et al., 2021; Cai et al., 2023). In bacterial taxonomy, common genetic markers encompass the 16S small subunit ribosomal RNA, a hypervariable region within the genome. Nevertheless, the utility of 16S rRNA genes in *Microcystis* taxonomy has demonstrated their limited informativeness, primarily attributed to the high sequence similarities (>97%) (Harke et al., 2016; Dick et al., 2021). Moreover, while the selected genetic markers may not accurately represent the entire genomic evolutionary landscape, whole-

genome sequencing offers a potential solution for establishing a stable taxonomy (Rodriguez-R et al., 2018; Thompson et al., 2021).

In this context, revisiting old taxonomy with modern tools is important to resolve taxonomic confusion. The recent research by Hongsan et al. (2020) on refining families of the Dothideomycetes expanded information on families in the Dothideomycetidae using new data and molecular tools. Furthermore, the paper provided a refined, updated document on orders and families *incertae sedis* of the Dothideomycetes. When considering phenotypic noise, morphological changes are more

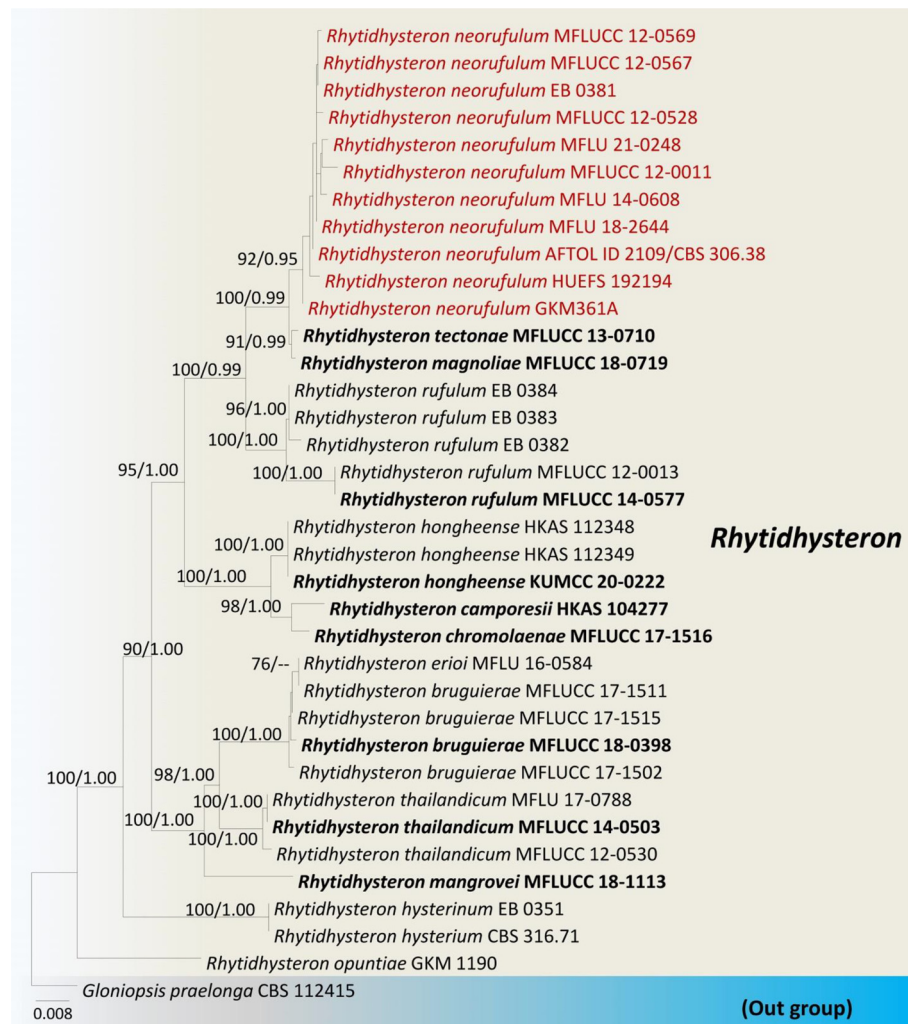


FIGURE 5

Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, ITS and *tef1* sequence data. The tree is rooted with *Gloniopsis praelonga* (CBS112415). The ex-type strains are in bold, and the *Rhytidhysterion neorufulum* isolates are in red. Bootstrap support values $\geq 75\%$ from the maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) values ≥ 0.95 are given above the nodes, respectively.

readily visible in macroorganisms than microorganisms. Therefore, the morphology of macroorganisms can tell a different story than their genotype. However, physiological characteristics are easily detectable in microorganisms, especially bacteria and viruses. As a result, in many group phenotypes (collectively morphology and physiology) express variations independently from genotypes, which can result in a conflicting taxonomy (Dusseux et al., 2018). However, deviations also can be seen in this regard, especially in yeast (Yurkov et al., 2015).

Taxa with large geographic distributions are comprised of cryptic species and phenotypic noise, thus leading to errors in a taxonomic system. For example, many marine taxa have an exceedingly wide geographical distribution (Knowlton, 1993, further discussion in the “Cryptic species–phenotypic noise and biodiversity section”). Hence, marine specimens from widely separated areas are possibly misidentified as a single species but later recognized as a cryptic species complex. Similarly, a huge geographical distribution can lead to phenotypic variations within a

single species, resulting in several different species names being applied. Until those different names for the same species are synonymized, probably following molecular phylogenetic analyses, they confuse taxonomic systems.

Integrated taxonomy (taxonomy based on characters from different sources (e.g., from morphology, molecules, ecology or distribution) plays a major in minimizing the effect of cryptic species and phenotypic noise (Padial and De la Riva, 2010; Jörger and Schrödl, 2013). Furthermore, using discriminate characters, based on their quality and suitability in species delineation, instead of adding more and more data is also important in developing a clear taxonomy. Hence, the taxonomic experts of a particular group are there to guide the respective set(s) of characters that will provide the best backbone for the diagnosis of a specific group (Jörger and Schrödl, 2013).

Although cryptic species are phenotypically similar and occur in most groups, those with a well-studied morphology and that contain specimens of a large size show this phenomenon to a lesser extent (Karanovic et al., 2016). Molecular studies or integrated

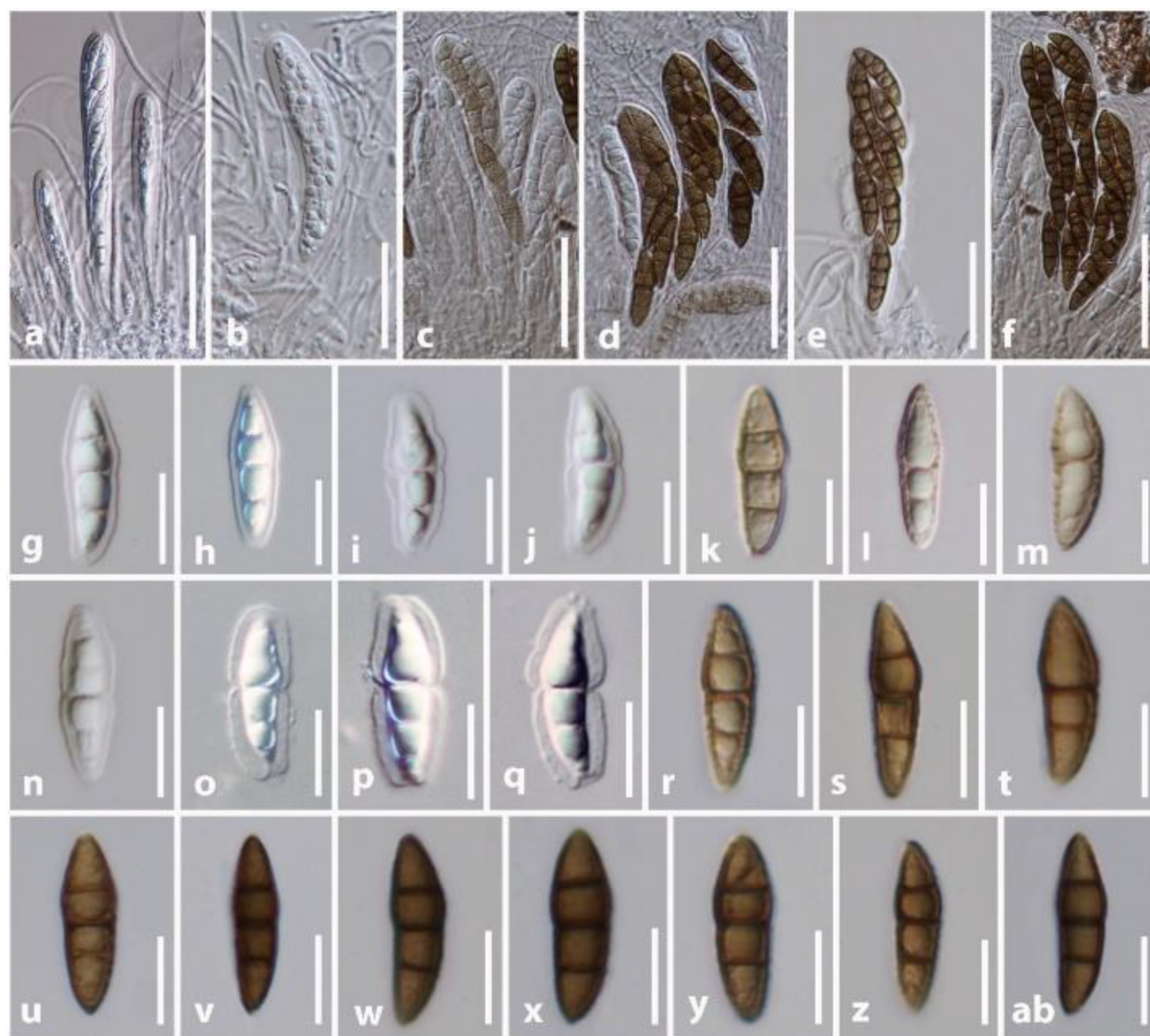


FIGURE 6
Ascospores variation in *Misturatosphaeria aurantiacinotata* (GKM 1238). (A–F) Asci. (G–AB) Ascospores. Scale bars: (A–F) = 50 μ m, (G–AB) = 10 μ m.

taxonomy of morphology and genetics can resolve this situation and incorporate cryptic species into their component species (Karanovic et al., 2016). For example, four possible cryptic species were found after barcoding 260 North American bird species (Hebert et al., 2004). In contrast, numerous cryptic species were found in subterranean faunas based on molecular analyses with deep morphological examinations (Guzik et al., 2011). In the case of phenotypic noise, molecular phylogenetic tools are essential for the identification of species phylogeny and assigning these to their correct taxonomic ranks.

4 Cryptic species - phenotypic noise and evolution

Cryptic species contribute to taxonomic incompleteness, impacting the elucidation of evolutionary events and significantly

influencing the delineation and classification of a given taxon. Moreover, these cryptic species obscure the evolutionary trajectories of traits within a specific lineage, thereby complicating species delineation efforts (Karanovic et al., 2016).

Cryptic species are in different evolutionary states. Some cryptic species are in the transition stage from populations to species. They are evolutionarily young, and their morphological descendants have not yet diverged (Delić et al., 2017). Other cryptic species are reproductively isolated from each other, with strong biological barriers between them. These cryptic species are evolutionarily old, and their morphologies provide delimiting characters when examined in detail (Delić et al., 2017). Both of these cryptic species and their evolutionary states are important to develop well resolved evolutionary relationships.

Stochasticity in gene expression causes phenotypic noise (Raser and O'shea, 2005). Within a cell or an individual organism, gene expression can be affected by either extrinsic or intrinsic factors,

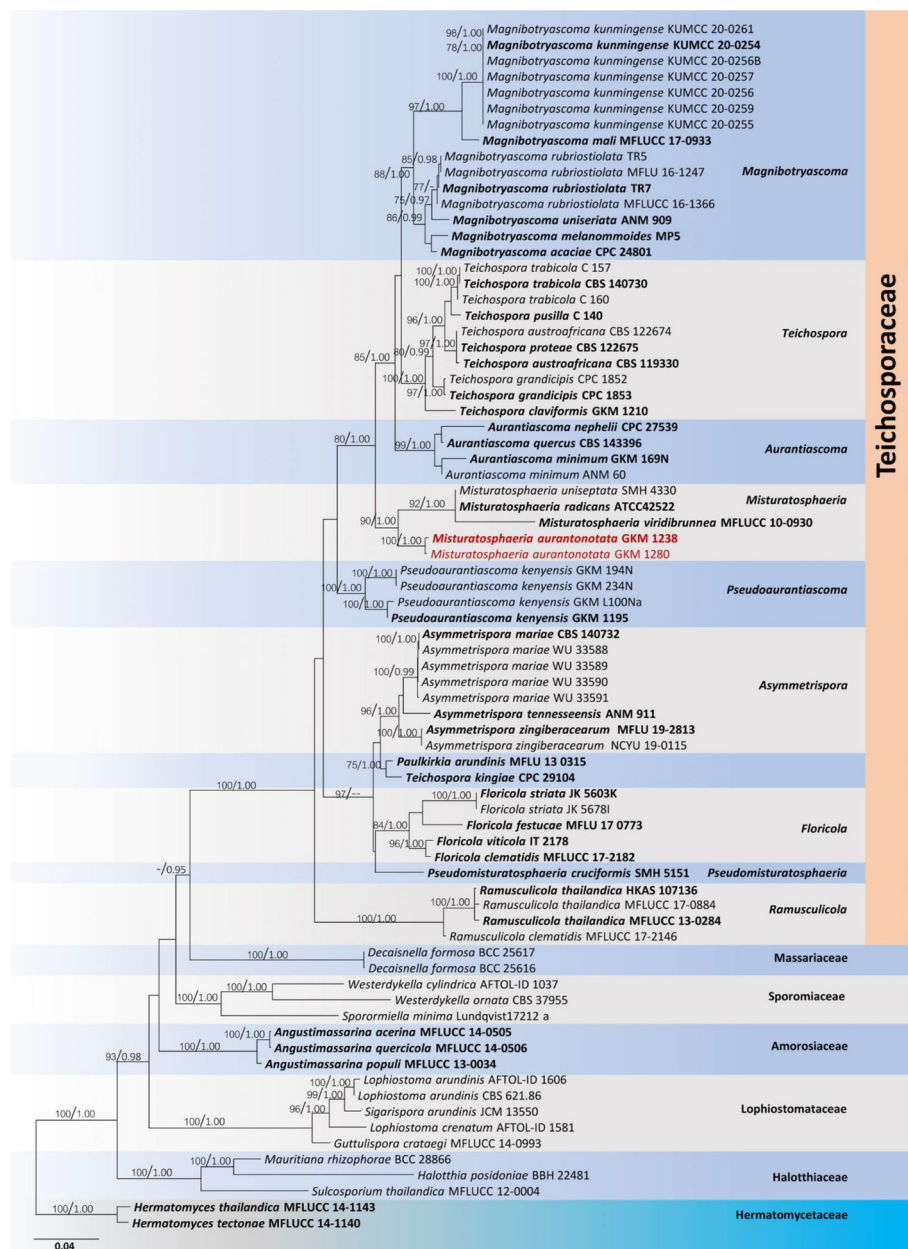


FIGURE 7

Phylogram generated from a maximum likelihood analysis based on combined LSU, SSU, ITS, *tef1* and *rpb2* sequence data. The tree is rooted with *Hermatomyces tectonae* (MFLUCC 14-1140) and *H. thailandica* (MFLUCC 14-1143). The ex-type strains are in bold, and the *Misturatosphaeria aurantiacina* isolates are in red. Bootstrap support values $\geq 75\%$ from the maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) values ≥ 0.95 are given above the nodes, respectively.

resulting in gene expression variations (Kaneko and Furusawa, 2008). Intrinsic factors are cell signaling molecules and minor genetic alterations. Examples for those genetic alterations include such things as haploinsufficiency and epistasis (Deutschbauer et al., 2005; Eckardt, 2008; Wang et al., 2014). These genetic alterations are inheritable and cause significant variations in the phenotype. The extrinsic factors are environmental factors. These factors are not significant enough to cause a particular species to separate into different species as described in the species concept (Raser and O'shea, 2005; Eckardt, 2008; Wang et al., 2014); hence it results fluctuations in phenotypes, which are phenotypic noises. These

phenotypic noises are at sub-species level of a taxonomically described species. They are actually environmentally sensitive subpopulations of the same genotype. Hence, it is assumed that the actual genotype is being shielded by the external environment, which results in a conflict between genotype and phenotype (de Vienne, 2021). Consequently, correct identification of the distinct phenotypic and genotypic state of a particular species is essential for the understanding of its evolutionary relationships.

Moreover, this is the initial stage of the evolution of isogenic populations with phenotypic noise. This is a deviation from the general evolution process based on the inheritance of beneficial

mutations of an organism in a population. Environmentally sensitive subpopulations characterized by phenotypic noise can adapt to a variety of environmental conditions without any genetic base, however with changes in their pattern of gene expression influenced by the environment. This spontaneous adaptation by noise can later be developed to a new environmentally well adapted isogenic population, when the deviation of gene expression level is significant enough to separate them from the main population (Kaneko and Furusawa, 2008).

5 Cryptic species - phenotypic noise and ecology

The physiological characters of a species are even more related to ecology. As such, phenotypic noise species are highly influenced by their environment. Although cryptic species are phenotypically undifferentiated, their ecology provides more possibilities for their discovery. As an example, Peintner et al. (2019) examined the use of ecological factors in resolving cryptic species in the fungal genus *Fomes*, and they concluded that volatile organic compounds serve a better outcome in species delimitation and discovering cryptic species in the future (Peintner et al., 2019). In addition, Bickford et al. (2007) mentioned that cryptic species are more common in insects and reptiles, and they tend to occur more in the tropics than temperate regions.

Cryptic species are common and can be found in any kingdom of the Tree of Life (McGuire, 2018). However, cryptic plant species are comparatively unclear and rare because genetic and molecular tools used to identify cryptic species in other organisms do not really fit with plants (Shneyer and Kotseruba, 2015). Furthermore, more records of cryptic plant species can be determined with more detailed examination of plants and with the improvement of new tools such as nuclear ribosomal DNA sequencing (Okuyama and Kato, 2009; Shneyer and Kotseruba, 2015). Okuyama and Kato (2009) examined the Asian endemic perennial lineage of *Mitella* (*Asimitellaria*; Saxifragaceae) based on nuclear ribosomal DNA sequences and discovered ten distinct biological species, including two new species. A multidisciplinary study, which combined cytogenetic analyses and phylogenetic analyses on two plastid and five nuclear genes, revealed the taxonomic separation of three distinct species in the *Brachypodium distachyon* complex, a model grass for cereals (Catalán et al., 2012). Moreover, cryptic species in plants can be differentiated by the secondary metabolites they produce. These secondary metabolites associated with pest and disease susceptibility, stress tolerance, interactions with soil microbes, attraction of pollinators, and palatability to herbivores or simply the ecological niche of a particular plant species (Shneyer and Kotseruba, 2015).

Gene expression variations associated with non-genetic factors are closely connected with ecology. Hence, phenotypic noise species are adaptive subpopulations to a particular habitat. The long-term ecological influences on those subpopulations or specific phenotypic noise also increase the evolution rate of the population. Moreover, those specific phenotypic noise species bear acquired characters, which can be referred to as adaptations

to their environments. In multicellular organisms, acquired characters would be in germplasm (genotype) or somatic cells (phenotype) (de Vienne, 2021). Somatic cell DNA mutations can impact individuals but not their progeny (Hurle, 2022). However, recent agricultural advances in tissue culture techniques and somatic cell genetics allow the regeneration of plants from cells in culture. In somatic cell genetics, even the changes in somatic cells can be transferred to the next generation (NCBI, 2022).

Agricultural research and ecology-based applications are more affected by cryptic species. One of the best examples is the development of biocontrol agents. The success of a biocontrol agent is based on a solid understanding of the biocontrol agent and the target organism, the details of their life cycle, preferred habitats, and foods (Walter, 2003; McGuire, 2018). The presence of cryptic species within either the pest population or the biocontrol agent population can fail the control method for no apparent reason (Walter, 2003; McGuire, 2018). Distinct species react differently to control methods. Correct identification and a better understanding of host and pathogen species are very important in developing control strategies (Walter, 2003; McGuire, 2018). This applies not only to biological control but also to any of the pathogen control methods affected by cryptic species.

The ecological niche of the organism is very important in discovering cryptic species within pathogenic taxa. More studies on pathogen-host-environment interactions are required to increase the understanding of the ecology of cryptic species (Jung et al., 2017). Also, there is a higher possibility of misleading species identification by phenotypic noise. As the plant host plays a major role in speciation, the physiological characters of the pathogenic organism have also turned out to be species-specific (Peintner et al., 2019). Hence, the same species can have different phenotypes within different hosts. Conversely, host interactions can use as a delimiting character to resolve cryptic species. An example for the host and cryptic species relationship showed up in an analysis of anther smuts (Genus *Antherospora*) on *Muscari* spp. The molecular phylogeny revealed three distinct lineages that were correlated with host plants with slight morphological differences. These lineages were assigned to three cryptic species: *Antherospora hortensis* sp. nov. on *Muscari armeniacum*, *A. muscari-botryoidis* comb. nov. (syn. *Ustilago muscari-botryoidis*) on *M. botryoides*, and *A. vaillantii* s. str. on *M. comosum* and *M. tenuiflorum* (Piątek et al., 2013). Furthermore, new cryptic species of *Teratosphaeria*, which is a serious pathogen of *Eucalyptus* were discovered from the phylogenetic analyses of gene regions of Internal transcribed spacer (ITS), β -tubulin (tub2), and translation elongation factor (tef1) (Andjic et al., 2016). Crespo and Lumbsch (2010) also examined cryptic species on lichens.

Furthermore, species complexes such as *Microcystis* are ecologically much bounded. The taxa within complexes vary across the environmental and seasonal conditions (Frangeul et al., 2008; Cai et al., 2023). Hence, the phenotypic characters become useless in species delimitation, and molecular tools provide promising results. However, the high genome variability of *Microcystis* due to high genome plasticity and horizontal gene transfer causes the use of genetic markers to be inadequate for species delimitation. In such cases, advanced molecular tools such as pangenomics reveal cryptic diversity within those complexes (Cai et al., 2023).

6 Cryptic species - phenotypic noise and biodiversity

The “species concept” or “species level” concept plays a fundamental role in biodiversity (Lefébure et al., 2006). Estimating species numbers is an important criterion for both fundamental and applied biodiversity perspectives. Determining the boundaries of species is essential for species number and diversity estimations (Sites and Marshall, 2003). Although many species boundaries exist, their delimitation has no clear and operational criterion (Lefébure et al., 2006). The presence of cryptic species or failures in detecting existing species results in an underestimation of true levels of biodiversity (Lefébure et al., 2006; Padial et al., 2010). Conversely, the same species can be identified by different names as it has many phenotypic noise species, which falsely increases the number of species.

Moreover, biodiversity conservation has also been disturbed by cryptic species. This is because there are species hidden within cryptic species complexes, and these are often rare, have low populations, are seriously threatened, and thus are highly endangered and require urgent conservation efforts. However, conservation programs for undiscovered and unavailable species cannot be carried out. In addition, the formal naming of cryptic species is essential to enable them to be added to conservation policies and faunal listings (Delić et al., 2017). Recent research on the South European cryptic complex of the subterranean amphipod *Niphargus stygius sensu lato* used uni- and multilocus delineation methods. Molecular analyses showed that the newly discovered species came from several different subgroups within the genus, and that these subgroups coexist while showing no signs of lineage sharing. Those newly discovered cryptic species have increased the number of subterranean endemics in Slovenia by 12 and in Croatia by four previously underestimated species. Moreover, the new taxonomic additions renewed the national Red Lists, as it previously included mostly species with large ranges but omitted critically endangered single-site endemics (Delić et al., 2017).

True cryptic species within a species complex cause huge errors in biodiversity analyses, as discussed above. However, the morphological analyses have considerable limitations in discriminating species and need to be more robust for describing biodiversity at the species level. Therefore, nonmorphological techniques such as genetic analyses and investigations of behavioral, physiological, and other traits must be employed (Lajus et al., 2015). Furthermore, as some studies have included thorough morphological and molecular examinations to detect cryptic species, they do not lead to species descriptions (Pante et al., 2015). This results in an inflation of alpha diversity estimation (Chessman et al., 2007; Morrison et al., 2009; Karanovic et al., 2016). In this context, the phylogenetic species concept is essential in recognizing a far greater number of much less inclusive units. Moreover, the phylogenetic species concept closely examines the same group of organisms, resulting in different species identities, species ranges, and number of individuals (Agapow et al., 2004). Literature reviewing and analyzing organisms that were categorized

under phylogenetic and nonphylogenetic concepts show a significant difference, in analyses based on a phylogenetic species concept showing 48% more species than nonphylogenetic concept analyses (Agapow et al., 2004).

The traditional molecular techniques faced challenges when dealing with extensive sample analyses, which often hindered conducting large-scale biodiversity assessments. As a result, researchers outside the taxonomy field have adopted metabarcoding tools for specimen identification. Metabarcoding also holds promise for tasks such as the detection of new species, the revelation of cryptic species, the identification of non-native or invasive species, and the assessment of taxonomically meaningful variations within species that have broad or geographically scattered distributions (De Luca et al., 2021; McInnes et al., 2021; Semmouri et al., 2021). Marine protists have been perceived as having low diversity and a widespread distribution. However, recent research has revealed that many protist species are, in fact, intricate assemblages of cryptic species, often confined to specific biogeographic regions. Nevertheless, the detection of these cryptic species is frequently hindered by limitations in sampling coverage and the application of methods, such as phylogenetic trees, which are less suited for identifying relatively recent divergence and ongoing gene flow (De Luca et al., 2021). In such scenarios, metabarcoding emerges as an ideal solution. De Luca et al. (2021), successfully unraveled the complexities within the *Chaetoceros curvisetus* (Bacillariophyta) species complex by employing two complementary metabarcoding datasets. Species are diverse and common, especially in marine habitats (Knowlton, 1993). *In situ* observation of marine taxa is difficult. Furthermore, many marine invertebrate taxa require *in situ* detailed observations of live specimens by experts or well preserved and fixed specimens to be properly identified, conditions that are hard to fulfill, especially when it comes to large-scale marine inventories. Moreover, since it is possible to spread larvae/adults in a homogenous sea by ocean currents, marine organisms have a wider geographical distribution than terrestrial ones (Knowlton, 1993; Sundberg et al., 2009). This huge distribution of marine organisms results in errors in identifications and later gives rise to cryptic species complexes. Misidentification of the same species based on its phenotypic noise is even possible. Some taxa are affected by these factors more than others, such as small, soft-bodied, marine invertebrates with few morphological characters useful for taxonomy (Knowlton, 1993; Sundberg et al., 2009). Furthermore, certain organisms remain concealed within vast ecosystems because of their unculturable nature and significantly smaller population sizes (He et al., 2015; Ryberg, 2015). However, studies on fungi inhabiting unique environments have revealed that numerous fungi previously considered ‘unculturable’ can be cultivated on specific substrates and under particular conditions. Additionally, high-throughput amplicon sequencing, shotgun metagenomics, single-cell genomics, and Molecular Operational Taxonomic Units (MOTUs) serve as a valuable means to unveil these hidden organisms and offer a promising tool for delineating species boundaries and quantifying biodiversity (He et al., 2015; Ryberg, 2015; Wu et al., 2019).

7 Discussion

This paper discusses the two major concepts, cryptic species and phenotypic noise, which affect species concepts. The species concept is the basis of taxonomy, evolution, ecology, and biodiversity. As the kingdom of fungi has made many contributions to biotechnology and applied sciences, this review is expected to improve the identification of fungal species and aid in developing future fungal research applications.

Several branches of species concept are currently used, *viz.* morphological species concept, biological species concept, evolutionary species concept, and phylogenetic species concept (Taylor et al., 2000). A better understanding of each concept is essential for investigations on cryptic species and phenotypic noise concepts. Cronquist (1978) adopted the morphological species concept and refers to a community with distinctive morphological characters sufficient to entitle them to a specific name (Aldhebiani, 2018).

The biological species concept applies to a group with a distinct ecological niche that is reproductively isolated (Mayr, 1982), while an evolutionary species refers to a single lineage of ancestor-descendant populations with a unique evolutionary history (Simpson, 1961; Wiley, 1981; Paris et al., 1989). The phylogenetic species concept is outside of cladistic analysis and focuses on possible evolutionary processes contributing to species formation, including biotic and abiotic (even random) factors (Wheeler, 1999). A new concept being proposed is the modern species concept, which is focused on the relationship (phenetic or phylogenetic) between individuals (Aldhebiani, 2018).

However, there is no clear congruence between intraspecific variations and species boundaries since some taxonomic studies and many identifications are still based on morphological characters. This simply results in cryptic species, which are phenotypically similar but genotypically distinct, as well as phenotypic noise from species that are phenotypically distinct but genotypically identical. Hence, the cryptic species represent genetic variations of a population. Non-genetic variations are phenotypic variations that arise from different expressions of a genotype. Phenotypic variations are persuaded by varying environmental cues and categorized into two types: phenotypic plasticity and developmental noise (Saito et al., 2023). Phenotypic plasticity is a common act of a biological population or among individuals of a single species as a response to their recent environment by changing their morphology, physiology, or behavior. This is the foundation of how organisms learn, interact with their environment, and adapt to their environment during their lifetime (Matthey-Doret et al., 2020). Therefore, phenotypic plasticity refers to expressing traits that depend on the environment. However, the traits that change unpredictably in different environments are usually said to be 'noisy' rather than plastic (Gomulkiewicz and Stinchcombe, 2022). Hence, in this article, we are addressing the concept of phenotypic plasticity using the term phenotypic noises.

Nowadays, DNA sequence information has become the only truly reliable factor for species identification. However, morphological data still play a crucial role in taxonomy,

biodiversity, and ecological studies as these data are the primary key to identifying a species, and sometimes there are practical difficulties in obtaining molecular data. Moreover, the concept of phenotypic noise can also result in the occurrence of different individuals or groups of individuals within an isogenic population (Engl, 2019). Hence, using a combination of molecular phylogenetic approaches and morphological and physiological data make a fine-scale taxonomy possible (Jörger and Schrödl, 2013). Moreover, once cryptic species are discovered as a result of the availability of molecular data, there must then be a detailed examination and well documented description of newly discovered taxa using visible external or internal differences to avoid misidentifications in the future.

Most traditional taxonomic studies for some fungal groups, especially those that are unable or difficult to grow on artificial growth media, such as lichenized fungi and discomycetes, are still based on morphological characteristics. Hence, this results in classifying different species under the same taxonomic name. Later, these can be recognized as cryptic species, which causes a major conflict in natural classification systems. Furthermore, setting up evolutionary relationships is difficult with cryptic species. Generally, for most organisms, genetic changes tend to happen slowly than morphological changes (Hanken and Carl, 1996; Carroll, 2005; Hall and Strickberger, 2008). However, when genetic changes happen more rapidly than morphological changes, we see only the phylogenetic differences whereas the morphological changes are not yet discernible. The concept of phenotypic noise results in morphologically and physiologically diverse individuals within isogenic populations (Engl, 2019). Furthermore, Fungi have a unique mode of growth that allows them to adapt quickly to changing environments. Unlike other multicellular organisms (animals and plants), fungi evolved complex multicellularity through filamentous intermediate stages (Nagy et al., 2017). Hence they can exhibit a wide range of phenotypic or functional plasticity in response to environmental cues (Nagy et al., 2017). These changes can occur rapidly and do not always require genetic changes (Naranjo-Ortiz and Gabaldón, 2019). Moreover, since genetic changes can contribute to morphological changes in fungi, it is not accurate to say that genetic changes happen more rapidly than morphological changes in this group of organisms. The relationship between genetic and morphological changes in fungi is complex and varies depending on the specific context (Naranjo-Ortiz and Gabaldón, 2019). This would also result in confusion not only in taxonomy and evolution but also in biodiversity and ecology (Korshunova et al., 2019).

Here, we provide three fungal examples to understand the cryptic species concept and phenotypic noises concept clearly. Around 2–11 million estimated species and around 3 million predicted species are in kingdom fungi, and only around 150,000, a tiny fraction of the total estimation, have been formally described (Phukhamsakda et al., 2022). Compared to plants and animals, fungi have simple body plans that are often morphologically and ecologically concealed and continuously challenging for precise identifications and species delimitations (Lücking et al., 2020). Plant pathogenic fungi cover one of the most significant fractions of total estimated fungi and play an important ecological and

economic role; hence, they are directly involved with the development of effective food security, crop protection, and pest risk assessments (Cai et al., 2011; Li et al., 2020). For example, rust and smut fungi collectively cover 10% of all known fungi. Therefore, DNA sequence data have recently been extensively employed in developing stable taxonomy for plant pathogenic fungi (Cai et al., 2011). The next crucial ecological group of fungi is saprobes for the earth's nutrient cycling. The examples provided here are saprobes, which evolve fast with environmental stimuli (Naranjo-Ortiz and Gabaldón, 2019). This causes fast-evolving species concepts and delimitations. Hence, the fungi kingdom needs extensive analyses and comprehensive species identification methods. Recently, a conceptual framework was analyzed and suggested for identifying fungi (Lücking et al., 2020). Further, the approach encourages the integrative (polyphasic) taxonomy for species delimitation, which combines phylogeny, phenotype, and reproductive biology. This facilitates evaluating a wide range of diagnostic characteristics, either phenotypic, molecular, or both (Lücking et al., 2020). However, polyphasic approaches are being limited by the availability of data and analyzing capacity of particular groups, while it is well suited for some groups. The effectiveness of polyphasic approaches for identifying yeast as a case study was widely investigated (Yurkov et al., 2015; Boekhout et al., 2021). The advantage of polyphasic approaches over traditional morphological character-based approaches is that species ultimately depend on species recognition approaches and the number of characters in traditional methods. Considering the dichotomous key-like approach, species recognition is based on a few characteristics, such as categorical (spore types, color) or numerical range variables (spore size). In polyphasic approaches, taxonomy based on many characteristics [e.g., assimilation tests for yeasts (Yurkov et al., 2015; Boekhout et al., 2021)] and results in an in-depth understanding of the phenotype and/or niche concepts which aid to recognize cryptic species and phenotypic noises and minimize the confliction in stable systematics.

Phylogeny-based clade-specific evolutionary histories are mostly used to verify species identifications, a pivotal step in taxonomy. However, no single tool for identifying fungi and internal transcribed spacer (ITS) is still considered the first diagnosis, particularly in metabarcoding studies. Secondary DNA barcodes are long-established for resolving taxonomy and for precise identifications where they do not provide sufficient distinctness (Lücking et al., 2020). When considering the molecular markers for resolving cryptic species, it is important to know that identifying “what we call cryptic” depends on the markers used. Therefore, it is more important to ensure congruence between the different markers. Simply adding more and more increasingly variable gene loci can identify more lineages in a population, but the lineages may not necessarily represent species. This problem of applying species names to lineages within a species is becoming more common in the literature with respect to the diversity of fungi lineages being described as species. These pitfalls can be resolved using single-copy orthologs genes and coalescence-based methods (Shen et al., 2016; Maharachchikumbura et al., 2021). Single-copy orthologs are genes that are present only once in the genome of each species, and

have descended from a single ancestral gene present in their last common ancestor (Aguileta et al., 2008; Ren et al., 2016; Shen et al., 2016; Maharachchikumbura et al., 2021). Applications of whole genome sequencing are becoming increasingly common in fungal studies, as complete genomes provide in-depth knowledge and comparisons on the evolvability of the genomes and, consequently, reveal the species' true ancestry. Furthermore, using complete genome information is more beneficial than drafting genomes, as it provides a better understanding of species and their genome and genetic dynamics (Boekhout et al., 2021). The species recognition in some groups of fungi is tightly connected to species concepts of their hosts and vectors. This is especially important for parasitic fungi, depending on their host species. Some plant-pathogenic fungi have a broad host range, while the fungal family *Erysiphaceae* causes considerable damage to many crop plants, including Grapevine, *Eucalyptus*, Rubber, cucumber, tomato, onion, pepper and potato (Ekanayaka et al., 2019), some are highly limited in the range of plant species [e.g. Fungal family *Cyrtariaceae* is host specific on *Nothofagus* spp (Ekanayaka et al., 2019)] or even cultivars that they cause diseases (strict host-parasite association) (Li et al., 2020). Several recent taxonomic studies of fungi include the host of fungi collected into their descriptions, and the host used as a character in species recognition (Hongsan et al., 2020; Hyde et al., 2020), which would be helpful in accurate species identification. Furthermore, Cryptic sibling species can be specialized in different ecological conditions and often become host-specific. For instance, the fungus *Microbotryum violaceum* causes another smut disease in Caryophyllaceae plants, and the degree of specialization and gene flow between strains on different hosts are becoming controversial. Molecular phylogenetic analyses using single-copy nuclear genes help resolve taxonomic confusion in *M. violaceum* from 23 host species and different geographic origins (Le Gac et al., 2007). Hence, molecular phylogenetic analyses play a crucial role in identifying cryptic species. Moreover, host specificity indexes, such as S_{TD} , which measures the average taxonomic distinctness among the host species used by a parasite, weighted for the parasite's prevalence in the different hosts (Poulin and Mouillot, 2005) and specific analyses such as Global and individual ParaFit tests to construct a plant-pathogen evolutionary association network (Zeng et al., 2019; Chen et al., 2022) can be applied to analyze to investigate host and fungal relationship and their coevolution. This also provides a broad spectrum of data on those fungal groups, specifically in niche concepts.

Correct fungal species identification is vital as the kingdom of fungi plays a crucial role in applied sciences. For instance, disease diagnosis and medical prescriptions are based on correct species identifications in the pharmaceutical industry and medical sciences. Cryptic *Aspergillus* species cause cryptic aspergillosis and are becoming more prevalent in humans and can cause significant morbidity and mortality in immunocompromised individuals. Moreover, cryptic *Aspergillus* species are causing invasive diseases and are usually more resistant to common antifungal therapies. Therefore, the correct identification and characterization of these fungi have both epidemiological and clinical implications when evaluating the impact of such species in aspergillosis (Fernandez-Pittol et al., 2022; Ninan et al., 2022). Furthermore, accurate species

identification can reveal important information regarding possible biochemical properties of a particular species, which can be explicitly applied to drug discoveries (Raja et al., 2017).

Cryptic species represent a portion of the missing fraction of biodiversity (Vodā et al., 2015). Consequently, resolving cryptic species is important as it reveals the true level of biodiversity and thus contributes to conservation efforts. Some cryptic species may be seriously threatened but unavailable for conservation programs as they remain undescribed (Delić et al., 2017). The evolutionary state of the cryptic species (See cryptic species and evolution) is important with respect to conservation programs. However, the biological properties and their relationship to the recent ancestors relevant for the conservation of cryptic species (i.e., sexual reproduction, spore germination, etc.) are often not known (Delić et al., 2017). Inappropriate management of endangered species can result in these being more threatened than previously thought (Niemiller et al., 2013; Brodersen and Seehausen, 2014; Delić et al., 2017).

During the analyses of highly diverse species, mislabeling and insufficient investigations lead to identifying the same species in different names because of phenotypic plasticity. Later, this can be corrected in taxonomic revisions, and the misidentifications are synonymized under the correct names. However, having many synonyms for a single species still needs to be more accurate. In this regard, conducting taxonomic revisions [e.g. (Cruz-Morales et al., 2019; El-Banhawy et al., 2021)] and taxonomic outlines [e.g. (Wijayawardene et al., 2020)] that provide recent taxonomic updates on taxa at one place is very important and helpful for precise species identifications.

Resolving cryptic species complexes and identifying phenotypic plasticity of a species are highly demanding but critically important in taxonomy and taxonomic applications in other areas such as evolution, ecology, and biodiversity. It provides a better understanding of species, their distribution patterns in the Tree of Life, and the underlying story of their evolution. We hope this review will provide an overall understanding of cryptic species, phenotypic noise, and their effects. Also, this should encourage more detailed analyses of morphological characters with the combination of molecular phylogenetic analyses, which can provide fine scale taxonomy.

8 Conclusions

“Cryptic species” refers to a situation in which two or more species are morphologically indistinguishable in their original descriptions and have been erroneously classified under the same scientific name. “Phenotypic noise” refers to different phenotypic states of the same isogenic population. Both are contributing to evolution but in two opposite ways.

Cryptic species and phenotypic noise create confusion in taxonomic structure and leave huge gaps in describing the flow of the evolutionary process. Molecular tools are the only way to detect them easily. However, a detailed examination of macro-, micro-, external, and internal morphological characters also provides a

better endorsement of taxonomic status. Both concepts hide the real biodiversity and inhibit a clear understanding of species evolution and their ecological significance.

Although members of a particular species may appear to be similar, several to many distinct species can be present. In other words, many unrecognized species may be hidden as cryptic species. Many important taxa may need to be protected but are already extinct within cryptic species complexes. Conversely, species limitations must be well identified as the same species could be differently identified based on its phenotypic plasticity for different environmental conditions. Cryptic species and phenotypic variations of the same species also affect studies of ecology and agricultural research, especially in relation to plant pathology and developing biocontrol agents, where these concepts can pose significant obstacles.

Cryptic species result from the species evolution process. Discovering cryptic species and identifying the phenotypic noise of particular species helps develop a fine-scale taxonomic system and improve its applications in areas such as evolution, ecology, and biodiversity.

Author contributions

AE: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. SK: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. ST: Formal analysis, Methodology, Software, Visualization, Writing – review & editing. AD: Formal analysis, Methodology, Software, Writing – review & editing. DT: Formal analysis, Methodology, Writing – review & editing. AK: Formal analysis, Methodology, Writing – review & editing. EC: Formal analysis, Methodology, Writing – review & editing. DD: Formal analysis, Methodology, Writing – review & editing. SS: Formal analysis, Methodology, Validation, Writing – review & editing. SM: Formal analysis, Investigation, Methodology, Writing – review & editing. CL: Formal analysis, Funding acquisition, Methodology, Resources, Writing – review & editing. AP: Formal analysis, Investigation, Methodology, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY

Sinang Hongsanan,
Shenzhen University, China

REVIEWED BY

Marasinghe Wadige Diana Sandamali,
Smith College, United States
Chihiro Kadooka,
Sojo University, Japan

*CORRESPONDENCE

Dong-Qin Dai

✉ cicaidongqin@gmail.com

Nalin N. Wijayawardene

✉ nalinwijayawardene@yahoo.com

†These authors have contributed equally to this work

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Fungi from *Malus* in Qujing, China: two new species, three new records, and insights into potential host jumping and lifestyle switching

Gui-Qing Zhang^{1,2,3†}, Zhu-Mei Li^{1†}, Xin-Lei Fan⁴, Qi-Rui Li^{5,6}, Jaturong Kumla^{2,3}, Nakarin Suwannarach^{2,3}, Abdallah M. Elgorban⁷, Ihab M. Moussa⁸, Dong-Qin Dai^{1*} and Nalin N. Wijayawardene^{1,9*}

¹Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biology and Food Engineering, Qujing Normal University, Qujing, China, ²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ³Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai, Thailand, ⁴The Key Laboratory for Silviculture and Conservation of the Ministry of Education, Beijing Forestry University, Beijing, China, ⁵Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang, Guizhou, China, ⁶The High Efficacy Application of Natural Medicinal Resources Engineering Centre of Guizhou Province (The Key Laboratory of Optimal Utilization of Natural Medicine Resources), School of Pharmaceutical Sciences, Guizhou Medical University, Gui'an, Guizhou, China, ⁷Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia, ⁸Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, ⁹Tropical Microbiology Research Foundation, Colombo, Sri Lanka

Apple trees [*Malus domestica* Borkh. (*Rosaceae*)] are one of the important temperate fruit crops in China. In comparison to other temperate fruits, such as grapes and pears, fungal studies (in Yunnan) associated with *M. domestica* are fewer in number. In the present study, we investigated fungi associated with *M. domestica* in Qujing City, Yunnan Province, China. Samples were collected from apple gardens in different locations. Single spore isolation was carried out to isolate saprobic fungi, while the surface sterilization method was carried out to isolate endophytic fungi. Molecular analyses were carried out to determine the phylogenetic placement of the new collections. Based on the combined methods of morphology and phylogeny, *Cytospora qujingensis* sp. nov. and *Hypoxyton malongense* sp. nov. are introduced as novel saprobic and endophytic taxa, respectively. Moreover, *Aureobasidium pullulans* and *Cytospora schulzeri* are reported as new geographical records from southwestern China. *Allocreptovalsa castaneae* is reported on *M. domestica* in China for the first time. The checklist of fungi associated with *M. domestica* in China is presented.

KEYWORDS

apple tree, fungal diversity, life mode, phylogeny, taxonomy, two novel species

1 Introduction

Yunnan Province, located in the interior low-latitude plateau in southwestern China, features a complex geography and unique climate. These attributes, in conjunction with its favorable ecological condition, contribute to the high quality of fruit yields in the region (Dong et al., 2023). Yunnan has a diverse range of fruits, which hosts 287 fruit plant species belonging to 118 genera and 49 families from tropical to temperate species, ranking first in China (Zhao et al., 2018). According to the Department of Agriculture and Rural Affairs of Yunnan Province (2021) (accessed 01 April 2024), over 11 million tons of fruits (mostly blueberries, strawberries, raspberries, oranges, and apples) were harvested in 2020.

Apple is one of the four major fruits in the world, rich in minerals and vitamins, with high nutritional value (Sachini et al., 2020). However, large amounts of chemical fertilizers are applied to increase productivity. Extensive use of chemicals causes serious environmental impacts. Farmers are fond of using fungicides since fruits are susceptible to diseases caused by fungal pathogens. *Cytospora* canker is one of the most widespread canker diseases of *Malus domestica* (Wang et al., 2024a). For example, 1) *Cytospora mali* causes severe necrosis of the branches and stems globally (Wang et al., 2007), and 2) *Cytospora parasitica* is reported to cause canker on apples in China (Ma et al., 2018).

Fungi are diverse in terms of shape, color, lifestyle, and distribution throughout any ecosystem (Bhunjun et al., 2022). Researchers estimated the diversity of fungal species ranging from half a million (May, 2000) to more than 12.5 million (Wu et al., 2019), even reaching 19.35 million (Tedersoo et al., 2021). Hitherto, Species Fungorum (2024) (accession date: 04 September 2024) lists all accepted species of fungi, currently 161,348 species, with only approximately 40,000 named fungal species in GenBank (Hawksworth and Lücking, 2017). An accurate estimate of the number of fungi will provide a better understanding of fungal diversity and biogeography (Hawksworth, 1991; Bhunjun et al., 2022). In traditional taxonomy, species amount was provided based on the host or morphological characters (sexual morph: ascomata, pseudoparaphyses, asci, ascospores; asexual morph: conidiomata, ostioles, conidiophores, conidiogenous cells, conidia). Meanwhile, with the application of DNA sequencing technologies, the reliability of introducing species based on morphological characters was questioned by taxonomists (Wijayawardene et al., 2021b). A vague “dark taxa” poses challenges to researchers in estimating fungal populations; therefore, it is more important to use different molecular approaches to accurately delineate species and to estimate species diversity (Cai et al., 2009; Wijayawardene et al., 2023), such as DNA barcoding (Seifert, 2009), environmental DNA (eDNA) metabarcoding (Tedersoo et al., 2014), metagenomics (Tedersoo et al., 2018), genome sequencing (Baldrian et al., 2022), and phylogenomic analysis (Wijayawardene et al., 2023). Estimating species numbers is critical for species conservation and ecosystem management since changes in fungal diversity can

influence ecosystem performance (Genevieve et al., 2019). Our understanding of host specificity is unclear, particularly as new plant species are found and several habitats remain unexplored. Moreover, the knowledge about lifestyle switching between and within species remains unclear and thus needs extensive studies. Furthermore, the possibility of revealing novel fungal species and new host or country records creates more opportunities to discover new compounds, such as antimicrobial agents and enzymes (Suetrong et al., 2023). Researchers demonstrated the importance of revealing novel fungal species from different habitats and substrates, as well as reports of new host or country records. This allows us to enhance our understanding of lifestyle switching of one species in different hosts or the same host in different geographic regions and whether the same species produces new compounds to sustain an individual's existence when it survives in different habitats, such as from the stratosphere (Wainwright et al., 2003) to the bottom of the Dead Sea (Oren and Gunde-Cimerman, 2012), from the Antarctic glaciers (Freeman et al., 2009) to torrid deserts (Gonçalves et al., 2016), and from the gut of flies (Blackwell, 2017) to deep oceanic sediments (Nagahama et al., 2011), and prevents the occurrence of fungal diseases. For example, 1) *Alternaria alternata* is a widespread fungus that is regularly isolated from plants as both endophyte and pathogen and in the soil as a saprophyte (Thomma, 2003; DeMers, 2022); and 2) *Aureobasidium pullulans* is one of the well-adapted saprophytes in the phyllosphere (McCormack et al., 1995; Dik and Elad, 1999) and postharvest diseases (Ippolito and Nigro, 2000) and also reported as an endophyte in symptomless plant organs (Loi et al., 2000).

In previous studies, more than 200 fungal species (including new species and known species) were reported on *M. domestica* from China (Supplementary Table S1). *Alternaria* spp. and *Penicillium* spp. are the most common pathogens associated with the diseases reported from apples (Basson et al., 2019). *Cytospora* is considered one of the most important pathogenic genera, which can cause canker and dieback disease in apple-producing areas in China, such as Gansu, Shanxi, Liaoning, and Yunnan provinces (Gui et al., 2015). During our field surveys in Qujing City (from January to September 2021), significant disease symptoms on fruits, leaves, and stems have not been observed. Hence, we investigated whether these plants harbor any taxa (endophytes or saprobes) that have been reported as pathogens in previous studies. It is well-established that some species could change their lifestyle due to other factors, such as nutritional requirements and host specificity (Rai and Agarkar, 2016). Furthermore, it was predicted that well-studied host plants in less-studied geographical regions could harbor more novel taxa (Wijayawardene et al., 2022a, b).

In the present study, based on the morphological characteristics and DNA sequence-based phylogenetic analyses, we introduce two novel species, i.e., *Cytospora qujingensis* sp. nov. (as a saprobe) and *Hypoxyton malongense* sp. nov. (as an endophyte), respectively. *Aureobasidium pullulans* (as an endophyte) and *Alloccryptovalsa castaneae* and *Cytospora schulzeri* (as saprobes) are reported as new geographical or host records in southwestern China.

2 Materials and methods

2.1 Sampling and fungal isolation

Sampling was conducted during the fall season, using a random sampling method. Specifically, healthy and dead leaves and leaf litter of *M. domestica* were collected to assess fungal presence across different plant tissues from different apple orchards in Qujing City, Yunnan Province, China. The orchards under study were managed by local farmers who applied fungicides regularly as part of their standard pest and disease control practices. All the samples were stored in polythene bags and brought to the microbiology laboratory at Qujing Normal University.

Single spore isolation (for saprobes) was conducted as described by Dai et al. (2017). Stromata or conidiomata were sectioned by hand using a razor blade. The hymenium containing ascospores or conidia mass was removed and placed on a drop of sterile water on a flamed concave microscope slide and separated by sterile needles. The spore suspension was placed drop by drop on potato dextrose agar (PDA) plates, each containing a standard concentration of 39 g/L PDA, and incubated overnight in incubators at 28°C. The germinated spores were photographed and then transferred to a new PDA plate. Mycelium grew within 2 weeks, and the hyphal were transferred to three new PDA plates to get the pure culture for DNA extraction.

The surface sterilization method (for endophytes) was performed following Senanayake et al. (2020) but with a slight change. Plant leaves were cleaned with distilled water and then air-dried for 1 min. The leaf surface was sterilized by soaking in 75% ethanol for 30 s and then 1% sodium hypochlorite for 60 s. Finally, it was rinsed in sterile demineralized distilled water three times. Presterilized forceps were used to transfer the cleaned leaves onto the PDA after they had been chopped into fragments (approximately 5 mm × 5 mm) using a sterile scalpel blade on a sterile glass Petri dish under aseptic conditions within a laminar flow hood to minimize contamination. As for overnight growth in incubators at 28°C, the PDA plates were checked regularly, and individual colonies were transferred to a new PDA plate until pure strains were available for DNA extraction experiments.

2.2 Morphology and preserving

Morphological characteristics were observed and studied using a Leica DM1000 microscope and photographed by differential interference contrast (DIC). A Leica S8AP0 stereomicroscope with an HDMI 200C camera (Leica Corporation, Germany) was used for examining fruiting bodies. An Olympus BX53 compound microscope (Olympus Corporation, Japan) with differential interference contrast (Olympus BX53 DIC compound microscope with an Olympus DP74 camera, Japan) was used to observe and photograph the morphological characteristics. Colonies on PDA were observed and photographed using a Leica S8AP0 stereomicroscope with an HDMI 200C camera under appropriate lighting conditions to capture detailed morphological characteristics. A razor blade was used to obtain thin sections of stromata and conidiomata by hand.

Specimens were preserved at the Mycological Herbarium of Zhongkai University of Agriculture and Engineering (MHZU). Cultures were deposited at the Zhongkai University of Agriculture and Engineering (ZHKUCC). Duplicates of the specimens and type cultures were deposited at the Herbarium of Guizhou Medical University, Guiyang, China (GMB), and Guizhou Medical University Culture Collection (GMBCC) in Guiyang, China, respectively.

2.3 Registration of novel taxa

Index Fungorum Registration Identifiers were obtained from Index Fungorum (2024) (<https://www.indexfungorum.org>).

2.4 DNA extraction, PCR amplification, and sequencing

Fresh mycelium was cultured on PDA in incubators at 28°C overnight for 15 days. The genomic DNA was extracted from fresh cultures according to the specifications of the Biospin Fungal Genomic DNA Extraction Kit (bioflux®). Each gene region was separately amplified using both forward and reverse primers, which are provided in Table 1. A final volume of polymerase chain reaction (PCR) was prepared at 25 µL, including 1 µL of DNA template, 1 µL of each forward and reverse primer, 12.5 µL of 2× Taq PCR Master Mix, and 9.5 µL of double-distilled water (ddH₂O) as described by Dai et al. (2017). The PCR thermal cycling procedure for amplifying ITS, LSU, and *rpb2* was conducted as explained by Ma et al. (2022), while *tef1-α*, *tub*, and *act* genes were conducted as explained by Jia et al. (2024), respectively. The PCR products were obtained in Shanghai Majorbio Bio-Pharm Technology Co., Ltd., and BGI Tech Solutions Co., Ltd. (BGI-Tech, China) for sequencing.

2.5 Phylogenetic analyses

Closely related sequences were downloaded from GenBank based on blast similarity and recent publications (Fan et al., 2020;

TABLE 1 Partial gene regions and primers used in this study.

Loci	PCR primers (forward/reverse)	References
ITS	ITS5/ITS4	White et al. (1990)
LSU	LR0R/LR5	Vilgalys and Hester (1990)
<i>rpb2</i>	fRPB2-5f/fRPB2-7cr	Liu et al. (1999)
<i>tef1-α</i>	EF1-728F/EF1-1567R	Rehner and Buckley (2005)
<i>tub</i>	T1/T22	O'Donnell and Cigelnik (1997)
	T1/Bt2b	Glass and Donaldson (1995)
<i>act</i>	ACT-512F/ACT-783R	Carbone and Kohn (1999)

Maharachchikumbura et al., 2022; Wu et al., 2023; Zhang et al., 2024c) (Supplementary Tables S2–S5). Mafft v.7.215 (<http://mafft.cbrc.jp/alignment/server/index.html>) (Katoh and Standley, 2013) was used to align the single gene sequence, automatic cutting was done in Trimal.v1.2rev59, and the final improvements were performed in BioEdit v.7.0.5.2 (Hall, 2004) by hand. The combined gene regions of ITS, *tef1- α* , *rpb2*, *tub*, and *act* (*Cytospora*); ITS and *tub* (*Allocryptovalsa*); ITS and LSU (*Aureobasidium*); and ITS, LSU, *rpb2*, and *tub* (*Hypoxylon*) regions were performed in BioEdit v. 7.0.5.2 (Hall, 2004) manually. The combined datasets in FASTA format were converted to PHYLIP and NEXUS formats by using ALTER (Alignment Transformation Environment online, <http://sing.ei.uvigo.es/ALTER/>) (Glez-Peña et al., 2010). The online tool FindModel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) was used to determine the best nucleotide substitution model for each partition data (Dai et al., 2022). The phylogenetic trees were constructed by maximum likelihood (ML) and Bayesian analyses.

ML analysis was carried out via the online portal CIPRES Science Gateway v. 3.3 (Miller et al., 2010), using RAXML-HP v.8 on XSEDE (8.2.12) tool, with the default settings but adapted with the GAMMA nucleotide substitution model and 1,000 rapid bootstrap replicates.

Bayesian analysis was carried out by MrBayes v. 3.0b4 (Ronquist and Huelsenbeck, 2003), and the model of evolution was estimated with MrModeltest v. 2.2 (Nylander, 2004). The posterior probabilities (PP) (Rannala and Yang, 1996; Zhaxybayeva and Gogarten, 2002) were determined by the following Markov chain Monte Carlo sampling (MCMC) in MrBayes v.3.0b4 (Huelsenbeck and Ronquist, 2001). Six simultaneous Markov chains were run for 1,000,000 generations, with trees sampled every 100th generation. The preburn was set to 0.25 and the run was automatically stopped when the mean standard deviation of the split frequency reached below 0.01 (Maharachchikumbura et al., 2015).

Trees were viewed by FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) (Rambaut, 2006), and the phylogram was edited by Microsoft Office PowerPoint 2016 (Microsoft Inc., Redmond, WA, USA) and converted to jpg. file by the Adobe PhotoShop CC 2018 software (Jiang et al., 2021).

3 Results and discussion

3.1 Phylogenetic analyses

3.1.1 Multigene analyses for *Cytospora* (Valsaceae, Diaporthales, and Sordariomycetes)

The combined gene regions of ITS, *tef1- α* , *rpb2*, *tub*, and *act* contained 300 isolates, which comprised 2,249 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Diaporthe vaccinii* (CBS 160.32) was used as the outgroup taxon. The best-scoring RAXML tree with a final likelihood value of $-53,458.778097$ is presented in Figure 1. The matrix had 1,755 distinct alignment patterns, with 23.31%

of undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.243243, C = 0.273232, G = 0.242722, and T = 0.229822; the substitution rates were AC = 1.503463, AG = 3.421602, AT = 1.349798, CG = 1.091741, CT = 6.308732, and GT = 1.000000; and the gamma distribution shape parameter alpha = 0.483398. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis.

In the phylogenetic analysis, our new isolates [ZHKUCC 23-0978 (ex-type), ZHKUCC 23-0979, and GMBCC1004] formed a monophyletic clade (82% ML, 1.00 PP) sister to *Cytospora sophoricola* [CFCC 89595 (ex-type) and CFCC 89596] and *C. sophorae* (CFCC 89598, CFCC 50048, and CFCC 50047). Hence, the taxon which is represented by ZHKUCC 23-0978, ZHKUCC 23-0979, and GMBCC1004 is introduced as a novel species in *Cytospora* s. str. as *C. qujingensis* (see taxonomy section for further details). Furthermore, two new strains, ZHKUCC 23-0980 and GMBCC1005, were grouped with *C. schulzeri* (CFCC 50040 and CFCC 50042) with high statistical values (100% ML, 1.00 PP). Based on phylogenetic analyses and morphological comparisons, we confirmed that these two strains represent *C. schulzeri* (see taxonomy section for morphological comparison).

3.1.2 Multigene analyses for *Allocryptovalsa* (Diatrypaceae, Xylariales, and Sordariomycetes)

The combined gene regions of ITS and *tub* contained 19 isolates, which comprised 1,221 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Eutypella australiensis* (STEU-8248) was used as the outgroup taxon. The best-scoring RAXML tree with a final likelihood value of 3,671.144837 is presented in Figure 2. The matrix had 287 distinct alignment patterns, with 25.59% of undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.224076, C = 0.274997, G = 0.237980, and T = 0.262947; the substitution rates were AC = 1.266141, AG = 2.802018, AT = 1.131189, CG = 1.718083, CT = 4.262046, and GT = 1.000000; and the gamma distribution shape parameter alpha = 0.821838. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic tree, our two new strains of *Allocryptovalsa* (ZHKUCC 23-0981 and ZHKUCC 23-0982) were clustered with *Allocryptovalsa castaneae* [CFCC 52428 (ex-type) and CFCC 52427] with high statistical support (84% ML, 0.99 PP).

3.1.3 Multigene analyses for *Aureobasidium* (Saccotheciaceae, Dothideales, and Dothideomycetes)

The combined gene regions of LSU and ITS contained 34 isolates, which comprised 1,410 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Sydowia polyspora* (CBS 750.71) was used as the outgroup taxon. The best-scoring RAXML tree with a final likelihood value of $-6,098.421647$ is presented in Figure 3. The matrix had 298 distinct alignment patterns, with 21.36% of undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.256565, C = 0.222028, G = 0.279511, and T =

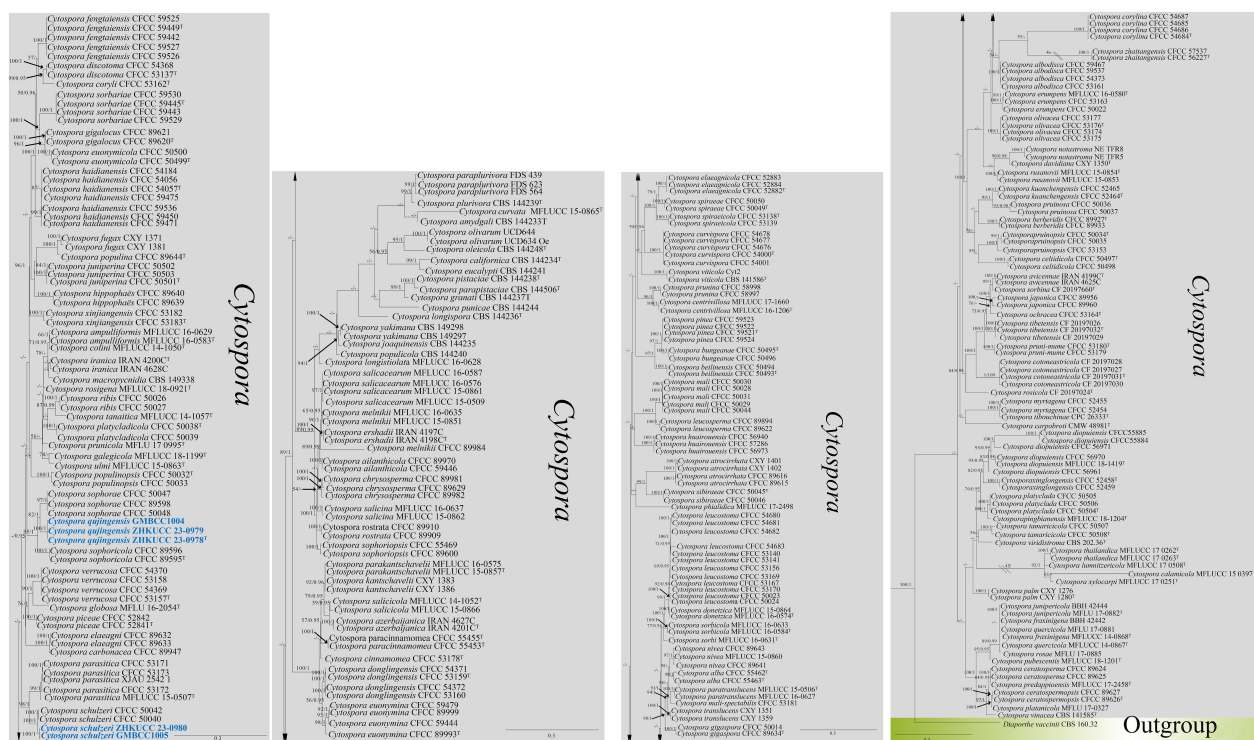


FIGURE 1

The phylogenetic tree from the best scoring of the RAxML analysis based on combined gene regions (ITS, *tef1-α*, *rpb2*, *tub*, and *act*) is rooted to *Diaporthe vaccinii* (CBS 160.32). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95 are given at the respective branches. A hyphen (-) means a value lower than 50% (ML) or 0.95 (PP). New isolates are labeled in blue bold and ex-types are indicated in "T" (Supplementary Table S2).

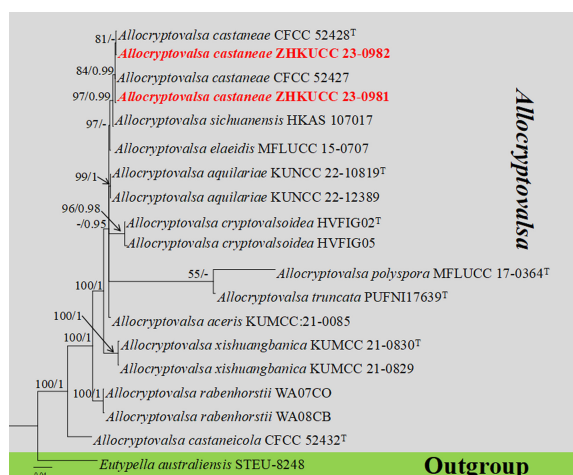


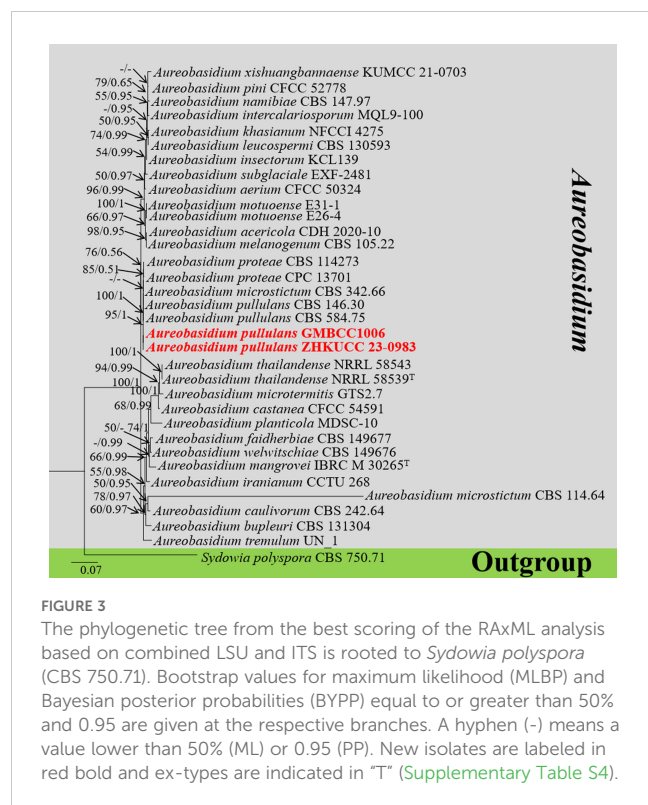
FIGURE 2

The phylogenetic tree from the best scoring of the RAxML analysis based on combined gene regions (ITS and *tub*) is rooted to *Eutypella australiensis* (STEU-8248). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95 are given at the respective branches. A hyphen (-) means a value lower than 50% (ML) or 0.95 (PP). New isolates are labeled in red bold and ex-types are indicated in "T" (Supplementary Table S3).

0.241896; the substitution rates were AC = 1.507336, AG = 2.554163, AT = 1.625168, CG = 1.056662, CT = 4.675945, and GT = 1.000000; and the gamma distribution shape parameter alpha = 2.840594. The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic tree, two newly generated strains of *Aureobasidium* (ZHKUCC 23-0983 and GMBCC1006) were clustered in the clade that comprises *Au. pullulans* (CBS 584.75 and CBS 146.30), *Au. proteae* (CBS 114273 and CPC13701), and *Au. microstictum* (CBS342.66) with high statistical values (91% ML, 1.00 PP). The placement of the abovementioned species agrees with Humphries et al. (2017) and Wu et al. (2023), who performed their analysis based on LSU and ITS gene regions. Thus, we compared the conidial morphologies of the new collection against the other three species and confirmed that our collection belongs to *Au. pullulans* (see taxonomic key in the taxonomy section).

3.1.4 Multigene analyses for Hypoxylon (Hypoxylaceae, Xylariales, and Sordariomycetes)

The combined gene regions of ITS, LSU, *rpb2*, and *tub* contained 150 strains in the sequence analysis, which comprise 3,728 characters with gaps. Single gene analysis was carried out and compared with each species, to compare the topology of the tree



and clade stability. *Xylaria arbuscula* (CBS 126415), *X. hypoxylon* (CBS 122620), and *Biscogniauxia nummularia* (MUCL 51395) are set as the outgroup taxa. The best-scoring RAXML tree with a final likelihood value of $-84,270.809729$ is presented. The matrix had 2,144 distinct alignment patterns, with 33.59% of undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.232029, C = 0.266223, G = 0.261601, and T = 0.240148; the substitution rates were AC = 1.022186, AG = 4.161361, AT = 1.213956, CG = 0.910957, CT = 5.597031, and GT = 1.000000; and the gamma distribution shape parameter $\alpha = 0.280100$ (Figure 4). The GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. Overall tree topologies based on ML and Bayesian inference (BI) analyses were similar and not significantly different. In the phylogenetic analysis (Figure 4), our new collections [ZHKUCC 23-0984 (ex-type) and GMBCC1007] clustered in clade *Hypoxylon* and formed an independent lineage sister to *Hypoxylon himmuleum* [MUCL 3621 (ex-type), DSM:107932, and DSM:107926] with relatively high statistical support (100% ML, 1.00 PP). Hence, we introduced *H. malongense* to accommodate our new collection from *M. domestica* (see taxonomy section for further details).

3.2 Taxonomy

Cytospora Ehrenb., Sylv. mycol. berol. (Berlin): 28

Index Fungorum Registration Identifier: 7904

Type species: *Cytospora chrysosperma* (Pers.) Fr., Syst. mycol. (Lundae) 2(2): 542

Cytospora Ehrenb. was typified by *C. chrysosperma* (Pers.) Fr. and the members were reported as important plant pathogens, saprobes, and endophytes on branches and twigs of a broad range of plants with a worldwide distribution (Fan et al., 2020). Currently, 696 epithets of *Cytospora* have been listed in Index Fungorum (2024) (accessed 23 June 2024), but many species lack herbarium materials, ex-type, and molecular data. The asexual morph of *Cytospora* is characterized by pycnidial locules, single or labyrinthine, conidiophores filamentous, conidia hyaline and allantoid; however, the sexual morph shows clavate to elongate obovoid asci, with four or eight hyaline, allantoid ascospores (Spielman, 1983, 1985). In the traditional taxonomy, host-based methods, such as morphological characteristics of the host and shape and size of conidia, were mainly used to define the species of *Cytospora* (Fan et al., 2020). However, in the past two decades, morphological identification and phylogenetic species recognition concepts have led to the description of several additional new species of *Cytospora* (Lawrence et al., 2017, 2018; Fan et al., 2020). In this study, we collected two *Cytospora* species from apples. Morpho-molecular analyses confirmed that one of them is a novel taxon of *Cytospora*; thus, we introduced our collections [(ZHKUCC 23-0978 (ex-type), ZHKUCC 23-0979, and GMBCC1004] as a novel species, viz., *C. qujingensis* and collections (ZHKUCC 23-0980 and GMBCC1005) were confirmed as the first record of *C. schulzeri* on *M. domestica* from Yunnan Province, southwestern China.

Cytospora qujingensis G.Q. Zhang, Wijayaw., & X.L. Fan, sp. nov.

Index Fungorum Registration Identifier: IF902662

Figure 5

Etymology: Named after the location "Qujing" where the new taxon was first discovered.

Diagnosis: *Cytospora qujingensis* differs from other *Cytospora* species by its asci with "J"-apical ring and larger size of asci ($60\text{--}85 \times 10\text{--}16 \mu\text{m}$) and ascospores ($10\text{--}25 \times 4\text{--}6 \mu\text{m}$).

Holotype: MHZU 23-0265

Description: Saprobiic on branches of *M. domestica* in China.

Sexual morph: Stromata $390\text{--}550 \mu\text{m}$ in length and $160\text{--}330 \mu\text{m}$ in width (av. $450 \times 230 \mu\text{m}$, $n = 30$), circular to ovoid, brown to black, usually scattered, initially immersed in the bark, slightly to strongly erumpent through the surface of bark when mature. Conceptacle absent. Ectostromatic disc inconspicuous, usually with a single ostiolar neck, $75\text{--}100 \mu\text{m}$ diam. Ostioles $140\text{--}170 \mu\text{m}$ diam., centric or lateral, slightly papillate, dark brown to black. Perithecia $420\text{--}530 \times 285\text{--}300 \mu\text{m}$ (av. $470 \times 290 \mu\text{m}$, $n = 5$), solitary, flask-shaped to spherical, black, arranged circularly. Paraphyses may be lacking at maturity but usually present, often collapsed and broad. Asci, $60\text{--}85 \times 10\text{--}16 \mu\text{m}$ (av. $70 \times 12.5 \mu\text{m}$, $n = 10$), eight-spored, unitunicate, clavate to elongate, with an indistinct pedicel, apically rounded and thinned, "J"-apical ring in Melzer's reagent, refractive, refractive truncated at the top. Ascospores $10\text{--}25 \times 4\text{--}6 \mu\text{m}$ (av. $15 \times 4.5 \mu\text{m}$, $n = 25$), biserial to overlapping, or irregularly arranged, elongate-allantoid, straight to slightly curved, slightly constricted at both ends, normally rounded, hyaline, smooth, thin-walled. **Asexual morph:** not observed.

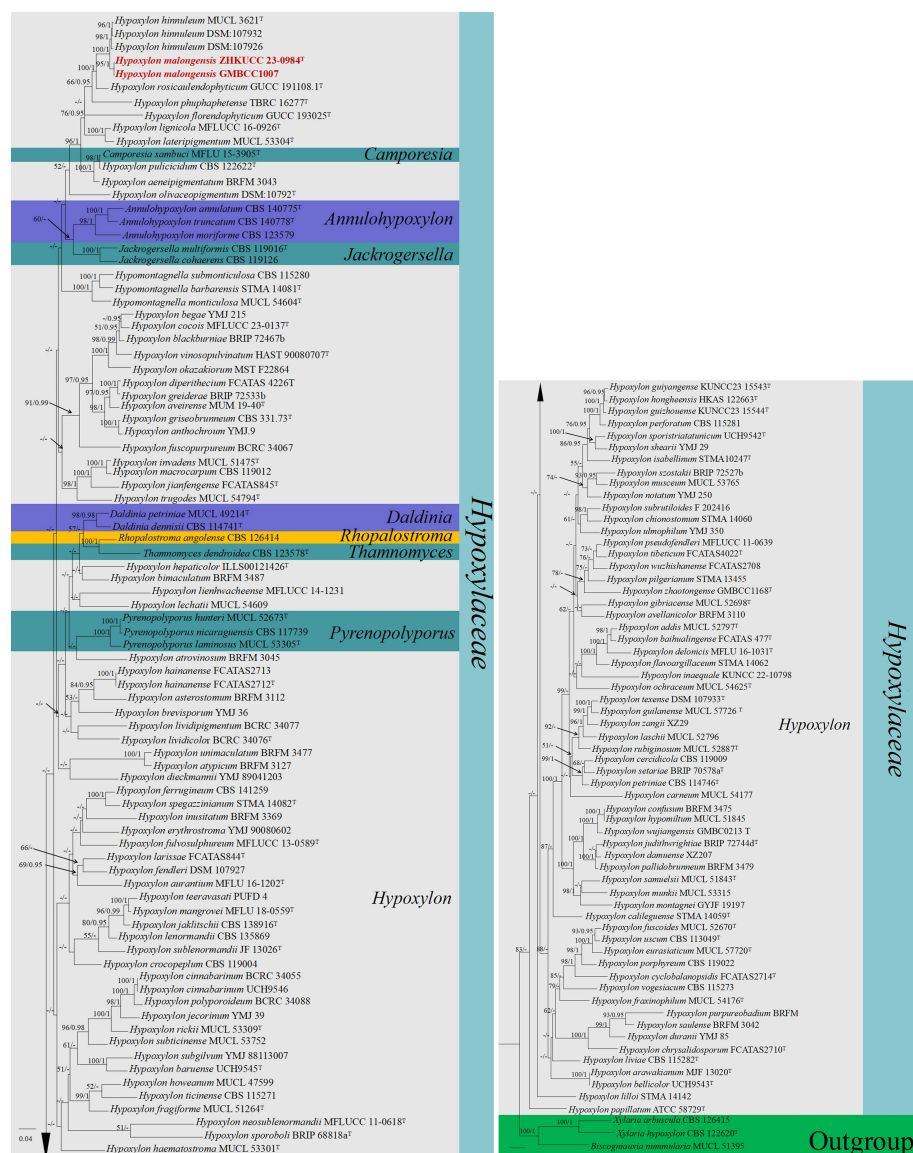


FIGURE 4

The phylogenetic tree from the best scoring of the RAxML analysis based on combined gene regions (ITS, LSU, *rpb2*, and *tub*) is rooted to *Xylaria arbuscula* (CBS 126415), *X. hypoxylon* (CBS 122620), and *Biscogniauxia nummularia* (MUCL 51395). Bootstrap values for maximum likelihood (MLBP) and Bayesian posterior probabilities (BYPP) equal to or greater than 50% and 0.95 are given at the respective branches. A hyphen (-) means a value lower than 50% (ML) or 0.95 (PP). New isolates are labeled in red bold and ex-types are indicated in "T" (Supplementary Table S5).

Culture characteristics: Ascospores germinating on PDA, producing germ tubes from both ends within 24 h. Colonies growing on PDA, reaching 6–9 cm diam. after 7 days at 28°C, white surface, growing up to 6 cm diam. with irregular margins, covering the 9-cm plate after 14 days. In reverse, the cultures are the same as the upper color after 3 days, becoming isabelline to umber after 7–14 days. Colonies are felty with a heterogeneous texture, lacking aerial mycelium.

Material examined: CHINA, Yunnan Province, Qujing City, 25°47'77"N, 62°34'96"E, on the dead branch of *M. domestica*, 1 October 2021, Guiqing Zhang, Z4 = MHZU 23-0265 (holotype), ex-type: ZHKUCC 23-0978; *Ibid.* Z4-1 = GMB1004 (isotype), ex-isotype: GMBCC 1004; *Ibid.* Z14 living culture: ZHKUCC 23-0979.

Notes: *Cytospora qujingensis* was collected as a saprobe on a dead branch of *M. domestica*. In the phylogenetic analysis, *C. qujingensis* grouped with *C. sophorae* and *C. sophoricola* (Figure 1), two asexually typified taxa (Fan et al., 2013). Among these two species, our new species shows a closer phylogenetic relationship with *C. sophorae* (CFCC 50047, CFCC 50048, and CFCC 89598), supported by 82% ML and 1.00 PP statistical support values (Figure 1). However, the representative strains of *C. sophorae* are not type strains but have been used in the phylogenetic analyses by Jia et al. (2024). The reference strains were isolated from *Styphnolobium japonicum* (Fabaceae) and *Magnolia grandiflora* (Magnoliaceae) in the Gansu and Shanxi provinces in China (Fan et al., 2020), whereas our new strains originate from *M. domestica* (Rosaceae) in Yunnan Province, southwestern China.

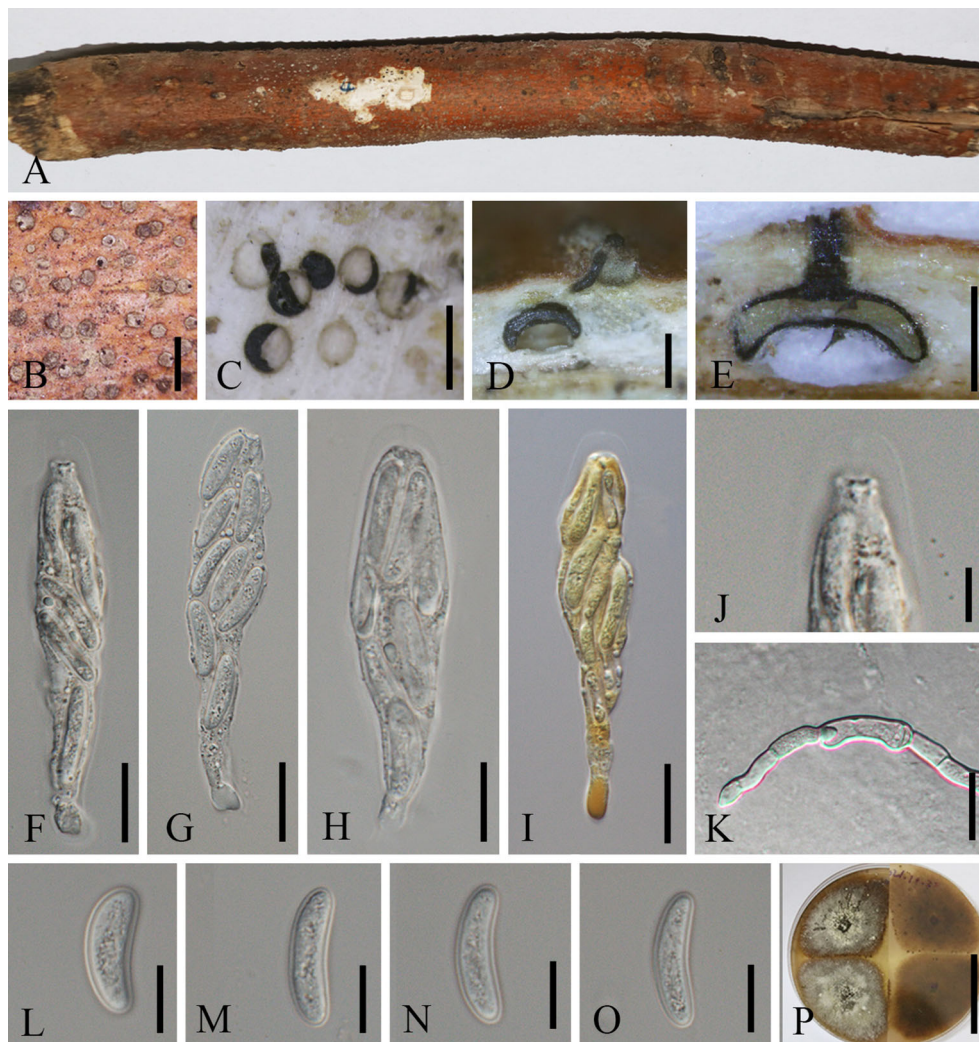


FIGURE 5

Cytospora qujingensis (MHZU 23-0265, holotype). (A, B) Habit of stromata on a branch. (C) Transverse section through stroma. (D, E) Longitudinal section through stroma. (F–H) Asci in water. (I) Asci in Melzer's reagent shows "J-" apical apparatus. (J) Apical apparatus of an ascus. (K) Germinating ascospore. (L–O) Ascospores. (P) Colonies on PDA from above and below. Scale bars: (B) = 1 mm, (C–E) = 0.5 mm, (F–I) = 15 μ m, (J–O) = 5 μ m, and (P) = 5 cm.

Comparative analysis of the base pairs of ITS, *tef1- α* , *rpb2*, *tub*, and *act* gene regions, our isolate [ZHKUCC 23-0978, (ex-type)] show significant differences with *C. sophorae* (CFCC 89598, CFCC 50048, and CFCC 50047): 3/489 bp (0.6%), 20/721 bp (2.8%), 23/260 bp (8.8%, including three gaps), 41/508 bp (8.1%, including six gaps), and 4/237 bp (1.7%), respectively. These phylogenetic incongruities, alongside ecological distinctions, form the basis for proposing that ZHKUCC 23-0978 (ex-type), ZHKUCC 23-0979, and GMBCC1004 belong to a new species, *Cytospora qujingensis*.

Morphologically, *C. qujingensis* shares similarities with type species *C. chrysosperma*, and *C. schulzeri* in terms of asci and ascospore characteristics. However, our isolates can distinguish *C. chrysosperma* and *C. schulzeri* (teleomorph *Valsa malicola*) by their remarkable features, which our isolates with a single perithecial stromata, asci with a "J-" apical ring, refractive, while *C. chrysosperma* characterized by 4–8 perithecia arranged circinate in black entostromata, and *C. schulzeri* with 5–14 perithecia, both

are not noted as having an apical ring (Fan et al., 2015, 2020) (more details are shown in Table 2). Phylogenetically, both *C. chrysosperma* and *C. schulzeri* are not closely related to our new isolates. Recently, Wang et al. (2024a) introduced three novel species of *Cytospora*, viz. *C. hejingensis* R. Ma & Ning Jiang, *C. kunsensis* R. Ma & Ning Jiang, and *C. jilongensis* R. Ma & Ning Jiang, the first two of which are revealed for their sexual morph while the last one was reported as its asexual morph. However, these three species are not included in our phylogenetic tree. The phylogenetic tree in Wang et al. (2024a) shows that these three species are not closely related to our novel isolates and are accommodated in a separate clade. Furthermore, the morphology among them supported by our new isolates is different from them (Table 2). The inability to obtain the asexual morph of our new isolates under laboratory conditions is a limitation of our study. Future discovery of the sexual morphs of *C. sophorae* and *C. sophoricola* would provide insights into whether these species

TABLE 2 Diagnostic characteristics of *Cytospora* species.

Morphological and colony characters	Species name and references				
	<i>C. chrysosperma</i> (Fan et al., 2022)	<i>C. hejingensis</i> (Wang et al., 2024a)	<i>C. qujingensis</i> (This study)	<i>C. kunsensis</i> (Wang et al., 2024a)	<i>C. schulzeri</i> (Fan et al., 2024)
Stromata	With 4–8 perithecia arranged circately, 0.86–1.19 mm diam.	With 4–9 perithecia arranged irregularly, 400–1,250 µm diam.	Single perithecia, 390–550 µm diam.	With 5–11 perithecia arranged circularly, 750–1,350 µm diam.	With 5–14 perithecia arranged circularly or irregularly, 980–1,600 µm diam.
Ectostromatic disc and ostiole	Ectostromatic disc was obscured by tightly ostiolar necks, 0.20–0.34 mm diam., ostioles numerous	Ectostromatic disc inconspicuous, surrounded by tightly aggregated ostiolar necks, 100–350 µm diam., ostioles numerous, concentrated, 35–90 µm diam.	Ectostromatic disc inconspicuous, with single ostiolar necks 75–100 µm diam., ostioles centric or lateral, slightly papillate, 140–170 µm diam.	Ectostromatic disc surrounded by tightly aggregated ostiolar necks, 100–350 µm diam., ostioles numerous, concentrated, 40–90 µm diam.	Ectostromatic disc surrounded by tightly ostiolar necks, 285–465 µm diam., ostioles numerous, concentrated, 56–122 µm diam.
Perithecia	Flask-shaped to spherical, 0.26–0.34 mm diam.	Spherical, 120–300 µm diam.	Flask-shaped to spherical, solitary, 420–530 µm diam.	Spherical, 180–420 µm diam.	Flask-shaped to spherical, 235–350 µm diam.
Asci	Asci clavate to elongate obovoid, 38.6–43.8 × 5.1–6.2 µm	Asci clavate, 38–77 × 7–12.5 µm	Asci clavate to elongate, 60–85 × 10–16 µm, with an indistinct pedicel, with “J-” apical ring in Melzer’s reagent	Asci clavate, 38–86 × 7.5–13.5 µm	Asci clavate to elongate obovoid, 22.5–49 × 4–10 µm
Ascospores	Elongate-allantoid, 8.3–13.1 × 2.0–2.8 µm	Biseriate, allantoid, 6.5–9 × 2–2.5 µm	Elongate-allantoid, straight to slightly curved, 10–25 × 4–6 µm	Biseriate, allantoid, 10–19.5 × 2–2.5 µm	Ascospores not observed

should be considered distinct or a single species. Until then, we recommend treating them as two distinct species.

Cytospora schulzeri Sacc. & P. Syd., Syll. fung. (Abellini) 14 (2): 918

Index Fungorum Registration Identifier: 140461

Figure 6

Description: *Saprobic* on a dead branch of *M. domestica* in China. **Sexual morph:** See Fan et al. (2020). **Asexual morph:** *Pycnidial stromata* ostiolate, scattered, immersed in bark, erumpent through the surface of bark, flat, discoid, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* 250–450 µm diam., circular to ovoid, brown, with one to five ostioles per disc. *Ostioles* numerous, 55–105 µm diam., arranged circularly, black, at the same level as the disc. *Locules* numerous, 900–1550 µm diam., irregular, arranged circularly with common walls. *Conidiomata wall* comprising a few layers of cells of *textura angularis*, with innermost layer brown, outer layer brown to dark brown. *Conidiophores* 10–20 × 1.5–2.0 µm, hyaline, unbranched, filamentous, thin-walled. *Conidiogenous cells* 9–16 × 1–2 µm (av. = 13.5 × 1.5 µm, *n* = 20), enteroblastic polyphialidic. *Conidia* 4.5–7 × 1–2 µm (av. = 5.5 × 1.5 µm, *n* = 20), allantoid, aseptate, hyaline, smooth, thin-walled.

Culture characteristics: Conidia germinating on PDA, producing germ tubes from both ends within 24 h. Colonies fast growing, reaching up to 6 cm in diam. after 7 days and entirely covering the 9-cm plate after 14 days, centrally white and olivaceous gray at the margin, becoming olivaceous black at the center. Colonies are slightly fluffy, thin with a uniform texture; sterile.

Material examined: CHINA, Yunnan Province, Qujing City, 25°47′ 77″N, 62°34′96″E, on a dead branch of *M. domestica*, 1 October 2021, Guiqing Zhang, Z6 = MHZU 23-0266, living culture: ZHKUCC 23-0980; *Ibid.* Z6-1 = GMB1005, living culture: GMBCC1005.

Known host and distribution: Known on *M. pumila* from Hebei, Ningxia, and Gansu provinces, China (Fan et al., 2020); on *Chestnut* from Hebei, China (Jiang et al., 2020); on *M. spectabilis* from Tibet, China (Li et al., 2024a); and on *M. domestica* from Yunnan Province, China (this study).

Notes: Our second cytospora-like taxon morphologically resembles *C. schulzeri* (CFCC 50040 and CFCC 50042), and this was confirmed in the phylogenetic analysis (Figure 1, ZHKUCC 23-0980 and GMBCC1005). *Cytospora schulzeri* is a common pathogen that causes apple canker disease in China (Wei, 1979; Zhuang, 2005). *Cytospora schulzeri* was reported as a saprobe on *M. pumila* from Hebei, Ningxia, and Gansu provinces (Fan et al., 2020); as pathogens that caused canker disease in *Castanea mollissima* and branches of *M. spectabilis* from Hebei Province and Tibet, respectively (Jiang et al., 2020; Li et al., 2024a). In this study, samples were collected from branches of *M. domestica* and identified as a saprobic fungus. Hence, this is the first record of *C. schulzeri* on *M. domestica* from Yunnan Province, southwestern China.

Allocriptovalsa Senwanna, Phookamsak & K.D. Hyde, in Senwanna, Phookamsak, Doilom, Hyde & Cheewangkoon, Mycosphere 8(10): 1839

Index Fungorum Registration Identifier: 553857

Type species: *Allocriptovalsa polyspora* Senwanna, Phookamsak & K.D. Hyde, in Senwanna, Phookamsak, Doilom, Hyde & Cheewangkoon, Mycosphere 8(10): 1840

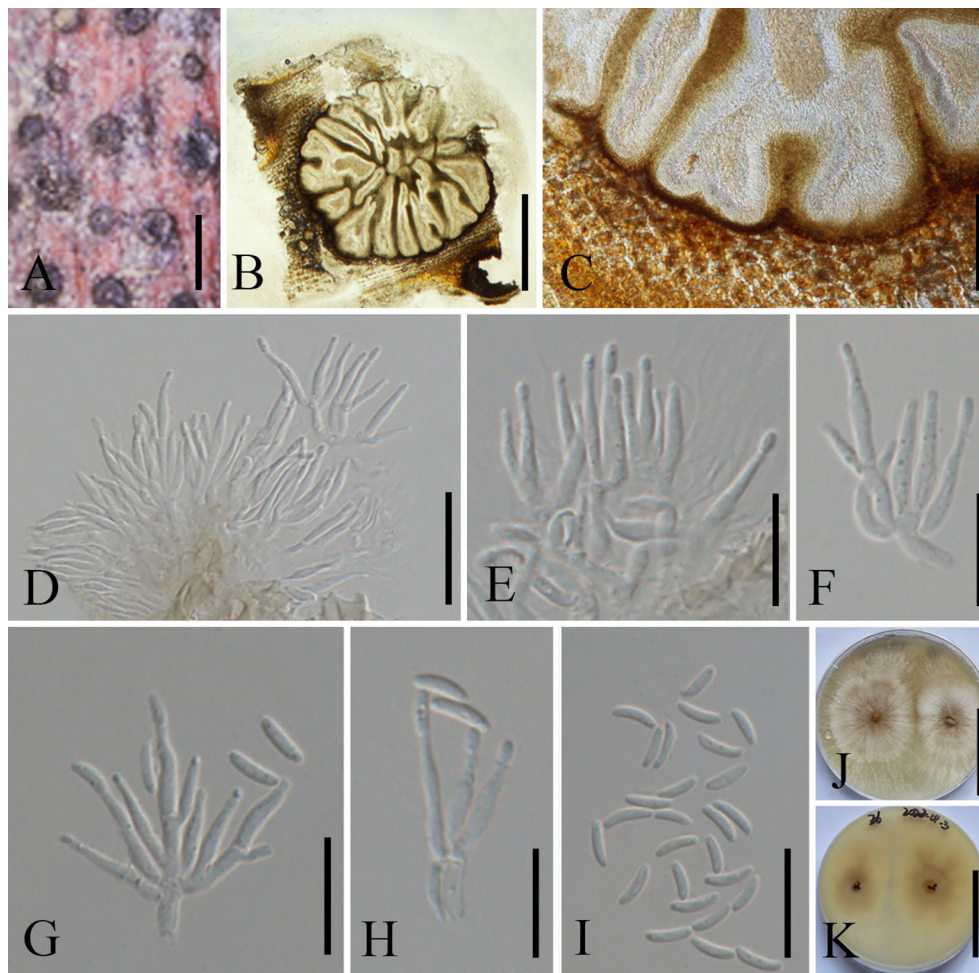


FIGURE 6

Cytospora schulzeri on a dead stem of *Malus domestica* (MHZU 23-0266). (A) Habit of conidiomata on a branch. (B) Transverse section through conidiomata. (C) Pycnidial wall. (D–H) Immature and mature conidia attached to conidiogenous cells. (I) Conidia. (J, K) Colonies on PDA from above and below (J: above, K: below). Scale bars: (A) = 300 μ m, (B) = 2 mm, (C) = 100 μ m, (D–H) = 15 μ m, (I) = 10 μ m, and (J, K) = 5 cm.

Allocryptovalsa Senwanna, Phookamsak & K.D. Hyde, typified with *A. polyspora* Senwanna, Phookamsak & K.D. Hyde. Zhu et al. (2021) regarded that the members of *Allocryptovalsa* show a cosmopolitan distribution (i.e., in Australia, China, Germany, India, Thailand, and the United States). *Allocryptovalsa* was originally introduced to accommodate two species, i.e., *A. cryptovalsoidea* and *A. rabenhorstii* (basonym: *Valsa rabenhorstii* Nitschke), which was characterized by immersed perithecia, polysporous asci, and allantoid ascospores (Senwanna et al., 2017). Subsequently, Konta et al. (2020) and Hyde et al. (2020) introduced *A. elaeidis* from *Elaeis guineensis* and *A. truncata* isolated from decaying twigs of unidentified plants, respectively. Zhu et al. (2021) introduced two new species within this genus, *A. castaneae* and *A. castaneicola*, which were isolated from *C. mollissima* in China. Hitherto, three additional new species have been reported in *Allocryptovalsa*. *Allocryptovalsa xishuangbanica* was discovered on dead branches collected from China (Maharachchikumbura et al., 2022); *A. aceris* was isolated on dead twigs of *Acer palmatum* (Aceraceae) from China (Senanayake et al., 2023); and *A. aquilariae* was isolated from dead twigs of *Aquilaria sinensis* from China

(Chethana et al., 2023). In this study, we collected *A. castaneae* and it is the first host record in China.

Allocryptovalsa castaneae N. Jiang & X.L. Fan, *Frontiers in Microbiology* 12(no. 646262): 5

Index Fungorum Registration Identifier: 837777

Figure 7

Description: Saprobic on branches of *M. domestica* in China.

Sexual morph: Stromata 2.5–3.5 mm diam., scattered to gregarious, immersed in the bark, erumpent through the surface of bark, with 8–13 perithecia arranged irregularly. Ectostromatic disc 0.2–0.6 mm diam., circular to oblong, brown, with more than eight ostioles arranged circularly per disc. Ostioles numerous, 100–250 μ m diam., gregarious, umbilicate, 4-sulcate dark brown to black, at the same level as the disc. Perithecia 210–280 μ m diam., outer surface coated with yellow, powdery entostroma, black, flask-shaped, perithecial necks erumpent in groups. Asci 120–220 $\times$ 12–24 μ m (av. = 155 $\times$ 18 μ m, n = 10), unitunicate, polysporous, clavate to elongate obovoid, long pedicellate, apically rounded, thin-walled. Ascospores 7.5–10.5 $\times$ 2.5–3.5 μ m (av. = 9 $\times$ 3 μ m, n = 20),

aseptate, elongate-allantoid, slightly curved, pale yellowish to pale brown at maturity, smooth, thin-walled. **Asexual morph:** undetermined.

Culture characteristics: Colonies are initially white, uniform, becoming dark after 2 weeks.

Material examined: CHINA, Yunnan Province, Qujing City, 25° 47'77"N, 62°34'96"E, 1 October 2021, on a dead branch of *M. domestica*, Guiqing Zhang, Z7 = MHZU 23-0264, living culture: ZHKUCC 23-0981; *Ibid.* Z1, living culture: ZHKUCC 23-0982.

Known host and distribution: Known on *C. mollissima* from Hebei Province and on *Juglans regia* in Yunnan Province, Chuxiong Yi Autonomous Prefecture (Zhu et al., 2021); *M. domestica* in Yunnan province, southwestern China (this study).

Notes: *Allocriptovalsa castaneae* is a taxon with a distinctive orange ectostromatic disc, and short pedicellate asci, which was described by Zhu et al. (2021). However, our new collection has black ectostromatic disc and slightly broader asci with a longer pedicel. Nevertheless, based on phylogenetic analysis (Figure 2), we confirmed that the new collections (i.e., ZHKUCC 23-0981 and ZHKUCC 23-0982) are *A. castaneae*. *Allocriptovalsa castaneae* has been known on *C. mollissima* in Hebei and on *J. regia* in Chuxiong Yi Autonomous Prefecture, Yunnan Province, China. Therefore, we report a new host record of *A. castaneae* on *M. domestica* from Qujing City, Yunnan, China.

Aureobasidium Viala & G. Boyer, Rev. gén. Bot. 3: 371

Index Fungorum Registration Identifier: 7297

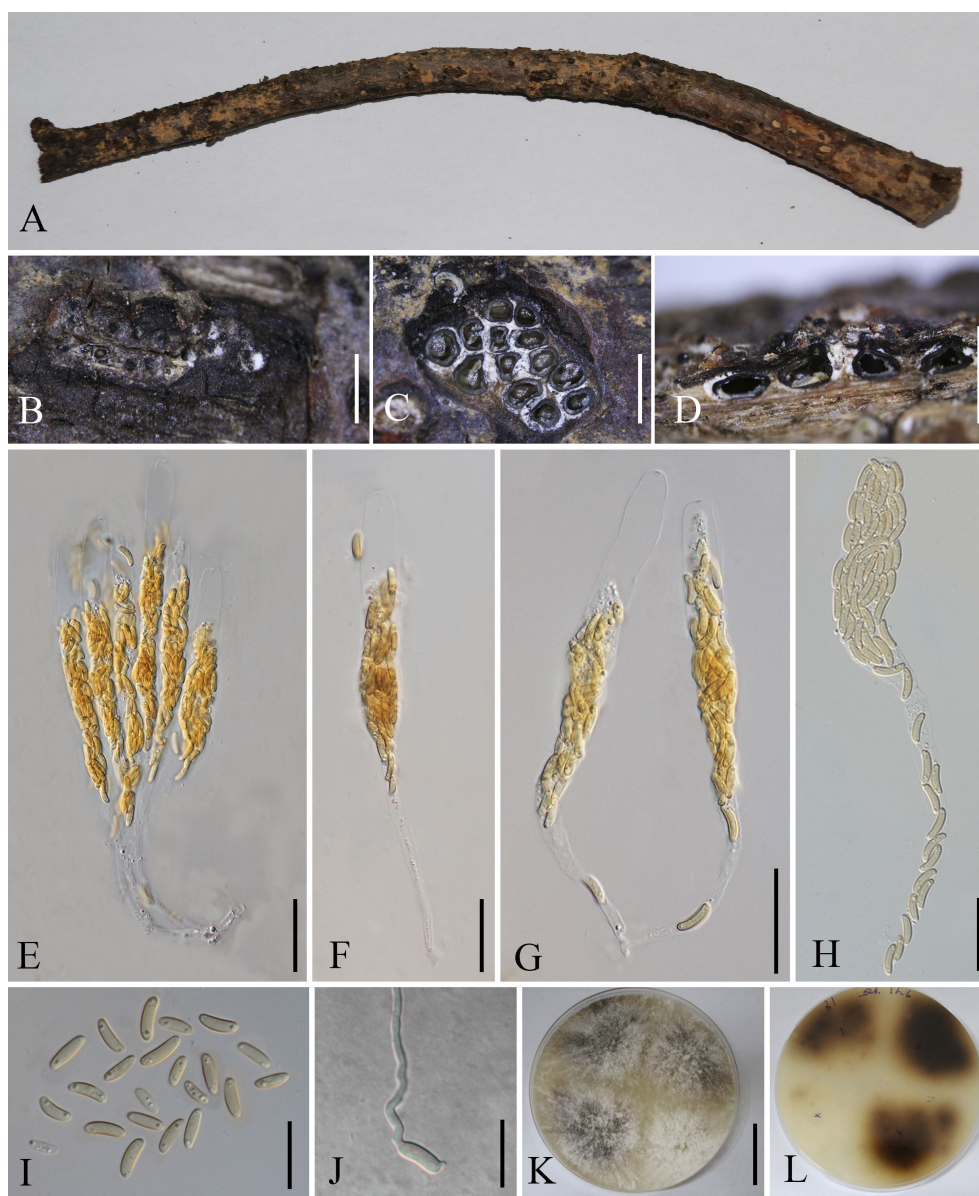


FIGURE 7

Allocriptovalsa castaneae (MHZU 23-0264). (A) *Malus domestica* branch. (B) Habit of stromata on a branch. (C) Transverse section through stroma. (D) Longitudinal section through the stroma. (E–H) Asci. (I) Ascospores. (J) Germinating ascospore. (K, L) Colonies on PDA from above and below [(K) above, (L) below]. Scale bars: (B) = 2 mm, (C) = 250 μ m, (D) = 1.2 mm, (E–H) = 30 μ m, (I, J) = 5 μ m, and (K, L) = 4 cm.

Type species: *Aureobasidium vitis* Viala & G. Boyer, Rev. gén. Bot. 3: 371

Aureobasidium Viala & G. Boyer, typified with *Au. vitis* [current name: *Au. pullulans* (de Bary & Löwenthal) G. Arnaud]. *Aureobasidium* species are often called black yeast because they produce melanin during growth, and species of *Aureobasidium* with a yeast-like morph have a wide range of distribution and diverse life modes, including saprobes, endophytes, and pathogens (Lee et al., 2021; Wang et al., 2022). Recently, based on morphology characters, phylogenetic analysis, and biochemistry, more species of this genus have been introduced. So far, 66 epithets have been recorded in *Index Fungorum* (2024) (accessed 23 June 2024). *Aureobasidium pullulans* can produce pullulan polysaccharides, which are with the properties of water retention, barrier formation, regeneration, whitening, hydrating, and repairing, and it also serves as an ingredient in cosmetic formulas (Wu et al., 2023). In this study, we collected an *Aureobasidium* species from *M. domestica*. Morpho-molecular analyses confirmed that it is *Au. pullulans*.

Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud, *Annals d'École National d'Agric. de Montpellier*, Série 2 16(1-4): 39
Index Fungorum Registration Identifier: 101771

Figure 8

Description: Endophytic in a leaf of *M. domestica* in China. **Sexual morph:** not observed. **Asexual morph on PDA:** Vegetative hyphae hyaline to brown, smooth to slightly roughened, thin-walled, 4–12 μm wide, constricted at septa, disarticulating to dark brown chlamydospores, 7–13 $\times$ 8–10 μm , intercalary or terminal. *Conidiogenous cells* undifferentiated, intercalary or terminal, or arising as short lateral branches on hyaline hyphae. *Conidia* 5–10 $\times$ 3–5 μm (av. = 8 $\times$ 4 μm , $n = 20$), one-celled, ellipsoidal and variable in shape and size, hyaline homogeneous to sectorized, yeast-like to filamentous growth, smooth. Secondary conidia smaller. *Endoconidia* 5 $\times$ 3 μm , occasionally produced in an intercalary cell and released into a neighboring empty cell.

Culture characteristics: Colonies on PDA at 28°C attaining approximately 50 mm diam. after 7 days, appearing smooth and slimy due to abundant sporulation, pinkish, reverse pinkish. Initially white, uniform, becoming pink after 2 weeks. *Hyphae* are hyaline, smooth, thin-walled, with transverse septa.

Material examined: CHINA, Yunnan Province, Qujing City, *Malus* plantation, 25°47'77"N, 62°34'96"E, on the leaves of *M. domestica*, 1 October 2021, Guiqing Zhang, Z24, living culture: ZHKUCC 23-0983. *Ibid.* Z36, living culture: GMBCC1006

Known host and distribution: Known on *M. domestica* in Poland, Europe (Mirzwa-Mróz and Wińska-Krysiak, 2011); *M.*

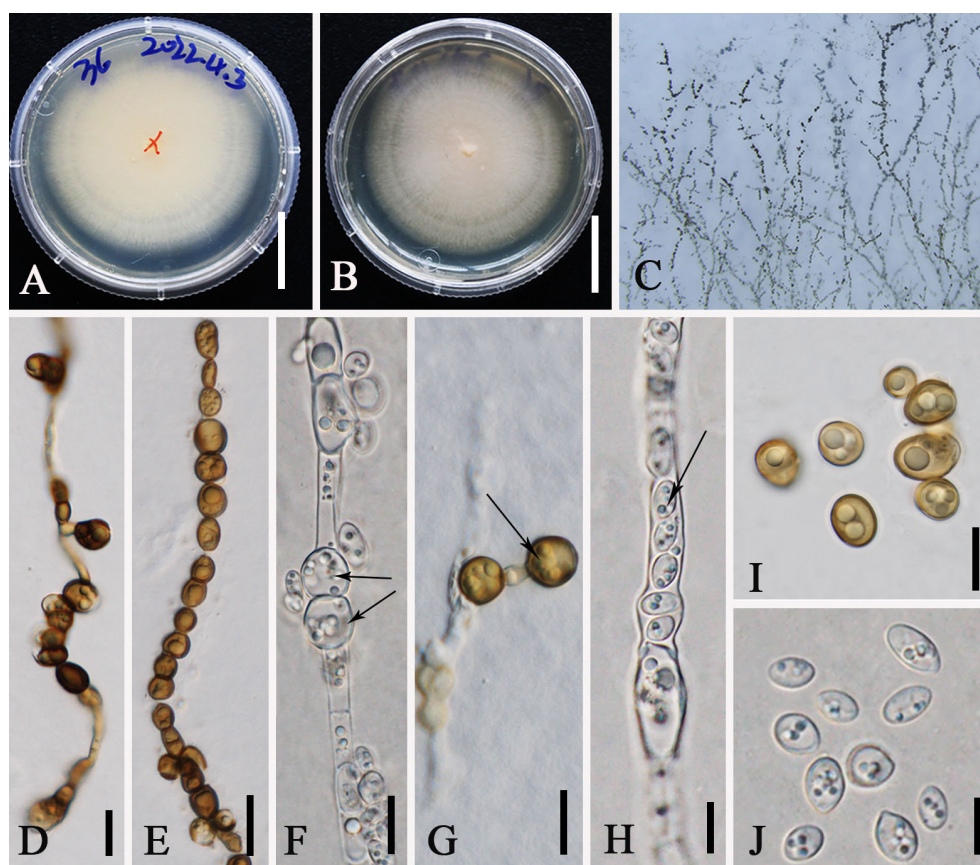


FIGURE 8

Aureobasidium pullulans (ZHKUCC 23-0983). (A, B) Colonies on PDA from above and below [(A) below, (B) above] (C) Melanized hyphae. (D) Melanized hyphae developing into arthrospores and chlamydospores. (E) Melanized hyphae/chlamydospores. (F) Intercalary chlamydospores. (G) Terminal chlamydospores. (H) Endoconidia. (I) Chlamydospores. (J) Conidia. Scale bars: (A, B) = 3 cm, (D, G) = 15 μm , (E) = 20 μm , and (F–J) = 10 μm .

pumila in Canada (Ginns, 1986); *Hylocereus polyrhizus* and *Hylocereus undatus* (Taylor and Hyde, 2003; Ding et al., 2021); *Pinus thunbergii* (Jayawardena et al., 2018); *Trachycarpus fortunei* (Wu et al., 2017); *Vitis* sp (Grabowski, 2007); *Pinus* in China (Ding et al., 2021); and *M. domestica* in Yunnan Province, China (this study).

Notes: In this study, *Au. pullulans* was isolated as an endophyte in the leaves of *M. domestica*. Previously, it was reported from the leaves of *M. domestica* which was grown at an experimental orchard in Germany (Rühmann et al., 2013). Moreover, it shows a broad range of geographical distribution from cold to warm climates and wet/humid regions to arid ones (Bozoudi and Tsaltas, 2018). Phylogenetically, our new collections (ZHKUCC 23-0983, GMBCC1006) clustered in the clade that comprises *Au. pullulans*, *Au. proteae*, and *Au. microstictum*. Morphologically, our collections closely resemble *Au. pullulans*, in having yeast-like colonies covered with a slimy mass of spores and same-size conidia [$5\text{--}10 \times 3\text{--}5\text{ }\mu\text{m}$ (av. = $8 \times 4\text{ }\mu\text{m}$, $n = 20$)] and possess chlamydospores and endoconidia. However, our collections can be distinguished from *Au. proteae* and *Au. microstictum* by the presence of chlamydospores and endoconidia (Yoshikawa and Yokoyama, 1987; Crous et al., 2011). Thus, we confirmed our new collections (ZHKUCC 23-0983 and GMBCC1006) to belong to *Au. pullulans*, and this is the first report of *Au. pullulans* from Yunnan, southwestern China.

Hypoxylon Bull., Hist. Champ. Fr. (Paris) 1(1): 168

Index Fungorum Registration Identifier: 2456

Type species: *Hypoxylon coccineum* Bull., Hist. Champ. Fr. (Paris) 1(1): 174

Species of *Hypoxylon* are often isolated as saprobes and endophytes of angiospermous plants (Halecker et al., 2020; Ma

et al., 2022). The sexual morph of *Hypoxylon* is characterized by hemispherical, cushion-shaped stromata, immersed locules, periphysate ostiolate opening, eight-spored, uniseriate asci with an amyloid apical ring, one-celled ascospores (Rogers, 2018), while asexual morphs are characterized by different branching patterns of conidia, conidiogenous structure with virgariella-like branching patterns on natural substrate and artificial medium (Ju and Rogers, 1996). In this study, we collected a *Hypoxylon* species from apples. Morpho-molecular analyses confirmed that it is a novel taxon of *Hypoxylon*; thus, we introduce *H. malongense*.

Hypoxylon malongense G.Q. Zhang, Wijayaw., & Q.R. Li, sp. nov.

Index Fungorum Registration Identifier: IF902663

Figure 9

Etymology: Named after the location “Malong” where the new taxon was first discovered.

Ex-type: ZHKUCC 23-0984

Description: Endophytic in the leaf of *M. domestica* in China.

Sexual morph: not observed. **Asexual morph on PDA:** Conidiogenous structure on PDA with nodulisporium-like branching patterns to unbranched. **Conidiophores** simple, septate, thin, sparingly branched or unbranched, straight to slightly curved, hyaline to yellow-brown, smooth walled to sometimes verruculose. **Conidiogenous cells** $9\text{--}19 \times 2\text{--}4\text{ }\mu\text{m}$ (av. = $15 \times 3\text{ }\mu\text{m}$, $n = 10$), enteroblastic, phialidic, terminal or lateral, subcylindrical, straight or slightly curved, hyaline to pale yellow-brown, smooth to finely verruculose, denticulate and protuberant conidiogenous loci, thickened. **Conidia** $4.1\text{--}7 \times 2.0\text{--}3.0\text{ }\mu\text{m}$ (av. = $5.0 \times 2.5\text{ }\mu\text{m}$, $n = 20$), aseptate, rarely oblong, ellipsoidal to subglobose, apex obtuse,

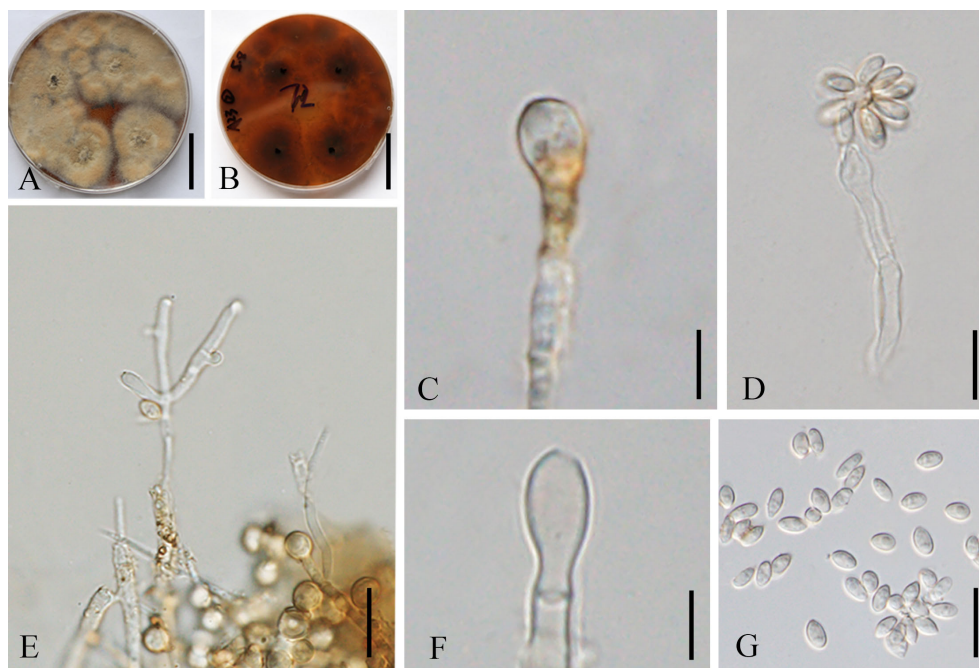


FIGURE 9

Hypoxylon malongense (ZHKUCC 23-0984, ex-type). (A, B) Colonies on PDA from above and below [(A) below, (B) above]. (C, E, F) Conidiophores and conidiogenous cells. (D) Anamorph from structure with nodulisporium-like branching patterns. (G) Conidia. Scale bars: (A, B) = 3 cm, (C) = 5 μm , (D, F, G) = 10 μm , and (E) = 15 μm .

base truncate or bluntly rounded, straight or slightly curved, hyaline, smooth, mostly with minute guttules.

Culture characteristics: Colonies on PDA covering a 9-cm Petri dish after 3 weeks at 28°C, under 24 h dark, at first whitish becoming pale brown powdery, with scarce white mycelium that form rays toward the margins; yellowish-brown from below. Sporulating regions scattered over the entire surface of the colony.

Material examined: CHINA, Yunnan Province, Qujing City, *M. domestica* plantation, 25°47'77"N, 62°34'96"E, endophytic in the living leaves of *M. domestica*, 1 October 2021, Guiqing Zhang, Z60, ex-type: ZHKUCC 23-0984. *Ibid.* Z72, isotype: GMBCC1007.

Notes: The multilocus phylogenetic analyses indicate that our new isolates *H. malongense* [ZHKUCC 23-0984 (ex-type) and GMBCC1007] form an independent lineage sister to *H. hinnuleum* [MUCL 3621 (ex-type), DSM:107932, and DSM:107926] with high statistical support (100% ML, 1.00 PP) (Figure 4). Morphologically, *H. malongense* can be distinguished from *H. hinnuleum* by its distinct differences in conidiogenous structure and conidia (Table 3). Our isolation is characterized by having nodulisporium-like branching patterns or unbranched conidiogenous structure, with ellipsoidal to subglobose conidia. However, *H. hinnuleum* is characterized by virgariella-like, nodulisporium-like, and periconiella-like branching patterns, with ellipsoid conidia. Therefore, more morphological differences between the new taxon and *H. hinnuleum* are listed in Table 3. Based on both morphology and phylogeny, we established this species as a novel taxon within *Hypoxylon*.

TABLE 3 Diagnostic characteristics of *Hypoxylon* species.

Morphological and colony characters	Species name and references	
	<i>H. hinnuleum</i> (Se et al., 2019)	<i>H. malongense</i> (This study)
Conidiogenous structure	With virgariella-like, nodulisporium-like, and periconiella-like branching patterns	With nodulisporium-like branching patterns to unbranched
Conidiophores	Hyaline, smooth to slightly roughened	Hyaline to yellow-brown, septate, smooth-walled to sometimes verruculose, sparingly branched or unbranched
Conidiogenous cells	Hyaline, 9.1–20.8 × 1.7–2.4 μm	Hyaline to pale yellow-brown, 9–19 × 2–4 μm, phalidic
Conidia	Ellipsoid, hyaline to pale brown, smooth to rough, 3.6–5.1 × 2.1–3.1 μm	Rarely oblong, ellipsoidal to subglobose, apex obtuse, base truncate or bluntly rounded, 4.1–7 × 2.0–3.0 μm
Culture characteristics	Colonies on OA covering a 6-cm Petri dish after 3 weeks, at first whitish becoming apricot powdery with scarce white mycelium that forms rays toward the margins; reverse, orange, sienna	Colonies on PDA covering a 9-cm Petri dish after 3 weeks at 28°C, under 24 h dark, at first whitish becoming pale brown powdery, with scarce white mycelium that forms rays toward the margins; yellowish-brown from below

3.3 Discussion

3.3.1 Rich and underexplored fungal diversity in Yunnan

Yunnan Province in China is rich in fungal diversity, and annually, a significant number of species are introduced (e.g., Feng and Yang, 2018; Wijayawardene et al., 2021a, b; He and Zhao, 2022; Hongsanan et al., 2023; Du et al., 2023; Tian et al., 2024). These novel species have been reported from well-studied hosts (e.g., 1. from coffee *fide* Lu et al., 2022; 2. from bamboo *fide* Dai et al., 2022; Han et al., 2024a, b; 3. from Pará Rubber *fide* Xu et al., 2024; 4. from *Macadamia* *fide* Zhang et al., 2024a), well-studied microhabitats (e.g., freshwater fungi *fide* Shen et al., 2022; Li et al., 2024b), and unusual or understudied microhabitats (e.g., fungi from bats *fide* Liu et al., 2023; fungi from dead American bullfrog larvae *fide* Yang et al., 2023a). Furthermore, some species have been introduced from well-studied and complex genera, such as *Colletotrichum*, and confirmed the unexplored and rich fungal diversity in the region (e.g., *Colletotrichum gardeniae* Q. Zhang et al. *fide* Zhang et al., 2023).

Extensive exploration of fungal diversity in Qujing City, Yunnan Province has been carried out since 2019 and more than 16 novel fungal species have been introduced from different hosts in different studies (Yasanthika et al., 2020; Doilom et al., 2021; Monkai et al., 2021; Dissanayake et al., 2022; Wijayawardene et al., 2022b; Yang et al., 2023b, 2024; Wang et al., 2024b; Zhang et al., 2024b; Zhou et al., 2024). The present study is a continuation of a long-term study of discovering fungal diversity in an understudied geographical region,

Qujing City. *Malus* species are an important fruit crop that is widely cultivated in Qujing City and its adjacent villages (e.g., Tongquan Town, Maguohe Town, and Wangjiashizhuang Town). There are several varieties of *Malus* species commonly cultivated in this region. Even though *Malus* species are extensively cultivated, there are no proper studies carried out to understand the mycobiota inhabiting its phyllosphere and mycosphere. This study has been carried out to fulfil this requirement. We have not observed any significant diseases; thus, we focused on identifying common saprobic and endophytic taxa associated with *Malus* species. In total, we have isolated 60 species from different localities of Qujing, and most taxa belong to *Alternaria* sp. and *Fusarium* sp.

In the present study, two novel species (*C. qujingensis* and *H. malongense*) and three new records (*C. schulzeri*, *A. castaneae*, and *Au. pullulans*) have been compiled. Based on detailed morphological studies, complemented by phylogenetic analyses based on ITS, *tef1-α*, *rpb2*, *tub*, and *act* sequence data (Figure 1), *C. qujingensis* is introduced as a novel saprobe taxon, while collections (ZHKUCC 23-0980 and GMBCC1005) revealed to be hitherto known species, namely, *C. schulzeri*, which was first reported from southwestern China. However, *H. malongense* was isolated as novel endophytic fungi on *M. domestica* from Qujing, Yunnan, based on morphological descriptions coupled with phylogenetic analyses (ITS, LSU, *rpb2*, and *tub* loci regions). Furthermore, Zhu et al. (2021) reported *A. castaneae* on *C. mollissima* and *J. regia* from Hebei and Yunnan provinces, respectively. Herein, we report our collections (ZHKUCC 23-0981

and ZHKUCC 23-0982) as a new host record of *A. castaneae* isolated from *M. domestica*. Moreover, the ITS and LSU regions are used to construct the phylogenetic tree of *Aureobasidium*, and the placements in Figure 3 in our study agree with Humphries et al. (2017) and Wu et al. (2023), who performed their analysis based on LSU and ITS gene regions. The morphological characteristics of each related species in the phylogenetic tree are compared in our study. Thus, we regard our collections (ZHKUCC 23-0983 and GMBCC1006) as the first report of *Au. pullulans* on *M. domestica* from Yunnan, China. Both the geographical and host distribution of fungus provide new insights into its ecological preferences and contribute to our knowledge of its regional distribution (Li et al., 2024b).

3.3.2 Species that can switch life modes; insights from this study

Fungi play key roles in ecosystems as saprobes, endophytes, and pathogens, but the role of an individual species in nature is still unknown (Schmit and Mueller, 2007). Recently, more research focused on the properties of endophytic fungi. For example, the secondary metabolites produced by endophytes can be employed in biotechnology such as in the pharmaceutical industry (Strobel and Daisy, 2003; Mapook et al., 2022). Mattoo and Nonzom (2021) mentioned that the endophyte could switch to be a pathogen lifestyle when it became more widespread upon the host getting old (e.g., *Colletotrichum tropicale* fide Rojas et al., 2010). For example, *Pestalotiopsis palmarum*, *Ceratocystis paradoxa*, and *Ganoderma lucidum* have been reported as endophytes, pathogens, or saprobes on coconut, which suggested that those fungi may switch their lifestyles from endophytes to pathogens or saprobes (Bhunjun et al., 2022; Tian et al., 2024). Furthermore, Knapp et al. (2018) claimed that a saprobic ancestral lifestyle of endophytes would be the reason for its ability to produce enzymes. Bhunjun et al. (2024) argued that the endophytic lifestyle is the ancestor of fungi, implying that some endophytes are host-specific, while others are connected to a wide range of hosts. A better understanding of fungi lifestyle switching could help us fill the gaps on fungi diversity and secondary metabolite diversity produced by fungi. Glauser et al. (2009) predicted that the production of secondary metabolite can be triggered by the presence of other fungi and pathogens.

In the present study, we isolated two *Cytospora* species, and both of them are isolated as saprobic taxa on *M. domestica*. More than 100 species of *Cytospora* are causal agents (or associated with) of the stem canker and dieback of woody and coniferous plants (e.g., *Populus* and *Salix*) (Fan et al., 2020). Initially, *C. schulzeri* was reported as a pathogen that can cause black spots on the infected apple trees in China (Wei, 1979; Zhuang, 2005). Recently, it was reported as a saprobe on *M. pumila* and a pathogen on *C. mollissima* and *M. spectabilis* in China (Fan et al., 2020; Jiang et al., 2020; Li et al., 2024a). However, we have not observed any disease symptoms that are similar to symptoms caused by *C. schulzeri* on *Malus* spp. in our study areas. Thus, we conclude that *C. schulzeri* is only a saprobic taxon in this region. More collections are needed to confirm whether *C. qujingensis* and *C. schulzeri* can exist as endophytes. Furthermore, pathogenicity tests need to be carried out to check whether both species are latent pathogens of *M. domestica*.

Species of *Diatrypaceae* were less reported as pathogens (Trouillas et al., 2011) or endophytes in petioles and woody tissue (Carroll, 1986; de Errasti et al., 2010), but most of them were predominantly saprobes inhibiting the wood and bark of various angiosperms. Hitherto, *Allocryptovalsa* species have not been reported as pathogens. Nevertheless, we conclude that further collection of different hosts in this region would provide insightful data to conclude the host switching and life mode changes of the taxon.

Aureobasidium pullulans was initially found as a saprobic yeast-like taxon but subsequently reported as an endophyte on the flesh of sweet cherries (Scheda et al., 2003) and a pathogen isolated from the environment of a patient's bone marrow (Liu et al., 2020). Ginns (1986) isolated this species on *M. pumila* from Canada, and later, Mirzwa-Mróz and Wińska-Krysiak (2011) recorded *Au. pullulans* on *M. domestica* from Europe. Previously, Ding et al. (2021) reported *Au. pullulans* from *P. thunbergii* (which causes brown spot needle blight) in China. In our study, we report *Au. pullulans* as an endophyte on *M. domestica* from China for the first time. Furthermore, many yeast species, including the yeast-like species, *Au. pullulans*, have been reported as effective antagonists against postharvest diseases in fruit. *Aureobasidium pullulans* is an effective biocontrol agent against postharvest diseases in various fruits, including apples (Castoria et al., 2001).

Hypoxyton malongense was isolated as an endophyte, from *M. domestica* in Qujing in the present study. *Hypoxyton* species are generally regarded as saprobe, but some of them are known to have an endophytic phase in their life cycle (viz., *H. rubiginosum*, *H. guilanense*) (Halecker et al., 2020). For example, *H. monticulosum* and *H. submonticulosum* have been isolated as endophytes from the stems of *Litsea akoensis* var. *chitouchiaoensis* (Lauraceae) and raspberry (*Rubus idaeus*), respectively (Burgess et al., 2017; Cheng et al., 2020). Furthermore, *H. fuscum*, *H. truncatum*, and *H. investiens* have been reported to exist with an endophytic lifestyle and produce abundant secondary metabolites (Gu et al., 2007; Basnet et al., 2019; Yuan et al., 2019). Some species in this genus are found as pathogens on woody plants. For example, *H. macrocarpum* was reported as the causal agent of wood rot (Hu and Wright, 2022). More studies indicated that *Hypoxyton* can depend on a wide range of hosts (Ma et al., 2022; Zhu et al., 2023). However, whether there is lifestyle switching, more samples are needed to demonstrate the mechanisms of switching between and within species. Anyway, illustrations and morphological descriptions of the asexual morph of this genus in the future are also needed. More research is still needed to figure out the species of our collections whether they contain secondary metabolites to further determine their classification placements. However, we believe it is essential to document and make an inventory of the distribution of species to understand the biogeographical and evolutionary patterns.

3.3.3 Necessity of inventorying and continuous updating of *Malus*-associated fungi against the geographical distribution

Nevertheless, the fungal diversity and impact of fungal pathogens on the agricultural and forest industries are poorly studied in China. As Shen et al. (2018) summarized, fungal diversity will be affected by plant species, environmental conditions, sampling time, and isolation and extraction methods.

Shen et al. (2018) indicated the remarkable fungal diversity on the apple surfaces. Dai et al. (2021) listed all pathogenic fungi on apples (containing apple root, shoot, leaf, flower, and fruit). There exist 65 types of apple disease in China, and 46 of them are caused by 149 different kinds of pathogenic fungal species. A search involving the keywords “*Malus domestica*” and “China” retrieved 47 fungi species present in the USDA Fungal Databases (accessed on 20 September 2024). Furthermore, extra retrieval involving the keywords “*Malus domestica*” and “China” retrieved 19 titles of research articles that had been published since 2018 in the Scopus Database (accessed on 20 December 2024). We scanned more than 100 available articles about fungi species (including saprobes, pathogens, and endophytes) associated with *M. domestica* reported in China in Chinese and English from 2000 to present. Hitherto known fungi species isolated on *M. domestica* from China, including saprobic, pathogenic, and endophytic fungi, was presented in a checklist (Supplementary Table S1), which will expand our knowledge of fungi associated with *M. domestica* and will prove timely and informative to a researcher’s future work.

Some culturable taxa like *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* have been identified by previous studies (Magnani et al., 2007; Tadych et al., 2012). Granado et al. (2008) pointed out that the molecular approach, involving ribosomal DNA sequencing, to identify yeasts and sterile fungi, allows to spot unculturable fungi on apple fruits in the future. Bulgari et al. (2012) reported some uncultured species on *Malus*, viz., *Bradyrhizobium*, *Bradyrhizobium*, and *Legionella*. Our current study showed that *Malus* species are rich in culturable fungal diversity. However, we have not regarded the unculturable fungal diversity in this study. Hence, we conclude that more studies need to be carried out to collect more data to recognize the distribution of *Malus* taxa, and for precise identification based on the morpho-molecular analyses, barcoding current pathogens and potential pathogens would also be essential. Furthermore, more samples are required to determine whether fungi exist as a saprobe or endophyte and have the potential to be pathogens as well. In the future, studies based on omics approaches would be helpful in discovering novel taxa including unculturable taxa (Wijayawardene et al., 2023).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

G-QZ: Conceptualization, Data curation, Methodology, Software, Writing – original draft. Z-ML: Formal analysis, Methodology, Writing – review & editing. X-LF: Investigation, Writing – review & editing. Q-RL: Investigation, Writing – review & editing. JK: Visualization, Writing – review & editing. NS: Visualization, Writing – review & editing. AE: Visualization, Writing – review & editing. IM: Visualization, Writing – review

& editing. D-QD: Conceptualization, Supervision, Writing – review & editing. NW: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2025.1517908/full#supplementary-material>

SUPPLEMENTARY TABLE 1

Checklist of fungi associated with *Malus domestica*/*Malus pumila* in China.

SUPPLEMENTARY TABLE 3

The names, isolate numbers, and corresponding GenBank accession numbers of the taxa used in Figure 1.

SUPPLEMENTARY TABLE 4

The names, isolate numbers, and corresponding GenBank accession numbers of the taxa used in Figure 2.

SUPPLEMENTARY TABLE 5

The names, isolate numbers, and corresponding GenBank accession numbers of the taxa used in Figure 3.

SUPPLEMENTARY TABLE 6

The names, isolate numbers, and corresponding GenBank accession numbers of the taxa used in Figure 4.

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EDITED BY

Sinang Hongsan,
Shenzhen University, China

REVIEWED BY

Sudhir Navathe,
Agharkar Research Institute, India
Khalid Hameed,
Duke University, United States

*CORRESPONDENCE

Abdollah Ahmadpour
✉ a.ahmadpour@urmia.ac.ir
Nakarin Suwannarach
✉ suwan_462@hotmail.com

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Morphological and phylogenetic analyses of *Bipolaris* species associated with Poales and Asparagales host plants in Iran

Abdollah Ahmadpour ^{1*}, Zeinab Heidarian ¹,
Youbert Ghosta ², Zahra Alavi ², Fatemeh Alavi ²,
Dimuthu S. Manamgoda ³, Jaturong Kumla ^{4,5,6},
Samantha C. Karunarathna ⁷, Pabulo Henrique Rampelotto ⁸
and Nakarin Suwannarach ^{4,5,6*}

¹Higher Education Center of Shahid Bakeri, Urmia University, Miyandoab, Iran, ²Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran, ³Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Sri Lanka, ⁴Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand, ⁵Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai, Thailand, ⁶Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ⁷Center for Yunnan Plateau Biology Resources Protection and Utilization, College of Biology and Food Engineering, Qujing Normal University, Qujing, Yunnan, China, ⁸Bioinformatics and Biostatistics Core Facility, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Bipolaris species exhibit various ecological roles, including plant pathogens, epiphytes, saprophytes, or endophytes, primarily associated with poaceous hosts, including cultivated cereals. Iran is known for its diverse climates and rich flora, which serve as a hotspot for fungal diversity. In this study, to determine the species diversity of *Bipolaris* associated with members of the Poales and Asparagales plant orders, samples with leaf and stem lesion symptoms were collected from these plants across various locations in Iran between 2010 and 2022. Based on the morphological characteristics and multi-locus phylogeny (ITS–rDNA, *GAPDH*, and *TEF1*), nine *Bipolaris* species were identified: *Bipolaris avrinica* sp. nov., *Bipolaris azarbaijanica* sp. nov., *Bipolaris banihashemii* sp. nov., *Bipolaris hedjaroudei* sp. nov., *Bipolaris hemerocallidis* sp. nov., *Bipolaris iranica* sp. nov., *Bipolaris persica* sp. nov., *Bipolaris crotonis*, and *Bipolaris salkadehensis*. *B. crotonis* represents a new record for Iran's fungi, while *B. salkadehensis* has been documented on several new hosts globally. The study provides detailed morphological descriptions and illustrations of all identified species, along with insights into their habitats, distributions, and phylogenetic relationships within the *Bipolaris* genus. This study also emphasizes the need for further research into fungal biodiversity in Iran and provides significant data on the distribution and host range of *Bipolaris* species.

KEYWORDS

helminthosporioid fungi, morphology, seven novel species, phylogeny, Pleosporaceae, taxonomy

1 Introduction

The genus *Bipolaris* was established by Shoemaker (1959) with *Bipolaris maydis* as the type species and belongs to the family Pleosporaceae (Pleosporales, Dothideomycetes, Ascomycota) (Manamgoda et al., 2011, 2012, 2014; Raza et al., 2019; Bhunjun et al., 2020). *Bipolaris* is a dematiaceous hyphomycetous genus, characterized by the production of sympodial conidiophores, straight or curved, and distoseptate conidia with the germination of end cells (Sivanesan, 1987; Manamgoda et al., 2011, 2012, 2014). Although the sexual state name *Cochliobolus* predates *Bipolaris*, a proposal to conserve the latter name was made (Rossman et al., 2013).

Bipolaris species exhibit diverse ecological roles as plant pathogens, epiphytes, saprophytes, or endophytes, often associated with grasses and cultivated cereals. These fungi are globally distributed and are significant plant pathogens causing diseases, like leaf spots, foliar blights, and root/foot rots, in various crops (Ellis, 1971; Sivanesan, 1987; Zhang and Li, 2009; Manamgoda et al., 2011, 2012, 2014; Tan et al., 2016; Raza et al., 2019; Bhunjun et al., 2020; Jayawardena et al., 2021; Ferdinandez et al., 2022; Khan et al., 2023; Farr et al., 2024). Certain *Bipolaris* species cause economically important plant diseases in cereal crops, such as rice brown spot (*Bipolaris oryzae*), barley and wheat common root rot or spot blotch (*Bipolaris sorokiniana*), and southern corn leaf blight diseases (*B. maydis*) (Manamgoda et al., 2014; Bhunjun et al., 2020; Jayawardena et al., 2021). In addition to grasses and cereals, *Bipolaris* species have been reported on over 60 other genera from various plant families, including Anacardiaceae, Araceae, Euphorbiaceae, Fabaceae, Malvaceae, Rutaceae, and Zingiberaceae, either as saprobes or pathogens (Ellis, 1971; Sivanesan, 1987; Manamgoda et al., 2011, 2012, 2014; Jayawardena et al., 2021; Farr et al., 2024). Furthermore, *Bipolaris cynodontis*, *B. oryzae*, and *Bipolaris setariae* have been identified as causative agents of human infections, such as lung and skin infections, allergic sinusitis, onychomycosis, keratitis, and central nervous system infections, particularly in immunocompromised individuals (da Cunha et al., 2012; Wang et al., 2016; Sharma and Nonzom, 2021). The ecological adaptability of *Bipolaris* is notable, as it thrives across a broad range of hosts, including grasses, cereals, and different dicotyledonous plants. This adaptability highlights the genus' capability to colonize diverse environments and exploit varying ecological conditions.

The taxonomy of the *Bipolaris* genus has historically presented challenges due to its morphological variability and overlapping characteristics with other genera within the family Pleosporaceae. Early classifications were primarily based on morphological traits such as conidial shape, septum ontogeny, germination patterns, hilum morphology, and sexual morph characteristics (Ellis, 1971; Sivanesan, 1987; Alcorn, 1988; Manamgoda et al., 2011, 2012, 2014; Amaradasa et al., 2014; Tan et al., 2014; Hernández-Restrepo et al., 2018). However, the advent of molecular phylogenetics has revolutionized our understanding of *Bipolaris* taxonomy uncovering cryptic species complexes and providing new insights into the evolutionary relationships within the genus. Historically, the genera *Bipolaris*, *Curvularia*, *Exserohilum*, *Johalcornia*, *Porocercospora*, and *Pyrenophora* were classified under the

helminthosporioid fungi or graminicolous *Helminthosporium* (Sivanesan, 1987; Alcorn, 1988; Manamgoda et al., 2011, 2012, 2014, 2015; Amaradasa et al., 2014; Tan et al., 2014; Hernández-Restrepo et al., 2018; Marin-Felix et al., 2020). Recent advancements in molecular biology and phylogenetics have led to substantial taxonomic revisions within this group resulting in the recognition of new genera in the family Pleosporaceae (Amaradasa et al., 2014; Manamgoda et al., 2014; Tan et al., 2014). The genus *Bipolaris* is morphologically similar to *Curvularia* and shares the same sexual morph, *Cochliobolus*, which makes their differentiation challenging (Manamgoda et al., 2011, 2014, 2015; Marin-Felix et al., 2017a, b, 2020). However, *Bipolaris* conidia are generally longer and maintain a uniform curvature along their length, unlike the conidia of *Curvularia*. Additionally, the *Bipolaris* species lack stromata structures, as documented in several studies (Manamgoda et al., 2014, 2015; Marin-Felix et al., 2017a, b, 2020). For these reasons, integrating morphological observations with molecular methods is crucial for accurately delineating helminthosporioid fungi, identifying species, and recognizing cryptic species within *Bipolaris* (Manamgoda et al., 2014, 2015; Tan et al., 2014, 2016; Marin-Felix et al., 2017a, b, 2020; Raza et al., 2019; Ferdinandez et al., 2022). At present, the Index Fungorum (<http://www.indexfungorum.org>, accessed on 20 October 2024) lists 146 names under the genus *Bipolaris*, of which approximately 70 species have been reported from the orders Poales and Asparagales, as well as from other monocotyledonous plants (Manamgoda et al., 2014; Farr et al., 2024).

Iran, with its diverse climatic zones and rich flora, represents a hotspot for fungal biodiversity. Despite this ecological importance, the fungal diversity in Iran remains relatively underexplored. In recent years, efforts to study fungal communities in Iran have accelerated driven by advancements in molecular biology and an increasing recognition of Iran's critical role in global biodiversity conservation. To date, 11 species of *Bipolaris* have been recorded in Iran (Ahmadpour et al., 2011, 2012a, 2012b, 2013, 2014, 2018; Ershad, 2022). However, many of these species were identified based solely on morphological traits raising questions about their accuracy in light of recent molecular taxonomic revisions of *Bipolaris* species from other regions. This study aims to identify *Bipolaris* species associated with Poales and Asparagales hosts in Iran by integrating morphological characteristics, ecological observations, and molecular data including ITS-rDNA, *GAPDH*, and *TEF1* sequences.

2 Materials and methods

2.1 Sample collection and fungal isolation

A total of 130 samples exhibiting leaf and stem lesions were collected from various host plants in the orders Poales and Asparagales across different locations in Iran (Isfahan, Mazandaran, and West Azarbaijan Provinces) between 2010 and 2022, and the important collection information was recorded (Rathnayaka et al., 2024). Subsequently, they were brought to the laboratory for further analysis. Small sections, approximately 0.5 ×

0.5 cm², were cut from the interface between healthy and diseased tissue. These sections were disinfected by submerging them in a diluted bleach solution (2% sodium hypochlorite) for 2 min, followed by three thorough rinses in sterile distilled water, and then blotted dry on sterile filter paper. The disinfected sections were then transferred to Potato Dextrose Agar (PDA, 39 g/L, Merck, Germany) plates supplemented with streptomycin sulfate and penicillin G (150 ppm each). The plates were incubated at 23 ± 2°C under cool white fluorescent light with a 12-h photoperiod for 5 days. Fungi growing out from the margins of plant sections were transferred into new PDA plates and purified via single-spore or hyphal tip methods. Furthermore, infected plant samples were incubated in moist chambers at 25°C until formation of conidial mass was observed. The incubated samples were inspected under a stereomicroscope, and single spores were then transferred to PDA at 23°C–25°C using a fine sterile needle. All identified isolates were deposited as pure cultures in the fungal culture collections at the Iranian Research Institute of Plant Protection (IRAN) and Urmia University (FCCUU).

2.2 Morphological characterization

Mycelial disks (5 mm in diameter) were excised from the actively growing margins of 7-day-old cultures and placed on fresh PDA, Corn Meal Agar (CMA, 17 g/L, Quelab, Montreal, Canada), and Malt Extract Agar (MEA, 50 g/L, Quelab, Montreal, Canada) media plates. The plates were incubated in the dark at 25°C for 7 days. Subsequently, the characteristics of the colonies, including color, pattern, and diameter, were observed and recorded. The color of the colonies was recorded using Rayner's (1970) color charts. The micro-morphological characteristics were observed using 10- to 14-day-old cultures on tap water agar plates with autoclaved wheat straw (TWA–wheat straw) or leaves of the host plant. The cultures were subjected to near-ultraviolet light on a 12-h diurnal cycle at 23°C–25°C, as described by Sivanesan (1987) and Hernández-Restrepo et al. (2018). Fungal structures, such as hyphae, conidiophores, conidiogenous cells, conidia, ascocarps, asci, and ascospores, were measured (20–50 measurements per structure) and photographed using an Olympus AX70 microscope with differential interference contrast (DIC) illumination from slide mounts prepared with either clear lactic acid or lactophenol cotton blue staining solutions. Images were edited with Adobe Photoshop 2020 v. 2.10.8 software (Adobe Inc., San Jose, California). Taxonomic novelties were registered in MycoBank (www.MycoBank.org; Crous et al., 2004).

2.3 DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from the mycelial mass of each isolate harvested from 10-day-old PDA Petri dishes using the method described by Ahmadpour et al. (2021). The internal transcribed spacer (ITS–rDNA) region, parts of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and the translation elongation factor-1 alpha (*TEF1*) genes were amplified using the primer pairs

ITS1/ITS4 (White et al., 1990), *gpd1/gpd2* (Berbee et al., 1999), and TEF1-983F/TEF1-2218R (Rehner and Buckley, 2005), respectively. Polymerase chain reaction (PCR) was performed in the SimpliAmpTM Thermal Cycler (Applied BiosystemsTM, Thermo Fisher Scientific Corp., USA) with a final volume of 30 µl. The PCR mixture comprised of 0.4 µM of each primer, 10 µl of a ready master mix (Taq DNA Polymerase 2× Master Mix Red, 2 mM MgCl₂, Ampliqon Company, Denmark), and approximately 10 ng of DNA. The PCR amplification conditions were as follows: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 62°C–57°C (annealing temperature decreased by 0.5°C per cycle in the first 12 cycles) for 45 s, extension at 72°C for 45 s, and a final extension step at 72°C for 7 min. Amplicons were visualized on a 1% agarose gel stained with FluoroVueTM Nucleic Acid Gel Stain (SMOBIO Technology Inc., China), and the sizes of amplicons were determined using a FluoroBandTM 100 bp+3K Fluorescent DNA Ladder (SMOBIO Technology Inc., China). The amplified products were cleaned and sequenced by Macrogen Corp. (Seoul, South Korea) using the same primer sets that were used for PCR amplification. The sequences derived from this study were submitted to GenBank (Table 1).

2.4 Sequence alignments and phylogenetic analyses

The initial identification of the isolates involved utilizing newly generated sequences of ITS–rDNA, *GAPDH*, and *TEF1* with the NCBI Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/blast/). Subsequently, pairwise sequence comparisons were performed between novel species and their closely related taxa using the same BLAST tool. DNA sequences from the type or representative species were obtained from GenBank (Table 1) and used in the analyses. A multi-locus phylogenetic analysis was conducted on a combined dataset comprising the three genes/regions (ITS–rDNA + *GAPDH* + *TEF1*). Multiple sequence alignment was done using the online alignment tool MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>) (Katoh et al., 2019). The best-fit substitution models were determined with the Akaike Information Criterion (AIC) in MrModeltest 2.3 (Nylander, 2004). The maximum likelihood (ML) and maximum parsimony (MP) analyses were conducted via the CIPRES Science Gateway portal (accessible at <https://www.phylo.org/>) (Miller et al., 2012) using RAXML-HPC BlackBox v. 8.2.12 (utilizing the GTR + GAMMA model and 1,000 bootstrapping iterations) (Stamatakis, 2014) and PAUP on ACCESS v. 4.0a168 (using the heuristic search option and branch swapping with the tree-bisection–reconnection (TBR) algorithm with 1,000 bootstrapping replicates) (Swofford, 2002) tools, respectively. Descriptive tree statistics [tree length (TL), consistency index (CI), retention index (RI), and homoplasy index (HI)] were calculated for trees generated in the parsimony analysis. Bayesian phylogenetic inference (BI) and Bayesian posterior probabilities (BPP) were conducted in MrBayes v. 3.2.7 (Ronquist et al., 2012) with the Markov chain Monte Carlo (MCMC) method (four chains, 1,000,000 generations, 1,000

TABLE 1 GenBank and culture collection accession numbers of *Bipolaris* isolates used in this study.

Species	Isolate/culture collection ^{a,b}	Host/Substratum	Country	GenBank accessions			References
				ITS	GAPDH	TEF1	
<i>Bipolaris adikaramae</i>	USJCC-0008 ^T	<i>Panicum maximum</i>	Sri Lanka	MN535176	MT497479	MT548605	Ferdinandez et al., 2022
<i>B. adikaramae</i>	USJCC-0017	<i>Panicum maximum</i>	Sri Lanka	MT509431	MT497473	MT548601	Ferdinandez et al., 2022
<i>B. austrostipae</i>	BRIP 12490 ^T	<i>Austrostipa verticillata</i>	Australia	KX452442	KX452408	KX452459	Tan et al., 2016
<i>B. avrinica</i>	IRAN 4806C ^T	<i>Setaria</i> sp.	Iran	PP799772	PP806864	PP806836	This study
<i>B. avrinica</i>	FCCUU 1012	<i>Setaria</i> sp.	Iran	PP799773	PP806865	PP806837	This study
<i>B. axonopicola</i>	BRIP 11740 ^T	<i>Axonopus fissifolius</i>	Australia	KX452443	KX452409	KX452460	Tan et al., 2016
<i>B. azarbaijanica</i>	IRAN 4776C ^T	<i>Setaria</i> sp.	Iran	PP799774	PP806866	PP806838	This study
<i>B. azarbaijanica</i>	FCCUU 1010	<i>Setaria</i> sp.	Iran	PP799775	PP806867	PP806839	This study
<i>B. bamagaensis</i>	BRIP 13577 ^T	<i>Brachiaria subquadrifera</i>	Australia	KX452445	KX452411	KX452462	Tan et al., 2016
<i>B. bamagaensis</i>	BRIP 10711	<i>Dactyloctenium aegyptium</i>	Australia	KX452444	KX452410	KX452461	Tan et al., 2016
<i>B. banihashemii</i>	IRAN 3389C ^T	<i>Setaria</i> sp.	Iran	PP799777	PP806869	PP806840	This study
<i>B. banihashemii</i>	IRAN 3388C	<i>Setaria</i> sp.	Iran	PP799778	PP806870	PP806841	This study
<i>B. banihashemii</i>	IRAN 3387C	<i>Setaria</i> sp.	Iran	PP799779	PP806871	PP806842	This study
<i>B. bicolor</i>	CBS 690.96	Unknown	Unknown	KJ909762	KM042893	KM093776	Manamgoda et al., 2014
<i>B. brachiariae</i>	CPC 28819 ^T	<i>Brachiaria mutica</i>	Thailand	MF490806	MF490828	MF490850	Marin-Felix et al., 2017b
<i>B. chloridis</i>	BRIP 10965 ^T	<i>Chloris gayana</i>	Australia	KJ415523	KJ415423	KJ415472	Tan et al., 2014
<i>B. chusqueae</i>	SGO 166370 ^T	<i>Chusquea cumingii</i>	Chile	OM914401	OM912808	–	Lebeuf et al., 2023
<i>B. clavata</i>	BRIP 12530 ^T	<i>Dactyloctenium radulans</i>	Australia	KJ415524	KJ415422	KJ415471	Tan et al., 2014
<i>B. coffeana</i>	BRIP 14845 ^{IsoT}	<i>Coffea arabica</i>	Kenya	KJ415525	KJ415421	KJ415470	Tan et al., 2014
<i>B. cookei</i>	MAFF 51191	<i>Sorghum bicolor</i>	Japan	KJ922392	KM034834	KM093778	Manamgoda et al., 2014
<i>B. cookei</i>	AR5185	<i>Sorghum</i> sp.	Japan	KJ922391	KM034833	KM093777	Manamgoda et al., 2014
<i>B. crotonis</i>	CBS 274.91 ^{IsoT}	<i>Eleusine indica</i>	Australia	KJ909768	KM034820	KM093758	Manamgoda et al., 2014
<i>B. crotonis</i>	IRAN 4807C	<i>Eleusine indica</i>	Iran	PP799776	PP806868	–	This study
<i>B. cynodontis</i>	CBS 109894 ^{ET}	<i>Cynodon dactylon</i>	Hungary	KJ909767	KM034838	KM093782	Manamgoda et al., 2014
<i>B. distoseptata</i>	CGMCC 3.19361 ^T	<i>Saccharum officinarum</i>	China	MN215628	MN264064	MN263922	Raza et al., 2019
<i>B. drechsleri</i>	CBS 136207 ^T	<i>Microstegium vimineum</i>	USA	KF500530	KF500533	KM093760	Crous et al., 2013; Manamgoda et al., 2014
<i>B. drechsleri</i>	FIP 373	Ornamental grass	USA	KF500531	KF500534	KM093759	Crous et al., 2013
<i>B. fujianensis</i>	CGMCC 3.2088 ^T	<i>Cunninghamia lanceolata</i>	China	MN595057	MW051017	MT966888	Zhang et al., 2024
<i>B. fujianensis</i>	cfsb5	<i>Cunninghamia lanceolata</i>	China	MT974094	MT993889	MW051019	Zhang et al., 2024
<i>B. gigantea</i>	NRRL 66763	<i>Microstegium vimineum</i>	USA	KM507761	–	MN894581	Lane et al., 2020
<i>B. gossypina</i>	BRIP 14840 ^T	<i>Gossypium</i> sp.	Kenya	KJ415528	KJ415418	KJ415467	Tan et al., 2014
<i>B. hedjaroudei</i>	IRAN 4805C ^T	<i>Setaria</i> sp.	Iran	PP799788	PP806880	PP806851	This study

(Continued)

TABLE 1 Continued

Species	Isolate/culture collection ^{a,b}	Host/Substratum	Country	GenBank accessions			References
				ITS	GAPDH	TEF1	
<i>B. hedjaroudei</i>	FCCUU 1013	<i>Setaria</i> sp.	Iran	PP799789	PP806881	PP806852	This study
<i>B. heliconiae</i>	BRIP 17186 ^T	<i>Heliconia psittacorum</i>	Australia	KJ415530	KJ415417	KJ415465	Tan et al., 2014
<i>B. hemerocallidis</i>	IRAN 4774C ^T	<i>Hemerocallis fulva</i>	Iran	PP799780	PP806872	PP806843	This study
<i>B. hemerocallidis</i>	FCCUU 1011	<i>Hemerocallis fulva</i>	Iran	PP799781	PP806873	PP806844	This study
<i>B. heveae</i>	CBS 241.92 ^T	<i>Hevea</i> sp.	Nigeria	KJ909763	KM034843	KM093791	Manamgoda et al., 2014
<i>B. iranica</i>	IRAN 4775C ^T	<i>Cynodon dactylon</i>	Iran	PP799782	PP806874	PP806845	This study
<i>B. iranica</i>	FCCUU 1005	<i>Sorghum halepense</i>	Iran	PP799783	PP806875	PP806846	This study
<i>B. iranica</i>	FCCUU 1006	<i>Arundo donax</i>	Iran	PP799784	PP806876	PP806847	This study
<i>B. iranica</i>	FCCUU 1007	<i>Echinochloa colona</i>	Iran	PP799785	PP806877	PP806848	This study
<i>B. iranica</i>	FCCUU 1008	<i>Hordeum vulgare</i>	Iran	PP799786	PP806878	PP806849	This study
<i>B. iranica</i>	FCCUU 1009	<i>Triticum aestivum</i>	Iran	PP799787	PP806879	PP806850	This study
<i>B. louisemackiae</i>	BRIP 14812b ^T	Unknown	Australia	OR271904	OR269435	–	Tan and Shivas, 2023
<i>B. luttrellii</i>	BRIP 14643 ^T	<i>Dactyloctenium aegyptium</i>	Australia	AF071350	AF081402	KJ415464	Yun et al., 1999; Tan et al., 2014
<i>B. marantae</i>	COAD 2068 ^T	<i>Maranta leuconeura</i>	Brazil	KX365749	KX907136	–	Lourenço et al., 2017
<i>B. mariehareliae</i>	BRIP 75357a ^T	<i>Cycas candida</i>	Australia	OR271905	OR269436	OR269441	Tan and Shivas, 2023
<i>B. maryandersoniae</i>	BRIP 72520b ^T	<i>Leersia hexandra</i>	Australia	OR271906	OR269434	OR269442	Tan and Shivas, 2023
<i>B. maydis</i>	CBS 137271/C5 ^{NT}	<i>Zea mays</i>	USA	AF071325	KM034846	KM093794	Berbee et al., 1999; Manamgoda et al., 2014
<i>B. maydis</i>	AR5182	<i>Sorghum bicolor</i>	Japan	KM230388	KM034844	KM093792	Manamgoda et al., 2014
<i>B. microconidica</i>	CGMCC 3.1936 ^T	<i>Saccharum officinarum</i>	China	MN215630	MN264066	MN263924	Raza et al., 2019
<i>B. microconidica</i>	LC12040	<i>Saccharum officinarum</i>	China	MN215631	MN264067	MN263925	Raza et al., 2019
<i>B. microlaenae</i>	BRIP 15613 ^T	<i>Microlaena stipoides</i>	Australia	JN601032	JN600974	JN601017	Manamgoda et al., 2011
<i>B. microstegii</i>	CBS 132550 ^T	<i>Microlaena vimineum</i>	USA	JX089579	JX089575	KM093756	Manamgoda et al., 2014
<i>B. microstegii</i>	AR5192	<i>Microlaena vimineum</i>	USA	KM230391	KM034819	KM093757	Manamgoda et al., 2014
<i>B. omanensis</i>	SQUCC 13928 ^T	<i>Hibiscus</i> sp.	Oman	MK072962	MK089803	–	Al Dughhaishi et al., 2018
<i>B. oryzae</i>	MFLUCC 10-0715 ^{NT}	<i>Oryza sativa</i>	Thailand	JX256416	JX276430	JX266585	Manamgoda et al., 2012
<i>B. oryzae</i>	MAFF 235499	<i>Oryza sativa</i>	Japan	KJ922383	KM042897	KM093789	Manamgoda et al., 2014
<i>B. panici-miliacei</i>	CBS 199.29 ^{LT}	<i>Panicum miliaceum</i>	Japan	KJ909773	KM042896	KM093788	Manamgoda et al., 2014
<i>B. peregianensis</i>	BRIP 12790 ^T	<i>Cynodon dactylon</i>	Australia	JN601034	JN600977	JN601022	Manamgoda et al., 2011

(Continued)

TABLE 1 Continued

Species	Isolate/culture collection ^{a,b}	Host/Substratum	Country	GenBank accessions			References
				ITS	GAPDH	TEF1	
<i>B. peregrinensis</i>	DAOM 221998	<i>Cynodon dactylon</i>	Australia	KJ922393	KM034849	KM093797	Manamgoda et al., 2011
<i>B. persica</i>	IRAN 4777C ^T	<i>Cynodon dactylon</i>	Iran	PP799790	PP806882	PP806853	This study
<i>B. persica</i>	FCCUU 1004	<i>Cynodon dactylon</i>	Iran	PP799791	PP806883	PP806854	This study
<i>B. petchii</i>	USJCC-0007 ^T	<i>Ischaemum</i> sp.	Sri Lanka	MN535174	MT497476	MT548603	Ferdinandez et al., 2022
<i>B. petchii</i>	USJCC-0018	<i>Ischaemum</i> sp.	Sri Lanka	MT509432	MT497475	MT548602	Ferdinandez et al., 2022
<i>B. pluriseptata</i>	BRIP 14839 ^{IsolT}	<i>Eleusine coracana</i>	Zambia	KJ415532	KJ415414	KJ415461	Tan et al., 2014
<i>B. sacchari</i>	ICMP 6227	<i>Oplismenus imbecillis</i>	New Zealand	KJ922386	KM034842	KM093785	Manamgoda et al., 2014
<i>B. saccharicola</i>	CBS 155.26 ^T	<i>Saccharum officinarum</i>	Unknown	KY905674	KY905686	KY905694	Marin-Felix et al., 2017a
<i>B. saccharicola</i>	CBS 324.64	<i>Saccharum officinarum</i>	Unknown	HE792932	KY905692	KY905699	Marin-Felix et al., 2017a
<i>B. salkadehensis</i>	IRAN 3382C	<i>Scirpus acutus</i>	Iran	PP799794	PP806886	PP806857	This study
<i>B. salkadehensis</i>	IRAN 3383C	<i>Sorghum halepense</i>	Iran	PP799795	PP806887	PP806858	This study
<i>B. salkadehensis</i>	FCCUU 1001	<i>Arundo donax</i>	Iran	PP799796	PP806888	PP806859	This study
<i>B. salkadehensis</i>	FCCUU 1002	<i>Setaria</i> sp.	Iran	PP799797	PP806889	PP806860	This study
<i>B. salkadehensis</i>	FCCUU 1003	<i>Hordeum vulgare</i>	Iran	PP799798	PP806890	PP806861	This study
<i>B. salkadehensis</i>	Bi 1= IRAN 3385C ^T	<i>Sparganium erectum</i>	Iran	AB675490	PP806891	PP806862	Ahmadpour et al., 2012a; This study
<i>B. salkadehensis</i>	Bi 4 = IRAN 3386C	<i>Cladium mariscus</i>	Iran	AB675491	PP806892	PP806863	Ahmadpour et al., 2012a; This study
<i>B. salviniae</i>	IMI 228224 ^{ET}	<i>Salvinia auriculata</i>	Brazil	KJ922390	KM034829	KM093772	Manamgoda et al., 2014
<i>B. salviniae</i>	BRIP 16571 ^{LT}	<i>Salvinia auriculata</i>	Brazil	KJ415535	KJ415411	KJ415457	Tan et al., 2014
<i>B. secalis</i>	BRIP 14453 ^{IsolT}	<i>Secale cereale</i>	Argentina	KJ415537	KJ415409	KJ415455	Tan et al., 2014
<i>B. setariae</i>	CPC 28802	<i>Imperata cylindrica</i>	Thailand	MF490811	MF490833	–	Marin-Felix et al., 2017b
<i>B. setariae</i>	CBS 141.31	Unknown	Unknown	EF452444	EF513206	–	Andrie et al., 2008
<i>B. setariae</i>	LC12047	<i>Saccharum officinarum</i>	China	MN215632	MN264068	MN263926	Raza et al., 2019
<i>B. shoemakeri</i>	BRIP 15929 ^T	<i>Ischaemum rugosum</i> var. <i>segetum</i>	Australia	KX452453	KX452419	KX452470	Tan et al., 2016
<i>B. shoemakeri</i>	BRIP 15806	<i>Ischaemum rugosum</i> var. <i>segetum</i>	Australia	KX452452	KX452418	KX452469	Tan et al., 2016
<i>B. simmondsii</i>	BRIP 12030 ^T	<i>Zoysia macrantha</i>	Australia	KX452454	KX452420	KX452471	Tan et al., 2016
<i>B. sivanesaniana</i>	BRIP 15847 ^T	<i>Paspalidium distans</i>	Australia	KX452455	KX452421	KX452472	Tan et al., 2016
<i>B. sivanesaniana</i>	BRIP 15822	<i>Setaria sphaecelata</i>	Australia	KX452456	KX452422	KX452473	Tan et al., 2016
<i>B. sorokiniana</i>	CBS 480.74	<i>Tribulus terrestris</i>	South Africa	KJ909771	KM034827	KM093768	Manamgoda et al., 2014
<i>B. sorokiniana</i>	CBS 110.14	<i>Hordeum</i> sp.	USA	KJ922381	KM034822	KM093763	Manamgoda et al., 2014
<i>B. stenospila</i>	CBS 156.36	Unknown	Unknown	MH855749	–	–	Vu et al., 2019
<i>B. subramanianii</i>	BRIP 16226 ^T	<i>Setaria sphaecelata</i>	Australia	KX452457	KX452423	KX452474	Tan et al., 2016

(Continued)

TABLE 1 Continued

Species	Isolate/culture collection ^{a,b}	Host/Substratum	Country	GenBank accessions			References
				ITS	GAPDH	TEF1	
<i>B. urochloae</i>	ATCC 58317	<i>Urochloa panicoides</i>	Australia	KJ922389	KM230396	KM093770	Manamgoda et al., 2014
<i>B. variabilis</i>	CBS 127716 ^T	<i>Pennisetum clandestinum</i>	Argentina	KY905676	KY905688	KY905696	Marin-Felix et al., 2017a
<i>B. variabilis</i>	CBS 127736	<i>Pennisetum clandestinum</i>	Brazil	KY905677	KY905689	-	Marin-Felix et al., 2017a
<i>B. victoriae</i>	CBS 327.64 ^T	<i>Avena sativa</i>	USA	KJ909778	KM034811	KM093748	Manamgoda et al., 2014
<i>B. victoriae</i>	DAOM 147449	<i>Avena sativa</i>	USA	KJ909785	KM034812	KM093749	Manamgoda et al., 2014
<i>B. woodii</i>	BRIP 12239 ^T	<i>Paspalidium caespitosum</i>	Australia	KX452458	KX452424	KX452475	Tan et al., 2016
<i>B. yamadae</i>	CBS 202.29 ^{ET}	<i>Panicum miliaceum</i>	Japan	KJ909779	KM034830	KM093773	Manamgoda et al., 2014
<i>B. zeae</i>	BRIP 11512 ^{IsoPT}	<i>Zea mays</i>	USA	KJ415538	KJ415408	KJ415454	Tan et al., 2014
<i>B. zeae</i>	DAOM 211267	<i>Triticum</i> sp.	Canada	KJ909787	KM034818	KM093755	Manamgoda et al., 2014
<i>B. zeicola</i>	FIP 532 ^{ET}	<i>Zea mays</i>	USA	KM230398	KM034815	KM093752	Manamgoda et al., 2014
<i>B. zeicola</i>	AR5166	<i>Sorghum</i> sp.	USA	KJ909788	KM034813	KM093750	Manamgoda et al., 2014
<i>Curvularia affinis</i>	CBS 154.34 ^T	unknown	Indonesia	KJ909780	KM230401	KM196566	Manamgoda et al., 2015
<i>C. lunata</i>	CBS 730.96 ^T	Human lung biopsy	USA	JX256429	JX276441	JX266596	Manamgoda et al., 2012

Newly generated sequences are in bold.

^aCulture collections: ATCC American Type Culture Collection, Virginia, USA; BRIP Queensland Plant Pathology Herbarium, Queensland, Australia; CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; COAD Coleção Octávio de Almeida Drumond housed at Universidade Federal de Viçosa; CPC Working collection of P.W. Crous, housed at the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; AR ans FIP Isolates housed in Mycology and Nematology Genetic Diversity and Biology Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland; FCCUU the fungal culture collections of Urmia University, Iran; ICMP International Collection of Micro-organisms from Plants, Landcare Research, Auckland, New Zealand; IMI International Mycological Institute, Kew, UK; IRAN Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; LC: Personal culture collection of Prof. Lei Cai housed in State Key Laboratory of Mycology, Institute of Microbiology, Beijing, China; MAFF Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; MFLUCC Mae Fah Luang University Culture Collection, Thailand; NRRL USDA Agricultural Research Service Culture Collection, USA; SQUCC Sultan Qaboos University Culture Collection, Muscat, Oman; USJCC University of Sri Jayewardenepura Culture Collection, Sri Lanka. ^b T, ET, IsoT, IsoLT, IsoPT, LT and NT indicate ex-type, ex-epitype, ex-isotype, ex-isolectotype, ex-isoparatype, ex-lectotype and ex-neotype strains, respectively.

sampling frequency, and 25% burn-in phase). In all phylogenetic analyses, *Curvularia affinis* (CBS 154.34) and *Curvularia lunata* (CBS 730.96) were used as the outgroup taxa (Manamgoda et al., 2014; Tan et al., 2016; Bhunjun et al., 2020; Fernandez et al., 2022). The generated phylogenetic trees were viewed using FigTree v. 1.4.4 (Rambaut, 2019) and further edited using graphic design software, Adobe Illustrator[®] CC 2020.

2.5 Genealogical Concordance Phylogenetic Species Recognition analysis

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) was used to test for significant recombinant events (Quaedvlieg et al., 2014). Three-locus concatenated datasets (ITS + rDNA + GAPDH + TEF1) with closely related species were used for the analyses. The data were analyzed using SplitsTree 5 software

employing the pairwise homoplasy index (PHI or Φw) test (Bruen et al., 2006; Huson and Bryant, 2006). PHI test results indicating a value less than 0.05 (Φw < 0.05) suggest the presence of significant recombination within the dataset. To visualize the relationships between novel taxa and their closely related counterparts, split graphs were constructed using concatenated datasets. The LogDet transformation and split decomposition options were used for this purpose.

3 Results

3.1 Phylogenetic analyses

A total of 85 isolates were obtained from various hosts (Poales and Asparagales plants). All isolates were examined based on their morphology. Representative isolates were then selected from

various plant hosts for phylogenetic analyses. PCR amplifications produced DNA fragments of approximately 540 bp for ITS-rDNA, 545 bp for *GAPDH*, and 850 bp for *TEF1*. A total of 104 ITS-rDNA, 102 *GAPDH*, and 95 *TEF1* sequences were subjected to multiple sequence alignment (nucleotides + gaps) resulting in 505-, 494-, and 898-character datasets, respectively. A combination of three gene sequences from 104 strains yielded a dataset with 1,897 characters, of which, 1,472 characters were constant, 112 characters were variable and parsimony uninformative, and 313 were parsimony informative. The most parsimonious tree yielded the following metrics: TL = 937, CI = 0.574, RI = 0.842, HI = 0.426. The nucleotide substitution model GTR + I + G was identified by MrModeltest 2.3 for all ITS-rDNA, *GAPDH*, and *TEF1* datasets. The ML, MP, and BI phylogenetic analyses produced trees with similar topology and showed no significant conflicts. The combined dataset analysis of RAXML generated the best-scoring tree (Figure 1) with a final ML optimization likelihood value of $-8,235.239283$. Estimated base frequencies were as follows: A = 0.229913, C = 0.302740, G = 0.236579, T = 0.230767; substitution rates AC = 1.001686, AG = 2.714677, AT = 1.255930, CG = 0.832074, CT = 6.025850, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.731765$. Based on morphological characteristics and multi-locus phylogeny (ITS-rDNA, *GAPDH*, and *TEF1*), nine *Bipolaris* species were identified: *Bipolaris avrinica* sp. nov., *B. azarbaijanica* sp. nov., *B. banihashemii* sp. nov., *B. hedjaroudei* sp. nov., *B. hemerocallidis* sp. nov., *B. iranica* sp. nov., *B. persica* sp. nov., *Bipolaris crotonis*, and *B. salkadehensis*. *B. crotonis* is a new record for Iran's fungi. Also, the phylogenetic relationship of *B. salkadehensis* with related species was re-defined using sequences from three genomic regions, and several new hosts were identified for this species worldwide. All identified taxa clustered with high statistical support values in the phylogenetic tree (Figure 1). Each species was thoroughly illustrated, described, and discussed in terms of morphology, habitat, distribution, and phylogenetic relationships with other *Bipolaris* species.

3.2 Taxonomy

Bipolaris avrinica A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 2).

Mycobank No: MB 854730

Etymology: The name refers to Avrin Mountain, located in Khoy County, West Azarbaijan Province, where the holotype was collected.

Diagnosis: Differs from *Bipolaris adikaramae* and *B. yamadae* by the abundant production of secondary conidiophores and conidia in culture media.

Type: IRAN, West Azarbaijan Province, Khoy County, on infected leaves of *Setaria* sp. (Poaceae, Poales), 10 September 2020, A. Ahmadpour, (IRAN 18493F, **holotype**, dried culture; **ex-type** culture IRAN 4806C).

Description: Lesions on infected leaves of *Setaria* sp., 1- to 10-mm long, gray color at the center with dark brown margins. Sexual morph: Undetermined. Asexual morph: On TWA Hyphae 2- to 5- μ m wide, pale brown to brown, smooth, septate, branched.

Conidiophores (125–)185–500(–600) $\times$ 4–6 μ m ($\bar{x} \pm SD = 342.5 \pm 157.5 \times 5 \pm 1 \mu$ m, $n = 50$), mononematous, semi- to macronematous, arising singly or rarely in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, rarely swollen at the base. Secondary conidiophores are frequently formed in culture media and conidia attached to primary conidiophores. *Conidiogenous cells* (6–)8–21(–16) $\times$ 4–7 μ m ($\bar{x} \pm SD = 14.5 \pm 6.5 \times 5.5 \pm 1.5 \mu$ m, $n = 50$), mono- to polytretic, sympodial proliferation, integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth-walled, with thickened and darkened scars. *Conidia* (45–)50–87(–100) $\times$ 10–13 μ m ($\bar{x} \pm SD = 68.5 \pm 18.5 \times 12 \pm 1 \mu$ m, $n = 50$), pale brown to brown, smooth walled, straight to curved, fusoid to cylindrical, occasionally ellipsoidal, tapering toward rounded ends, (6–)7–10(–11)-distoseptate, germinated mono- or bipolar; hila 1.5- to 2.5- μ m wide, inconspicuous, flat, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 50 mm in diameter after 7 days at 25°C in the dark, circular, margin irregular, cottony appearance, gray with white to gray aerial mycelia; reverse gray olivaceous. Colonies on MEA reaching 38-mm diameter, circular, margin irregular, cottony appearance, white with white aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on CMA reaching 52 mm in diameter, circular, margin entire, hairy appearance with concentric rings, gray with sparse white to gray aerial mycelia; reverse olivaceous brown at the center and a hyaline margin.

Additional material examined: IRAN, West Azarbaijan Province, Khoy County, on infected leaves of *Setaria* sp. (Poaceae, Poales), 10 September 2020, A. Ahmadpour, isolate FCCUU 1012.

Host and distribution: *Setaria* sp. in Iran (this study).

Notes: Based on the phylogenetic analyses, *B. avrinica* is closely related to *B. adikaramae* and *B. yamadae* (MLBS/MPBS/BIPP = 100/98/1.0) (Figure 1). A comparison of nucleotide differences in ITS-rDNA, *GAPDH*, and *TEF1* indicates that *B. avrinica* (IRAN 4806C) differs from *B. adikaramae* (USJCC-0008) by 1/511 bp [0.19%, with one gap (0%)] in ITS-rDNA, 3/550 bp (0.54%) in *GAPDH*, and 3/763 bp (0.39%) in *TEF1* and from *B. yamadae* (CBS 202.29) by 1/511 bp [0.54%, with one gap (0%)] in ITS-rDNA, 4/480 bp (0.83%) in *GAPDH*, and 4/763 bp (0.52%) in *TEF1*. The PHI analysis confirms that *B. avrinica* shows no significant genetic recombination with closely related species ($\Phi_w = > 0.05$, Figure 3). *Bipolaris avrinica* can be differentiated by its abundant production of secondary conidiophores and conidia in cultures, a feature absent in *B. adikaramae* and *B. yamadae*. Additionally, *B. avrinica* has smaller conidia [(45–)50–87(–100) $\times$ 10–13 μ m] compared to *B. yamadae* [(60–)65–100(–120) $\times$ (12–)14–18 μ m] (Manamgoda et al., 2014; Fernandez et al., 2022). The production of secondary conidiophores and secondary conidia has been observed in *B. cookei* and *B. microstegii* grown on culture media (Manamgoda et al., 2014). However, *B. avrinica* is phylogenetically distinct from these species (Figure 1). *Bipolaris yamadae* has been reported from several hosts, including *Oryza* sp., *Euphorbia* sp., *Panicum* spp. (*P. capillare*, *P. implicatum*, *P. maximum*, and *P. miliaceum*), *Saccharum officinarum*, and *Setaria plicata*.

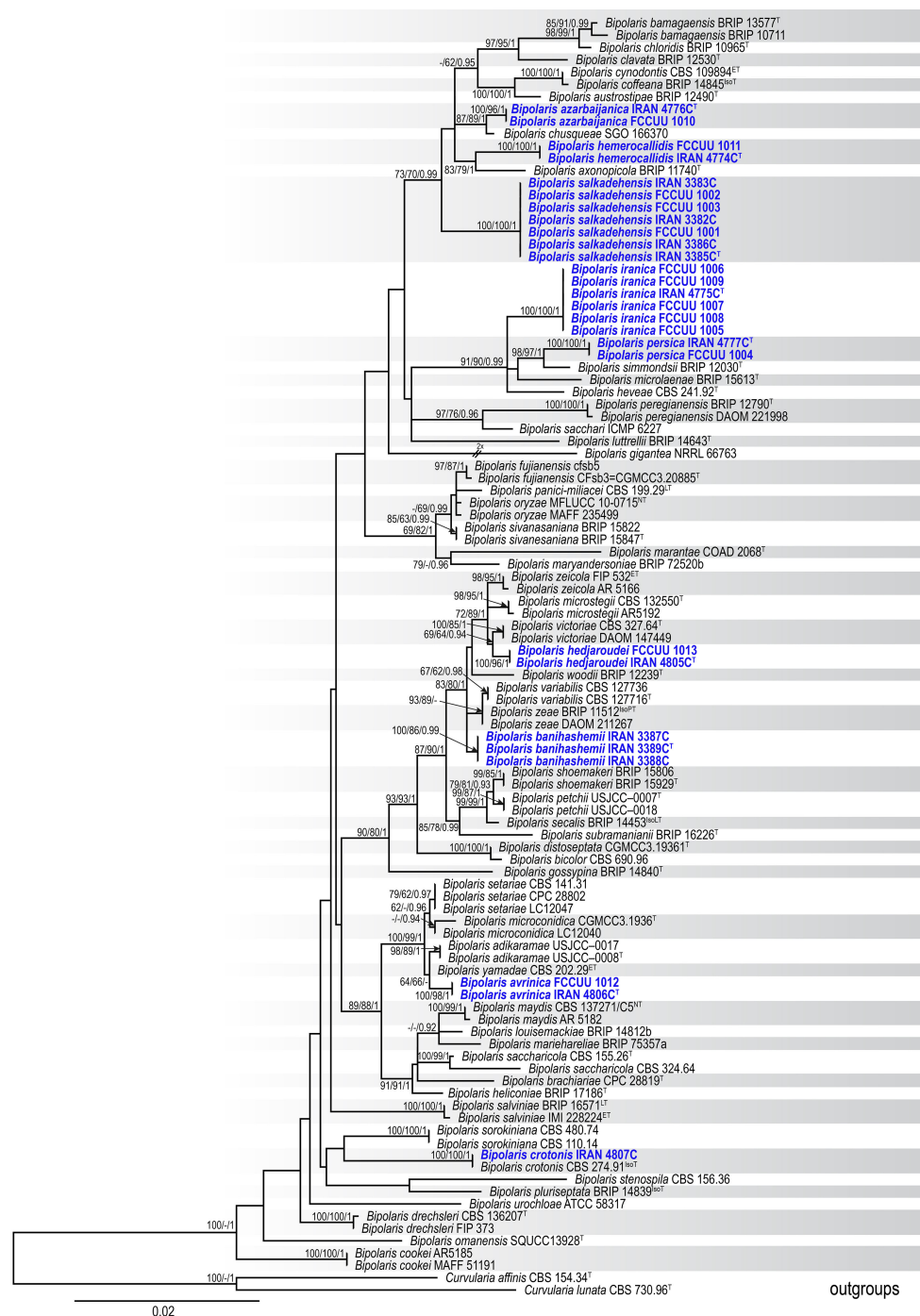


FIGURE 1

Maximum likelihood (ML) tree of *Bipolaris* species based on the dataset of ITS-rDNA, *GAPDH*, and *TEF1*. Bootstrap support values of the ML and maximum parsimony (MP) (MLBS/MPBS) values $\geq 60\%$ and Bayesian posterior probabilities (BIPP) ≥ 0.90 are given at the nodes. The tree is rooted with *Curvularia affinis* (CBS 154.34) and *C. lunata* (CBS 730.96), and new species are indicated in blue boldface. The scale bar indicates the number of nucleotide substitutions. T, ET, IsoT, IsoLT, IsoPT, LT and NT indicate ex-type, ex-epitype, ex-isotype, ex-isolectotype, ex-isoparatype, ex-lectotype, and ex-neotype strains, respectively.

(Manamgoda et al., 2014; Marin-Felix et al., 2017a; Farr et al., 2024). *Bipolaris adikaramae* has been isolated from yellow lesions on the leaf of *Panicum maximum* in Sri Lanka (Ferdinandez et al., 2022). Based on morphological and molecular evidence, we propose *B. avrinica* as a new species.

Bipolaris azarbaijanica A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 4).

Mycobank No: MB 854731

Etymology: The name refers to the West Azarbaijan Province, where the holotype was collected.

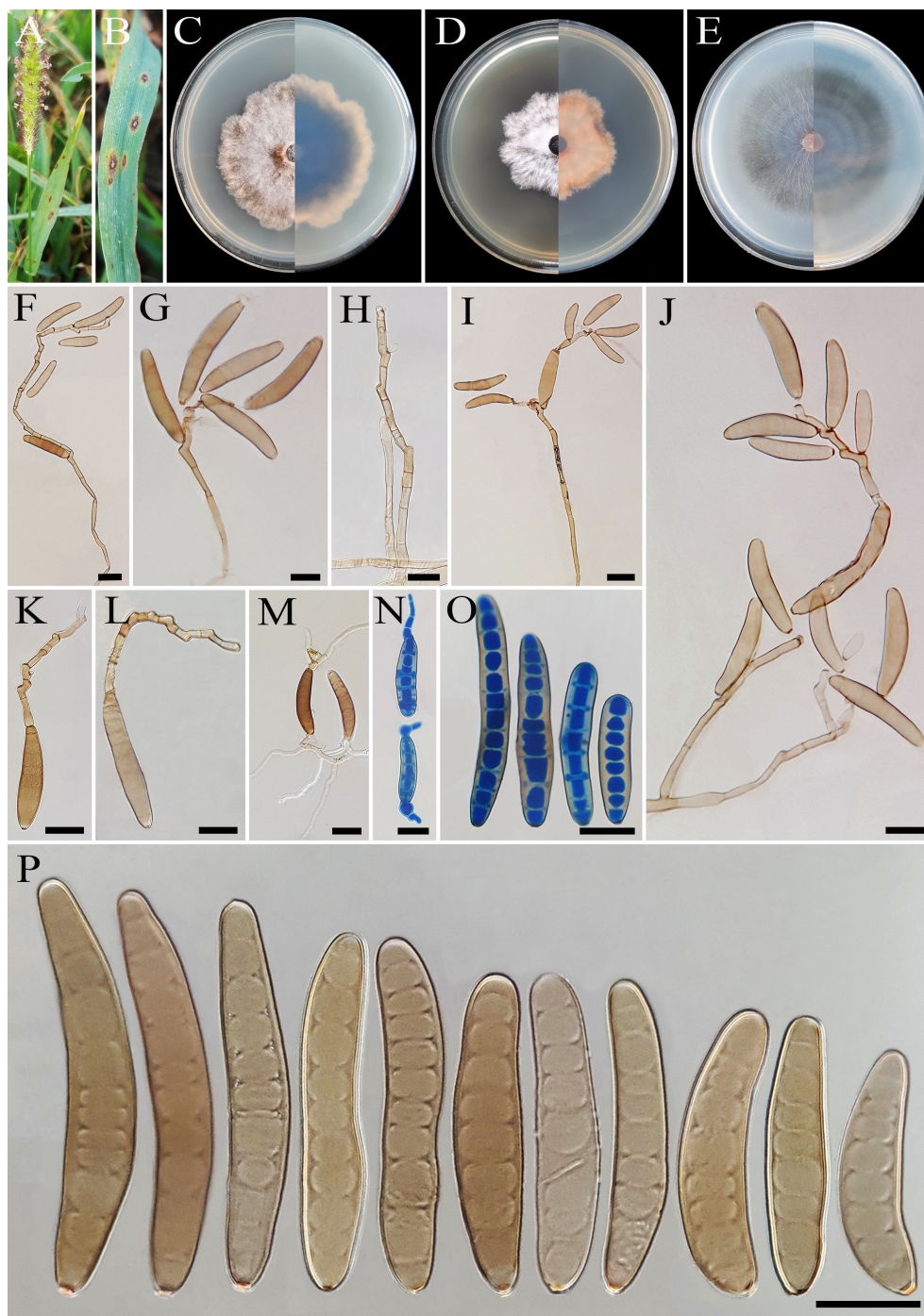


FIGURE 2

Bipolaris avrinica (IRAN 4806C). (A, B) Lesions on host leaf (*Setaria* sp.). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after 7 days. (F–H) Conidiophores. (I–L) Conidia with secondary sporulation. (M, N) Germinated conidia. (O, P) Conidia. Scale bars: (F–P) = 20 μ m.

Diagnosis: Differs from *Bipolaris chusqueae* by the shape (fusoid to cylindrical) and size (longer and wider) of conidia.

Type: IRAN, West Azarbaijan Province, Salmas County, on leaves of *Setaria* sp. (Poaceae, Poales), 10 September 2015, A. Ahmadpour/Z. Heidarian, (IRAN 18208F, **holotype**, dried culture; **ex-type** culture IRAN 4776C).

Description: Leaf spots on *Setaria* sp., 1- to 10-mm long, gray at the center with a red-brown margin. Sexual morph: Undetermined.

Asexual morph: On TWA: *Hyphae* 3- to 5- μ m wide, pale brown to brown, smooth, septate, branched. *Conidiophores* (112–)140–300(–450) $\times$ 5–7 μ m ($\bar{x} \pm SD = 220 \pm 80 \times 6 \pm 1 \mu$ m, $n = 50$), mononematous, semi- to macronematous, arising singly or in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, swollen at the base. *Conidiogenous cells* (8–)10–22(–25) $\times$ 5–8 μ m ($\bar{x} \pm SD = 16 \pm 6 \times 6.5 \pm 1.5 \mu$ m, $n = 50$), mono- to polytretic, sympodial proliferation,

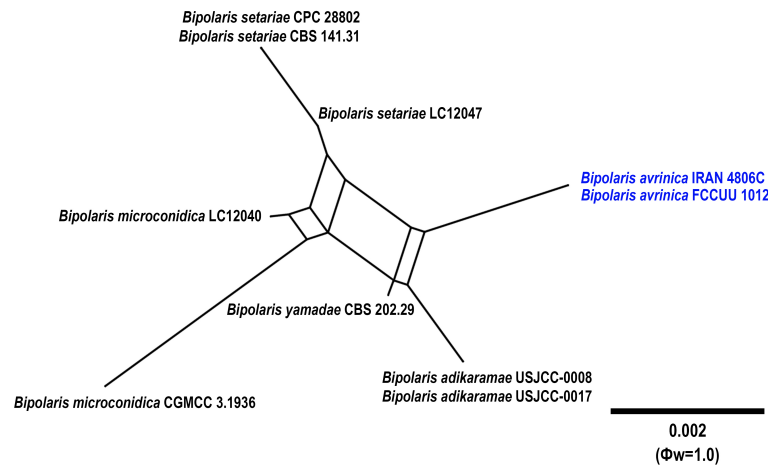


FIGURE 3

Split graphs showing the results of PHI test of *Bipolaris avrinica* with their most closely related species ($\Phi w = 1.0$). The new taxa are shown in bold blue.

integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* $(44\text{--}50\text{--}80\text{--}84) \times 11\text{--}15 \mu\text{m}$ ($\bar{x} \pm \text{SD} = 65 \pm 15 \times 13 \pm 2 \mu\text{m}$, $n = 50$), pale brown to brown, smooth walled, straight to slightly curved, broadly fusoid to cylindrical, occasionally ellipsoidal to clavate, tapering toward the rounded ends, apical and basal cells paler than the median cells, $(4\text{--})5\text{--}9\text{--}(10)\text{-distoseptate}$, germination mono- or bipolar; hila 2- to 3- μm wide, flat to slightly protuberant, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 73 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, gray at the center with white to gray aerial mycelia, white at the margin; reverse olivaceous gray at the center, margin pale brown. Colonies on MEA reaching 68 mm in diameter, circular, margin entire, cottony appearance, gray at the center, white at the margin with white aerial mycelia; reverse brown to pale brown. Colonies on CMA reaching 65 mm in diameter, circular, margin entire, hairy appearance, olivaceous gray with sparse white to gray aerial mycelia; reverse olivaceous gray at the center and a hyaline margin.

Additional material examined: Iran, West Azarbaijan Province, Salmas County, on leaves of *Setaria* sp. (Poaceae, Poales), 10 September 2015, A. Ahmadpour/Z. Heidarian, isolate FCCUU 1010.

Host and distribution: *Setaria* sp. in Iran (this study).

Notes: *Bipolaris azarbaijanica* is phylogenetically closely related to *B. chusqueae* (MLBS/MPBS/BIPP = 100/96/1.0) (Figure 1). The pairwise DNA sequence comparison revealed that *B. azarbaijanica* is distinct from *B. chusqueae*. A comparison of nucleotide differences in ITS-rDNA and *GAPDH* indicates that *B. azarbaijanica* (IRAN 4776C) differs from *B. chusqueae* (SGO 166370) by 3/525 bp (0.57%) in ITS-rDNA and 6/531 bp (1.12%) in *GAPDH*. The PHI analysis confirms that *B. azarbaijanica* has no significant genetic recombination with closely related species ($\Phi w = > 0.05$, Figure 5). Morphologically, *B. azarbaijanica* can be

differentiated by the shape of the conidia (broadly fusoid to cylindrical vs. subcylindrical to narrowly clavate in *B. chusqueae*), and longer and wider conidia $[(44\text{--})50\text{--}80\text{--}(84) \times 11\text{--}15 \mu\text{m}$ vs. $(17\text{--})26\text{--}50\text{--}(68) \times 10\text{--}12\text{--}(15) \mu\text{m}$ in *B. chusqueae*] (Lebeuf et al., 2023). *Bipolaris chusqueae* has been reported from *Chusquea cumingii* (Bambusoideae, Poales) in Chile (Lebeuf et al., 2023).

Bipolaris banihashemii A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 6).

Mycobank No: MB 854732

Etymology: Named in honor of Dr. Zia Banihashemi, emeritus Professor of Shiraz University, Iran, who significantly contributed to the knowledge of mycology and plant pathology in Iran.

Diagnosis: Differs from *Bipolaris variabilis* and *B. zeae* by the size of conidiophores and the shape and size of the conidia.

Type: IRAN, West Azarbaijan Province, Khoy County, on infected leaves of *Setaria* sp. (Poaceae, Poales), 20 September 2010, A. Ahmadpour, (IRAN 18244F, **holotype**, dried culture; **ex-type** IRAN 3389C).

Description: Leaf spots on *Setaria* sp., 1- to 5-mm long, gray at the center with red-brown margins. Sexual morph: Undetermined. Asexual morph: On TWA *Hyphae* 3- to 5- μm wide, pale brown to brown, smooth, septate, branched. *Conidiophores* $(150\text{--})260\text{--}400\text{--}(450) \times 5\text{--}7 \mu\text{m}$ ($\bar{x} \pm \text{SD} = 330 \pm 70 \times 5 \pm 1 \mu\text{m}$, $n = 50$), mononematous, semi- to macronematous, arising singly or in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, swollen at the base. *Conidiogenous cells* $(7\text{--})9\text{--}23\text{--}(28) \times 5\text{--}8 \mu\text{m}$ ($\bar{x} \pm \text{SD} = 16 \pm 7 \times 6.5 \pm 1.5 \mu\text{m}$, $n = 50$), mono- to polytretic, sympodial proliferation, integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* $(28\text{--})38\text{--}62\text{--}(68) \times 9\text{--}13 \mu\text{m}$ ($\bar{x} \pm \text{SD} = 50 \pm 18.5 \times 11 \pm 2 \mu\text{m}$, $n = 50$), golden brown, smooth walled, straight, cylindrical to fusoid, occasionally ellipsoidal, tapering toward rounded ends, end cells often cut off by a thick dark septum, $(4\text{--})5\text{--}8\text{--}(9)\text{-distoseptate}$, germination mono- or bipolar; hila 2- to 3- μm wide, truncate, slightly protruding,

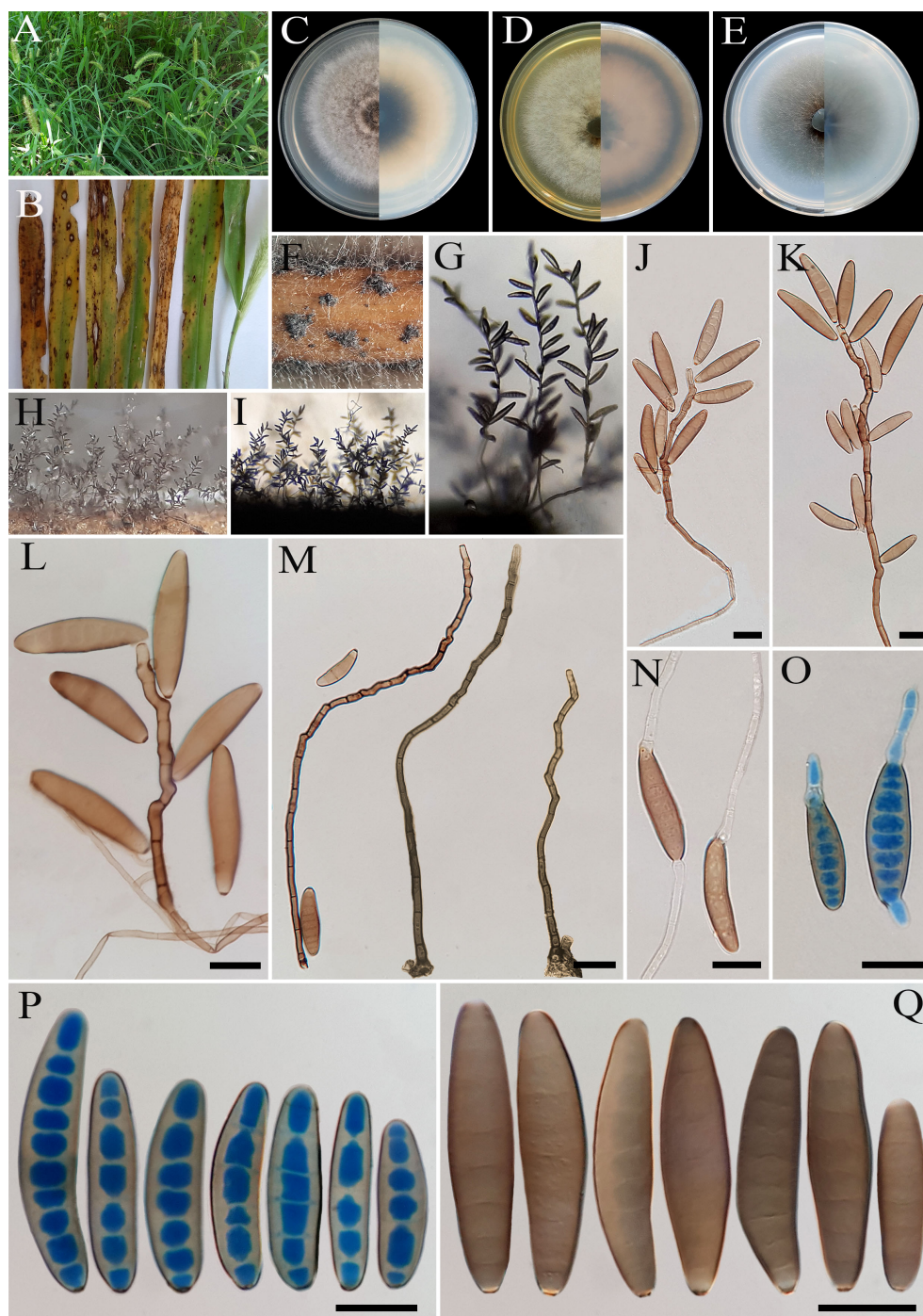


FIGURE 4

Bipolaris azarbaijanica (IRAN 4776C). (A, B) Lesions on host leaf (*Setaria* sp.). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after 7 days. (F–I) Sporulation pattern on TWA medium. (J–M) Conidiophores. (N, O) Germinated conidia. (P, Q) Conidia. (J–Q) Scale bars = 20 μ m.

thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 67 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, olivaceous green at the center, white at the margin with white to gray aerial mycelia; reverse gray olivaceous to olivaceous black with a hyaline margin. Colonies on MEA reaching 35 mm in diameter, circular, margin

irregular, cottony appearance, white with white aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on CMA reaching 62 mm in diameter, circular, margin entire, hairy appearance, olivaceous gray with sparse white to gray aerial mycelia; reverse olivaceous gray at the center and a hyaline margin.

Additional materials examined: IRAN, West Azarbaijan Province, Khoy County, on infected leaves of *Setaria* sp. (Poaceae,

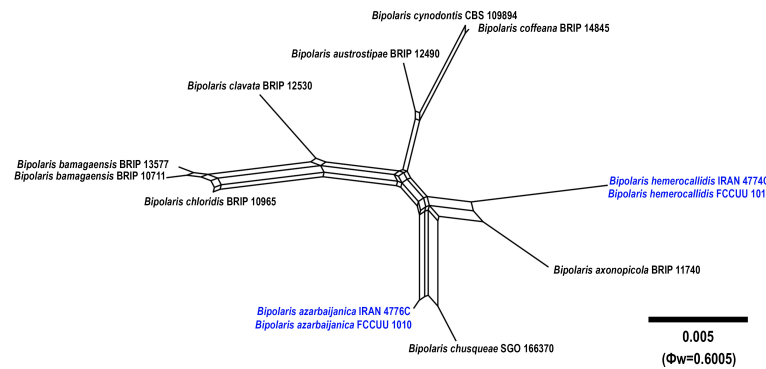


FIGURE 5

Split graphs showing the results of PHI test of *Bipolaris azarbaijanica* and *B. hemerocallidis* with their most closely related species ($\Phi w = 0.6005$). The new taxa are shown in bold blue.

Poales), 20 September 2010, A. Ahmadpour, isolate IRAN 3388C; *ibid.* on infected leaves of *Setaria* sp. (Poaceae, Poales), 20 September 2010, A. Ahmadpour, isolate IRAN 3387C.

Host and distribution: *Setaria* sp. in Iran (this study).

Notes: Based on multi-locus phylogenetic analyses, *B. banihashemii* clustered closely with *B. variabilis* and *B. zeae* (MLBS/MPBS/BIPP = 100/86/0.99) (Figure 1). A comparison of nucleotide differences in ITS-rDNA, *GAPDH*, and *TEF1* indicates that *B. banihashemii* (IRAN 3389C) differs from *B. variabilis* (CBS 127716) by 1/548 bp [0.18%, with one gap (0%)] in ITS-rDNA, 4/577 bp (0.69%) in *GAPDH*, and 1/642 bp (0.15%) in *TEF1* and from *B. zeae* (BRIP 11512) by 3/577 bp (0.52%) in *GAPDH* and 2/712 bp (0.28%) in *TEF1*. The PHI analysis confirms that *B. banihashemii* has no significant genetic recombination with closely related species ($\Phi w = > 0.05$, Figure 7). *Bipolaris variabilis* can be differentiated by having longer conidiophores (up to 1,600 μm vs. up to 450 μm in *B. banihashemii*), shape of conidia (verruculose walled, straight or slightly curved, globose to obclavate conidia vs. smooth walled, straight, cylindrical to fusoid conidia in *B. banihashemii*), and wider conidia (10–19.5 vs. 9–13 μm in *B. banihashemii*) (Marin-Felix et al., 2017a). *Bipolaris zeae* differs from *B. banihashemii* in producing shorter conidiophores (up to 370 vs. 450 μm in *B. banihashemii*) and longer and wider conidia [(30–)40–80(–120) $\times$ 12–18(–21) μm vs. (28–)38–62(–68) $\times$ 9–13 μm in *B. banihashemii*] (Sivanesan, 1987; Manamgoda et al., 2014).

Bipolaris hedjaroudei A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 8).

Mycobank No: MB 854733

Etymology: Named in honor of Dr. Ghorbanali Hedjaroud, emeritus Professor of Tehran University, who significantly contributed to the knowledge of mycology in Iran.

Diagnosis: Differs from *Bipolaris microstegii*, *B. victoriae*, *B. zeicola*, and *B. woodii* by having longer conidiophores, smaller conidia, and production of sexual morph (homothallic species) in culture media.

Type: IRAN, West Azarbaijan Province, Khoy County, on infected leaves of *Setaria* sp. (Poaceae, Poales), 10 September

2020, A. Ahmadpour, (IRAN 18492F, **holotype**, dried culture; **ex-type** IRAN 4805C).

Description: On infected leaves of *Setaria* sp., leaf lesions 1- to 10-mm long, gray at the center and red-brown at the margins. Sexual morph: On sterile leaves of *Setaria* sp. in TWA medium *Ascomata* pseudothecial, (300–)400–550(–600) $\times$ (290–)300–500(–550) μm ($\bar{x} \pm \text{SD} = 475 \pm 75 \times 400 \pm 100 \mu\text{m}$, $n = 20$), solitary, scattered, superficial or slightly embedded, globose to subglobose or oval, dark brown to black, unilocular with a short ostiolate neck, with long brown setae and conidiophores bearing conidia developing on the upper part of the ascoma. *Ostiolar neck* 10–20 $\times$ 8–12 μm ($\bar{x} \pm \text{SD} = 15 \pm 5 \times 10 \pm 2 \mu\text{m}$, $n = 20$), conical, central, filled with masses of hyaline cells frequently covering the apex of the neck. *Peridium* (ascomata wall) 30- to 35- μm wide, composed of layers of pigmented thick-walled cells. *Pseudoparaphyses* 2- to 3- μm wide, hyaline, septate, filamentous, simple to branched. *Asci* (136–)150–200(–212) $\times$ (15–)17–20 (–22) μm ($\bar{x} \pm \text{SD} = 150 \pm 50 \times 18.5 \pm 1.5 \mu\text{m}$, $n = 20$), with eight ascospores coiled in a tight helix, bitunicate, cylindrical to clavate, occasionally obclavate–fusoid, straight or curved, with short pedicel. *Ascospores* 170–250 $\times$ 5–7 μm ($\bar{x} \pm \text{SD} = 210 \pm 40 \times 6 \pm 1 \mu\text{m}$, $n = 50$), hyaline, filiform to flagelliform, tapering toward the rounded ends, tightly coiled inside the ascus, 7–13 septate, with a thin mucilaginous sheath visible in water mounts. Asexual morph: On TWA *Hyphae* 2- to 5- μm wide, pale brown to brown, smooth, septate, branched. *Conidiophores* (125–)175–250(–325) $\times$ 5–7 μm ($\bar{x} \pm \text{SD} = 212.5 \pm 37.5 \times 6 \pm 1 \mu\text{m}$, $n = 50$), mononematous, semi- to macronematous, arising mostly singly or rarely in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, swollen at the base. *Conidiogenous cells* (6–)8–22(–25) $\times$ 5–8 μm ($\bar{x} \pm \text{SD} = 15 \pm 7 \times 6.5 \pm 1.5 \mu\text{m}$, $n = 50$), mono- to polytretic, sympodial proliferation, integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* (25–)32–60(–62) $\times$ 15–17 μm ($\bar{x} \pm \text{SD} = 46 \pm 14 \times 16 \pm 1 \mu\text{m}$, $n = 50$), brown to dark brown, smooth walled, straight to slightly curved, broadly fusiform, occasionally ellipsoidal to obclavate, tapering toward the

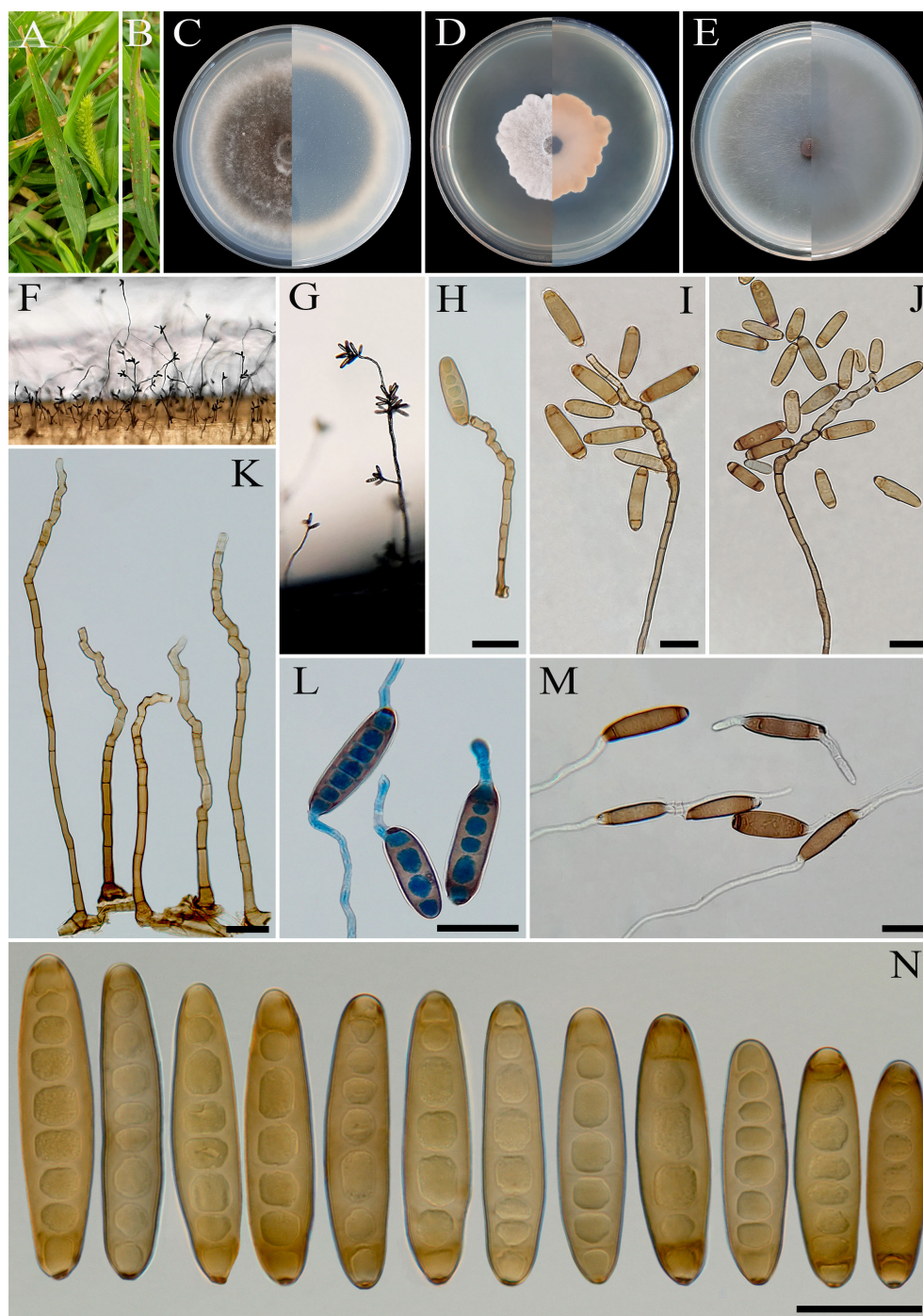


FIGURE 6

Bipolaris banhashemii (IRAN 3389C). (A, B) Lesions on host leaf (*Setaria* sp.). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after 7 days. (F, G) Sporulation pattern on TWA medium. (H–K) Conidiophores. (L, M) Germinated conidia. (N) Conidia. (H–N) Scale bars = 20 μ m.

rounded ends, apical and basal cells paler than the median cells, end cells often cut off by a thick dark septum, (4–)5–8(–9)-distoseptate, germinated mono- or bipolar; hila 2- to 3- μ m wide, conspicuous, brown, slightly protuberant, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 65 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, velvety, gray at the center and white at the margin, with gray to

white aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on MEA reaching 61 mm in diameter, circular, margin entire, cottony appearance, white with white aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on CMA reaching 61 mm in diameter, circular, margin entire, hairy appearance, gray with sparse white to gray aerial mycelia; reverse olivaceous brown at the center and a hyaline margin.

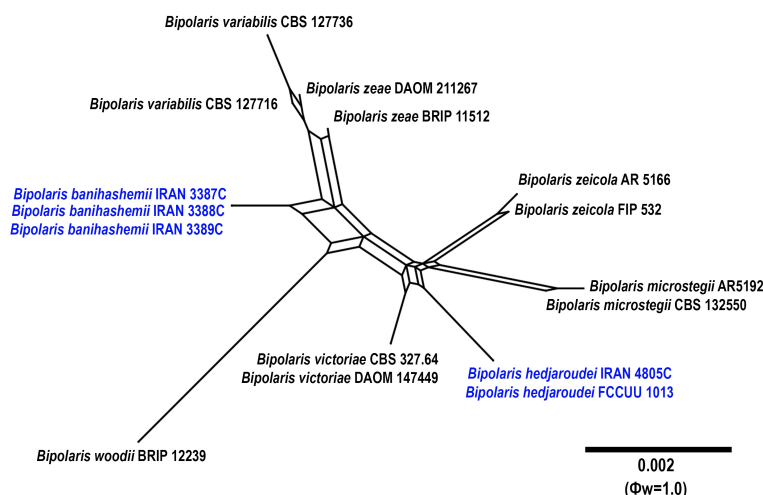


FIGURE 7

Split graphs showing the results of PHI test of *Bipolaris banihashemii* and *B. hedjaroudei* with their most closely related species ($\Phi_w = 1.0$). The new taxa are shown in bold blue.

Additional material examined: Iran, West Azarbaijan Province, Khoy County, on infected leaves of *Setaria* sp. (Poaceae, Poales), 10 September 2020, A. Ahmadpour, isolate FCCUU 1013.

Host and distribution: *Setaria* sp. in Iran (this study).

Notes: *Bipolaris hedjaroudei* is phylogenetically closely related to *B. victoriae*, *B. microstegii*, *B. zeicola*, and *B. woodii* (Figure 1). Pairwise sequence similarity analyses of three genomic regions in *B. hedjaroudei* distinguished it from closely related taxa. A comparison of nucleotide differences in ITS-rDNA, *GAPDH*, and *TEF1* indicates that *B. hedjaroudei* (IRAN 4805C) differs from *B. microstegii* (CBS 132550) by 2/507 bp (0.39%) in ITS-rDNA, 5/539 bp (0.92%) in *GAPDH*, and 8/763 bp [1.04%, with two gaps (0%)] in *TEF1*; from *B. victoriae* (CBS 327.64) by 1/480 bp (0.20%) in *GAPDH* and 6/763 bp [0.78%, with two gaps (0%)] in *TEF1*; from *B. woodii* (BRIP 12239) by 2/512 bp (0.39%) in ITS-rDNA, 9/550 bp [1.63%, with one gap (0%)] in *GAPDH*, and 8/761 bp [1.05%, with two gaps (0%)] in *TEF1*; and from *B. zeicola* (FIP 532) by 2/437 bp [0.45%, with one gap (0%)] in ITS-rDNA, 1/480 bp (0.20%) in *GAPDH*, and 6/763 bp [0.78%, with two gaps (0%)] in *TEF1*. The PHI analysis confirms that *B. hedjaroudei* has no significant genetic recombination with closely related species ($\Phi_w = > 0.05$, Figure 7). *Bipolaris hedjaroudei* can be differentiated by having longer conidiophores (up to 325 μm vs. up to 250 μm in *B. victoriae*, up to 270 μm in *B. zeicola*, up to 250 μm in *B. woodii*) and smaller conidia [(25–)32–60(–62) $\times$ 15–17 μm vs. (25–)55–90(–110) $\times$ (10–)12–16(–19) μm in *B. victoriae*, (45–)65–90(–105) $\times$ (10–)15–19(–22) μm in *B. zeicola*, (60–)69–76(–86) $\times$ (10–)12.5–13.5(–15) μm in *B. woodii*] (Manamgoda et al., 2014; Tan et al., 2016). *Bipolaris microstegii* differs from *B. hedjaroudei* in producing secondary conidiophores and conidia, longer conidiophores (up to 750 μm vs. up to 325 μm in *B. hedjaroudei*), and accentuated septa (Manamgoda et al., 2014). *Bipolaris victoriae* and *B. zeicola* have been reported on various poaceous hosts and caused destructive diseases in oat and maize, respectively (Manamgoda et al., 2014; Farr et al., 2024). *Bipolaris hedjaroudei* is a homothallic

species that forms sexual morph abundantly on TWA medium containing host leaves after 21–30 days. In contrast, *B. victoriae* and *B. zeicola* are heterothallic species, and the sexual morph of *B. microstegii* and *B. woodii* has not been recorded yet (Manamgoda et al., 2014; Tan et al., 2016).

Bipolaris hemerocallidis A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 9).

Mycobank No: MB 854734

Etymology: Named after the host genus, *Hemerocallis*, from which the holotype was collected.

Diagnosis: Differs from *Bipolaris axonopicola* by having longer conidiophores.

Type: IRAN, Isfahan Province, Isfahan County, Flower Garden, on leaves of *Hemerocallis fulva* (Asphodelaceae, Asparagales), 7 October 2013, A. Ahmadpour/Z. Heidarian, (IRAN 18206F, **holotype**, dried culture; **ex-type** IRAN 4774C).

Description: Associated with leaves of *Hemerocallis fulva*. Sexual morph: Undetermined. Asexual morph: On TWA *Hyphae* 2- to 5- μm wide, pale brown to brown, smooth, septate, branched. *Conidiophores* (180–)230–550(–600) $\times$ 5–7 μm ($\bar{x} \pm \text{SD} = 390 \pm 160 \times 6 \pm 1 \mu\text{m}$, $n = 50$), mononematous, semi- to macronematous, arising singly or in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, swollen at the base. *Conidiogenous cells* (5–)7–21(–25) $\times$ 5–8 μm ($\bar{x} \pm \text{SD} = 14 \pm 7 \times 6.5 \pm 1.5 \mu\text{m}$, $n = 50$), mono- to polytretic, sympodial proliferation, integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* (38–)40–52(–60) $\times$ 9–11 μm ($\bar{x} \pm \text{SD} = 46 \pm 6 \times 10 \pm 1 \mu\text{m}$, $n = 50$), pale brown to brown, smooth walled, straight to slightly curved, broadly fusoid to cylindrical, occasionally ellipsoidal to clavate, tapering toward the rounded ends, apical and basal cells paler than the median cells, (4–)5–9(–10)-distoseptate, germinated mono- or bipolar; hila 2- to 3- μm wide, truncate, slightly protruding, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

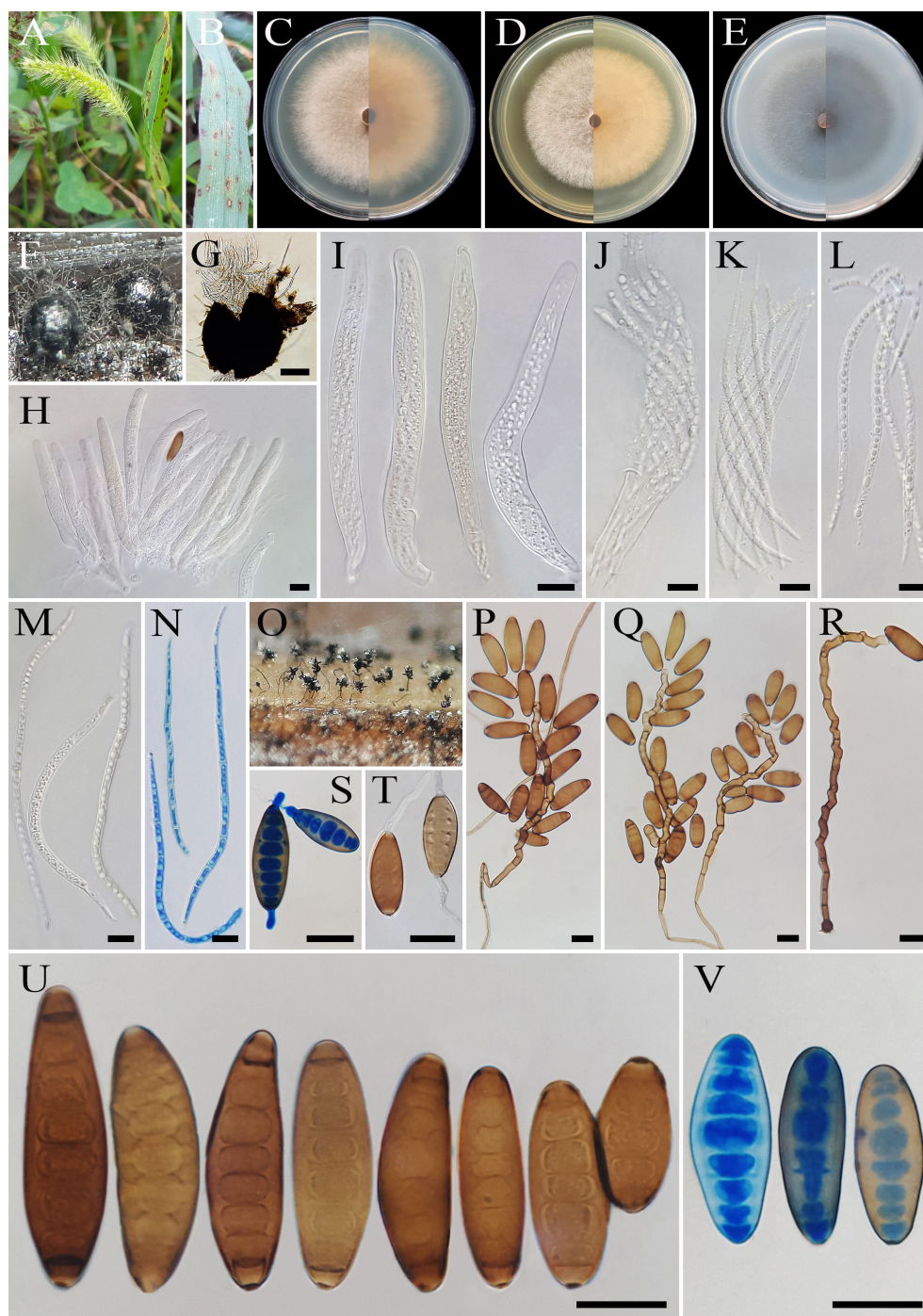


FIGURE 8

Bipolaris hedjaroudei (IRAN 4805C). (A, B) Lesions on host leaf (*Setaria* sp.). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after 7 days. (F, G) Ascomata on TWA medium containing leaves of the host plant. (H–N) Asci and ascospores. (O) Sporulation pattern on TWA medium. (P–R) Conidiophores. (S, T) Germinated conidia. (U, V) Conidia. (G) Scale bars = 100 µm. (H–V) Scale bars = 20 µm.

Culture characteristics: Colonies on PDA reaching 65 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, olivaceous green at the center with white to gray aerial mycelia, white at the margin; reverse olivaceous gray to olivaceous black. Colonies on MEA reaching 55 mm in diameter, circular, margin entire, cottony appearance, olivaceous gray to gray, with white to gray aerial mycelia; reverse brown to pale brown from the center to

the margin. Colonies on CMA reaching 60 mm in diameter, circular, margin entire, hairy appearance, olivaceous gray with sparse white to gray aerial mycelia; reverse olivaceous brown at the center with a hyaline margin. Sterile ascomata were produced on TWA medium containing leaves of the host plant. However, these structures remained sterile (without asci and ascospores) after 3–6 months of incubation.

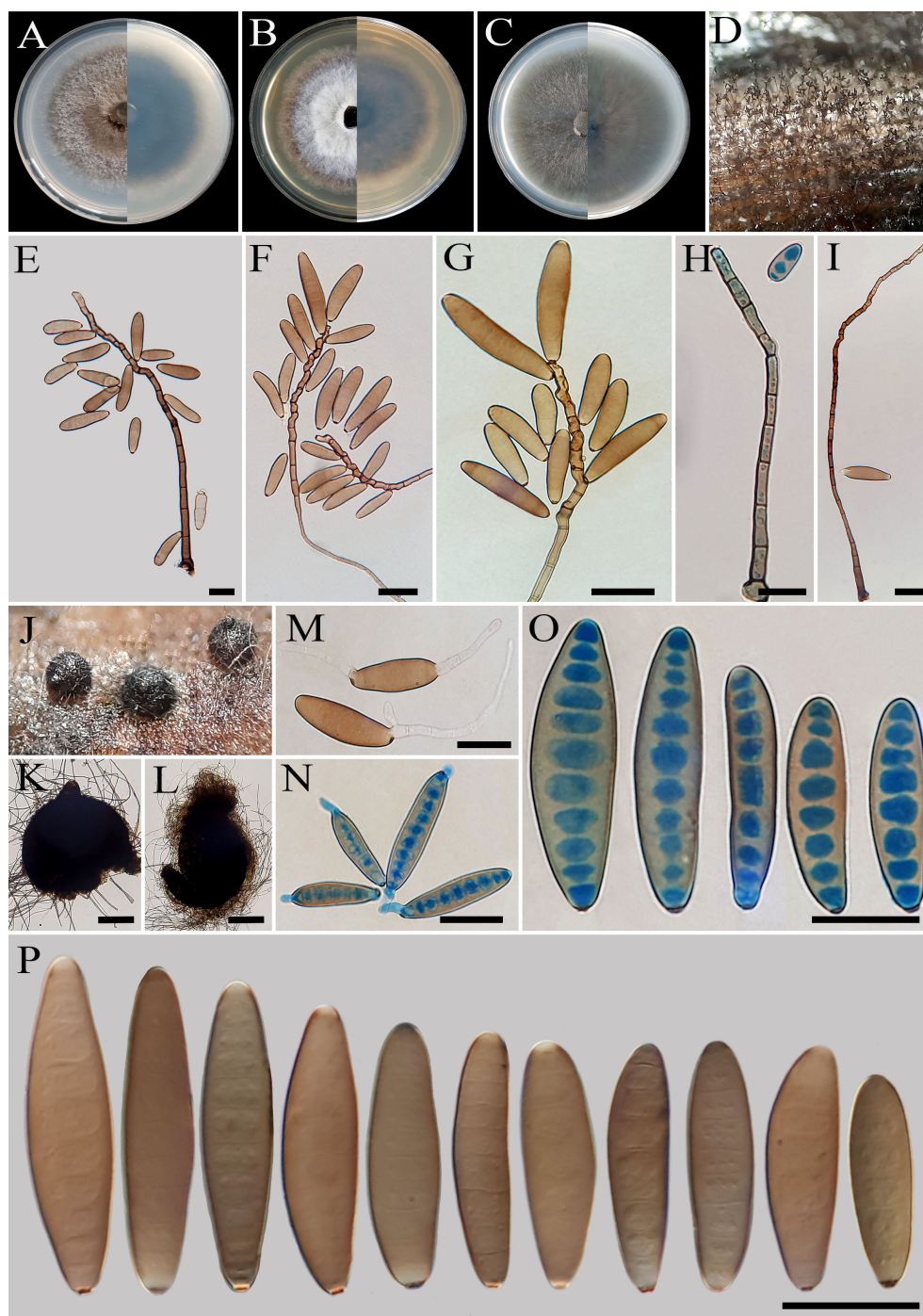


FIGURE 9

Bipolaris hemerocallidis (IRAN 4774C). (A–C) Colonies (front and reverse) on PDA (A), MEA (B), and CMA (C) media after 7 days. (D) Sporulation pattern on TWA medium. (E–I) Conidiophores. (J–L) Sterile ascomata on TWA medium containing leaves of the host plant. (M, N) Germinated conidia. (O, P) Conidia. (K, L) Scale bars = 50 μ m, (E–I), (M–P) Scale bars = 20 μ m.

Additional material examined: IRAN, Isfahan Province, Isfahan County, Flower Garden, on leaves of *Hemerocallis fulva* (Asphodelaceae, Asparagales), 7 October 2013, A. Ahmadpour/Z. Heidarian, isolate FCCUU 1011.

Host and distribution: Associated with leaves of *Hemerocallis fulva* in Iran (this study).

Notes: *Bipolaris hemerocallidis* is phylogenetically close to *B. axonopicola* (MLBS/MPBS/BIPP = 100/100/1.0) (Figure 1). The pairwise DNA sequence comparison revealed that *B. hemerocallidis* is distinct from *B. axonopicola*. A comparison of nucleotide differences in ITS-rDNA, GAPDH, and TEF1 indicates that *B. hemerocallidis* (IRAN 4774C) differs from *B. axonopicola*

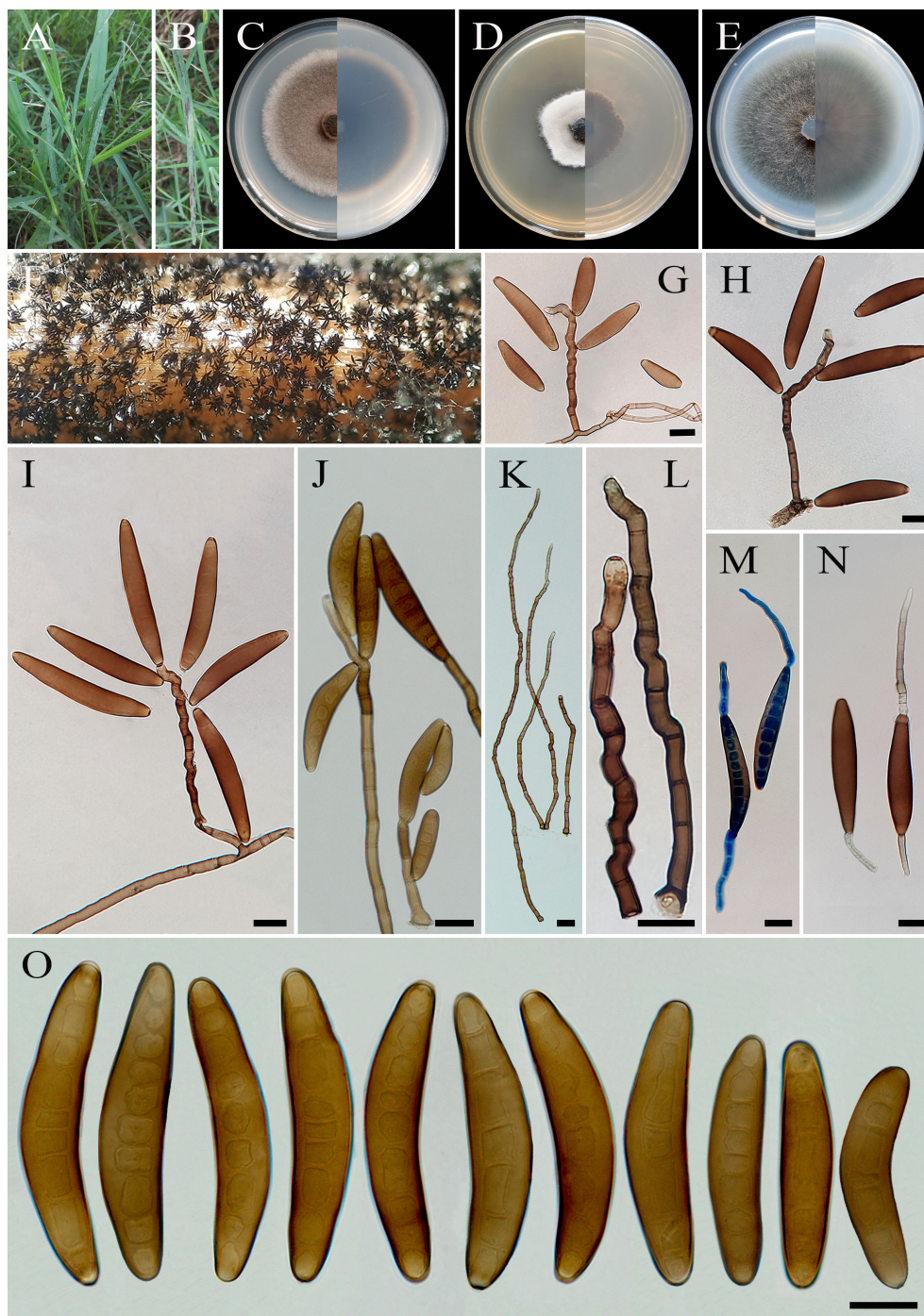


FIGURE 10

Bipolaris iranica (IRAN 4775C). (A, B) Lesions on host leaf (*Cynodon dactylon*). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after 7 days. (F) Sporulation pattern on TWA medium. (G–L) Conidiophores. (M, N) Germinated conidia. (O) Conidia. (J–O) Scale bars = 20 μ m.

(BRIP 11740) by 6/532 bp [1.12%, with four gaps (0%)] in ITS –rDNA, 17/546 bp (3.11%) in *GAPDH* and 4/788 bp (0.50%) in *TEF1*. The PHI analysis confirms that *B. hemerocallidis* has no significant genetic recombination with closely related species ($\Phi_w = > 0.05$, Figure 5). *Bipolaris hemerocallidis* can be differentiated by having longer conidiophores (up to 600 μ m vs. up to 250 μ m in *B. axonopicola*) (Tan et al., 2016). *Bipolaris axonopicola* is only known on *Axonopus fissifolius* (Poaceae) in Australia (Tan et al.,

2016). In this study, *B. hemerocallidis* was isolated from the leaves of *Hemerocallis fulva* (Asphodelaceae, Asparagales) in the greenhouse.

Bipolaris iranica A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 10).

Mycobank No: MB 854735

Etymology: Named after the country “Iran” where the holotype was collected.

Diagnosis: Differs from *Bipolaris heveae*, *B. microlaenae*, and *B. simmondsii* by having much longer conidiophores and accentuated transverse septa.

Type: IRAN, West Azarbaijan Province, Khoy County, on infected leaves of *Cynodon dactylon* (Poaceae, Poales), 20 September 2010, A. Ahmadpour, (IRAN 18207F, **holotype**, dried culture; **ex-type** IRAN 4775C).

Description: Leaf lesions on *Arundo donax*, *Cynodon dactylon*, *Echinochloa colona*, *Hordeum vulgare*, *Sorghum halepense*, and *Triticum aestivum*, 1- to 10-mm long. Sexual morph: Undetermined. Asexual morph: On TWA *Hyphae* 2- to 5- μ m wide, pale brown to brown, smooth, septate, branched. *Conidiophores* (125–)187–480(–550) $\times$ 7–8 μ m ($\bar{x} \pm SD = 337.5 \pm 146.5 \times 7.5 \pm 0.5 \mu$ m, $n = 50$), mononematous, macronematous, arising singly or in groups, simple, straight to flexuous, septate, geniculate, with cell walls thicker than vegetative hyphae, pale brown to brown, paler toward the apex, basal cell swollen and darker than the other cells, up to 10 μ m in diameter. *Conidiogenous cells* (8–)10–24(–28) $\times$ 6–8 μ m ($\bar{x} \pm SD = 17 \pm 7 \times 7 \pm 1 \mu$ m, $n = 50$), mono- to polytretic, proliferating sympodially, integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to dark brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* (70–)85–100(–110) $\times$ 15–20 μ m ($\bar{x} \pm SD = 92.5 \pm 7.5 \times 17.5 \pm 2.5 \mu$ m, $n = 50$), brown to dark brown, smooth walled, straight to curved, mostly navicular to fusoid, rarely cylindrical to clavate, taper toward rounded ends, apical and basal cells paler than the median cells, septa accentuated at maturity, (6–)8–11(–13)-distoseptate, germinated mono- or bipolar; hila 2- to 3- μ m wide, flat to slightly protuberant, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 58 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, olivaceous gray at the center with white to gray aerial mycelia, white at the margin; reverse olivaceous gray to olivaceous black with a hyaline margin. Colonies on MEA reaching 42 mm in diameter, circular, margin entire, cottony appearance, white with white aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on CMA reaching 66 mm in diameter, circular, margin

entire, olivaceous gray with sparse gray aerial mycelia; reverse olivaceous gray at the center and a hyaline margin.

Additional materials examined: IRAN, West Azarbaijan Province, Miyandoab County, on infected leaves of *Sorghum halepense* (Poaceae, Poales), 11 July 2013, A. Ahmadpour/Z. Heidarian, isolate FCCUU 1005; *ibid.* on infected leaves of *Echinochloa colona* (Poaceae, Poales), 23 September 2014, A. Ahmadpour/Z. Heidarian, isolate FCCUU 1007; West Azarbaijan Province, Urmia County, on infected leaves of *Arundo donax* (Poaceae, Poales), 20 September 2014, A. Ahmadpour/Z. Heidarian, isolate FCCUU 1006; West Azarbaijan Province, Bukan County, on infected leaves of *Hordeum vulgare* (Poaceae, Poales), 20 October 2014, A. Ahmadpour/Z. Heidarian, isolate FCCUU 1008; West Azarbaijan Province, Khoy County, on infected leaves of *Triticum aestivum* (Poaceae, Poales), 22 May 2021, A. Ahmadpour, isolate FCCUU 1009.

Hosts and distribution: *Arundo donax*, *Cynodon dactylon*, *Echinochloa colona*, *Hordeum vulgare*, *Sorghum halepense*, and *Triticum aestivum* in Iran (this study).

Notes: Based on the results of phylogenetic analyses (Figure 1), *B. iranica* isolates clustered well in a separate lineage with 100% ML, 100% MP bootstrap, and 1.0 BI posterior probability values, representing a new taxon. The pairwise DNA sequence comparison revealed that *B. iranica* is distinct from related taxa, *B. heveae*, *B. microlaenae*, and *B. simmondsii*. A comparison of nucleotide differences in ITS-rDNA, *GAPDH*, and *TEF1* indicates that *B. iranica* (IRAN 4775C) differs from *B. heveae* (CBS 241.92) by 5/539 bp [0.92%, with four gaps (0%)] in ITS-rDNA, 11/490 bp (2.24%) in *GAPDH* and 6/770 bp (0.77%) in *TEF1*; from *B. microlaenae* (BRIP 15613) by 2/534 bp (0.37%) in ITS-rDNA, 11/543 bp (2.02%) in *GAPDH*, and 12/788 bp (1.52%) in *TEF1*; and from *B. simmondsii* (BRIP 12030) by 3/536 bp [0.55%, with one gap (0%)] in ITS-rDNA, 13/536 bp (2.42%) in *GAPDH*, and 7/788 bp (0.88%) in *TEF1*. The PHI analysis confirms that *B. iranica* has no significant genetic recombination with closely related species ($\Phi_w = > 0.05$, Figure 11). *Bipolaris iranica* is morphologically similar to *B. heveae*, *B. microlaenae*, and *B. simmondsii*; however, it can be distinguished by its much longer conidiophores (up to 550 μ m vs.

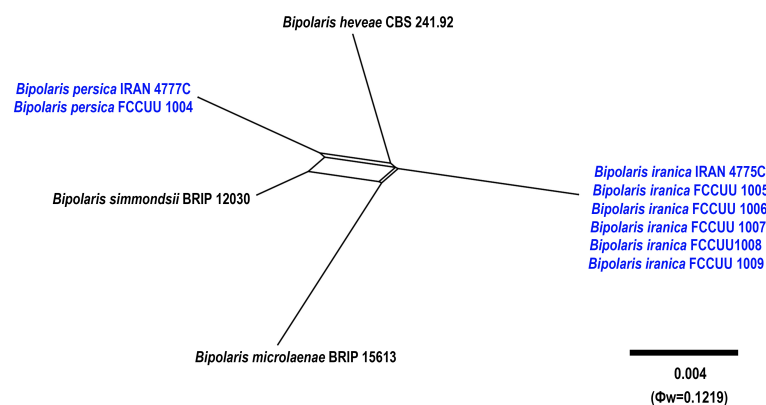


FIGURE 11

Split graphs showing the results of PHI test of *Bipolaris iranica* and *B. persica* with their most closely related species ($\Phi_w = 0.1219$). The new taxa are shown in bold blue.

up to 335 μm in *B. heveae*, and up to 240 μm in *B. simmondsii*), and its accentuated septa, which are absent in *B. heveae*, *B. microlaenae*, and *B. simmondsii* (Manamgoda et al., 2014; Tan et al., 2016). *Bipolaris heveae* is known to cause diseases on rubber trees (*Hevea brasiliensis*) across various tropical countries, including Cambodia, the Dominican Republic, Ghana, Guatemala, Haiti, Honduras, Indonesia, Mexico, Nigeria, the Philippines, Sri Lanka, and the United States. Unlike *B. iranica*, this pathogen does not infect grass species and is restricted to its specific host plant (Manamgoda

et al., 2014; Farr et al., 2024). Furthermore, *B. microlaenae* and *B. simmondsii* have only been documented in Australia, where they cause leaf spots on *Zoysia macrantha* (Tan et al., 2016).

Bipolaris persica A. Ahmadpour, Z. Heidarian, Y. Ghosta, Z. Alavi & F. Alavi, sp. nov. (Figure 12)

Mycobank No: MB 854736

Etymology: The name refers to the old name of Iran, Persia, from where the holotype was collected.

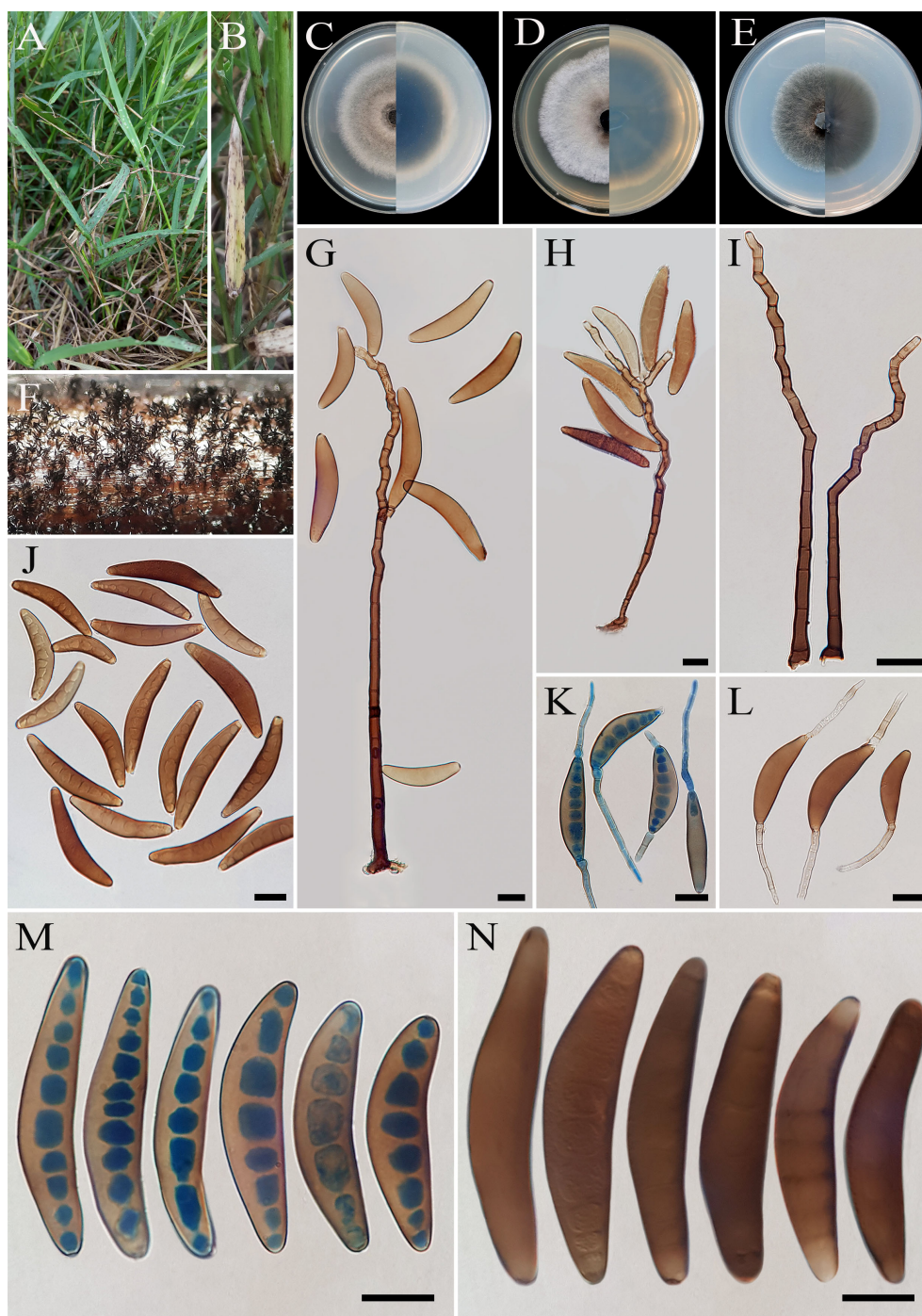


FIGURE 12

Bipolaris persica (IRAN 4777C). (A, B) Lesions on host leaf (*Cynodon dactylon*). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after seven days. (F) Sporulation pattern on TWA medium. (G–I) Conidiophores. (K, L) Germinated conidia. (J, M, N) Conidia. (G–N) Scale bars = 20 μm .

Diagnosis: Differs from *Bipolaris heveae*, *B. microlaenae*, and *B. simmondsii* by having longer conidiophores and accentuated transverse septa.

Type: IRAN, West Azarbaijan Province, Mahabad County, on infected leaves of *Cynodon dactylon* (Poaceae, Poales), 20 June 2015, A. Ahmadpour, (IRAN 18209F, **holotype**, dried culture; **ex-type** IRAN 4777C).

Description: Leaf spots on *Cynodon dactylon*, 1- to 10-mm long, with dark brown spots. Sexual morph: Undetermined. Asexual morph: On TWA *Hyphae* 2- to 5- μ m wide, pale brown to brown, smooth, septate, branched. *Conidiophores* (210–)250–330(–350) $\times$ 6–7 μ m ($\bar{x} \pm SD = 290 \pm 40 \times 6.5 \pm 0.5 \mu$ m, $n = 50$), mononematous, macronematous, arising singly or in groups, simple, straight to flexuous, septate, geniculate, with cells wall thicker than those of vegetative hyphae, pale brown to brown, paler toward the apex, basal cell swollen and darker than the other cells, up to 10 μ m in diameter. *Conidiogenous cells* (7–)10–25(–30) $\times$ 6–8 μ m ($\bar{x} \pm SD = 17.5 \pm 7.5 \times 7 \pm 1 \mu$ m, $n = 50$), mono- to polytetric, proliferating sympodially, integrated, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* (62–)80–95(–100) $\times$ 13–20 μ m ($\bar{x} \pm SD = 87.5 \pm 7.5 \times 16.5 \pm 3.5 \mu$ m, $n = 50$), brown to dark brown, smooth walled, curved, mostly navicular to fusoid, tapering toward the rounded ends, apical and basal cells paler than the median cells, septa accentuated at maturity, (6–)7–10 (–11)-distoseptate, germinated mono- or bipolar; hila 2- to 3- μ m wide, flat, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 68 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, olivaceous gray with white to gray aerial mycelia, sporulation abundant; reverse olivaceous gray at the center with hyaline margin. Colonies on MEA reaching 58 mm in diameter, circular, margin entire, cottony appearance, gray at the center, white at the margin with white to gray aerial mycelia; reverse olivaceous gray with hyaline margin. Colonies on CMA reaching 53 mm in diameter, circular, margin entire, olivaceous green with white to gray aerial mycelia; reverse olivaceous gray to olivaceous black and a hyaline margin.

Additional material examined: IRAN, West Azarbaijan Province, Mahabad County, on infected leaves of *Cynodon dactylon* (Poaceae, Poales), 20 June 2015, A. Ahmadpour, isolate FCCUU 1004.

Host and distribution: *Cynodon dactylon* in Iran (this study).

Notes: *Bipolaris persica* is phylogenetically closely related to *B. heveae*, *B. iranica*, *B. microlaenae*, and *B. simmondsii* (Figures 1, 11). Pairwise DNA sequence comparison revealed that *B. persica* is distinct from its closely related taxa. A comparison of nucleotide differences in ITS-rDNA, *GAPDH*, and *TEF1* indicates that *B. persica* (IRAN 4777C) differs from *B. heveae* (CBS 241.92) by 1/500 bp [0.20%, with one gap (0%)] in ITS-rDNA, 13/481 bp (2.70%) in *GAPDH*, and 7/731 bp (0.95%) in *TEF1*; from *B. iranica* (IRAN 4775C) by 4/500 bp [0.80%, with three gaps (0%)] in ITS-rDNA, 15/504 bp (2.97%) in *GAPDH*, and 8/744 bp (1.07%) in *TEF1*; from *B. microlaenae* (BRIP 15613) by 4/500 bp [0.80%, with three gaps

(0%)] in ITS-rDNA, 17/516 bp (3.29%) in *GAPDH*, and 8/744 bp (1.07%) in *TEF1*; and from *B. simmondsii* (BRIP 12030) by 4/500 bp [0.80%, with three gaps (0%)] in ITS-rDNA, 9/545 bp (1.65%) in *GAPDH*, and 3/744 bp (0.40%) in *TEF1*. The PHI analysis further confirms that *B. iranica* has no significant genetic recombination with closely related species ($\Phi_w = > 0.05$, Figure 11). Morphologically, *Bipolaris persica* can be differentiated from closely related taxa by its longer conidiophores (up to 350 μ m vs. up to 240 μ m in *B. simmondsii*), and by its accentuated septa, which are absent in *B. heveae*, *B. microlaenae*, and *B. simmondsii* (Manamgoda et al., 2014; Tan et al., 2016). However, *B. persica* shares overlapping morphological characteristics and the same host with *B. iranica*, complicating their differentiation. Unlike *B. persica*, *B. iranica* has a broader host range, including *Arundo donax*, *Cynodon dactylon*, *Echinochloa colona*, *Hordeum vulgare*, *Sorghum halepense*, and *Triticum aestivum* (this study). Consequently, using molecular tools is essential for accurately distinguishing *Bipolaris* species and identifying any cryptic species.

Bipolaris crotonis Sivan., Trans. Br. mycol. Soc. 84(3): 404 (1985) (Figure 13).

Description: Leaf spots on *Eleusine indica*, gray at the center and dark brown margins. Sexual morph: Undetermined. Asexual morph: On TWA *Hyphae* 3- to 6- μ m wide, pale brown to brown, smooth, septate, branched. *Conidiophores* (60–)100–300(–325) $\times$ 5–7 μ m ($\bar{x} \pm SD = 200 \pm 100 \times 6 \mu$ m, $n = 50$), mononematous, semi- to macronematous, arising singly or mostly in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, swollen at the base. *Conidiogenous cells* (9–)11–26 (–30) $\times$ 5–8 μ m ($\bar{x} \pm SD = 18.5 \pm 7.5 \times 6.5 \pm 1.5 \mu$ m, $n = 50$), mono- to polytetric, proliferating sympodially, integrated, terminal or intercalary, subcylindrical to slightly swollen, hyaline to pale brown, smooth walled to slightly verruculose, with thickened and darkened scars. *Conidia* (62–)75–100(–120) $\times$ 17–25 μ m ($\bar{x} \pm SD = 87.5 \pm 12.5 \times 21 \pm 4 \mu$ m, $n = 50$), straight, brown to dark golden brown, smooth walled, broadly ellipsoidal, fusoid to obclavate, tapering toward the rounded ends, apical and basal cells paler than the median cells, (5–)7–9(–11)-distoseptate, germinated mono- or bipolar; hila 2- to 3- μ m wide, truncate, slightly protruding, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 45 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, velvety, gray at the center and white at the margin, with sparse gray aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on MEA reaching 40 mm in diameter, circular, margin entire, cottony appearance, gray at the center, white at the margin, with floccose aerial mycelia; reverse brown to pale brown from the center to the margin. Colonies on CMA reaching 50 mm in diameter, hairy appearance, olivaceous gray, with sparse gray aerial mycelia; reverse olivaceous gray at the center and a hyaline margin.

Material examined: IRAN, Mazandaran Province, Nour County, from leaf spots of *Eleusine indica* (Poaceae, Poales), 10 September 2022, Hashemlou E., living culture IRAN 4807C.

Hosts: *Croton* sp., *Eleusine indica* (Sivanesan, 1987; Manamgoda et al., 2014; Farr et al., 2024).

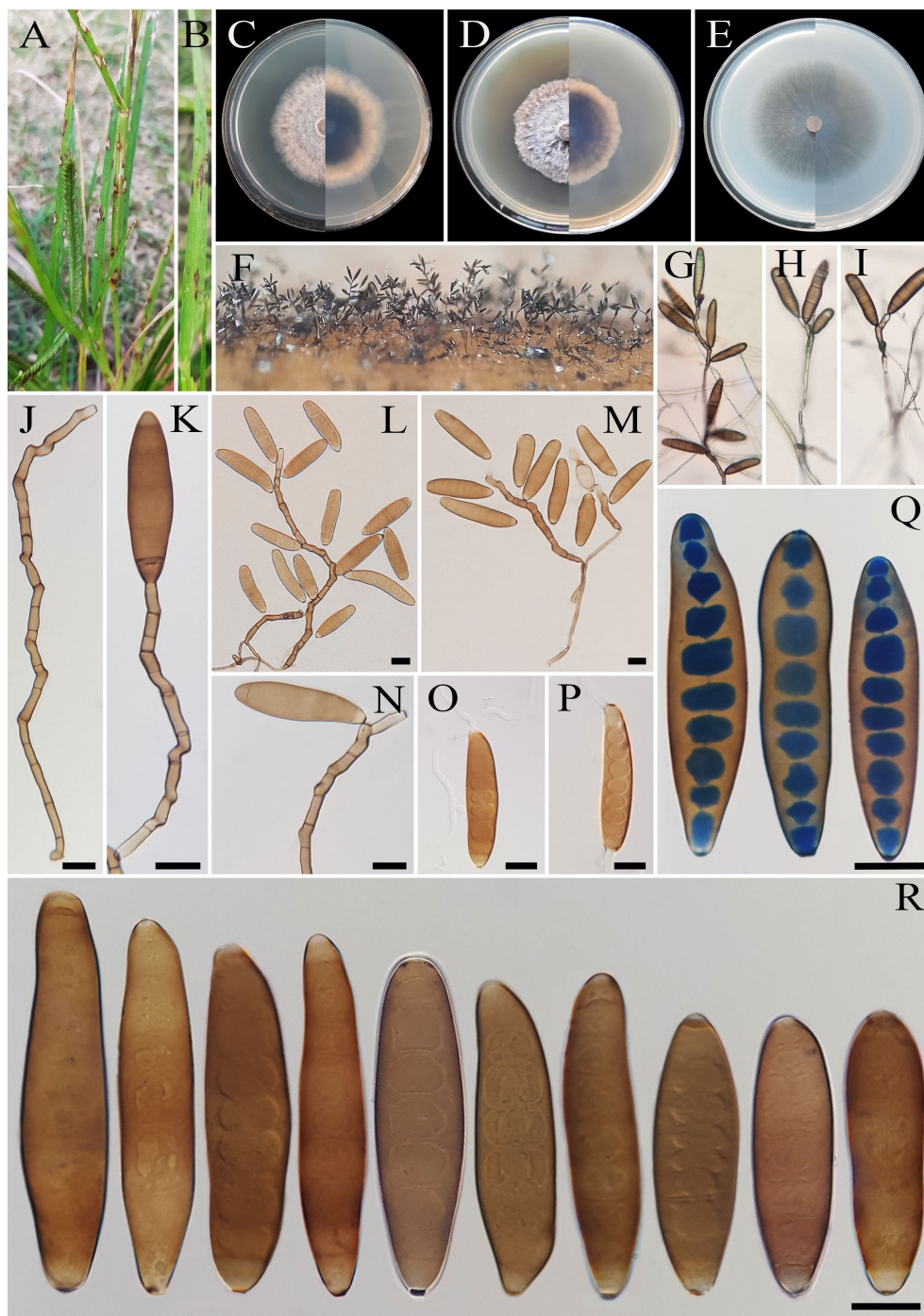


FIGURE 13

Bipolaris crotonis (IRAN 4807C). (A, B) Lesions on host leaf (*Eleusine indica*). (C–E) Colonies (front and reverse) on PDA (C), MEA (D), and CMA (E) media after 7 days. (F–I) Sporulation pattern on TWA medium. (J–N) Conidiophores. (O, P) Germinated conidia. (Q, R) Conidia. (J–R) Scale bars = 20 μm .

Distribution: Australia, Iran (this study), Papua New Guinea, Samoa, and Vanuatu (Sivanesan, 1987; Manamgoda et al., 2014; Farr et al., 2024).

Notes: *Bipolaris crotonis* is morphologically similar and phylogenetically related to *B. sorokiniana* (Figure 1) and can be differentiated by having longer conidia [(51–)60–110(–138) $\times$ (14–)20–25(–32) μm vs. (31–)40–72(–100) $\times$ 15–25(–27) μm in *B.*

sorokiniana] (Sivanesan, 1987; Manamgoda et al., 2014). This species is heterothallic, and sexual morph can be developed by crossing compatible isolates in Sach's agar medium (Manamgoda et al., 2014). Tan et al. (2014) reported that *B. eleusines* is phylogenetically similar to *B. crotonis* and synonymized it under *B. crotonis* based on nomenclatural priority. *Bipolaris crotonis* was first reported on decaying leaves of *Croton* sp. (Euphorbiaceae)

(Sivanesan, 1985; Manamgoda et al., 2014) and was later identified on *Eleusine indica* (Poaceae) (Tan et al., 2014; Bhunjun et al., 2020). This species has also been reported as the causal agent of black point disease in wheat on the North China Plain (Xu et al., 2018) and as an endophytic fungus associated with *Dillenia indica*, an ethnomedicinal plant (Prasher and Kumar, 2021). To the best of our knowledge, this is the first report of *B. crotonis* in Iran.

Bipolaris salkadehensis Ahmadpour & Heidarian, Mycotaxon 120: 302 (2012) (Figure 14).

Description: Culm spots on *Scirpus acutus*, 1- to 20-mm long, with dark brown spots. Sexual morph: Undetermined. Asexual morph: on TWA Hyphae 3- to 5- μ m wide, pale brown to brown, smooth, septate, branched. Conidiophores (225–)260–400(–590) $\times$ 6–7 μ m ($\bar{x} \pm SD = 330 \pm 70 \times 6.5 \pm 0.5 \mu$ m, $n = 50$), mononematous,

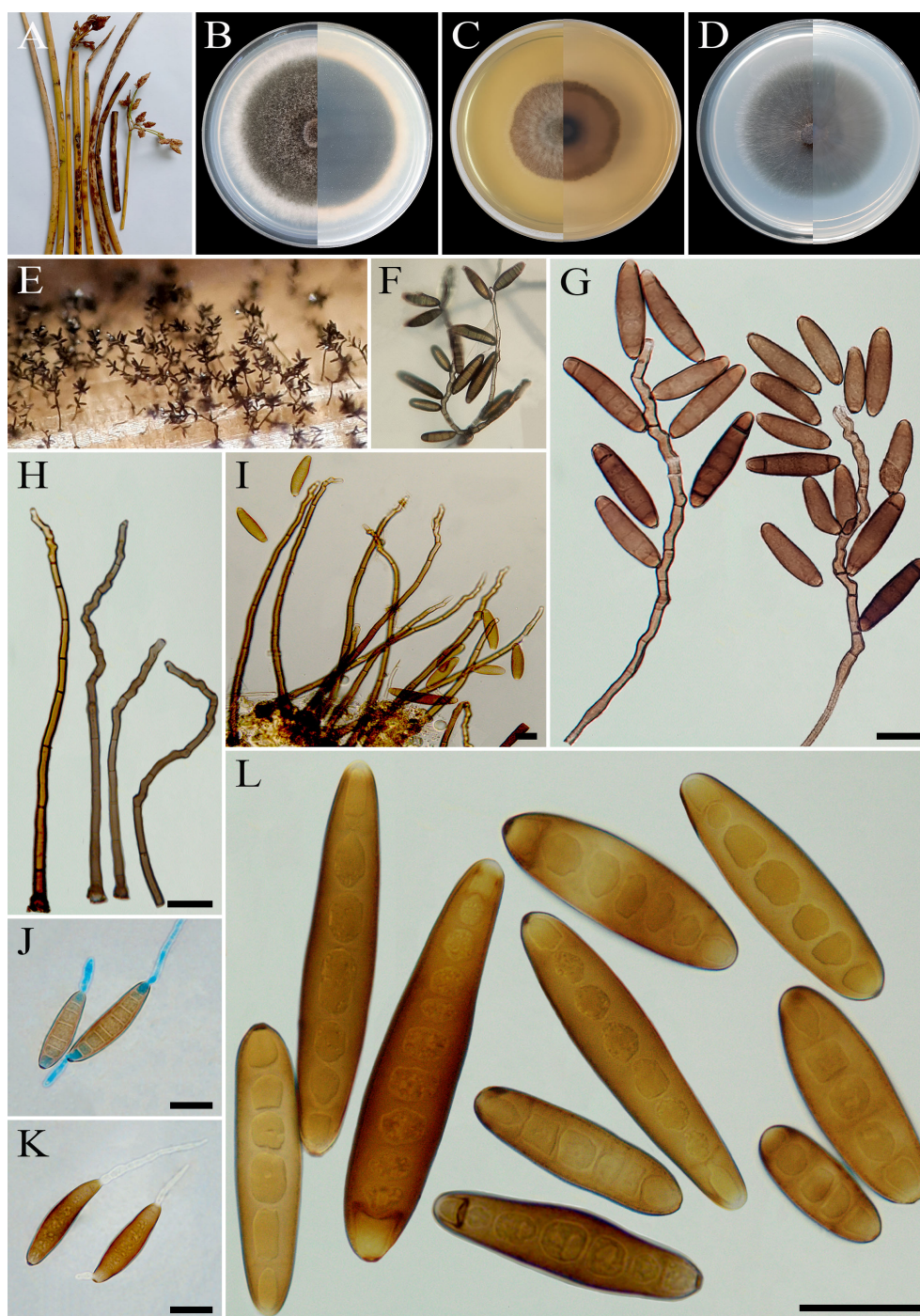


FIGURE 14

Bipolaris salkadehensis (IRAN 3382C). (A) Lesions on host culms (*Scirpus acutus*). (B–D) Colonies (front and reverse) on PDA (B), MEA (C), and CMA (D) media after 7 days. (E, F) Sporulation pattern on TWA medium. (G–I) Conidiophores. (J, K) Germinated conidia. (L) Conidia. (G–L) Scale bars = 20 μ m.

semi- to macronematous, arising singly or in groups, unbranched, straight to flexuous, septate, geniculate, pale brown to brown, paler toward the apex, swollen at the base. *Conidiogenous cells* (8–)10–24 (–30) $\times$ 5–8 μm ($\bar{x} \pm \text{SD} = 16 \pm 6 \times 6.5 \pm 1.5 \mu\text{m}$, $n = 50$), smooth walled, mono- to polytretic, proliferating sympodially, integrated, terminal or intercalary, subcylindrical to slightly swollen, subhyaline or pale brown to brown. *Conidia* (32–)52–70(–93) $\times$ 11–15 μm ($\bar{x} \pm \text{SD} = 61 \pm 9 \times 13 \pm 2 \mu\text{m}$, $n = 50$), brown to dark brown, smooth walled to slightly verruculose, straight to slightly curved, subcylindrical to fusoid, occasionally obclavate to clavate, tapering toward the rounded apex, median cells brown to dark brown, apical and basal cells paler than the median cells being subhyaline to pale brown, end cells often cut off by a thick dark septum, (3–)5–8(–10)-distoseptate, germinated mono- or bipolar; hila 2- to 4- μm wide, flat to slightly protuberant, thickened, and darkened. *Stroma*, *chlamydospores*, and *microconidiation* were not observed.

Culture characteristics: Colonies on PDA reaching 60 mm in diameter after 7 days at 25°C in the dark, circular, margin entire, olivaceous gray to olivaceous brown with white to gray aerial mycelia; reverse gray olivaceous to olivaceous black at the center, white at the margin. Colonies grow more slowly on MEA, reaching 40 mm in diameter, circular, with irregular margin, gray at the center, white at the margin, with floccose aerial mycelia; reverse brown. Colonies on CMA reaching 51 mm in diameter, hairy appearance, olivaceous gray, with sparse gray aerial mycelia; reverse olivaceous gray.

Materials examined: IRAN, West Azarbaijan Province, Khoy County, on infected culms of *Scirpus acutus* (Cyperaceae, Poales), 10 September 2020, A. Ahmadpour, living culture IRAN 3382C; *ibid.* on infected leaves of *Sparganium erectum* (Typhaceae, Poales), 20 September 2010, A. Ahmadpour, living culture BI 1 = IRAN 3385C; *ibid.* on infected leaves of *Cladium mariscus* (Cyperaceae, Poales), 20 September 2010, A. Ahmadpour, living culture BI 4 = IRAN 3386C; *ibid.* on infected leaves of *Setaria* sp. (Poaceae, Poales), 25 September 2013, A. Ahmadpour, living culture FCCUU 1002; West Azarbaijan Province, Miyandoab County, on infected leaves of *Sorghum halepense* (Poaceae, Poales), 10 July 2013, A. Ahmadpour/Z. Heidarian, living culture IRAN 3383C; *ibid.* on infected leaves of *Arundo donax* (Poaceae, Poales), 10 July 2013, A. Ahmadpour/Z. Heidarian, living culture FCCUU 1001; *ibid.* on infected leaves of *Hordeum vulgare* (Poaceae, Poales), 10 May 2014, A. Ahmadpour/Z. Heidarian, living culture FCCUU 1003.

Hosts: *Arundo donax*, *Cladium mariscus*, *Hordeum vulgare*, *Scirpus acutus*, *Setaria* sp., *Sorghum halepense*, and *Sparganium erectum* (Ahmadpour et al., 2012a; Farr et al., 2024; this study).

Distribution: Iran (Ahmadpour et al., 2012a; this study).

Notes: *Bipolaris salkadehensis* was originally reported from infected leaves of *Sparganium erectum* (Sparganiaceae) and *Cladium mariscus* (Cyperaceae) with brown oval to elliptical lesions based on morphological characteristics and molecular data obtained from ITS–rDNA sequences (Ahmadpour et al., 2012a). In this study, two additional genes, *GAPDH* and *TEF1*, were sequenced for the ex-type isolate (Bi 1 = IRAN 3385C) as well as for other isolates from various plant hosts (Table 1) and used in phylogenetic

analyses. *Bipolaris salkadehensis* isolates clustered well in a separate lineage with 100% ML, 100% MP bootstrap, and 1.0 BI posterior probability values (Figure 1). This species is morphologically similar to *B. cynodontis* and *B. setariae* (Sivanesan, 1987; Ahmadpour et al., 2012a). However, the conidia of *B. cynodontis* are smaller in size (30–75 $\times$ 10–16 μm), have three to nine (commonly seven to eight) distoseptate, and without cut-off in end cells (Sivanesan, 1987; Ahmadpour et al., 2012a). The conidia of *B. setariae* are fusoid to navicular, pale to mid golden brown, 5–10 distoseptate, 45–100 (mostly 50–70) $\times$ 10–15 μm , without cut-off in end cells, and lighter than those of *B. salkadehensis* (Sivanesan, 1987; Ahmadpour et al., 2012a). Zibani et al. (2025) have reported that *B. salkadehensis* exhibits low virulence as a pathogen of corn in Algeria. To the best of our knowledge, *Arundo donax*, *Hordeum vulgare*, *Scirpus acutus*, *Setaria* sp., and *Sorghum halepense* are newly identified hosts for this species.

4 Discussion

Advancements in molecular biology and phylogenetics have revolutionized our understanding of *Bipolaris* taxonomy and phylogeny. Techniques, such as DNA sequencing and phylogenetic analysis have enabled the identification of cryptic species and elucidated evolutionary relationships within the genus (Manamgoda et al., 2012, 2014; Tan et al., 2016; Raza et al., 2019; Bhunjun et al., 2020; Ferdinandez et al., 2022). Nevertheless, challenges persist in defining species boundaries and integrating morphological and molecular data. The results of this study revealed the presence of seven previously undocumented *Bipolaris* species from Iran, representing significant additions to the fungal biodiversity of the region. Comprehensive morphological characteristics and molecular phylogenetic analyses distinguish these new species from known taxa within the *Bipolaris* genus. Moreover, the study reports new records of *Bipolaris* species (*B. crotonis*) and identifies additional plant hosts for *B. salkadehensis*, highlighting the ecological diversity and host specificity of these fungi in Iranian ecosystems. Understanding their distribution, host range, and pathogenicity is crucial for developing effective disease management strategies and safeguarding agricultural crops in Iran.

Numerous *Bipolaris* species exhibit similar morphological characteristics making identification based solely on morphology unreliable and often ambiguous. Factors, such as environmental conditions, host plants, substrate, and culture media, further influence the morphological characteristics of *Bipolaris* species (Sivanesan, 1987; Alcorn, 1988; Manamgoda et al., 2012, 2014, 2015; Tan et al., 2014, 2016; Marin-Felix et al., 2017a, b, 2020). Accurate identification and understanding of genetic and pathogenic variability are essential for developing effective control measures and breeding programs. Molecular analyses, particularly using ITS–rDNA, *GAPDH*, and *TEF1* loci, have proven invaluable in addressing these challenges, offering more precise species delimitation within the *Bipolaris* genus (Berbee et al., 1999; Manamgoda et al., 2012, 2014, 2015; Tan et al., 2014, 2016; Marin-Felix et al., 2017a, b, 2020). While ITS–rDNA has limitations in distinguishing closely related species, *GAPDH* has

been shown to be a more informative genetic marker and is recommended as a critical locus and supplementary barcode for accurately identifying closely related *Bipolaris* species (Manamgoda et al., 2012, 2014, 2015; Madrid et al., 2014; Tan et al., 2014, 2016; Marin-Felix et al., 2017a, b, 2020; Bhunjun et al., 2020). Comparative analyses of taxa have consistently demonstrated that the *GAPDH* gene region provides greater resolution, as indicated by multiple prior investigations (Berbee et al., 1999; Manamgoda et al., 2012, 2014, 2015; Madrid et al., 2014; Tan et al., 2014, 2016; Fernandez et al., 2022). In our study, *B. persica* and *B. iranica* exhibit overlapping morphological features complicating their differentiation. Therefore, molecular tools are essential for accurate differentiation among *Bipolaris* species.

The sexual morph of the fungus *Bipolaris* is rare in natural environments but has been observed under controlled laboratory conditions (Sivanesan, 1987; Manamgoda et al., 2011, 2014). The majority of *Bipolaris* species are heterothallic, with their sexual reproduction determined by mating-type idiomorphs, *MAT1-1* and *MAT1-2* (Turgeon, 1998; Yun et al., 1999; Lu et al., 2011). In heterothallic species, the presence of both mating types is necessary for the development of sexual structures. The only previously known homothallic species within the *Bipolaris* genus is *B. luttrellii* (synonym: *Cochliobolus luttrellii*), which contains both *MAT1-1* and *MAT1-2* idiomorphs (Turgeon, 1998; Yun et al., 1999; Lu et al., 2011). This study identifies *B. hedjaroudei* as another homothallic species capable of forming a sexual morph on TWA medium supplemented with host leaves (Figure 8) making it the second known homothallic species in the genus. *MAT* genes are particularly useful for studying the evolution of reproductive strategies and sexual mechanisms, as they appear to evolve faster than ITS-rDNA and *GAPDH* sequence regions (Turgeon, 1998). Additionally, due to their high interspecies variability and low intraspecies variability, *MAT* genes have been proposed as potential markers for defining species boundaries (Yun et al., 1999). Phylogenetic analyses of *MAT* genes, along with ITS-rDNA and *GAPDH* sequences, provide valuable insights into the evolutionary history of reproductive strategies (Turgeon, 1998). Yun et al. (1999) also developed a specific PCR assay for amplifying *MAT* idiomorphs using the TAIL-PCR technique. Further research should focus on identifying and characterizing the mating type idiomorphs in *B. hedjaroudei* and other newly identified species in this study.

Most of the newly identified *Bipolaris* species in this study, including *Bipolaris avrinica*, *B. azarbaijanica*, *B. banihashemii*, and *B. hedjaroudei*, were isolated from *Setaria* species. This plant genus is among the most significant weeds affecting field crops in Iran. Some species of *Bipolaris* (*B. bicolor*, *B. setariae*, and *B. sorokiniana* on *Eleusine indica*; *B. euphorbiae* on *Euphorbia heterophylla*; *B. eleusines* on *Echinochloa crus-galli*; *B. sorghicola* on *Sorghum halepense*; *B. yamadae* on various poaceous plants; and *B. microstegii*, *B. panicumiliacei*, and *B. zeicola* on *Microstegium vimineum*) are known to cause diseases in weeds. These fungi have shown potential for use as herbicides and have been validated as effective mycoherbicides in several studies (Figliola et al., 1988; Winder and Van Dyke, 1990; Kleczewski and Flory, 2010; Manamgoda et al., 2011; Zhang et al., 2014, 2022; Omar and Naquiuddin, 2020; Tan et al., 2022, 2024; Xiao et al., 2022; Khan et al., 2023). Additional research is needed to investigate the host range, pathogenicity, and potential applications of these newly

discovered species as mycoherbicides on *Setaria* spp. and other plants within the Poaceae family. Recent studies suggest that certain phytopathogenic fungi can switch hosts and infect nearby plants (Rai and Agarkar, 2016). Weeds can serve as reservoirs for pathogens that threaten economically important crops. Additionally, environmental changes may drive certain fungi, previously considered mildly pathogenic, to evolve into more aggressive pathogens in new hosts (Manamgoda et al., 2011; Rai and Agarkar, 2016; Hernández-Restrepo et al., 2018). This study also revealed that *B. iranica* and *B. salkadehensis* have wide host ranges, being isolated from barley, wheat, and various weed species. Hence, the accurate identification of *Bipolaris* species associated with cereal crops and their weedy hosts is crucial for effective disease management and for ensuring stable crop production.

Understanding fungal biodiversity, such as *Bipolaris* species, is crucial for understanding diverse ecosystems globally. Exploring their diversity, distribution, and ecological functions enhances our understanding of plant health and ecosystem dynamics on a worldwide scale. This comprehensive study highlights the rich diversity of *Bipolaris* species and the complex relationships between fungi and their environments providing insights that are valuable for developing strategies to manage plant diseases and conserve fungal biodiversity in different regions.

5 Conclusions

This study analyzed 85 *Bipolaris* isolates collected from various hosts, in the Poales and Asparagales plant orders, across different locations in Iran between 2010 and 2022. Seven new species (*B. avrinica*, *B. azarbaijanica*, *B. banihashemii*, *B. hedjaroudei*, *B. hemerocallidis*, *B. iranica*, and *B. persica*) were discovered, along with two new records to Iran's funga (*B. crotonis* and *B. salkadehensis*), identified through a combination of morphological characteristics and multi-locus phylogenetic analyses (ITS-rDNA, *GAPDH*, and *TEF1*). Investigating the diversity, distribution, and ecology of *Bipolaris* species in Iran is crucial for understanding their role in plant diseases, ecosystem interactions, and the development of effective disease management strategies in agriculture. This detailed study provides valuable insights into the diverse *Bipolaris* species in Iran paving the way for future research into plant diseases and fungal biodiversity in the country.

Data availability statement

The datasets presented in this study can be found in the online repository <https://www.ncbi.nlm.nih.gov/genbank/> and the accession numbers are mentioned in Table 1.

Author contributions

AA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing, Funding

acquisition, Project administration, Resources, Supervision. ZH: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Formal analysis, Investigation, Methodology, Resources, Visualization. YG: Conceptualization, Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation, Methodology. ZA: Investigation, Methodology, Software, Formal analysis, Writing – review & editing. FA: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. DM: Data curation, Validation, Writing – review & editing, Formal analysis, Visualization. JK: Data curation, Writing – review & editing, Formal analysis. SK: Data curation, Writing – review & editing, Formal analysis, Funding acquisition, Visualization. PR: Data curation, Writing – review & editing, Formal analysis. NS: Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY

Ahmed M. Abdel-Azeem,
Suez Canal University, Egypt

REVIEWED BY

Danushka Sandaruwan Tennakoon,
Shenzhen University, China
Hiran Kanti Santra,
Vidyasagar University, India

*CORRESPONDENCE

Saisamorn Lumyong

✉ scboi009@gmail.com

Rungtiwa Phookamsak

✉ phookamsak@mail.kib.ac.cn

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Novel endophytic pestalotioid species associated with *Itea* in Thailand

Jutamart Monkai^{1,2}, Rungtiwa Phookamsak^{3,4,5,6*},
Darbhe Jayarama Bhat^{7,8}, Toe Swe Zin Ei², Jianchu Xu^{3,4,5,6}
and Saisamorn Lumyong^{2,9,10*}

¹Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand, ²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ³Centre for Mountain Futures (CMF), Kunming Institute of Botany, Kunming, Yunnan, China, ⁴Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China, ⁵Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China, ⁶The Center for International Forestry Research and World Agroforestry (CIFOR-ICRAF) China Program, World Agroforestry (ICRAF), Kunming, Yunnan, China, ⁷Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, ⁸Biology Division, Vishnugupta Vishwavidyapeetam, Gokarna, India, ⁹Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai, Thailand, ¹⁰Academy of Science, The Royal Society of Thailand, Bangkok, Thailand

Endophytic fungi are a well-known fascinating host-associated fungal group that can enhance plant growth and fitness by producing various bioactive secondary metabolites. They are an excellent source of industrial enzymes for potential secondary metabolite synthesis, which is useful in green agriculture, biotechnology, and pharmaceuticals. *Itea* is a valuable plant genus since it naturally contains rare sugar; however, endophytic fungi associated in this host have not yet been documented. In the present study, 11 strains of endophytic fungi were isolated and primarily identified as pestalotioid taxa based on morphological characteristics exhibited *in vitro*. Eleven strains of *Pestalotiopsis*-like taxa were isolated from the healthy leaves, stems, and roots of *Itea japonica* and *I. riparia* from Chiang Mai Province, Thailand. Species delimitation was based on morphology, multiloci phylogeny of a concatenated ITS, *tub2*, and *tef1-α* sequence data, and nucleotide polymorphism analyses. *Neopestalotiopsis iteae* and *Pseudopestalotiopsis iteae* are proposed as new species on *I. japonica* and *I. riparia*, respectively. *Neopestalotiopsis chrysea*, *N. haikouensis*, and *Pestalotiopsis jinchanghensis* are described as new records on *I. riparia*. Owing to the conspecific relationship based on multiloci phylogeny and identically nucleotide pairwise comparison of sufficient gene regions, several species are synonymized including *Neopestalotiopsis cercidicola* and *N. terricola* as *N. haikouensis*, *N. umbrinospora* as *N. chrysea*, and *Pestalotiopsis zhaoqingensis* as *P. jinchanghensis*. The updated phylogenetic trees, nucleotide comparisons, and morphological descriptions are herein provided and discussed for the taxonomic placements of these new species and records. This study is the first to investigate *Itea* endophytes in Thailand, and it reveals the intra- and interspecific relationships of pestalotioid species, which need to be further reevaluated because of ambiguous taxa.

KEYWORDS

Neopestalotiopsis, Pestalotiopsis, polyphasic taxonomic approach, Pseudopestalotiopsis, Sporocadaceae

1 Introduction

Pestalotioid fungi are generally referred to as appendage-bearing coelomycetes that have multi-septate and fusiform conidia in the family *Sporocadaceae* (*Amphisphaeriales*, *Sordariomycetes*), including genera *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis* (Maharachchikumbura et al., 2011; 2014; Peng et al., 2022; Razaghi et al., 2024). *Pestalotiopsis* and allied genera are taxonomically, chemically, and ecologically diverse, which have been associated with a broad range of host plants through their life cycle as endophytes, pathogens, and saprobes (Peng et al., 2022; Giri and Pradhan, 2023; Hsu et al., 2024). The natural classification of this group based on the conidial morphology suggests concolorous median cells in *Pestalotiopsis* and *Pseudopestalotiopsis*, and versicolorous median cells in *Neopestalotiopsis* (Maharachchikumbura et al., 2014; Peng et al., 2022; Sun et al., 2023). Compared with *Pestalotiopsis*, *Pseudopestalotiopsis* has darker colored median cells (Maharachchikumbura et al., 2014; Nozawa et al., 2018; Sun et al., 2023). However, the characteristics of conidia (e.g., color, size, and shape) and conidial appendages (e.g., number, length, shape, branched or unbranched, and presence or absence of knobbed tips) are overlapping (Maharachchikumbura et al., 2014; Liu et al., 2017; Tsai et al., 2021). The multiloci phylogeny of the concatenated ITS, *tub2*, and *tef1- α* coupled with morphology has proved to be reliable for the taxonomic circumscription of pestalotioid species (Maharachchikumbura et al., 2012; 2014; Nozawa et al., 2017; 2018; Norphanphoun et al., 2019; Sun et al., 2023; Hsu et al., 2024).

Ecologically, *Pestalotiopsis*-like species are important as phytopathogens, endophytes, and saprobes and have a wide range of distribution in the temperate regions and tropics (Maharachchikumbura et al., 2012; 2014; Gu et al., 2022; Peng et al., 2022; Giri and Pradhan, 2023; Hsu et al., 2024). As plant pathogens, they cause various diseases, resulting in significant loss in various economic plants such as avocado (Fiorenza et al., 2022), eucalyptus (Diogo et al., 2021), blueberry (Zheng et al., 2023), macadamia (Prasannath et al., 2021), mangrove-associated hosts (Norphanphoun et al., 2019), pine (Monteiro et al., 2022), rose (Peng et al., 2022), and tea (Liu et al., 2017; Chen et al., 2018; Tsai et al., 2021). Endophytic pestalotioid fungi are considered as a valuable source for producing secondary metabolites with diverse chemical structures and promising antibacterial, antifungal, and anticancer properties (Xu et al., 2010; Palwe et al., 2021; Wu et al., 2022; Giri and Pradhan, 2023). Since the discovery of the antitumor drug taxol from *Pestalotiopsis microspora*, which was earlier isolated from *Taxus wallachiana* (Strobel et al., 1996), more studies have focused on *Pestalotiopsis* and allied genera.

Itea is a plant genus in *Iteaceae*, including approximately 21 species, widely distributed in Africa, Asia, and North America (POWO, 2024; Song and Zhang, 2024). *Itea japonica* is an edible species, native to Japan, and located in the temperate regions (Ayers et al., 2014; POWO, 2024), whereas *I. riparia* is endemic to China,

Laos, Myanmar, Thailand, and Vietnam and is located in the tropical region (POWO, 2024; Song and Zhang, 2024). *Itea* species are recognized for their nutritional and medicinal value owing to the presence of rare sugars (e.g., D-allulose and allitol) and other bioactive compounds (e.g., coumarins, flavonoids, and terpenoids), which exhibited antioxidant, anti-hepatocellular carcinoma, anti-tuberculosis, and glycosidase inhibitory activities (Luo et al., 2014; Zeng et al., 2015; Lang et al., 2022; Feng et al., 2024). Previous studies have isolated some pathogenic and endophytic fungi from *Itea* species (Farr et al., 2021). However, fungi associated with *I. japonica* and *I. riparia* have not yet been reported. Therefore, it is worthy to investigate the fungi associated to this host plant as it could offer a promising resource for new discoveries of useful bioactive compounds and enzymes. As part of our study on endophytic fungi associated with *Itea* species, we recovered 11 *Pestalotiopsis*-like taxa from fresh tissues of *I. japonica* and *I. riparia* in Chiang Mai Province, Thailand. Therefore, the aims of this study are to identify these novel strains using morphological and molecular approaches and to examine their intra- and intergeneric affinities within the pestalotioid species.

2 Materials and methods

2.1 Collection and isolation of endophytic fungi

Fresh and healthy samples (viz., leaves, stems, and roots) of *Itea japonica* and *I. riparia* were collected from Chiang Mai Province, northern Thailand. The plant samples were initially rinsed with running tap water and cut into small sections (5 mm × 5 mm). The surface sterilization of plant tissues was carried out by immersing in distilled water for 1 min, 70% alcohol for 30 s, and 2% NaOCl for 30 s, in this order. Finally, the plant sections were thoroughly rinsed in sterile distilled water, air dried, and placed on potato dextrose agar (PDA) plates. After incubation at 25°C for 24–48 h, the hyphal tips grown on the margin of the plant tissues were picked up and inoculated onto new PDA plates to obtain the pure cultures. The cultures were incubated at 25°C for 1–2 months for the sporulation and morphological examination (Mattoo and Nonzom, 2022). The living cultures were deposited in the Culture Collection of Sustainable Development of Biological Resources Laboratory, Faculty of Science, Chiang Mai University, Chiang Mai Province, Thailand (SDBR-CMU) and the Kunming Institute of Botany Culture Collection, Kunming, China (KUNCC). To preserve the dried fungal samples (herbarium), cultures were dried in the hot air oven at 45–50°C for 1–2 days and deposited at the Herbarium of the Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai Province, Thailand (CMUB). The newly described taxa were registered for the Index Fungorum numbers (<https://indexfungorum.org/Names/IndexFungorumRegisterName.asp>; accessed on 13 November 2024).

2.2 Morphological observation and examination

The morphological characteristics of endophytic fungi (i.e., conidiomata, conidiophores, conidiogenous cells, and conidia) grown on PDA after 30 days were observed using a Nikon SMZ800N stereo microscope (Nikon Instruments Inc., Melville, NY, USA) and a Nikon Eclipse Ni compound microscope attached to a Nikon DS-Ri2 camera system (Nikon Instruments Inc., Melville, NY, USA). Permanent slides were prepared by adding lactoglycerol and sealed with clear nail polish. The measurements of fungal structures were carried out using a Tarosoft (R) Image Frame Work version 0.9.7 program. The photographic plates were edited and combined using Adobe Photoshop version 21.2.4 (Adobe Systems, San Jose, CA, USA).

2.3 DNA extraction, PCR amplification, and sequencing

The Biospin Fungus Genomic DNA Extraction Kit (BioFlux, Hangzhou, China) was used to extract DNA from 7-day-old colonies grown on PDA at 25°C. The internal transcribed spacer region of ribosomal DNA (ITS) was amplified using primers ITS5 and ITS4 (White et al., 1990); the beta-tubulin (*tub2*) was amplified using primers T1 and Bt2b (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997); and the translation elongation factor-1 alpha (*tef1-α*) was amplified using primers EF1-728F and EF2 (O'Donnell et al., 1998; Carbone and Kohn, 1999). The PCR conditions for the three loci were set up following Sun et al. (2023) and Gao et al. (2024). For *Pseudopectalotiopsis* species, the beta-tubulin (*tub2*) was additionally amplified using primers Bt2a and Bt2b (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997) with the PCR condition as described by Maharachchikumbura et al. (2012). The PCR amplification was performed in a 25-μL reaction volume, containing 12.5 μL of Master Mix (mixture of *EasyTaq*™ DNA Polymerase, dNTPs, and optimized buffer; Beijing TransGen Biotech Co., Ltd., Chaoyang District, Beijing, China), 8.5 μL of double-distilled water (ddH₂O), 2 μL of DNA template, and 1 μL of each forward and reverse primer (10 μM). The purification of PCR products and DNA sequencing were conducted by TsingKe Company (Kunming City, Yunnan Province, China).

2.4 Phylogenetic analyses

The sequences were checked and assembled using BioEdit version 7.0.5.3 (Hall, 1999). The homogeneous sequences were obtained from relevant publications (Li et al., 2022; Zhang et al., 2022; 2024; Sun et al., 2023; Dong et al., 2023; Hsu et al., 2024) and the results of BLAST searches that were downloaded from GenBank (www.ncbi.nlm.nih.gov/blast/; accessed on 1 September 2024) (Tables 1–3). Alignments for each locus were carried out using the MAFFT v7.307 online version (Katoh et al., 2019; <https://mafft.cbrc.jp/alignment/server/>, accessed on 1 September 2024)

and manually verified in BioEdit version 7.0.5.3 (Hall, 1999). To construct the phylogenetic analyses, maximum likelihood (ML) was previously applied for single locus, and further combined datasets of ITS, *tub2*, and *tef1-α* gene loci were analyzed by Bayesian inference (BI) and ML when the tree topology of each locus is congruent. ML analysis was generated with 1,000 bootstrap (BS) iterations and the GTRGAMMA model of nucleotide evolution using RAXML-HP2 on XSEDE (v.8.2.12) (Stamatakis, 2014) via the online web platform CIPRES Science Gateway V3.3 (Miller et al., 2010). The best-fit substitution models were evaluated using MrModeltest v.2.3 (Nylander, 2004). BI analysis was implemented with MrBayes on XSEDE v.3.2.7a (Huelsenbeck and Ronquist, 2001; Zhaxybayeva and Gogarten, 2002; Ronquist et al., 2012) via the same platform (Miller et al., 2010). Six simultaneous Markov chains were run for 10 million generations but were automatically terminated when the standard deviation of split frequency approached 0.01. The trees were sampled every 100th generation, and the first 25% of sampled trees were removed during the burn-in phase of analyses, which were evaluated by Tracer v. 1.7 (Rambaut et al., 2018). The phylograms were displayed and modified with the FigTree v1.4.0 program (Rambaut and Drummond, 2012) and Adobe Illustrator version 24.3 (Adobe Systems, San Jose, CA, the USA). The newly generated sequences were submitted to the NCBI database to obtain GenBank accession numbers.

3 Results

3.1 Multiloci phylogenetic analyses

Analysis 1: the combined sequence dataset of ITS, *tub2*, and *tef1-α* comprised 158 *Neopestalotiopsis* strains and two outgroup taxa, *Pestalotiopsis diversiseta* (MFLUCC 12-0287) and *P. spathulata* (CBS 356.86) (Figure 1). The aligned dataset consisted of a total 1,896 characters with gaps (ITS: 1–541 bp, *tub2*: 542–1340 bp, *tef1-α*: 1341–1896 bp). The best-scoring RAXML tree was selected to represent the relationships among taxa, with a final likelihood value of −11,777.047879. The matrix contained 944 distinct alignment patterns, with 17.98% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.235628, C = 0.271348, G = 0.215132, and T = 0.277892; substitution rates were AC = 0.918956, AG = 2.618624, AT = 1.144576, CG = 0.760024, CT = 3.458802, and GT = 1.000000; gamma distribution shape parameter α = 0.361998; Tree-Length = 1.334915. For BI analysis, the best-fitting model of each locus and the final average standard deviation of split frequencies are shown in Table 4. Similar tree topologies were acquired by ML and Bayesian posterior probability (BYPP) analyses. The phylogenetic tree revealed that a new species, *Neopestalotiopsis iteae* (SDBR-CMU515), formed an independent branch sister to *N. cocoës* (0.91 BYPP; Figure 1). The new strain, SDBR-CMU517, is closely related to *N. cercidicola*, *N. haikouensis*, and *N. terricola* (Figure 1), whereas the new strain, SDBR-CMU516, grouped with *N. chrysea* and *N. umbrinospora* (Figure 1).

Analysis 2: the combined sequence dataset of ITS, *tub2*, and *tef1-α* comprised 186 *Pestalotiopsis* strains and two outgroup taxa,

TABLE 1 GenBank accession numbers of *Neopestalotiopsis* strains used in phylogenetic analysis.

Taxa	Strains	GenBank accession number		
		ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754*	MK764272	MK764338	MK764316
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1755	MK764273	MK764339	MK764317
<i>Neopestalotiopsis alpapicalis</i>	MFLUCC 17-2544*	MK357772	MK463545	MK463547
<i>Neopestalotiopsis amomi</i>	HKAS 124563*	OP498012	OP752133	OP653489
<i>Neopestalotiopsis amomi</i>	HKAS 124564	OP498013	OP765913	OP753382
<i>Neopestalotiopsis aotearoa</i>	CBS 367.54*	NR163673	KM199454	KM199526
<i>Neopestalotiopsis areacearum</i>	COAD 2017*	MH463406	MH460830	MH460838
<i>Neopestalotiopsis areacearum</i>	COAD 2021	MH463409	MH460833	MH460841
<i>Neopestalotiopsis asiatica</i>	MFLUCC 12-0286*	JX398983	JX399018	JX399049
<i>Neopestalotiopsis australis</i>	CBS 114159*	KM199348	KM199432	KM199537
<i>Neopestalotiopsis brachiata</i>	MFLUCC 17-1555*	MK764274	MK764340	MK764318
<i>Neopestalotiopsis brasiliensis</i>	COAD 2166*	MG686469	MG692400	MG692402
<i>Neopestalotiopsis brasiliensis</i>	HGUP 191004	MZ724916	MZ724121	–
<i>Neopestalotiopsis camelliae-oleiferae</i>	CSUFTCC 81*	OK493585	OK562360	OK507955
<i>Neopestalotiopsis camelliae-oleiferae</i>	CSUFTCC 82	OK493586	OK562361	OK507956
<i>Neopestalotiopsis cavernicola</i>	KUMCC 20-0269*	MW545802	MW557596	MW550735
<i>Neopestalotiopsis cavernicola</i>	KUMCC 20-0332	MW581238	MW590328	MW590327
<i>Neopestalotiopsis chiangmaiensis</i>	MFLUCC 18-0113*	–	MH412725	MH388404
<i>Neopestalotiopsis chiangmaiensis</i>	MFLUCC 19-0048	MW248391	–	MW259070
<i>Neopestalotiopsis chrysea</i>	MFLUCC 12-0261*	JX398985	JX399020	JX399051
<i>Neopestalotiopsis chrysea</i>	MFLUCC 12-0262	JX398986	JX399021	JX399052
<i>Neopestalotiopsis chrysea</i>	SDBR-CMU516/KUNCC 24-18917	PQ521226	PQ560699	PQ529177
<i>Neopestalotiopsis chrysea</i> (=N. umbrinospora)	MFLUCC 12-0285	JX398984	JX399019	JX399050
<i>Neopestalotiopsis clavispora</i>	MFLUCC 12-0280	JX398978	JX399013	JX399044
<i>Neopestalotiopsis clavispora</i>	MFLUCC 12-0281*	JX398979	JX399014	JX399045
<i>Neopestalotiopsis cocoës</i>	MFLUCC 15-0152*	NR156312	–	KX789689
<i>Neopestalotiopsis coffeae-arabicae</i>	HGUP 4015	KF412647	KF412641	KF412644
<i>Neopestalotiopsis coffeae-arabicae</i>	HGUP 4019*	KF412649	KF412643	KF412646
<i>Neopestalotiopsis concentrica</i>	CFCC 55162/ROC 53*	OK560707	OM117698	OM622433
<i>Neopestalotiopsis concentrica</i>	ROC 137	OK560711	OM117702	OM622437
<i>Neopestalotiopsis cubana</i>	CBS 600.96*	KM199347	KM199438	KM199521
<i>Neopestalotiopsis cubana</i>	UMS01	OM320626	OM339539	OM339540
<i>Neopestalotiopsis dendrobii</i>	MFLUCC 14-0099	MK993570	MK975834	MK975828
<i>Neopestalotiopsis dendrobii</i>	MFLUCC 14-0106*	MK993571	MK975835	MK975829
<i>Neopestalotiopsis drenthii</i>	BRIP 72263a	MZ303786	MZ312679	MZ344171
<i>Neopestalotiopsis drenthii</i>	BRIP 72264a*	MZ303787	MZ312680	MZ344172
<i>Neopestalotiopsis egyptiaca</i>	CBS 140162*	KP943747	KP943746	KP943748

(Continued)

TABLE 1 Continued

Taxa	Strains	GenBank accession number		
		ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Neopestalotiopsis elaeagni</i>	HGUP10002/GUCC 21002*	MW930716	MZ683391	MZ203452
<i>Neopestalotiopsis elaeagni</i>	HGUP10004/GUCC 21006	ON597079	ON595537	ON595535
<i>Neopestalotiopsis elaeidis</i>	MFLUCC 15-0735*	ON650690	–	ON734012
<i>Neopestalotiopsis ellipsospora</i>	MFLUCC 12-0283*	JX398980	JX399016	JX399047
<i>Neopestalotiopsis ellipsospora</i>	MFLUCC 12-0284	JX398981	JX399015	JX399046
<i>Neopestalotiopsis eucalypticola</i>	CBS 264.37*	NR163670	KM199431	KM199551
<i>Neopestalotiopsis eucalyptorum</i>	CBS 147684*	MW794108	MW802841	MW805397
<i>Neopestalotiopsis eucalyptorum</i>	CBS 147685	MW794098	MW802831	MW805398
<i>Neopestalotiopsis foedans</i>	CGMCC 3.9123*	JX398987	JX399022	JX399053
<i>Neopestalotiopsis foedans</i>	CGMCC 3.9178	JX398989	JX399024	JX399055
<i>Neopestalotiopsis foedans</i>	CGMCC 3.9202	JX398988	JX399023	JX399054
<i>Neopestalotiopsis formicarum</i>	CBS 115.83	KM199344	KM199444	KM199519
<i>Neopestalotiopsis formicarum</i>	CBS 362.72*	KM199358	KM199455	KM199517
<i>Neopestalotiopsis fragariae</i>	ZHKUCC 22-0113*	ON553410	ON569075	ON569076
<i>Neopestalotiopsis fragariae</i>	ZHKUCC 22-0114	ON651145	ON685198	ON685196
<i>Neopestalotiopsis guajavae</i>	FMB 0026*	MF783085	MH460871	MH460868
<i>Neopestalotiopsis guajavae</i>	FMB 0027	MF783084	MH460872	MH460869
<i>Neopestalotiopsis guajavicola</i>	FMB 0129*	MH209245	MH460873	MH460870
<i>Neopestalotiopsis hadrolaeliae</i>	VIC 47180*	MK454709	MK465120	MK465122
<i>Neopestalotiopsis haikouensis</i>	SAUCC 212271*	OK087294	OK104870	OK104877
<i>Neopestalotiopsis haikouensis</i>	SAUCC 212272	OK087295	OK104871	OK104878
<i>Neopestalotiopsis haikouensis</i>	SDBR-CMU517/KUNCC 24-18918	PQ521227	PQ560700	PQ529178
<i>Neopestalotiopsis haikouensis</i> (= <i>N. cercidicola</i>)	CFCC 70632	PP784737	PP842614	PP842626
<i>Neopestalotiopsis haikouensis</i> (= <i>N. cercidicola</i>)	CFCC 70624	PP784738	PP842615	PP842627
<i>Neopestalotiopsis haikouensis</i> (= <i>N. cercidicola</i>)	CFCC 70623	PP784739	PP842616	PP842628
<i>Neopestalotiopsis haikouensis</i> (= <i>N. terricola</i>)	CGMCC 3.23553	OP082294	OP235982	OP204796
<i>Neopestalotiopsis haikouensis</i> (= <i>N. terricola</i>)	UESTCC 22.0034	OP082295	OP235983	OP204797
<i>Neopestalotiopsis hispanica</i>	CBS 147686*	MW794107	MW802840	MW805399
<i>Neopestalotiopsis honoluluana</i>	CBS 111535	KM199363	KM199461	KM199546
<i>Neopestalotiopsis honoluluana</i>	CBS 114495*	KM199364	KM199457	KM199548
<i>Neopestalotiopsis hydeana</i>	MFLUCC 20-0132*	MW266069	MW251119	MW251129
<i>Neopestalotiopsis hydeana</i>	MFLUCC 20-0136	MW266066	MW251116	MW251126
<i>Neopestalotiopsis hypericin</i>	KUNCC 22-12597*	OP498010	OP765908	OP713768
<i>Neopestalotiopsis hypericin</i>	KUNCC 22-12598	OP498009	OP737883	OP737880
<i>Neopestalotiopsis iberica</i>	CBS 147688*	MW794111	MW802844	MW805402
<i>Neopestalotiopsis iberica</i>	CBS 147689	MW794114	MW802847	MW805403
<i>Neopestalotiopsis iranensis</i>	CBS 137767	KM074045	KM074056	KM074053

(Continued)

TABLE 1 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Neopestalotiopsis iranensis</i>	CBS 137768*	KM074048	KM074057	KM074051
<i>Neopestalotiopsis iteae</i>	SDBR-CMU515/KUNCC 24-18919*	PQ521228	PQ560701	PQ529179
<i>Neopestalotiopsis javaensis</i>	CBS 257.31*	KM199357	KM199437	KM199543
<i>Neopestalotiopsis keteleeriae</i>	MFLUCC 13-0915*	KJ023087	KJ023088	KJ023089
<i>Neopestalotiopsis longiappendiculata</i>	MEAN 1315*	MW794112	MW802845	MW805404
<i>Neopestalotiopsis longiappendiculata</i>	MEAN 1316	MW794103	MW802836	MW805405
<i>Neopestalotiopsis lusitanica</i>	CBS 147692*	MW794110	MW802843	MW805406
<i>Neopestalotiopsis lusitanica</i>	MEAN 1318	MW794093	MW802826	MW805407
<i>Neopestalotiopsis macadamiae</i>	BRIP 63737c*	KX186604	KX186654	KX186627
<i>Neopestalotiopsis macadamiae</i>	BRIP 63748a	KX186612	KX186663	KX186636
<i>Neopestalotiopsis maddoxii</i>	BRIP 72266a*	MZ303782	MZ312675	MZ344167
<i>Neopestalotiopsis maddoxii</i>	BRIP 72272a	MZ303783	MZ312676	MZ344168
<i>Neopestalotiopsis maddoxii</i>	BRIP 72284a	MZ303785	MZ312678	MZ344170
<i>Neopestalotiopsis magna</i>	MFLUCC 12-0652*	KF582795	KF582793	KF582791
<i>Neopestalotiopsis mesopotamica</i>	CBS 299.74	KM199361	KM199435	KM199541
<i>Neopestalotiopsis mesopotamica</i>	CBS 336.86*	KM199362	KM199441	KM199555
<i>Neopestalotiopsis mianyangensis</i>	UESTCC 22.0006	OP082291	OP235979	OP204793
<i>Neopestalotiopsis mianyangensis</i>	CGMCC 3.23554 *	OP546681	OP672161	OP723490
<i>Neopestalotiopsis musae</i>	MFLUCC 15-0776*	NR156311	KX789686	KX789685
<i>Neopestalotiopsis musae</i>	MM3-2z9A	MW959799	MZ288737	MZ417508
<i>Neopestalotiopsis musae</i>	MM3-2z9C	MW959801	MZ288739	MZ417510
<i>Neopestalotiopsis natalensis</i>	CBS 138.41*	NR156288	KM199466	KM199552
<i>Neopestalotiopsis nebuloides</i>	BRIP 66617*	MK966338	MK977632	MK977633
<i>Neopestalotiopsis nebuloides</i>	BRIP 70567	OM417295	ON995131	ON624201
<i>Neopestalotiopsis olumideae</i>	BRIP 72273a*	MZ303790	MZ312683	MZ344175
<i>Neopestalotiopsis olumideae</i>	BRIP 72283a	MZ303791	MZ312684	MZ344176
<i>Neopestalotiopsis paeonia-suffruticosa</i>	CGMCC 3.23555 *	OP082292	OP235980	OP204794
<i>Neopestalotiopsis paeonia-suffruticosa</i>	UESTCC 22.0033	OP082293	OP235981	OP204795
<i>Neopestalotiopsis pandanicola</i>	MFLUCC 17-2261*	–	MH412720	MH388389
<i>Neopestalotiopsis pernambucana</i>	UFPE-URM 7148*	KJ792466	–	KU306739
<i>Neopestalotiopsis perukae</i>	FMB 0127*	MH209077	MH460876	MH523647
<i>Neopestalotiopsis perukae</i>	FMB 0130	MH208973	MH477871	MH523648
<i>Neopestalotiopsis petila</i>	MFLUCC 17-1737*	MK764275	MK764341	MK764319
<i>Neopestalotiopsis petila</i>	MFLUCC 17-1738	MK764276	MK764342	MK764320
<i>Neopestalotiopsis phangngaensis</i>	MFLUCC 18-0119*	MH388354	MH412721	MH388390
<i>Neopestalotiopsis photiniae</i>	MFLUCC 22-0129*	OP498008	OP752131	OP753368
<i>Neopestalotiopsis photiniae</i>	GUCC 21-0820	OP806524	OP896200	OP828691

(Continued)

TABLE 1 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Neopestalotiopsis piceana</i>	CBS 394.48*	KM199368	KM199453	KM199527
<i>Neopestalotiopsis piceana</i>	SAUCC 210112	OK149224	OK206434	OK206436
<i>Neopestalotiopsis protearum</i>	CBS 114178*	JN712498	KM199463	KM199542
<i>Neopestalotiopsis protearum</i>	CBS 111506	MH553959	MH554618	MH554377
<i>Neopestalotiopsis psidii</i>	FMB 0028*	MF783082	MH477870	MH460874
<i>Neopestalotiopsis rhapsidis</i>	GUCC 21501*	MW931620	MW980441	MW980442
<i>Neopestalotiopsis rhizophorae</i>	MFLUCC 17-1551*	MK764277	MK764343	MK764321
<i>Neopestalotiopsis rhododendri</i>	GUCC 21504	MW979577	MW980443	MW980444
<i>Neopestalotiopsis rhododendri</i>	GUCC 21505*	MW979576	MW980445	MW980446
<i>Neopestalotiopsis rhododendricola</i>	KUN-HKAS 123204*	OK283069	OK274147	OK274148
<i>Neopestalotiopsis rosae</i>	CBS 101057*	KM199359	KM199429	KM199523
<i>Neopestalotiopsis rosae</i>	ML1664	MT469940	MT469943	MT469946
<i>Neopestalotiopsis rosicola</i>	CFCC 51992*	KY885239	KY885245	KY885243
<i>Neopestalotiopsis rosicola</i>	CFCC 51993	KY885240	KY885246	KY885244
<i>Neopestalotiopsis samarangensis</i>	MFLUCC 12-0233*	NR120125	JQ968610	JQ968611
<i>Neopestalotiopsis saprophytica</i>	GD22-1	MK228998	MK360939	MK512492
<i>Neopestalotiopsis saprophytica</i>	MFLUCC 12-0282*	JX398982	JX399017	JX399048
<i>Neopestalotiopsis scalabiensis</i>	CAA1029*	MW969748	MW934611	MW959100
<i>Neopestalotiopsis sichuanensis</i>	CFCC 54338*	MW166231	MW218524	MW199750
<i>Neopestalotiopsis sichuanensis</i>	SM15-1C	MW166232	MW218525	MW199751
<i>Neopestalotiopsis siciliana</i>	AC46*	ON117813	ON209162	ON107273
<i>Neopestalotiopsis siciliana</i>	TAP18N016	LC427168	LC427169	LC427170
<i>Neopestalotiopsis sonneratae</i>	MFLUCC 17-1744*	MK764279	MK764345	MK764323
<i>Neopestalotiopsis steyaertii</i>	IMI 192475*	KF582796	KF582794	KF582792
<i>Neopestalotiopsis subepidermalis</i>	CFCC 55160/ROC 161*	OK560699	OM117690	OM622425
<i>Neopestalotiopsis subepidermalis</i>	CFCC 55161/ROC 169	OK560701	OM117692	OM622427
<i>Neopestalotiopsis suphanburiensis</i>	MFLUCC 22-0126*	OP497994	OP752135	OP753372
<i>Neopestalotiopsis surinamensis</i>	MFLUCC 22-0126	KM199351	KM199465	KM199518
<i>Neopestalotiopsis thailandica</i>	MFLUCC 17-1730*	MK764281	MK764347	MK764325
<i>Neopestalotiopsis thailandica</i>	MFLUCC 17-1731	MK764282	MK764348	MK764326
<i>Neopestalotiopsis vaccinii</i>	CAA1059*	MW969747	MW934610	MW959099
<i>Neopestalotiopsis vacciniicola</i>	CAA1054	MW969750	MW934613	MW959102
<i>Neopestalotiopsis vacciniicola</i>	CAA1055*	MW969751	MW934614	MW959103
<i>Neopestalotiopsis vheena</i>	BRIP 72293a*	MZ303792	MZ312685	MZ344177
<i>Neopestalotiopsis vheena</i>	BRIP 70210	MN114212	MN114214	MN114213
<i>Neopestalotiopsis vitis</i>	MFLUCC15-1265*	KU140694	KU140685	KU140676
<i>Neopestalotiopsis vitis</i>	MFLUCC 15-1270	KU140699	KU140690	KU140681

(Continued)

TABLE 1 Continued

Taxa	Strains	GenBank accession number		
		ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Neopestalotiopsis xishuangbannaensis</i>	KUMCC 21-0424*	ON426865	OR025934	OR025973
<i>Neopestalotiopsis xishuangbannaensis</i>	KUMCC 21-0425	ON426866	OR025935	OR025974
<i>Neopestalotiopsis zakeelii</i>	BRIP 72271a	MZ303788	MZ312681	MZ344173
<i>Neopestalotiopsis zakeelii</i>	BRIP 72282a*	MZ303789	MZ312682	MZ344174
<i>Neopestalotiopsis zimbabwana</i>	CBS 111495*	JX556231	KM199456	KM199545
<i>Neopestalotiopsis zimbabwana</i>	MEAN 1329	MW794095	MW802828	MW805418
<i>Neopestalotiopsis zingiberis</i>	HGUP10001/GUCC 21001*	MW930715	MZ683390	MZ683389
<i>Neopestalotiopsis zingiberis</i>	HGUP10005/GUCC 21007	ON597078	ON595538	ON595536
<i>Pestalotiopsis diversiseta</i>	MFLUCC 12-0287	NR_120187	JX399040	JX399073
<i>Pestalotiopsis spathulata</i>	CBS 356.86	KM199338	KM199423	KM199513

The newly generated sequences are indicated in bold and the new species are in red. The synonymized taxon is indicated in blue. The ex-type strains are represented as “*” and missing sequences are represented as “–”.

TABLE 2 GenBank accession numbers of *Pestalotiopsis* strains used in phylogenetic analysis.

Taxa	Strains	GenBank accession number		
		ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Neopestalotiopsis cubana</i>	CBS 600.96*	KM199347	KM199438	KM199521
<i>Neopestalotiopsis protearum</i>	CBS 114178*	JN712498	KM199463	KM199542
<i>Pestalotiopsis abietis</i>	CFCC 53011*	MK397013	MK622280	MK622277
<i>Pestalotiopsis adusta</i>	ICMP 6088*	JX399006	JX399037	JX399070
<i>Pestalotiopsis</i> aff. <i>jesteri</i>	WPF-54	KT000164	–	–
<i>Pestalotiopsis</i> aff. <i>jesteri</i>	WPF-55-12G	KT000165	–	–
<i>Pestalotiopsis aggestorum</i>	LC6301*	KX895015	KX895348	KX895234
<i>Pestalotiopsis anacardiacearum</i>	IFRDCC 2397*	KC247154	KC247155	KC247156
<i>Pestalotiopsis anacardiacearum</i>	FY10-12	MK228990	MK360931	MK512484
<i>Pestalotiopsis anhuiensis</i>	CFCC 54791*	ON007028	ON005056	ON005045
<i>Pestalotiopsis appendiculata</i>	CGMCC 3.23550*	OP082431	OP185516	OP185509
<i>Pestalotiopsis arceuthobii</i>	CBS 433.65	MH554046	MH554722	MH554481
<i>Pestalotiopsis arceuthobii</i>	CBS 434.65*	KM199341	KM199427	KM199516
<i>Pestalotiopsis arengae</i>	CBS 331.92*	KM199340	KM199426	KM199515
<i>Pestalotiopsis arengae</i>	MFTU12	MT952584	MT957914	MT957939
<i>Pestalotiopsis australasiae</i>	CBS 114126*	KM199297	KM199409	KM199499
<i>Pestalotiopsis australis</i>	CBS 114193*	KM199332	KM199383	KM199475
<i>Pestalotiopsis australis</i>	MEAN 1096	MT374684	MT374709	MT374696
<i>Pestalotiopsis biciliata</i>	CBS 124463*	KM199308	KM199399	KM199505
<i>Pestalotiopsis brachiata</i>	LC2988*	KX894933	KX895265	KX895150
<i>Pestalotiopsis brachiata</i>	LC8188	KY464142	KY464162	KY464152
<i>Pestalotiopsis brassicae</i>	CBS 170.26*	KM199379	–	KM199558

(Continued)

TABLE 2 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Pestalotiopsis camelliae</i>	MFLUCC 12-0277*	JX399010	JX399041	JX399074
<i>Pestalotiopsis camelliae</i>	NTUCC 18-001/BCRC FU31443	MT322016	MT321818	MT321917
<i>Pestalotiopsis camelliae-japonicae</i>	ZHKUCC 23-0826*	OR258040	OR251483	OR251480
<i>Pestalotiopsis camelliae-japonicae</i>	ZHKUCC 23-0827	OR258041	OR251484	OR251481
<i>Pestalotiopsis camelliae-oleiferae</i>	CSUFTCC 08*	OK493593	OK562368	OK507963
<i>Pestalotiopsis cangshanensis</i>	CGMCC 3.23544*	OP082426	OP185517	OP185510
<i>Pestalotiopsis castanopsidis</i>	CFCC 54430*	OK339732	OK358508	OK358493
<i>Pestalotiopsis castanopsidis</i>	CFCC 54384	OK339734	OK358510	OK358495
<i>Pestalotiopsis chamaeropsis</i>	CBS 186.71*	KM199326	KM199391	KM199473
<i>Pestalotiopsis chamaeropsis</i>	LC3609	KX894989	KX895320	KX895206
<i>Pestalotiopsis chamaeropsis</i>	NTUPPMCC 21-054	OR125062	OR126308	OR126315
<i>Pestalotiopsis changjiangensis</i>	CFCC 54314*	OK339739	OK358515	OK358500
<i>Pestalotiopsis changjiangensis</i>	CFCC 52803	OK339741	OK358517	OK358502
<i>Pestalotiopsis chaoyangensis</i>	CFCC 55549*	OQ344763	OQ410584	OQ410582
<i>Pestalotiopsis chaoyangensis</i>	CFCC 58805	OQ344764	OQ410585	OQ410583
<i>Pestalotiopsis chiangmaiensis</i>	MFLUCC 22-0127*	OP497990	OP752137	OP753374
<i>Pestalotiopsis chiaroscuro</i>	BRIP 72970*	OK422510	OK423752	OK423753
<i>Pestalotiopsis chinensis</i>	MFLUCC 12-0273*	JX398995	–	–
<i>Pestalotiopsis clavata</i>	MFLUCC 12-0268*	NR120182	JX399025	JX399056
<i>Pestalotiopsis colombiensis</i>	CBS 118553*	KM199307	KM199421	KM199488
<i>Pestalotiopsis cyclobalanopsidis</i>	CFCC 54328*	OK339735	OK358511	OK358496
<i>Pestalotiopsis cyclobalanopsidis</i>	CFCC 55891	OK339736	OK358512	OK358497
<i>Pestalotiopsis daliensis</i>	CGMCC 3.23548*	OP082429	OP185518	OP185511
<i>Pestalotiopsis dianellae</i>	CBS 143421*	NR156664	MG386164	–
<i>Pestalotiopsis digitalis</i>	ICMP 5434*	KP781879	KP781883	–
<i>Pestalotiopsis dilucida</i>	LC3232*	KX894961	KX895293	KX895178
<i>Pestalotiopsis dilucida</i>	LC8184	KY464138	KY464158	KY464148
<i>Pestalotiopsis diploclisiae</i>	CBS 115587*	KM199320	KM199419	KM199486
<i>Pestalotiopsis disseminata</i>	CBS 118552	MH553986	MH554652	MH554410
<i>Pestalotiopsis diversiseta</i>	MFLUCC 12-0287*	NR120187	JX399040	JX399073
<i>Pestalotiopsis doitungensis</i>	MFLUCC 14-0090*	MK993573	MK975836	MK975831
<i>Pestalotiopsis dracaenae</i>	HGUP 4037*	MT596515	MT598645	MT598644
<i>Pestalotiopsis dracaenae</i>	MFLU 19-2757	MW114334	–	MW192201
<i>Pestalotiopsis dracaenicola</i>	MFLUCC 18-0913*	MN962731	MN962733	MN962732
<i>Pestalotiopsis dracontomelonis</i>	MFLUCC 10-0149*	NR168755	–	KP781880
<i>Pestalotiopsis dracontomelonis</i>	MFLUCC 22-0122	–	OP762672	OP753375
<i>Pestalotiopsis eleutherococci</i>	HMJAU 60189*	NR182556	–	–

(Continued)

TABLE 2 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Pestalotiopsis eleuthero cocci</i>	HMJAU 60190	OL996127	OL898722	–
<i>Pestalotiopsis endophytica</i>	GUCC 21539	MZ477294	MZ868299	MZ868343
<i>Pestalotiopsis endophytica</i>	MFLUCC 18-0932*	NR172439	–	MW417119
<i>Pestalotiopsis ericacearum</i>	IFRDCC 2439*	KC537807	KC537821	KC537814
<i>Pestalotiopsis etonensis</i>	BRIP 66615*	MK966339	MK977634	MK977635
<i>Pestalotiopsis ficicola</i>	SAUCC230046*	OQ691974	OQ718749	OQ718691
<i>Pestalotiopsis ficicola</i>	SAUCC230042	OQ691972	OQ718747	OQ718689
<i>Pestalotiopsis ficicrescens</i>	GUCC 21556*	MZ477311	MZ868301	MZ868328
<i>Pestalotiopsis foliicola</i>	CFCC 54440*	ON007029	ON005057	ON005046
<i>Pestalotiopsis formosana</i>	NCYU 19-0353	MW114335	MW148260	MW192202
<i>Pestalotiopsis formosana</i>	NTUCC 17-009*	MH809381	MH809385	MH809389
<i>Pestalotiopsis formosana</i>	NTUCC 17-010/BCRC FU31632	MH809382	MH809386	MH809390
<i>Pestalotiopsis formosana</i>	NTUPPMCC 21-056	OR125064	OR126310	OR126317
<i>Pestalotiopsis furcata</i>	MFLUCC 12-0054*	NR120087	JQ683708	JQ683740
<i>Pestalotiopsis furcata</i>	LC6691	KX895030	KX895363	KX895248
<i>Pestalotiopsis fusioidea</i>	CGMCC 3.23545*	OP082427	OP185519	OP185512
<i>Pestalotiopsis gibbosa</i>	IFRD 411-014	KC537805	KC537819	KC537812
<i>Pestalotiopsis gibbosa</i>	NOF 3175*	LC311589	LC311590	LC311591
<i>Pestalotiopsis grevilleae</i>	CBS 114127*	KM199300	KM199407	KM199504
<i>Pestalotiopsis guangdongensis</i>	ZHKUCC 22-0016*	ON180762	ON221548	ON221520
<i>Pestalotiopsis guangdongensis</i>	ZHKUCC 22-0017	ON180763	ON221549	ON221521
<i>Pestalotiopsis guangxiensis</i>	CFCC 54308*	OK339737	OK358513	OK358498
<i>Pestalotiopsis guizhouensis</i>	CFCC 54803*	ON007035	ON005063	ON005052
<i>Pestalotiopsis hainanensis</i>	CNU060362	GQ869902	GQ869905	–
<i>Pestalotiopsis hainanensis</i>	PSHI2004Endo166*	DQ334863	DQ137861	–
<i>Pestalotiopsis hawaiiensis</i>	CBS 114491*	KM199339	KM199428	KM199514
<i>Pestalotiopsis hispanica</i>	CBS 115391*	NR161080	MH554640	MH554399
<i>Pestalotiopsis hispanica</i>	LS-1	OL441090	OL448307	OL448308
<i>Pestalotiopsis hispanica</i>	NTUPPMCC 18-162	OR125059	OR126305	OR126312
<i>Pestalotiopsis hollandica</i>	CBS 265.33*	KM199328	KM199388	KM199481
<i>Pestalotiopsis humicola</i>	CBS 336.97*	KM199317	KM199420	KM199484
<i>Pestalotiopsis hunanensis</i>	CSUFTCC 15*	OK493599	OK562374	OK507969
<i>Pestalotiopsis hydei</i>	MFLUCC 20-0135*	MW266063	MW251112	MW251113
<i>Pestalotiopsis iberica</i>	CAA 1004*	MW732248	MW759035	MW759038
<i>Pestalotiopsis inflexa</i>	MFLUCC 12-0270*	NR111789	JX399039	JX399072
<i>Pestalotiopsis intermedia</i>	MFLUCC 12-0259*	JX398993	JX399028	JX399059
<i>Pestalotiopsis italiana</i>	MFLUCC 12-0657*	KP781878	KP781882	KP781881

(Continued)

TABLE 2 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Pestalotiopsis jesteri</i>	CBS 109350*	KM199380	KM199468	–
<i>Pestalotiopsis jiangxiensis</i>	LC4399*	KX895009	KX895341	KX895227
<i>Pestalotiopsis jinchanghensis</i>	SDBR-CMU518/KUNCC 24-18920	PQ521229	PQ560702	PQ529180
<i>Pestalotiopsis jinchanghensis</i>	LC6636*	KX895028	KX895361	KX895247
<i>Pestalotiopsis jinchanghensis</i> (= <i>P. zhaoqingensis</i>)	ZHKUCC 23-0825	OR233336	OR239062	OR239061
<i>Pestalotiopsis kaki</i>	KNU-PT-1804*	LC552953	LC552954	LC553555
<i>Pestalotiopsis kandelicola</i>	NCYUCC 19-0354	MT560723	MT563100	MT563102
<i>Pestalotiopsis kenyana</i>	CBS 442.67*	KM199302	KM199395	KM199502
<i>Pestalotiopsis knightiae</i>	CBS 114138*	KM199310	KM199408	KM199497
<i>Pestalotiopsis krabiensis</i>	MFLUCC 16-0260*	NR168199	MH412722	MH388395
<i>Pestalotiopsis kunmingensis</i>	PSHI2002Endo766*	AY373376	DQ333576	–
<i>Pestalotiopsis leucadendri</i>	CBS 121417*	MH553987	MH554654	MH554412
<i>Pestalotiopsis licualacola</i>	HGUP 4057*	KC492509	KC481683	KC481684
<i>Pestalotiopsis lijiangensis</i>	CFCC 50738*	KU860520	–	–
<i>Pestalotiopsis lijiangensis</i>	CFCC 50739	MH880834	–	–
<i>Pestalotiopsis linearis</i>	MFLUCC 12-0271*	NR120183	JX399027	JX399058
<i>Pestalotiopsis linguae</i>	ZHKUCC 22-0159 *	OP094104	OP186108	OP186110
<i>Pestalotiopsis linguae</i>	ZHKUCC 22-0160	OP094103	OP186107	OP186109
<i>Pestalotiopsis lithocarpi</i>	CFCC 55100*	OK339742	OK358518	OK358503
<i>Pestalotiopsis lithocarpi</i>	CFCC 55893	OK339743	OK358519	OK358504
<i>Pestalotiopsis loeiana</i>	MFLUCC 22-0123*	OP497988	OP713769	OP737881
<i>Pestalotiopsis lushanensis</i>	LC4344*	KX895005	KX895337	KX895223
<i>Pestalotiopsis macadamiae</i>	BRIP 63738b*	KX186588	KX186680	KX186621
<i>Pestalotiopsis malayana</i>	CBS 102220*	KM199306	KM199411	KM199482
<i>Pestalotiopsis manyueyuanani</i>	NTUPPMCC 18-165*	OR125060	OR126306	OR126313
<i>Pestalotiopsis manyueyuanani</i>	NTUPPMCC 22-012	OR125061	OR126307	OR126314
<i>Pestalotiopsis menhaiensis</i>	CGMCC 3.18250*	KU252272	KU252488	KU252401
<i>Pestalotiopsis monochaeta</i>	CBS 144.97*	NR147554	KM199386	KM199479
<i>Pestalotiopsis montellica</i>	MFLUCC 12-0279	JX399012	JX399043	JX399076
<i>Pestalotiopsis nanjingensis</i>	CSUFTCC 16*	OK493602	OK562377	OK507972
<i>Pestalotiopsis nanningensis</i>	CSUFTCC 10*	OK493596	OK562371	OK507966
<i>Pestalotiopsis neolitseae</i>	MFLU 18-2536	MW114336	MW148261	MW192203
<i>Pestalotiopsis neolitseae</i>	NTUCC 17-011*	MH809383	MH809387	MH809391
<i>Pestalotiopsis novae-hollandiae</i>	CBS 130973*	NR147557	KM199425	KM199511
<i>Pestalotiopsis oryzae</i>	CBS 353.69*	KM199299	KM199398	KM199496
<i>Pestalotiopsis pallidotheae</i>	MAFF 240993*	NR111022	LC311584	LC311585
<i>Pestalotiopsis pandanicola</i>	MFLUCC 16-0255*	MH388361	MH412723	MH388396

(Continued)

TABLE 2 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Pestalotiopsis papuana</i>	CBS 331.96*	KM199321	KM199413	KM199491
<i>Pestalotiopsis papuana</i>	MFLU 19-2764	MW114337	MW296942	MW192204
<i>Pestalotiopsis parva</i>	CBS 265.37	KM199312	KM199404	KM199508
<i>Pestalotiopsis phoebes</i>	SAUCC230093*	OQ692028	OQ718803	OQ718745
<i>Pestalotiopsis phoebes</i>	SAUCC230092	OQ692027	OQ718802	OQ718744
<i>Pestalotiopsis photinicola</i>	GZCC 16-0028*	KY092404	KY047663	KY047662
<i>Pestalotiopsis pini</i>	MEAN 1092*	MT374680	MT374705	MT374693
<i>Pestalotiopsis pini</i>	CBS 127.80	MH553995	MH554664	MH554422
<i>Pestalotiopsis pinicola</i>	KUMCC 19-0183*	MN412636	MN417507	MN417509
<i>Pestalotiopsis piraubensis</i>	COAD 2165*	MH627381	MH643773	MH643774
<i>Pestalotiopsis portugallica</i>	CBS 393.48*	KM199335	KM199422	KM199510
<i>Pestalotiopsis portugallica</i>	NCYU 19-0352	MW114339	MW148263	MW192206
<i>Pestalotiopsis pyrrosiae-linguae</i>	ZHKUCC 23-0807*	OR199902	OR259258	OR259260
<i>Pestalotiopsis pyrrosiae-linguae</i>	ZHKUCC 23-0808	OR199903	OR259259	OR259261
<i>Pestalotiopsis rhizophorae</i>	MFLUCC 17-0416*	MK764283	MK764349	MK764327
<i>Pestalotiopsis rhododendri</i>	IFRDCC 2399*	KC537804	KC537818	KC537811
<i>Pestalotiopsis rhodomyrtus</i>	HGUP 4230*	KF412648	KF412642	KF412645
<i>Pestalotiopsis rosarioides</i>	CGMCC 3.23549*	OP082430	OP185520	OP185513
<i>Pestalotiopsis rosea</i>	MFLUCC 12-0258*	JX399005	JX399036	JX399069
<i>Pestalotiopsis sabal</i>	ZHKUCC 22-0031	ON180769	ON221555	ON221527
<i>Pestalotiopsis sabal</i>	ZHKUCC 22-0035*	ON180775	ON221561	ON221533
<i>Pestalotiopsis scoparia</i>	CBS 176.25*	KM199330	KM199393	KM199478
<i>Pestalotiopsis scoparia</i>	CBS 296.58	MH554026	MH554703	MH554461
<i>Pestalotiopsis sequoiae</i>	MFLUCC 13-0399*	KX572339	–	–
<i>Pestalotiopsis shaanxiensis</i>	CFCC 54958*	ON007026	ON005054	ON005043
<i>Pestalotiopsis shoreae</i>	MFLUCC 12-0314*	KJ503811	KJ503814	KJ503817
<i>Pestalotiopsis sichuanensis</i>	CGMCC 3.18244*	KX146689	KX146807	KX146748
<i>Pestalotiopsis silvicola</i>	CFCC 55296*	ON007032	ON005060	ON005049
<i>Pestalotiopsis smilacicola</i>	MFLUCC 22-0124	OP497989	OP762674	OP737879
<i>Pestalotiopsis smilacicola</i>	MFLUCC 22-0125*	OP497991	OP762673	OP753376
<i>Pestalotiopsis sonneratae</i>	CFCC 57394*	ON114184	ON086816	ON086812
<i>Pestalotiopsis sonneratae</i>	CFCC 57395	ON114185	ON086817	ON086813
<i>Pestalotiopsis spatholobi</i>	SAUCC231201*	OQ692023	OQ718798	OQ718740
<i>Pestalotiopsis spatholobi</i>	SAUCC231203	OQ692024	OQ718799	OQ718741
<i>Pestalotiopsis spathulata</i>	CBS 356.86*	KM199338	KM199423	KM199513
<i>Pestalotiopsis spathuliappendiculata</i>	CBS 144035*	MH554172	MH554845	MH554607
<i>Pestalotiopsis suae</i>	CGMCC 3.23546*	OP082428	OP185521	OP185514

(Continued)

TABLE 2 Continued

Taxa	Strains	GenBank accession number		
		ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Pestalotiopsis telopeae</i>	CBS 114161*	KM199296	KM199403	KM199500
<i>Pestalotiopsis terricola</i>	CBS 141.69*	NR161084	MH554680	MH554438
<i>Pestalotiopsis thailandica</i>	MFLUCC 17-1616*	NR164471	MK764352	MK764330
<i>Pestalotiopsis trachycarpicola</i>	IFRDCC 2440*	NR120109	JQ845945	JQ845946
<i>Pestalotiopsis trachycarpicola</i>	MFLU 18-2524	MW114340	MW148264	MW192207
<i>Pestalotiopsis trachycarpicola</i>	NTUCC 18-004/BCRC FU31445	MT322019	MT321821	MT321920
<i>Pestalotiopsis trachycarpicola</i>	NTUPPMCC 18-160	OR125058	OR126304	OR126311
<i>Pestalotiopsis trachycarpicola</i>	NTUPPMCC 21-055	OR125063	OR126309	OR126316
<i>Pestalotiopsis tumida</i>	CFCC 55158*	OK560610	OM158174	OL814524
<i>Pestalotiopsis unicolor</i>	MFLUCC 120275	JX398998	JX399029	JX399063
<i>Pestalotiopsis unicolor</i>	MFLUCC 12-0276*	JX398999	JX399030	–
<i>Pestalotiopsis verruculosa</i>	MFLUCC 12-0274*	NR120185	–	JX399061
<i>Pestalotiopsis yanglingensis</i>	LC4553*	KX895012	KX895345	KX895231
<i>Pestalotiopsis yanglingensis</i>	NTUCC 18-005/BCRC FU31446	MT322020	MT321822	MT321921
<i>Pestalotiopsis yunnanensis</i>	HMAS 96359*	AY373375	–	–
<i>Pestalotiopsis yunnanensis</i>	PSHI2002Endo8171	AY526872	–	–

The newly generated sequences are indicated in bold. The synonymized taxon is indicated in blue. The ex-type strains are represented as “*” and missing sequences are represented as “–”.

TABLE 3 GenBank accession numbers of *Pseudoestalotiopsis* strains used in phylogenetic analysis.

Taxa	Strains	GenBank accession number		
		ITS	<i>tub2</i>	<i>tef1-α</i>
<i>Pestalotiopsis linearis</i>	MFLUCC 12-0271*	JX398992	JX399027	JX399058
<i>Pestalotiopsis trachycarpicola</i>	IFRDCC 2240*	NR_120109	JQ845945	JQ845946
<i>Pseudoestalotiopsis ampullacea</i>	LC6618*	KX895025	KX895358	KX895244
<i>Pseudoestalotiopsis annellata</i>	NTUCC 17-030*	MT322087	MT321889	MT321988
<i>Pseudoestalotiopsis avicenniae</i>	MFLUCC 17-0434*	MK764287	MK764353	MK764331
<i>Pseudoestalotiopsis camelliae-sinesis</i>	LC3490*	KX894985	KX895316	KX895202
<i>Pseudoestalotiopsis celtidis</i>	GUCC 21599*	OL423535	OL439010	OL439012
<i>Pseudoestalotiopsis chinensis</i>	LC3011*	KX894937	KX895269	KX895154
<i>Pseudoestalotiopsis cocos</i>	CBS 272.29*	KM199378	KM199467	KM199553
<i>Pseudoestalotiopsis curvatipora</i>	MFLUCC 17-1722*	MK764288	MK764354	MK764332
<i>Pseudoestalotiopsis dawaina</i>	MM14 F0015*	LC324750	LC324751	LC324752
<i>Pseudoestalotiopsis elaeidis</i>	CBS 413.62*	MH554044	MH554720	MH554479
<i>Pseudoestalotiopsis gilvanii</i>	INPA 2913*	MN385951	MN385954	MN385957
<i>Pseudoestalotiopsis gilvanii</i>	INPA 2914	MN385952	MN385955	MN385958
<i>Pseudoestalotiopsis ignota</i>	NN 42909*	KU500020	–	KU500016
<i>Pseudoestalotiopsis indica</i>	CBS 459.78*	KM199381	KM199470	KM199560

(Continued)

TABLE 3 Continued

Taxa	Strains	GenBank accession number		
		ITS	tub2	tef1- α
<i>Pseudoestalotiopsis indocalami</i>	GUCC 21600*	OL423536	OL439011	OL439013
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU514/KUNCC 24-18921*	PQ521230	PQ560703	PQ529181
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU519/KUNCC 24-18922	PQ521231	PQ560704	PQ529182
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU520/KUNCC 24-18923	PQ521232	PQ560705	PQ529183
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU521/KUNCC 24-18924	PQ521233	PQ560706	PQ529184
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU522/KUNCC 24-18925	PQ521234	PQ560707	PQ529185
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU523/KUNCC 24-18926	PQ521235	PQ560708	PQ529186
<i>Pseudoestalotiopsis iteae</i>	SDBR-CMU524/KUNCC 24-18927	PQ521236	PQ560709	PQ529187
<i>Pseudoestalotiopsis iteae</i> (as <i>Ps. theae</i>)	NTUCC 18-067	MT322086	MT321888	MT321987
<i>Pseudoestalotiopsis ixorae</i>	NTUCC 17-001.1*	MG816316	MG816326	MG816336
<i>Pseudoestalotiopsis kawthaungina</i>	MM14 F0083*	LC324753	LC324754	LC324755
<i>Pseudoestalotiopsis kubahensis</i>	UMAS-KUB-P20*	KT006749	–	–
<i>Pseudoestalotiopsis myanmarina</i>	NBRC 112264*	LC114025	LC114045	LC114065
<i>Pseudoestalotiopsis rhizophorae</i>	MFLUCC 17-1560*	MK764291	MK764357	MK764335
<i>Pseudoestalotiopsis simitheae</i>	MFLUCC 12-0121*	KJ503812	KJ503815	KJ503818
<i>Pseudoestalotiopsis solicola</i>	CBS 386.97*	NR_161086	MH554715	MH554474
<i>Pseudoestalotiopsis</i> sp.	SD012	JQ683720	JQ683704	JQ683736
<i>Pseudoestalotiopsis</i> sp.	NBRC112267	LC114030	LC114050	LC114070
<i>Pseudoestalotiopsis</i> sp.	NBRC112268	LC114031	LC114051	LC114071
<i>Pseudoestalotiopsis</i> sp.	NBRC112258	LC114036	LC114056	LC114076
<i>Pseudoestalotiopsis</i> sp.	NBRC112259	LC114039	LC114059	LC114079
<i>Pseudoestalotiopsis</i> sp.	MAFF 238515	LC114040	LC114060	LC114080
<i>Pseudoestalotiopsis taiwanensis</i>	NTUCC 17-002.1*	MG816319	MG816329	MG816339
<i>Pseudoestalotiopsis thailandica</i>	MFLUCC 17-1724*	MK764292	MK764358	MK764336
<i>Pseudoestalotiopsis theae</i>	MFLUCC 12-0055*	JQ683727	JQ683711	JQ683743
<i>Pseudoestalotiopsis theae</i>	MFLUCC 22-0128	OP497993	OP752136	OP753377
<i>Pseudoestalotiopsis theae</i>	SC011	JQ683726	JQ683710	JQ683742
<i>Pseudoestalotiopsis vietnamensis</i>	NBRC 112252*	LC114034	LC114054	LC114074

The newly generated sequences are indicated in bold and the new species are in red. The synonymized taxon is indicated in blue. The ex-type strains are represented as “*” and missing sequences are represented as “–”.

Neopestalotiopsis cubana (CBS 114178) and *N. protearum* (ZHKUCC 23-0825) (Figure 2). The aligned dataset consisted of a total 1,959 characters with gaps (ITS: 1–604 bp, *tub2*: 605–1399 bp, *tef1- α* : 1400–1959 bp). The best-scoring RAXML tree was selected to represent the relationships among taxa, with a final likelihood value of –19,588.100995. The matrix contained 1,090 distinct alignment patterns, with 23.02% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.238520, C = 0.293691, G = 0.214954, and T = 0.252835; substitution rates were AC = 1.093468, AG = 2.897598, AT = 1.166594, CG = 1.024049, CT = 4.038506, and GT = 1.000000; gamma

distribution shape parameter α = 0.338893, Tree-Length = 2.486656. For BI, the best-fitting model of each locus and the final average standard deviation of split frequencies are shown in Table 4. Similar tree topologies were acquired from ML and BYPP analyses. In the phylogenetic tree (Figure 2), the new strain, SDBR-CMU518, clustered together with *Pestalotiopsis jinchanghensis* and *P. zhaoqingensis* (86% ML and 1.00 BYPP; Figure 2).

Analysis 3: the combined sequence dataset of ITS, *tub2*, and *tef1- α* comprised 44 *Pseudoestalotiopsis* strains and two outgroup taxa, *Pestalotiopsis linearis* (MFLUCC 12-0271) and *P. trachycarpicola* (IFRDCC 2240) (Figure 3). The aligned dataset

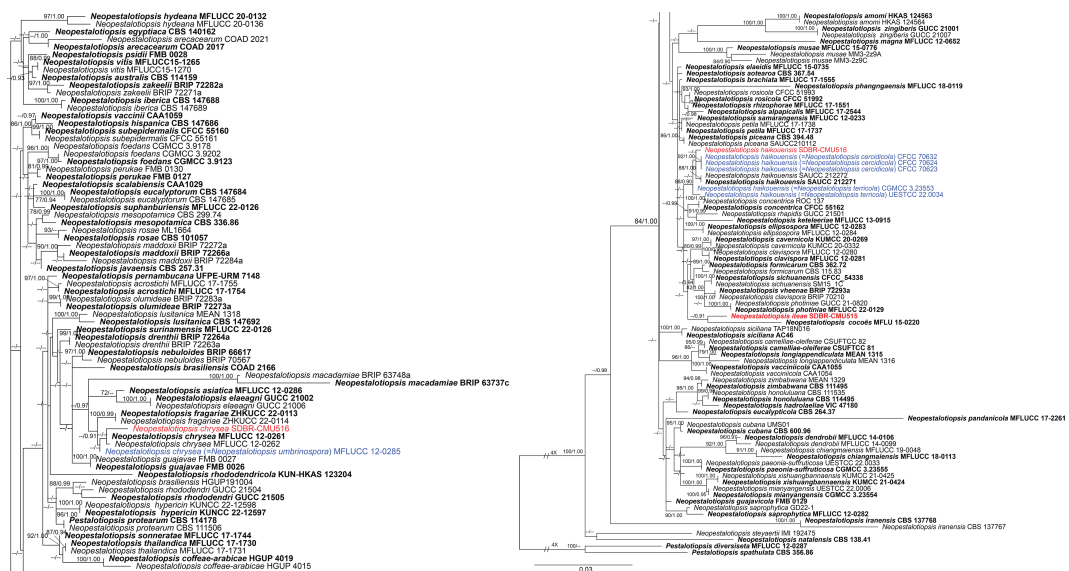


FIGURE 1
Phylogram constructed by maximum likelihood (ML) analyses of combined ITS, *tub2*, and *tef1- α* sequence dataset of *Neopestalotiopsis*. Bootstrap support values for ML and Bayesian posterior probabilities (BYPP) equal to or greater than 70% and 0.90 are shown above the nodes as ML/BYPP. The new isolates are indicated in red and the ex-type strains are in bold. The synonymized taxon is indicated in blue. The tree is rooted with *Pestalotiopsis diversiseta* (MFLUCC 12-0287) and *P. spathulata* (CBS 356.86).

consisted of a total 1,848 characters with gaps (ITS: 1–550 bp, *tefl- α* : 551–1067 bp, *tub2*: 1068–1848 bp). The best-scoring RAXML tree was selected to represent the relationships among taxa, with a final likelihood value of –6,642.700192. The matrix contained 554 distinct alignment patterns, with 16.44% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.243417, C = 0.274291, G = 0.213172, and T = 0.269120; substitution rates were

AC = 1.025029, AG = 2.296252, AT = 1.005669, CG = 0.871957, CT = 3.231208, and GT = 1.000000; gamma distribution shape parameter α = 0.306881, Tree-Length = 0.625052. For BI analysis, the best-fitting model of each locus and the final average standard deviation of split frequencies are shown in [Table 4](#). Similar tree topologies were acquired by ML and BYPP analyses. The phylogenetic tree showed that *Pseudopestalotiopsis itae* (SDBR-

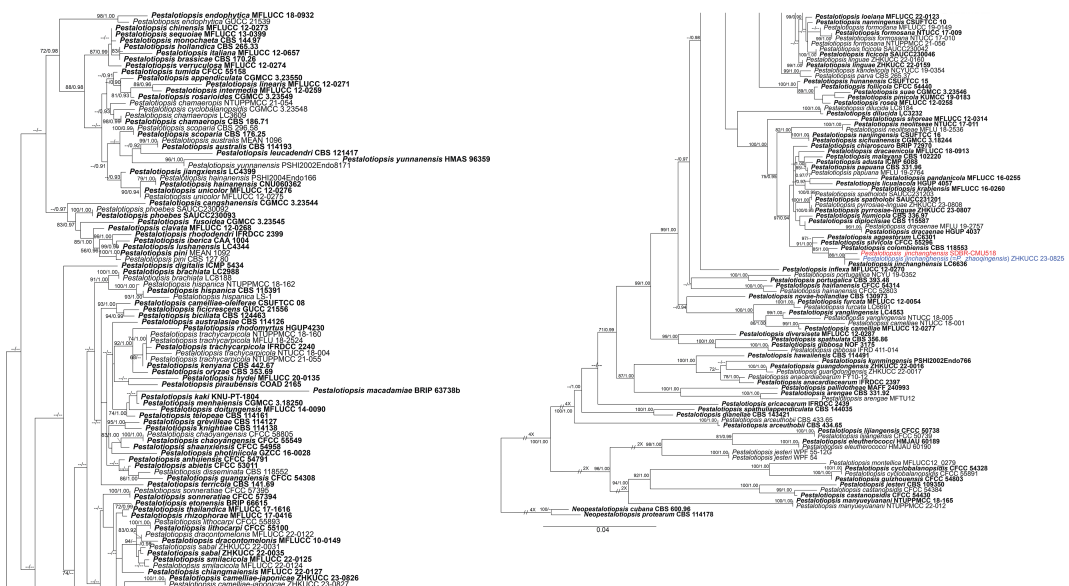


FIGURE 2
Phylogram constructed by maximum likelihood (ML) analyses of combined ITS, *tub2*, and *tef1- α* sequence dataset of *Pestalotiopsis*. Bootstrap support valued for ML and Bayesian posterior probabilities (BYPP) equal to or greater than 70% and 0.90 are shown above the nodes as ML/BYPP. The new isolates are indicated in red and the ex-type strains are in bold. The synonymized taxon is indicated in blue. The tree is rooted with *Neopestalotiopsis cubana* (CBS 114178) and *N. protearum* (ZHKUCC 23-0825).

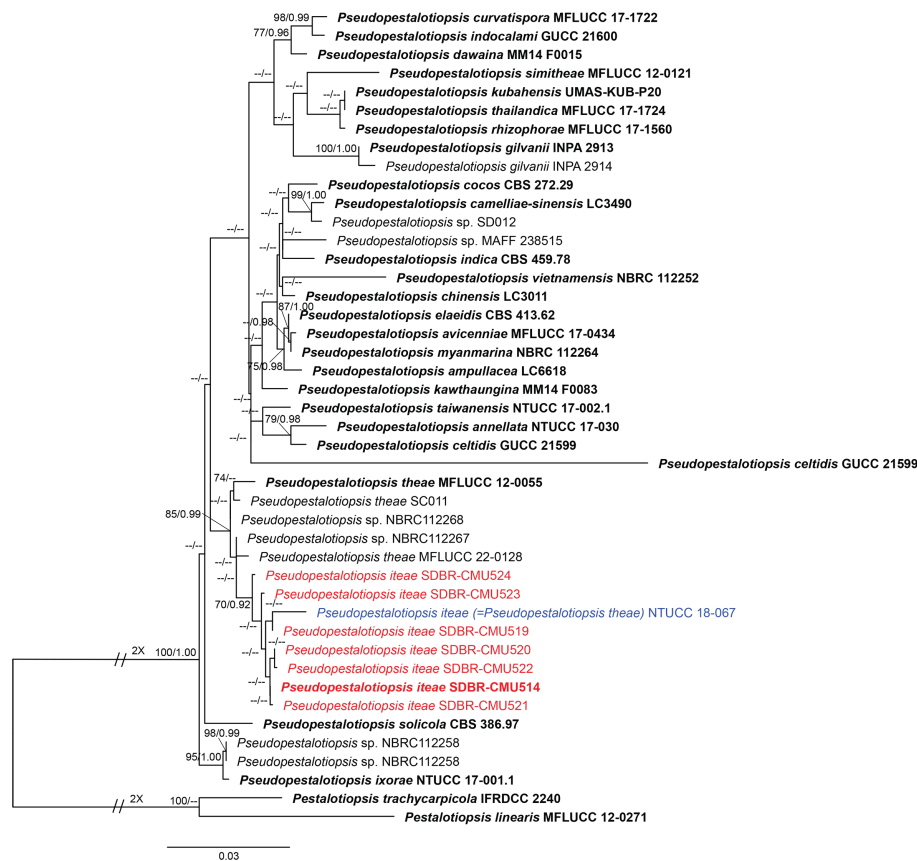


FIGURE 3
Phylogram constructed by maximum likelihood (ML) analyses of combined ITS, *tub2*, and *tef1-α* sequence dataset of *Pseudoestalotiopsis*. Bootstrap support valued for ML and Bayesian posterior probabilities (BYPP) equal to or greater than 70% and 0.90 are shown above the nodes as ML/BYPP. The new isolates are indicated in red and the ex-type strains are in bold. The synonymized taxon is indicated in blue. The tree is rooted with *Pestalotiopsis linearis* (MFLUCC 12-0271) and *P. trachycarpicola* (IFRDCC 2240).

CMU514, SDBR-CMU519, SDBR-CMU520, SDBR-CMU521, SDBR-CMU522, SDBR-CMU523, and SDBR-CMU524) grouped with *Ps. theae* strain NTUCC 18-067 (70% ML and 0.92 BYPP) and formed a distinct subclade basal to the ex-epitype of *Ps. theae* (MFLUCC 12-0055) (Figure 3).

3.2 Taxonomy

Neopestalotiopsis chrysea (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *Stud. Mycol.* 79: 138 (2014) (Figure 4).

≡ *Pestalotiopsis chrysea* Maharachch. and K.D. Hyde, *Fungal Divers.* 56(1): 107 (2012).
= *Neopestalotiopsis umbrinospora* (Maharachch. and K.D. Hyde) Maharachch., K.D. Hyde & Crous, *Stud. Mycol.* 79: 149 (2014).

Typification: CHINA, Guangxi Province, Shangsi, Shiwandashan, Wangle, dead leaves of unidentified plant, 2 January 1997, W.P. Wu (HMAS042855, holotype; MFLU 12-0411, isotype, ex-type culture NN042855 = MFLUCC 12-0261).

Description of the new collection: *Endophytic* on healthy roots of *Itea riparia*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* up to 600 μm diameter, globose to subglobose,

TABLE 4 The best-fit substitution model and the average standard deviation of split frequencies resulting from the Bayesian inference (BI) analysis. .

Analysis	Average standard deviation of split frequencies	Model		
		ITS	<i>tub2</i>	<i>tef1-α</i>
1. <i>Neopestalotiopsis</i>	0.009969	HKY+I+G	GTR+I+G	HKY+G
2. <i>Pestalotiopsis</i>	0.009998	GTR+I+G	HKY+I+G	GTR+I+G
3. <i>Pseudoestalotiopsis</i>	0.009889	HKY+G	HKY+G	GTR+I+G

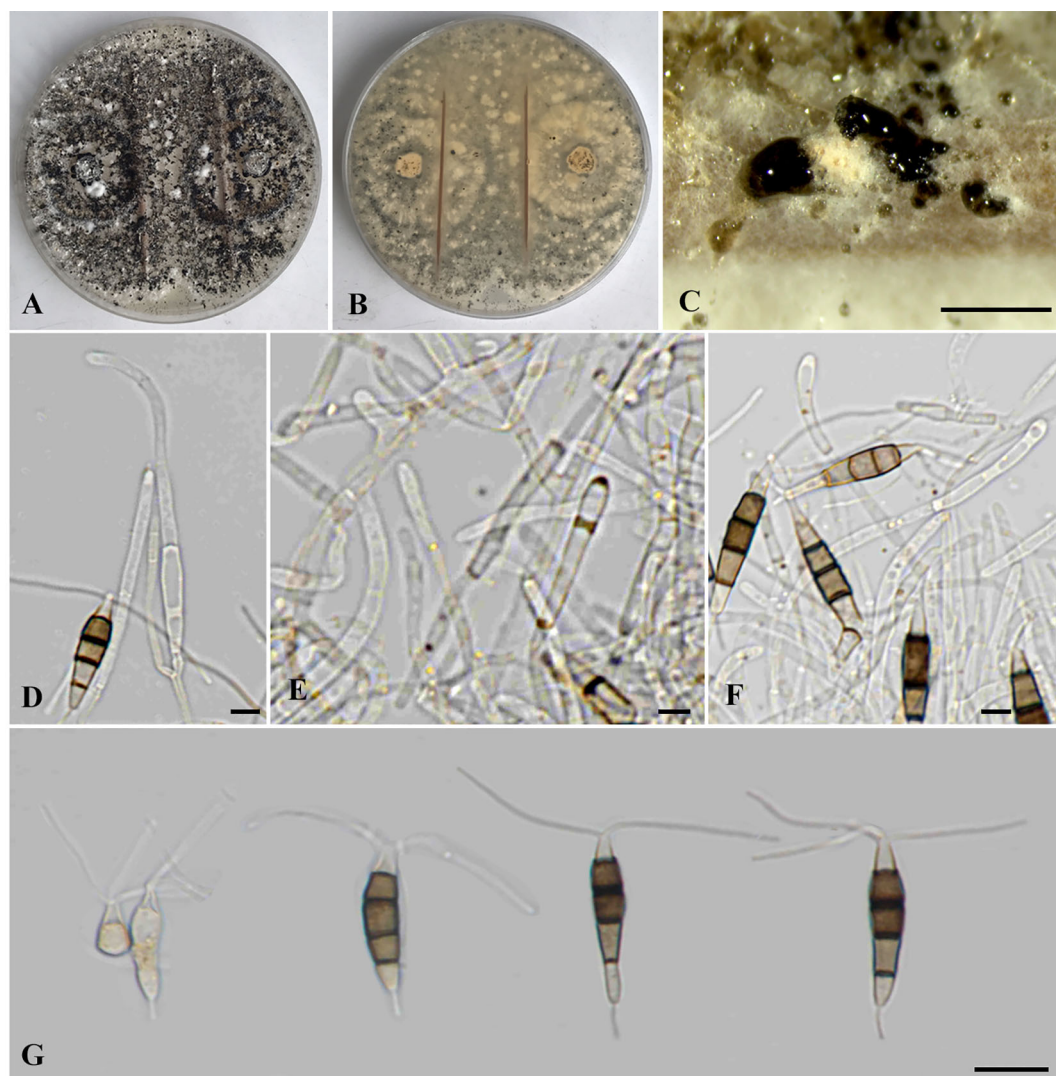


FIGURE 4

Neopestalotiopsis chrysea (SDBR-CMU516). (A) Surface of colonies on PDA. (B) Reverse of colonies on PDA. (C) Conidiomata and conidia masses. (D–F) Conidiophores, conidiogenous cells and conidia. (G) Conidia. Scale bars: (B, C) 500 μm , (D–F) 5 μm , and (G) 10 μm .

solitary to aggregated, black, semi-immersed on PDA, releasing globose, brown to black, slimy conidial masses. *Conidiophores* up to 65 μm long $\times$ 2–3 μm wide, hyaline, brown septa, branched. *Conidiogenous cells* 6–22 $\times$ 1.5–3 μm ($\bar{x}$ = 15.5 $\times$ 2 μm , n = 10), discrete, holoblastic, subcylindrical to lageniform, hyaline, smooth-walled. *Conidia* 18.5–27.5 $\times$ 4–6 μm ($\bar{x}$ = 22 $\times$ 5 μm , n = 30), fusiform to elongated fusiform, or ellipsoid, narrower towards the basal cells, straight to slightly curved, 4-septate; basal cell 3–7 μm long, subcylindrical to obconic, hyaline to pale brown, thin and smooth-walled; three median cells 12–16 μm long, sometimes hyaline to pale brown, one median cell when immature, pale brown to dark brown, with thick and darker brown septa, versicolored, second cell from base 4–4.5 μm , pale to light brown; third cell 4–4.5 μm , darker brown to olivaceous; fourth cell 4–4.5 μm , pale brown to olivaceous; apical cell 3–5 μm long, hyaline, obconic to conic; with two to three apical appendages, arising from

the apical crest, filiform, unbranched, 24–30 μm long; with a single basal appendage, filiform, unbranched, centric, 3–6 μm long.

Culture characteristics: *Colonies* on PDA reached at 7 cm diameter after 5 days at 25°C, irregular form, raised elevation, crenate edge, whitish, with sparse aerial mycelium on surface, yellowish to pale brown in reverse. Sporulation on PDA at 25°C after 30 days, with numerous black fruiting bodies.

Material examined: THAILAND, Chiang Mai Province, Mae Rim District, on healthy roots of *Itea riparia* (*Iteaceae*), 10 July 2023, J. Monkai, IT107 (CMUB 40073), living culture, SDBR-CMU516 = KUNCC 24-18917, dried culture permanently preserved in a metabolically inactive state, CMUB 40073.

GenBank number: PQ521226 (ITS), PQ560699 (*tub2*), and PQ529177 (*tef1- α*).

Habitats and host: Dead and living tissue of *Carya illinoensis*, *Itea riparia*, *Vaccinium ashei*, and unidentified plants

(Maharachchikumbura et al., 2012; 2014; Shi et al., 2017; Wu et al., 2021; this study).

Distribution: China (Anhui, Fujian, Guangxi and Hunan Provinces) and Thailand (Maharachchikumbura et al., 2012; 2014; Shi et al., 2017; Wu et al., 2021; this study).

Notes: Based on the nucleotide BLAST search of ITS sequence, the new isolate of *Neopestalotiopsis chrysea* (SDBR-CMU516) showed the closest similarity with *Neopestalotiopsis* sp. 15 SSNM-2014 strain CBS 177.25 (100%), *Pestalotiopsis* sp. LH162 (100%), and *N. clavispora* strain YZM-1 (100%). Based on the nucleotide BLAST search of *tub2* sequence, *N. chrysea* (SDBR-CMU516) showed the closest similarity with *Neopestalotiopsis* sp. strain LC3480 (99.35%), *N. asiatica* isolate JGGH5 (99.35%), and *N. chrysea* isolate LSCKS81 (99.35%). Based on the nucleotide BLAST search of *tef1-α* sequence, *N. chrysea* (SDBR-CMU516) showed the closest similarity with *Pestalotiopsis chrysea* strain MFLUCC12-0261 (99.59%), *Neopestalotiopsis* sp. strain LC3480 (99.59%), and *N. chrysea* strain FZX038 (99.38%).

Our new strain (SDBR-CMU516) was phylogenetically close to *Neopestalotiopsis chrysea* and *N. umbrinospora* (Figure 1). The nucleotide comparison between our new strain (SDBR-CMU516) and *N. chrysea* (MFLUCC 12-0261, ex-type) showed 0.9% (4/430) and 0.4% (2/487) bp difference in *tub2* and *tef1-α* (whereas those in ITS are identical). The nucleotide comparison between our new strain (SDBR-CMU516) and *N. umbrinospora* (MFLUCC 12-0285, ex-type) showed 0.9% (4/430) and 0.8% (4/487) bp difference in *tub2* and *tef1-α* (whereas those in ITS are identical). The nucleotide comparison between *N. chrysea* (MFLUCC 12-0261, ex-type) and *N. umbrinospora* (MFLUCC 12-0285, ex-type) showed 0.4% (2/487) in *tef1-α* (whereas those in ITS and *tub2* are identical).

Pestalotiopsis chrysea and *P. umberspora* were initially introduced by Maharachchikumbura et al. (2012), which transferred to *Neopestalotiopsis chrysea* and *N. umbrinospora* based on morphology and molecular phylogeny (Maharachchikumbura et al., 2014). *Neopestalotiopsis chrysea* was described by their distinct yellowish conidiogenous cells, conidia, and colony (Maharachchikumbura et al., 2012; 2014), whereas *N. umbrinospora* was characterized by its umber color of the median cells of the conidia (Maharachchikumbura et al., 2012; 2014). Both species were isolated from dead plant materials from Guangxi and Hunan China (Maharachchikumbura et al., 2012; 2014). Although there are only few base-pair differences between *N. umbrinospora* and *N. chrysea*, Maharachchikumbura et al. (2012; 2014) treated *N. umbrinospora* differently from the latter species due to its umber color and relatively wider conidia (9–25 × 6–8 μm vs. 20–24 × 5.5–7 μm), whereas our new strain (SDBR-CMU516) shares similar conidial shapes, color, and size to *N. chrysea* in producing darker brown to olivaceous conidia (18.5–27.5 × 4–6 μm vs. 20–24 × 5.5–7 μm) with three-tubular apical appendages (24–30 μm vs. 22–30 μm) (Maharachchikumbura et al., 2012; 2014). Therefore, we identified this strain as a new host record of *N. chrysea* on *Itea* based on morphological and phylogenetic lines of evidence. Furthermore, *N. umbrinospora* is synonymized under *N. chrysea* herein due to their conspecific relationship based on phylogenetic evidence coupled with the nucleotide pairwise comparison of the sufficient genes.

Neopestalotiopsis haikouensis Z.X. Zhang, J.W. Xia, and X.G. Zhang, *MycKeys* 88: 181 (2022) (Figure 5).

= *Neopestalotiopsis cercidicola* W.S. Zhang and X.L. Fan, *J. Fungi* 10: 475 (2024).

= *Neopestalotiopsis terricola* W.L. Li and Jian K. Liu, *J. Fungi* 8: 1175 (2022).

Typification: CHINA, Hainan Province, Haikou City: East Harbour National Nature Reserve, on diseased leaves of *Ilex chinensis*, 23 May 2021, Z.X. Zhang (HSAUP212271, holotype), ex-type living culture: SAUCC212271.

Description of the new collection: *Endophytic* on healthy stems of *Itea riparia*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* up to 500 μm diameter, pycnidial, globose to subglobose, solitary to aggregated, black, semi-immersed on PDA, releasing globose, brown to black, slimy conidial masses. *Conidiophores* up to 140 μm long × 2–3.5 μm wide, hyaline, dark brown septa, branched, sometimes giving rise to conidia. *Conidiogenous cells* 4–8.5 × 2.5–6 μm ($\bar{x}$ = 7 × 4 μm, n = 10), discrete, holoblastic, subcylindrical to ampulliform, hyaline, smooth-walled. *Conidia* 20–30 × 5–7 μm ($\bar{x}$ = 25 × 6 μm, n = 30), fusiform to elongated fusiform, or ellipsoid, sometimes slightly wider in the upper median cells, straight to slightly curved, 4-septate; basal cell 1–6 μm long, obconic to conic, hyaline, thin and smooth-walled; three median cells 13–18.5 μm long, pale brown when immature, pale brown to dark brown, darker at septa, versicolored, verruculose, second cell from base 4–6 μm, pale brown; third cell 4.5–5.5 μm, dark brown to olivaceous; fourth cell 3.5–6 μm, pale brown to olivaceous; apical cell 3.5–5 μm long, hyaline, subcylindrical to obconic; with two to three apical appendages (mostly three), arising from the apical crest, filiform, unbranched, 25–32.5 μm long; with a single basal appendages, filiform, unbranched, centric, 3–12 μm long.

Culture characteristics: *Colonies* on PDA reached at 7 cm diameter after 5 days at 25°C irregular form, raised elevation, crenate edge, whitish, cottony, with dense aerial mycelium on surface. Sporulation on PDA at 25°C after 30 days, with numerous black fruiting bodies.

Material examined: THAILAND, Chiang Mai Province, Mae Rim District, on living stems of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT92 (CMUB40074), living culture, SDBR-CMU517 = KUNCC 24-18918, dried culture permanently preserved in a metabolically inactive state, CMUB40074.

GenBank number: PQ521227 (ITS), PQ560700 (*tub2*), and PQ529178 (*tef1-α*).

Habitats and host: Dead and living tissue of *Castanea mollissima*, *Cercis chinensis*, *Ilex chinensis*, *Itea riparia*, *Paeonia suffruticosa*, and *Olea europaea* (Jiang et al., 2021; Li et al., 2022; Zhang et al., 2022; 2024; this study).

Distribution: China (Hainan, Sichuan and Yunnan Provinces) and Thailand (Jiang et al., 2021; Li et al., 2022; Zhang et al., 2022; 2024; this study).

Notes: Based on the nucleotide BLAST search of ITS sequence, the new isolate of *Neopestalotiopsis haikouensis* (SDBR-CMU517) showed the closest similarity with *N. saprophytica* (100%), *Hymenoplella hippophaeicola* isolate SF134_3_1 (100%), and

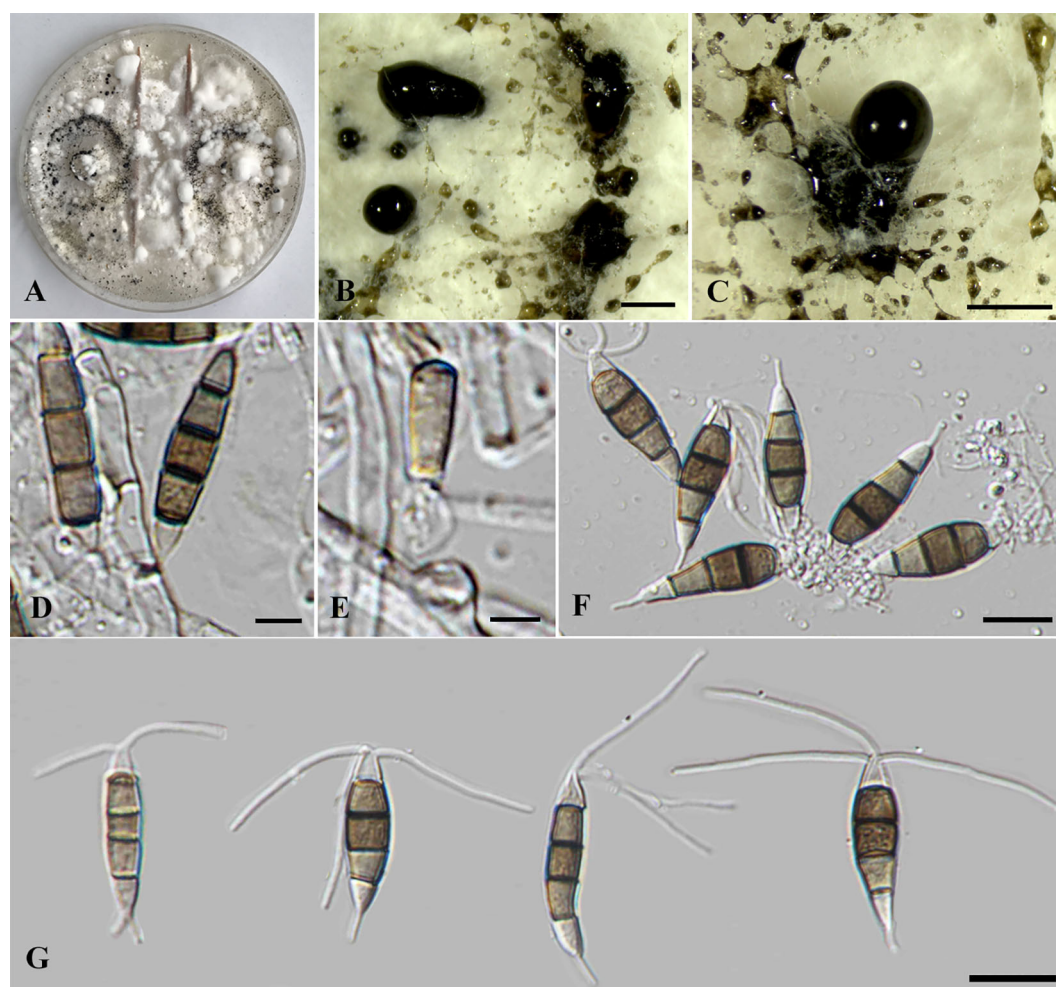


FIGURE 5

Neopestalotiopsis haikouensis (SDBR-CMU517). (A) Surface of colonies on PDA. (B, C) Conidiomata and conidia masses. (D–F) Conidiophores, conidiogenous cells, and conidia. (G) Conidia. Scale bars: (B, C) 500 μm , (D–F) 5 μm , and (G) 10 μm .

Pestalotiopsis microspora isolate WH55 (100%). Based on the nucleotide BLAST search of *tub2* sequence, *N. haikouensis* (SDBR-CMU517) showed the closest similarity with *Neopestalotiopsis* sp. strain PP026 (99.73%), *N. clavispora* isolate MCH21 (99.73%), and *N. clavispora* isolate SGP37 (99.73%). Based on the nucleotide BLAST search of *tef1- α* sequence, *N. haikouensis* (SDBR-CMU517) showed the closest similarity with *N. protearum* strain GBLZ16PE-007 (99.8%), *N. protearum* strain GUCC 23-0329 (99.8%), and *Neopestalotiopsis* sp. strain LC2945 (99.8%).

In the phylogenetic analyses (Figure 1), our new strain (SDBR-CMU517) clustered with *Neopestalotiopsis cercidicola*, *N. haikouensis*, and *N. terricola*. The nucleotide comparison among our new strain (SDBR-CMU517) and the ex-type strains of *N. cercidicola*, *N. haikouensis*, and *N. terricola* indicated low nucleotide difference (less than 1%) (Table 5). Zhang et al. (2022) introduced *N. haikouensis* as a leaf spot disease of *Ilex chinensis*. Lately, *N. terricola* and *N. cercidicola* were isolated as pathogens of *Paeonia suffruticosa* (Li et al., 2022) and *Cercis chinensis* (Zhang et al., 2024), respectively. Based on morphology, our new strain (SDBR-

CMU517) shares similar conidial color (pale brown) and size ($20\text{--}30 \times 5\text{--}7 \mu\text{m}$) to *N. haikouensis* ($16\text{--}22 \times 4.5\text{--}7 \mu\text{m}$) (Zhang et al., 2022) and *N. cercidicola* ($17.5\text{--}23.5 \times 5.5\text{--}8.5 \mu\text{m}$) (Zhang et al., 2024) (Table 6), whereas the conidia of *N. terricola* is wider and darker in the middle cell ($20\text{--}23 \times 8\text{--}9.5 \mu\text{m}$), compared to others (Li et al., 2022) (Table 6). Li et al. (2022) noted the variation of conidial color and size among strains of *N. terricola* depending on host substrates. The ex-type strain of *N. terricola* (CGMCC3.23553), which was isolated from diseased leaves of *Paeonia suffruticosa*, had darker and relatively wider conidia than other strains including the strain UESTCC 22.0034, isolated from infected olive leaves (see Figures 6 and 9 in Li et al., 2022), and two strains (CFCC 54337 and CFCC 54340) recovered from diseased leaves of *Castanea mollissima* (see Figure 17 in Jiang et al., 2021) (Table 6). Moreover, we could detect the varieties of conidial shape and size in our samples, which is shown in Figure 5G. Therefore, because of the morphological and phylogenetic consistencies, we synonymized *N. terricola* and *N. cercidicola* under *N. haikouensis* following the prior publication. Likewise, our new strain is reported as a new host

TABLE 5 Nucleotide differences among the closely related strains of *Neopestalotiopsis haikouensis*.

Compared strains	Gene region/locus		
	ITS	<i>tub2</i>	<i>tef1-α</i>
The new strain (SDBR-CMU517) vs. <i>N. haikouensis</i> (SAUCC 212271)	0.2% (1/503)	0.3% (2/747)	Identical
The new strain (SDBR-CMU517) vs. <i>N. haikouensis</i> (= <i>N. terricola</i>) (CGMCC 3.23553)	Identical	0.3% (2/727)	Identical
The new strain (SDBR-CMU517) vs. <i>N. haikouensis</i> (= <i>N. cercidicola</i>) (CFCC 7063)	0.4% (2/494)	0.3% (2/632)	0.5% (2/444)
<i>N. haikouensis</i> (SAUCC 212271) vs. <i>N. haikouensis</i> (= <i>N. terricola</i>) (CGMCC 3.23553)	Identical	0.1% (1/735)	Identical
<i>N. haikouensis</i> (SAUCC 212271) vs. <i>N. haikouensis</i> (= <i>N. cercidicola</i>) (CFCC 7063)	0.4% (2/489)	0.3% (2/632)	0.2% (1/439)
<i>N. terricola</i> (CGMCC 3.23553) vs. <i>N. haikouensis</i> (= <i>N. cercidicola</i>) (CFCC 7063)	0.2% (1/485)	0.5% (2/434)	0.3% (2/712)

The data are represented by the number of different nucleotides/numbers of all nucleotides (% base-pair difference).

record of *N. haikouensis* on *Itea* and it is also a new geographical record in Thailand.

Neopestalotiopsis iteae Monkai, Phookamsak, Bhat & S. Lumyong, sp. nov. (Figure 6).

Index Fungorum number: IF902438.

Etymology: Refers to the host genus, *Itea* from which the holotype was collected.

Endophytic on healthy stems of *Itea japonica*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* up to 400 μm diameter, globose to subglobose, solitary to aggregated, black, raising above surface of PDA. *Conidiophores* up to 50 μm long × 2–3 μm wide, hyaline, dark brown septa, branched. *Conidiogenous cells* 4–9.5 × 2.5–4 μm ($\bar{x}$ = 6 × 3 μm, n = 10), discrete, holoblastic, subcylindrical to ampulliform, hyaline, smooth-walled. *Conidia* 17.5–26 × 4.5–6.5 μm ($\bar{x}$ = 22.5 × 5.5 μm, n = 30), fusiform, ellipsoid, straight, 4-septate; basal cell 4–5.5 μm long, obconic to conic, hyaline, thin and smooth-walled; three median cells 12–17.5

μm long, initially hyaline with brown septa, becoming brown to pale brown, with darker brown septa, slightly constricted at septa, with second cell from base 4–6.5 μm, pale brown to olivaceous; third cell 3.5–6 μm, brown to olivaceous; fourth cell 4–6 μm, brown to olivaceous; apical cell 4.5–6 μm long, hyaline, subcylindrical to obconic; with two to three apical appendages, not arising from the apical crest, but each inserted at a different locus in the upper half of the apical cell, filiform, unbranched, 8.5–14 μm long; with a single basal appendages, filiform, unbranched, 4.5–6 μm long.

Culture characteristics: *Colonies* on PDA reached at 7 cm diameter after 5 days at 25°C, circular form, flat elevation, undulage edge, whitish to gray, with dense aerial mycelium on surface. Sporulation on PDA at 25°C after 30 days, with numerous black fruiting bodies.

Material examined: THAILAND, Chiang Mai Province, Hang Dong District, on living stems of *Itea japonica* (*Iteaceae*), 7 February 2023, J. Monkai, IT41 (CMUB40072, holotype), ex-type living culture, SDBR-CMU515 = KUNCC 24-18919, dried culture permanently preserved in a metabolically inactive state, CMUB40072.

GenBank number: PQ521228 (ITS), PQ560701 (*tub2*), and PQ529179 (*tef1-α*).

Notes: Based on the nucleotide BLAST search of ITS sequence, *Neopestalotiopsis iteae* sp. nov. (SDBR-CMU515) showed the closest similarity with *N. clavispora* isolate MI003 (100%), *N. saprophytica* (100%), and *Pestalotiopsis maculans* isolate RM1.18A.01 (100%). Based on the nucleotide BLAST search of *tub2* sequence, *N. iteae* (SDBR-CMU515) showed the closest similarity with *N. piceana* (99.12%), *N. piceana* strain CBS 225.30 (99.12%), and *Neopestalotiopsis* sp. strain PP026 (99.12%). Based on the nucleotide BLAST search of *tef1-α* sequence, *N. iteae* (SDBR-CMU515) showed the closest similarity with *Neopestalotiopsis* sp. isolate YJ11-0708 (98.57%), *N. clavispora* isolate SGP35 (98.57%), and *N. clavispora* isolate MCH27 (98.57%).

Phylogenetically, *Neopestalotiopsis iteae* formed a stable clade sister to *N. cocoës* (0.91 BYPP; Figure 1). The nucleotide comparison between *N. iteae* (SDBR-CMU515) and *N. cocoës* (MFLUCC 15-0152, ex-type) showed 0.4% (2/520) and 2.4% (6/253) bp differences in ITS and *tef1-α* (where *tub2* sequence data of *N. cocoës* were not available for comparison). It should be noted that the *tef1-α* sequence data of *N. cocoës* are very short (approximately 200–300 bp). The low support between *N. iteae* and *N. cocoës* might be

TABLE 6 The comparison of conidial morphology and host between the closely related strains of *Neopestalotiopsis haikouensis*.

Strains	Conidial size	Host	References
The new strain (SDBR-CMU517)	20–30 × 5.3–7 μm (25 × 6 μm)	Healthy stems of <i>Itea riparia</i>	This study
<i>N. haikouensis</i> (SAUCC 212271)	16.0–22.0 × 4.5–7.0 μm (20.0 × 5.5 μm)	Diseased leaves of <i>Ilex chinensis</i>	Zhang et al. (2022)
<i>N. haikouensis</i> (= <i>N. terricola</i>) (CGMCC 3.23553)	20–23 × 8–9.5 μm (21.5 × 8.5 μm)	Diseased branch of <i>Paeonia suffruticosa</i> and diseased leaf of <i>Olea europaea</i>	Li et al. (2022)
<i>N. haikouensis</i> (= <i>Neopestalotiopsis</i> sp.1) (CFCC 54337)	19.1–24.7 × 5.4–8.6 μm (21.6 × 6.7 μm)	Leaf spots of <i>Castanea mollissima</i>	Jiang et al. (2021)
<i>N. haikouensis</i> (= <i>Neopestalotiopsis</i> sp.2) (CFCC 54340)	21.4–26.2 × 5.1–8.7 μm (23.6 × 7 μm)	Leaf spots of <i>Castanea mollissima</i>	Jiang et al. (2021)
<i>N. haikouensis</i> (= <i>N. cercidicola</i>) (CFCC 7063)	17.5–23.5 × 5.5–8.5 μm (20.7 × 6.9 μm)	Leaf spots of <i>Cercis chinensis</i>	Zhang et al. (2024)

The new strain obtained in this study is indicated in black bold.

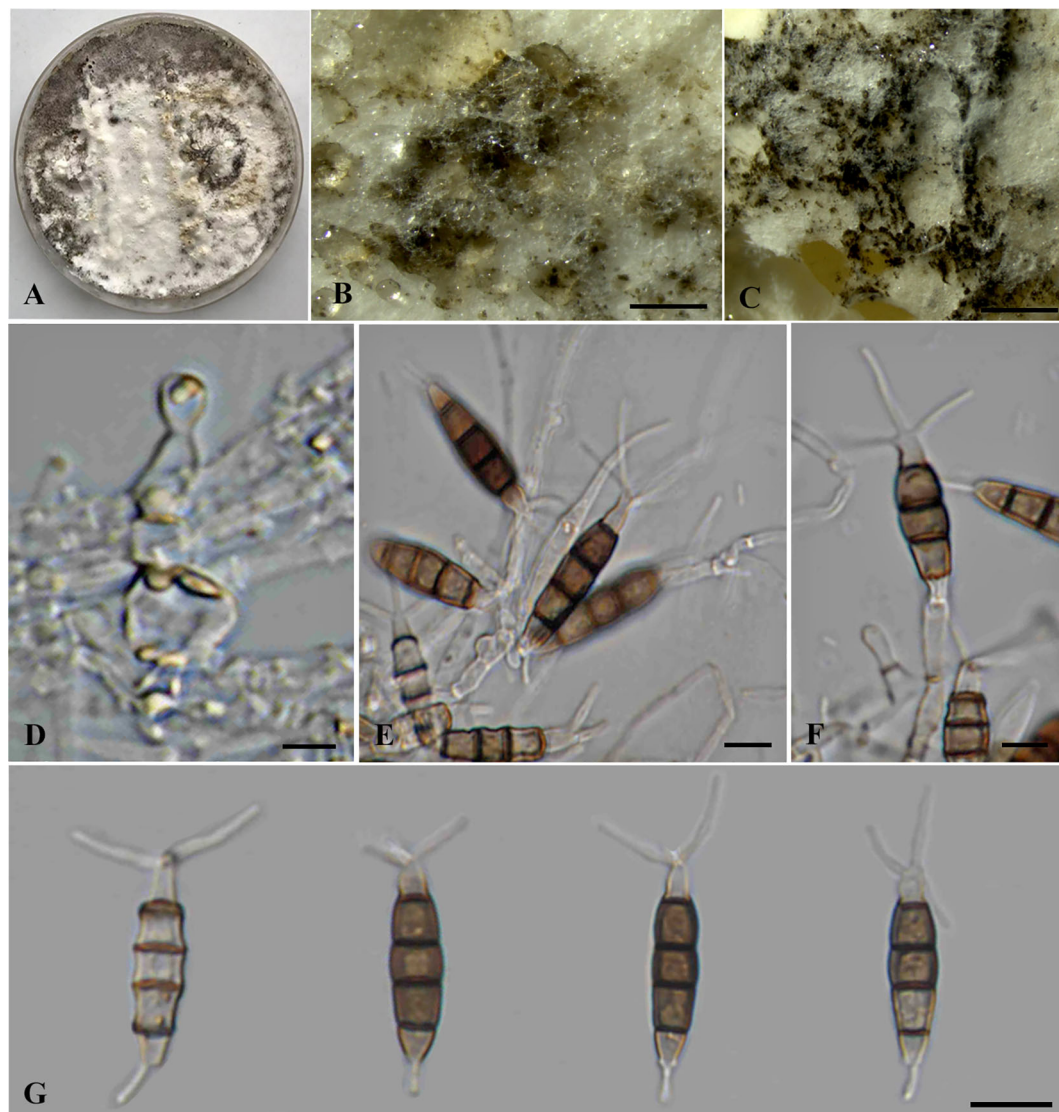


FIGURE 6

Neopestalotiopsis iteae (SDBR-CMU515, ex-type). (A) Surface of colonies on PDA. (B, C) Conidiomata. (D–F) Conidiophores, conidiogenous cells, and conidia. (G) Conidia. Scale bars: (B, C) 500 μm , (D–F) 5 μm , and (G) 10 μm .

coming from the uncompleted sequence data of *N. cocoës*. Morphologically, *N. iteae* can be distinguished from *N. cocoës* in having longer and narrower conidia ($17.5\text{--}26 \times 4.5\text{--}6.5 \mu\text{m}$ vs. $19\text{--}22.5 \times 7.5\text{--}9.5 \mu\text{m}$) and slightly constricted septa (Hyde et al., 2016). Based on the lines of evidence in morphology and phylogeny coupled with a significant nucleotide pairwise difference of *tef1- α* sequence, *N. iteae* was introduced as a novel species.

Pestalotiopsis jinchanghensis Liu, Hou, Raza and Cai, *Scientific Reports* 7 (no. 866): 8 (2017) (Figure 7).

= *Pestalotiopsis zhaoqingensis* H.J. Zhao and W. Dong, *Mycosphere* 14(1): 2238 (2023).

Typification: CHINA, Yunnan Province, Xishuangbanna, Jinchanghe, on leaves of *Camellia sinensis*, 20 April 2015, F. Liu, HMAS 247061 (holotype), ex-type living culture CGMCC 3.18158 (= LC6636).

Description of the new collection: *Endophytic* on healthy stems of *Itea riparia*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* up to 600 μm diameter, pycnidial, globose to subglobose, solitary, black, semi-immersed on PDA, releasing globose, brown to black, slimy conidial masses. *Conidiophores* often reduced to conidiogenous cells. *Conidiogenous cells* $4\text{--}10 \times 2.5\text{--}8.5 \mu\text{m}$ ($\bar{x} = 7.5 \times 6.5 \mu\text{m}$, $n = 10$), discrete, holoblastic, ampulliform to lageniform, hyaline, smooth-walled. *Conidia* $19\text{--}27 \times 4\text{--}6 \mu\text{m}$ ($\bar{x} = 23.5 \times 5 \mu\text{m}$, $n = 30$), fusiform to elongated fusiform, straight to slightly curved, 4-septate; basal cell $3.5\text{--}4.5 \mu\text{m}$ long, obconic to conic, hyaline, thin and smooth-walled; three median cells $12\text{--}16.5 \mu\text{m}$ long, sometimes hyaline, two median cells when immature, brown to olivaceous, septa and periclinal walls darker than the rest of the cell, concolorous, wall rugose, second cell from base $4\text{--}5 \mu\text{m}$; third cell $3.5\text{--}5.5 \mu\text{m}$; fourth cell $4\text{--}5.5 \mu\text{m}$;

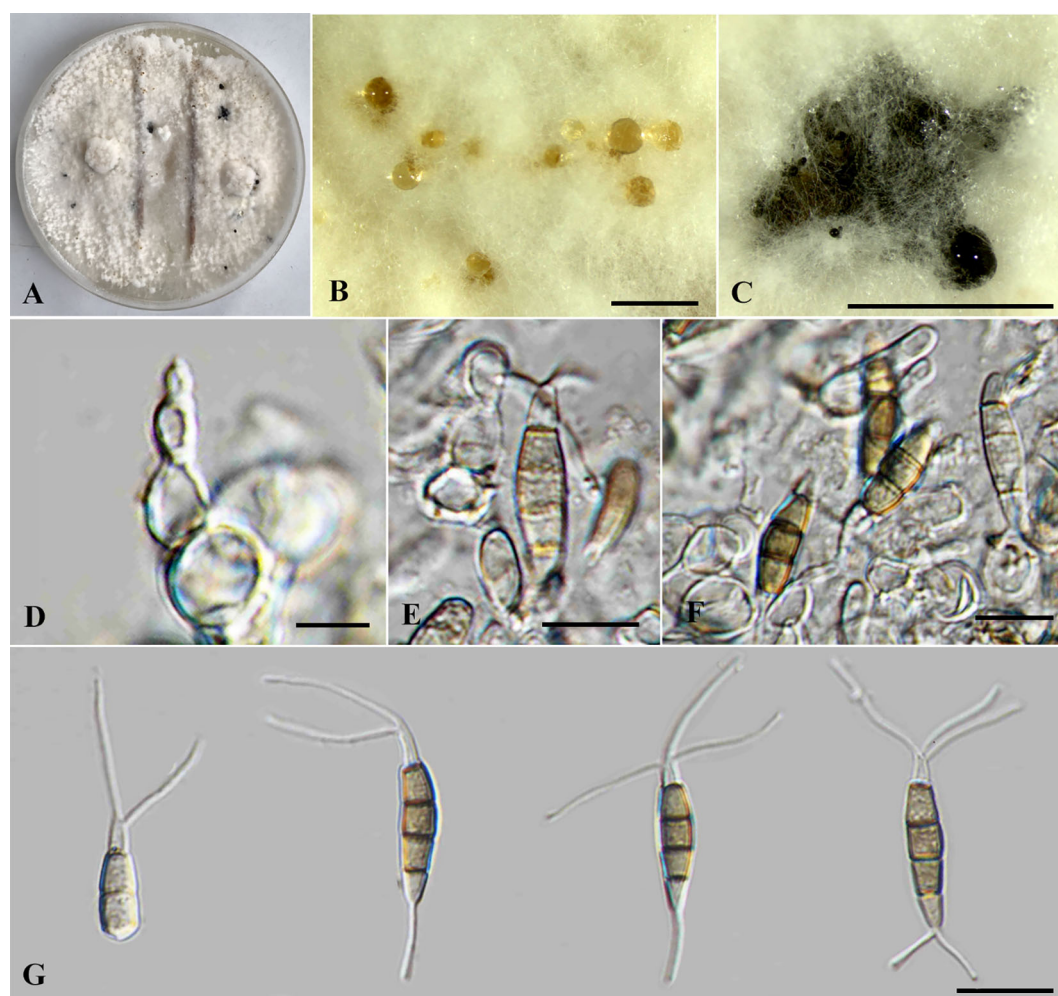


FIGURE 7

Pestalotiopsis jinchanghensis (SDBR-CMU518). (A) Surface of colonies on PDA. (B, C) Conidiomata and conidia masses. (D–F) Conidiogenous cells with attached conidia. (G) Conidia. Scale bars: (B, C) 500 µm and (D–G) 10 µm.

apical cell 3.5–4.5 µm long, hyaline, subcylindrical to obconic; with two to three tubular apical appendages, arising from the apical crest, filiform, unbranched, 14–27 µm long; with 1(–2) tubular basal appendages, filiform, unbranched, centric, 5–11.5 µm long.

Culture characteristics: Colonies on PDA reached at 7 cm diameter after 5 days at 25°C, irregular form, raised elevation, crenate edge, whitish, cottony, with dense aerial mycelium on surface. Sporulation on PDA at 25°C after 30 days, with few black fruiting bodies.

Material examined: THAILAND, Chiang Mai Province, Mae Rim District, on living stems of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT86 (CMUB40075), living culture, SDBR-CMU518 = KUNCC 24-18920, dried culture permanently preserved in a metabolically inactive state, CMUB40075.

GenBank number: PQ521229 (ITS), PQ560702 (*tub2*), and PQ529180 (*tef1-α*).

Habitats and host: Dead and living tissue of *Camellia sinensis*, *Itea riparia*, and unidentified plants (Liu et al., 2017; Dong et al., 2023; this study).

Distribution: China (Yunnan and Guangdong Provinces) and Thailand (Liu et al., 2017; Dong et al., 2023; this study).

Notes: Based on the nucleotide BLAST search of ITS sequence, the new isolate of *Pestalotiopsis jinchanghensis* (SDBR-CMU518) showed the closest similarity with *Pestalotiopsis* sp. NJ-2022e strain SAUCC230044 (99.83%), *Pestalotiopsis* sp. strain JMB08_3B2 (99.67%), and *P. malayana* strain SAUCC230483 (99.66%). Based on the nucleotide BLAST search of *tub2* sequence, *P. jinchanghensis* (SDBR-CMU518) showed the closest similarity with *P. zhaoqingensis* strain ZHKUCC 23-0825 (99.87%), *P. jinchanghensis* strain LC8190 (99.87%), and *P. jinchanghensis* strain LC8191 (99.87%). Based on the nucleotide BLAST search of *tef1-α* sequence, *P. jinchanghensis* (SDBR-CMU518) showed the closest similarity with *P. jinchanghensis* strain LC8191 (99.79%), *P. jinchanghensis* strain LC6636 (99.79%), and *P. jinchanghensis* strain LC8190 (99.79%).

The combined phylogenetic tree indicated that our new strain (SDBR-CMU518) clustered in the same clade with *Pestalotiopsis jinchanghensis* and *P. zhaoqingensis* with 86% ML, 1.00 BYPP

statistical support (Figure 2). The nucleotide comparison between our new strain (SDBR-CMU518) and *P. jinchanghensis* (CGMCC 3.18158, ex-type) showed 0.2% (1/475) base-pair difference in *tef1-α* (whereas those in ITS and *tub2* are identical). The nucleotide comparison between our new strain (SDBR-CMU518) and *P. zhaoqingensis* (ZHKUCC 23-0825, ex-type) showed 1% (6/563), 0.1% (1/127), and 0.2% (1/447) bp differences in ITS, *tef1-α*, and *tub2*. In addition, the nucleotide comparison between *P. jinchanghensis* (CGMCC 3.18158, ex-type) and *P. zhaoqingensis* (ZHKUCC 23-0825, ex-type) showed 1.2% (6/500) and 0.2% (1/449) bp differences in ITS and *tef1-α* (whereas those in *tub2* are identical). *Pestalotiopsis jinchanghensis* was identified as a disease on *Camellia sinensis* (Liu et al., 2017), and *P. zhaoqingensis* was isolated from dead unknown plants in China (Dong et al., 2023). Although there were relatively low nucleotide variations between *P. jinchanghensis* and *P. zhaoqingensis*, Dong et al. (2023) justified *P. zhaoqingensis* as distinct species based on morphology, wherein it produced comparatively shorter conidia ($17\text{--}24 \times 4\text{--}8 \mu\text{m}$ vs. $22\text{--}32 \times 5.5\text{--}8.5 \mu\text{m}$) and branched apical appendages. Our new strain (SDBR-CMU518) mostly resembles *P. jinchanghensis* in having unbranched apical appendages (Liu et al., 2017). However, the conidial size of our new isolate showed to be overlapping with both *P. jinchanghensis* and *P. zhaoqingensis* ($19\text{--}27 \times 4\text{--}6 \mu\text{m}$ vs. $22\text{--}32 \times 5.5\text{--}8.5 \mu\text{m}$ vs. $17\text{--}24 \times 4\text{--}8 \mu\text{m}$). Based on morphological and phylogenetic lines of evidence as well as an identical nucleotide pairwise, we treated the new strain as a new host record of *P. jinchanghensis* on *Itea* and the species is reported from Thailand for the first time, while *P. zhaoqingensis* is synonymized under *P. jinchanghensis* herein.

Pseudopestalotiopsis iteae Monkai, Phookamsak, Bhat & S. Lumyong, sp. nov. (Figure 8).

Index Fungorum number: IF902439.

Etymology: Refers to the host genus from which the holotype was collected, *Itea*.

Endophytic on healthy leaves and roots of *Itea riparia*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* up to 500 μm diameter, globose to subglobose, solitary to aggregated, black, semi-immersed on PDA, releasing globose, brown to black, slimy conidial masses. *Conidiophores* $34\text{--}52 \times 1.5\text{--}3 \mu\text{m}$ ($\bar{x} = 43 \times 2 \mu\text{m}$, $n = 10$), hyaline, septate, branched. *Conidiogenous cells* $9.5\text{--}16.5 \times 2\text{--}4 \mu\text{m}$ ($\bar{x} = 12.5 \times 3 \mu\text{m}$, $n = 10$), discrete, holoblastic, subcylindrical to ampulliform, hyaline, smooth-walled. *Conidia* $20\text{--}27 \times 4.5\text{--}7 \mu\text{m}$ ($\bar{x} = 24 \times 6 \mu\text{m}$, $n = 30$), fusiform to ellipsoid, straight to slightly curved in C-form or S-form, 4-septate; basal cell $3\text{--}4.5 \mu\text{m}$ long, obconic to conic, hyaline, thin and smooth-walled; three median cells $12\text{--}17 \mu\text{m}$ long, sometimes hyaline, one to three median cells when immature, brown to dark brown, with thick verruculose walls, constricted at septa, septa and periclinal walls darker than the rest of the cell, concolorous, second cell from base $4\text{--}5.5 \mu\text{m}$; third cell $4\text{--}5.5 \mu\text{m}$; fourth cell $4\text{--}6.5 \mu\text{m}$; apical cell $4\text{--}5.5 \mu\text{m}$ long, hyaline, subcylindrical to obconic; with two to three tubular apical appendages (mostly three), arising from the upper portion of apical cell, filiform, unbranched, knobbed, $18\text{--}21 \mu\text{m}$ long; with a single basal appendages (mostly one), filiform, unbranched, slightly knobbed, $4\text{--}7 \mu\text{m}$ long.

Culture characteristics: Colonies on PDA reached 7 cm diameter after 5 days at 25°C, irregular form, umbonate elevation, crenate edge, whitish, cottony, with dense aerial mycelium on surface, pale brown to white in reverse, Sporulation on PDA at 25°C after 30 days, with numerous black fruiting bodies.

Material examined: THAILAND, Chiang Mai Province, Mae Rim District, on living leaves of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT48 (CMUB40071, holotype), ex-type living culture, SDBR-CMU514 = KUNCC24-18921, dried culture permanently preserved in a metabolically inactive state, CMUB40071; *ibid.*, on living leaves of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT49, living culture, SDBR-CMU519 = KUNCC24-18922; *ibid.*, on living leaves of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT50, living culture, SDBR-CMU520 = KUNCC24-18923; *ibid.*, on living leaves of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT61, living culture, SDBR-CMU521 = KUNCC24-18924; *ibid.*, on living leaves of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT62, living culture, SDBR-CMU522 = KUNCC24-18925; *ibid.*, on living leaves of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT73, living culture, SDBR-CMU523 = KUNCC24-18926; *ibid.*, on living roots of *Itea riparia* (Iteaceae), 10 July 2023, J. Monkai, IT99, living culture, SDBR-CMU524 = KUNCC24-18927.

GenBank number: SDBR-CMU514; PQ521230 (ITS), PQ560703 (*tub2*), PQ529181 (*tef1-α*), SDBR-CMU519; PQ521231 (ITS), PQ560704 (*tub2*), PQ529182 (*tef1-α*), SDBR-CMU520; PQ521232 (ITS), PQ560705 (*tub2*), PQ529183 (*tef1-α*), SDBR-CMU521; PQ521233 (ITS), PQ560706 (*tub2*), PQ529184 (*tef1-α*), SDBR-CMU522; PQ521234 (ITS), PQ560707 (*tub2*), PQ529185 (*tef1-α*), SDBR-CMU523; PQ521235 (ITS), PQ560708 (*tub2*), PQ529186 (*tef1-α*), SDBR-CMU524; PQ521236 (ITS), PQ560709 (*tub2*), PQ529187 (*tef1-α*).

Notes: Based on the nucleotide BLAST search of ITS sequence, *Pseudopestalotiopsis iteae* sp. nov. (SDBR-CMU514) showed the closest similarity with *Pseudopestalotiopsis* sp. isolate ERS19.48.Le.D (99.63%), *Ps. theae* strain KU20018.104 (99.63%), and *Pestalotiopsis theae* isolate CPO/Pe (99.63%). Based on the nucleotide BLAST search of *tub2* sequence, *Ps. iteae* (SDBR-CMU514) showed the closest similarity with *Ps. theae* isolate TN07 (98.97%), *Ps. theae* strain GUCC 23-0406 (99.46%), and *Ps. theae* strain GUCC 23-0472 (99.86%). Based on the nucleotide BLAST search of *tef1-α* sequence, *Ps. iteae* (SDBR-CMU514) showed the closest similarity with *Ps. theae* strain GUCC 23-0406 (99.60%), *Pseudopestalotiopsis* sp. 2-KW-2016 strain: 10 (99.60%), and *Pseudopestalotiopsis* sp. 2-KW-2016 strain: 11 (99.39%).

Phylogenetic tree of combined ITS, *tub2*, and *tef1-α* sequence data revealed that *Pseudopestalotiopsis iteae* (SDBR-CMU514, SDBR-CMU519, SDBR-CMU520, SDBR-CMU521, SDBR-CMU522, SDBR-CMU523, and SDBR-CMU524) are clustered together with *Ps. theae* strain NTUCC 18-067, with significant support (70% ML, 0.92 BYPP), which are distant from the ex-epitype of *Ps. theae* (MFLUCC 12-0055) (Figure 3). The nucleotide comparison between *Ps. iteae* (SDBR-CMU514) and *Ps. theae* (MFLUCC 12-0055) showed 0.2% (1/491), 0.7% (3/426), and 1.8% (9/493) bp difference in ITS, *tub2*, and *tef1-α* (Table 7), respectively.

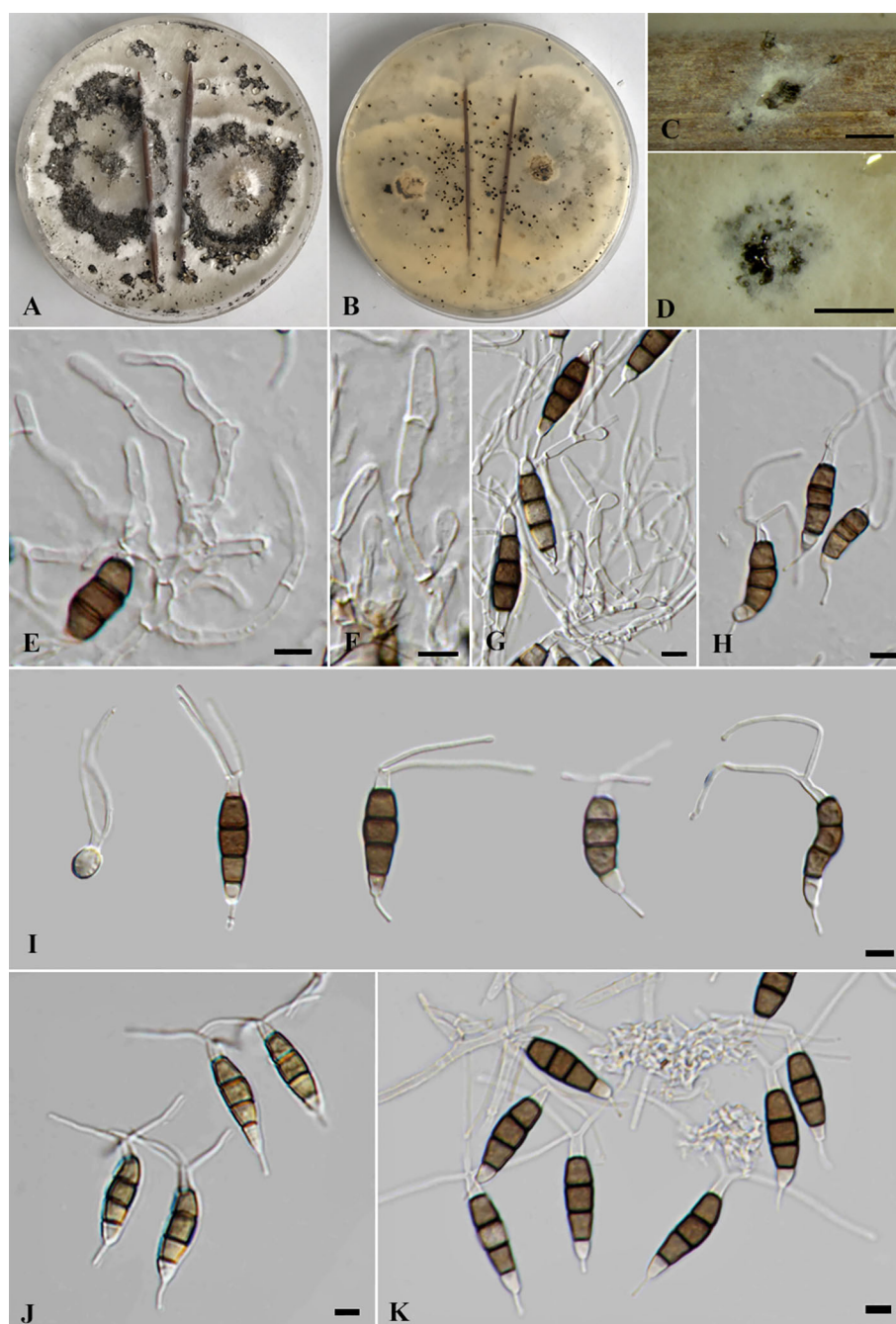


FIGURE 8

Pseudoestalotipsis iteae [SDBR-CMU514, ex-type (A–I)], SDBR-CMU523 (J), and SDBR-CMU524 (K). (A) Surface of colonies on PDA. (B) Reverse of colonies on PDA. (C, D) Conidiomata and conidia masses. (E–G) Conidiophores, conidiogenous cells, and conidia. (H–K) Conidia. Scale bars: (C, D) 500 µm and (E–K) 5 µm.

Moreover, the nucleotide differences between the epitype of *Ps. theae* and other *Ps. iteae* strains are provided in Table 7. The morphological characteristics of *Ps. iteae* resemble those of *Ps. theae* in having brown concolorous median cells, constricted at septa with knobbed apical appendages (Maharachchikumbura et al., 2013). However, *Ps. iteae* has narrower conidia (24×6 vs. 25.5×7.6 µm) and shorter apical appendages (18–21 µm vs. 22.5–31 µm) than *Ps. theae* (Maharachchikumbura et al., 2013). Thus, we introduced *Ps. iteae* as a new species based on the differences of nucleotide pairwise

comparison of the sufficient gene (*tef1-α*) and morphological characteristics.

We noticed that the conidial characteristics varied between the strains of *Pseudoestalotipsis iteae*. Compared with the type strain, strain SDBR-CMU523 produced shorter conidia with pale brown color (21×5 vs. 24×6 µm) and shorter appendages without knobbed tips (11–19 vs. 18–21 µm) (Figure 8J), while the strain SDBR-CMU524 has relatively longer conidia that are not constricted at septa (26×5 vs. 24×6 µm) and longer

TABLE 7 Nucleotide differences between the epitype of *Pseudopestalotiopsis theae* and *Ps. iteae* strains.

Gene region/ locus	The epitype of <i>Pseudopestalotiopsis theae</i> (MFLUCC 12-0055) compared with <i>Ps. iteae</i> strains							
	NTUCC 18-067	SDBR-CMU514 (ex-type)	SDBR-CMU519	SDBR-CMU520	SDBR-CMU521	SDBR-CMU522	SDBR-CMU523	SDBR-CMU524
ITS	0/489 (0%)	1/491 (0.2%)	1/491 (0.2%)	1/491 (0.2%)	1/491 (0.2%)	1/491 (0.2%)	1/491 (0.2%)	1/491 (0.2%)
<i>tub2</i>	2/424 (0.4%)	1/415 (0.2%)	1/413 (0.2%)	9/414 (2.1%)	1/404 (0.2%)	8/413 (1.9%)	3/402 (0.7%)	3/403 (0.7%)
<i>tef1-α</i>	11/473 (2.3%)	9/493 (1.8%)	11/509 (2.2%)	8/506 (1.6%)	8/502 (1.6%)	8/492 (1.6%)	9/493 (1.8%)	9/490 (1.8%)

The data represented by the number of different nucleotides/number of all nucleotides (% base-pair difference).

appendages (15–32 vs. 11–19 μ m) than the type strain (Figure 8K). In phylogeny, strain SDBR-CMU524 formed a separated branch to other strains of *Ps. iteae* (Figure 3). However, there are too few base-pair differences in ITS, *tub2*, and *tef1-α* (less than 1%) between the ex-type strain of *Ps. iteae* (SDBR-CMU514) and strain SDBR-CMU524. The strain SDBR-CMU524 isolated from healthy roots and other strains isolated from healthy leaves of *Itea* spp. should also be noted. It is indicated that *Ps. iteae* has also high intraspecific variation, similar to *Ps. theae*.

Pseudopestalotiopsis theae ($\equiv$ *Pestalotia theae*) was originally isolated from *Camellia sinensis* in Taiwan, then Maharachchikumbura et al. (2013) designated the epitype of *Ps. theae* (MFLUCC 12-0055) from a sample collected from the same host in Thailand. Tsai et al. (2021) isolated a strain of *Ps. theae* (NTUCC 18-067) as a pathogen on *C. sinensis* in Taiwan, representing it as the reference strain. Our phylogeny demonstrated that this strain is not consistent with *Ps. theae* and their nucleotide variation of *tef1-α* is greater (2.3% bp difference) (Table 7), regarding it as *Ps. iteae*. Also, the ex-epitype strain of *Ps. theae* (MFLUCC 12-0055) is closely related with *Ps. theae* strain SC011 and *Ps. theae* strain MFLUCC 22-0128, which are from Thailand (Maharachchikumbura et al., 2013; 2014; Sun et al., 2023), and *Pseudopestalotiopsis* sp. strains NBRC112267 and NBRC112268, which are from Myanmar (Nozawa et al., 2017). Thus, these strains could be treated as *Ps. theae*.

Additionally, we examined the few nucleotide differences of *tub2* (less than 1%) between the ex-type of *Pseudopestalotiopsis iteae*, epitype of *Ps. theae*, and strain NTUCC 18-067. The *tub2* sequence data of those strains lacked the first 300–400 base-pair positions in the alignment of all *Pseudopestalotiopsis* taxa. This may be caused by the different primers used for PCR amplification of *tub2*, which are BT2A/BT2B in those studies (Maharachchikumbura et al., 2013; Tsai et al., 2021), while a primer pair, T1/BT2B, was used in our study and other studies (i.e. Norphanphoun et al., 2019; Sun et al., 2023). To clarify this issue, we then amplified our strains with the BT2A/BT2B, and the results did not show any significant nucleotide variation. Thus, the epitype of *Ps. theae*, strain NTUCC 18-067, and other related strains should be further analyzed for *tub2* using T1/BT2B primers as it allows longer base-pair length (up to 800 bp).

4 Discussion

The nomenclature and classification of pestalotioid fungi are remarkably challenging. At the generic level, phylogenetic analyses

of combined ITS, *tub2*, and *tef1-α* provide sufficient distinction between *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis* (Maharachchikumbura et al., 2014; 2016; Norphanphoun et al., 2019; Sun et al., 2023), whereas the morphological circumscriptions of these genera remain inconclusive (Liu et al., 2017). At the species level, the combination of multi-locus phylogeny, morphology, and ecology has been employed for determining the interspecific variation (Maharachchikumbura et al., 2014; 2016; Tsai et al., 2018; 2021; Chen et al., 2018; Gu et al., 2022). According to their phenotypic plasticity that largely relies on environmental conditions and poor resolution derived from phylogenetic reconstruction, standard criteria for species delineation are still uncertain (Xiong et al., 2022; Guterres et al., 2023; Hsu et al., 2024). Recent studies recovered several undetermined pestalotioid taxa, most of which were not assigned a species name (Jiang et al., 2021; Tsai et al., 2021; Guterres et al., 2023; Hsu et al., 2024; Wang et al., 2024). During our taxonomic investigation of endophytic fungi associated with *Itea* spp., five *Pestalotiopsis*-like species were characterized using morphology, nucleotide pairwise comparison, and phylogenetic evidence. Two new species (*Neopestalotiopsis iteae* and *Pseudopestalotiopsis iteae*) and three hitherto known species (*Pestalotiopsis jinchangensis*, *N. chrysea*, and *N. haikouensis*) were reported for the first time on this host plant and were discussed accordingly.

The novel species, *Neopestalotiopsis iteae*, is phylogenetically adjacent to *N. cocoës* (Figure 1), but it produces constricted septate and longer conidia than the latter species. We also detected a significant nucleotide difference in *tef1-α* sequence and thus reported it as a new species. Our study pointed out that the phylogenetic placement of *N. iteae* received poor support value (Figure 1). This phenomenon was also observed in previous studies in which the phylograms of *Neopestalotiopsis* had unstable topology and mostly contained weak support (Liu et al., 2017; Tsai et al., 2021; Hsu et al., 2024), whereas a new record of *N. chrysea* was assigned based on the evidence of phylogenetic analyses and nucleotide differences of the sufficient genes. The novel strain (SDBR-CMU516) shared a close phylogenetic relationship with *N. chrysea* and *N. umbrinospora* (Figure 1), and it has overlapped conidia and apical appendage characteristics with *N. chrysea*, whereas *N. umbrinospora* produces umber-colored and comparatively wider conidia. Owing to the low nucleotide difference of ITS, *tub2*, and *tef1-α* sequences (below 1%) found among these strains, we synonymized *N. umbrinospora* under *N. chrysea*. Another new record of *Neopestalotiopsis* was described; the

strain SDBR-CMU517 was phylogenetically located in the same clade with *N. cercidicola*, *N. terricola*, and *N. haikouensis* (Figure 1). The nucleotide comparison of ITS, *tub2*, and *tef1- α* sequence among those strains was insignificant (lower than 1%) (Table 5). Moreover, the strains of *N. terricola* showed high variation of conidial dimensions and color intensities on different host substrates (Table 6). We also observed this phenotypic variation of conidia in the new strain (Figure 5G). Therefore, we designated our strain as *N. haikouensis* and synonymized *N. terricola* and *N. cercidicola* under *N. haikouensis* based on phylogenetic and morphological congruency.

Moreover, a new isolate of *Pestalotiopsis* was reported; the strain SDBR-CMU518 has a close phylogenetic relationship with *P. jinchanghensis* and *P. zhaoqingensis* (Figure 2). The conidial morphology of this isolate is more similar to the ex-type of *P. jinchanghensis*, whose apical appendages are not branched, whereas *P. zhaoqingensis* has branched apical appendages and relatively shorter conidia. Based on their nucleotide differences being not significant (below 1.5%) coupled with phylogenetic data, we considered our new strain to belong to *P. jinchanghensis* and synonymized *P. zhaoqingensis* under *P. jinchanghensis*.

Another new species described in this study is *Pseudopestalotiopsis iteae*, which is characterized by the production of conidia with brown concolorous median cells, constricted at septa with spatulate apical appendage. The novel strains (SDBR-CMU514, SDBR-CMU519, SDBR-CMU520, SDBR-CMU521, SDBR-CMU522, SDBR-CMU523, and SDBR-CMU524) constitute a distinct clade and sister to *Ps. theae*. Furthermore, the nucleotide variation of *tef1- α* among the ex-type strains of *Ps. theae* and *Ps. iteae* was greater than 1.5% (Table 7), validating it as a new species based on the recommendation of Jeewon and Hyde (2016) and Chethana et al. (2021). Notably, some strains of *Ps. iteae* exhibited highly variable conidial shapes and sizes (Figures 8I–K), which were also observed in *Ps. theae* strains.

In summary, the three-locus phylogeny (ITS, *tub2*, and *tef1- α*) does not provide a strong support for species boundaries of pestalotioid fungi. Hence, the morphological characteristics and nucleotide polymorphisms were herein applied to elucidate their interspecific differences. The morphological characteristics of pestalotioid fungi are always similar and difficult to distinguish from each other. Likewise, their morphological characteristics showed a high intraspecific variation under different conditions of fungal growth media, hosts, temperatures, and other environmental factors (personal observation). Thus, it is unreliable to define new pestalotioid species using host, culture, and morphological comparisons. The significant phenotypic and genetic variations among pestalotioid species and strains have been addressed in recent studies (Norphanphoun et al., 2019; Li et al., 2022; Sun et al., 2023; Koodalugodaarachchi et al., 2024), concurring with the present study. Our phylogeny also contained unclear phylogenetic lineages including *Neopestalotiopsis chrysea*, *N. haikouensis*, *Pestalotiopsis jinchanghensis*, *Pseudopestalotiopsis theae*, and *Ps. iteae*, representing them as cryptic species. Therefore, further collections and more cultures are required for the reevaluation of intra- and interspecific relationships among these species.

Moreover, additional informative loci, such as *rpb2*, and whole genome analysis are absolutely needed to evaluate the better taxonomic resolution of this fungal group.

The majority of pestalotioid taxa have been reported as plant pathogens (Maharachchikumbura et al., 2011; 2014; Peng et al., 2022; Giri and Pradhan, 2023; Hsu et al., 2024). Likewise, the species identified in this study—*Neopestalotiopsis chrysea*, *N. haikouensis*, and *Pestalotiopsis jinchanghensis*—were previously documented as causative agents of disease in significant plants (Liu et al., 2017; Shi et al., 2017; Jiang et al., 2021; Wu et al., 2021; Li et al., 2022; Zhang et al., 2022; 2024). Notably, some species exhibited distinct life modes including endophytes, saprobes, and phytopathogens. For example, *N. chrysea* was initially found as saprobes on unknown plants (Maharachchikumbura et al., 2012; 2014) and later reported as pathogens on blueberry and pecan in China (Shi et al., 2017; Wu et al., 2021). *Pestalotiopsis jinchanghensis* was introduced by Liu et al. (2017) as tea pathogen and was recently found as saprobes on unknown plants in China (Dong et al., 2023). Moreover, some species have a broad range of host association; for example, *N. haikouensis* could infect various plant taxa, such as *Castanea mollissima*, *Cercis chinensis*, *Ilex chinensis*, *Paeonia suffruticosa*, and *Olea europaea* (Jiang et al., 2021; Li et al., 2022; Zhang et al., 2022; 2024). These studies emphasize their abilities of lifestyle shift and host adaptation. Since we discovered endophytic pestalotioid fungi from *Itea*, further studies should be conducted to determine their pathogenicity as they may be a potential source of diseases.

To date, few fungi on the *Itea* species have been recorded (Farr et al., 2021), including only two pestalotioid species (*Pestalotiopsis acacia* on *Itea chinensis* var. *oblonga* from China and *P. gracilis* on *I. oldhamii* from Japan). Our study indicates that *Itea* contains a significant diversity of pestalotioid species. Moreover, we have observed that distinct pestalotioid species seem to prefer responsive tissues of *Itea*. Most strains of *Pseudopestalotiopsis* were found on leaves of *I. riparia*. Most *Pestalotiopsis* and *Neopestalotiopsis* species were found on stems of *I. japonica* and *I. riparia*. Only one species of *Pseudopestalotiopsis* and one *Neopestalotiopsis* species were found on roots of *I. riparia*. Our findings enhance the knowledge of taxonomic diversity of pestalotioid fungi in Thailand and highlight that *Itea* spp. could offer a high degree of undetermined fungal diversity. Since *Itea* is a significant source of rare sugar such as D-allulose, future studies are needed to examine the new endophytic fungal strains for the biosynthesis of rare sugar and other secondary metabolites. This would provide useful applications for these novel microbial resources in the pharmaceutical and food industries as well as in sustainable agriculture.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/genbank/> ITS: PQ521226, PQ521227, PQ521228, PQ521229,

PQ521230, PQ521231, PQ521232, PQ521233, PQ521234, PQ521235, PQ521236; tub2: PQ560699, PQ560700, PQ560701, PQ560702, PQ560703, PQ560704, PQ560705, PQ560706, PQ560707, PQ560708, PQ560709; *tefl-α*: PQ529177, PQ529178, PQ529179, PQ529180, PQ529181, PQ529182, PQ529183, PQ529184, PQ529185, PQ529186, PQ529187.

Author contributions

JM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. RP: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. DB: Investigation, Writing – original draft, Writing – review & editing. TE: Investigation, Methodology, Writing – review & editing. JX: Funding acquisition, Supervision, Writing – review & editing. SL: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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EDITED BY

Sinang Hongsanan,
Shenzhen University, China

REVIEWED BY

Wei Dong,
Zhongkai University of Agriculture and
Engineering, China
Satinee Suetrong,
National Biobank of Thailand, Thailand
Yuanpin Xiao,
Guizhou Institute of Technology, China

*CORRESPONDENCE

Napalai Chaiwan

✉ baimainapalaichaiwan@gmail.com;

✉ napalai.c@cmu.ac.th

Itthayakorn Promputtha

✉ ppam118@gmail.com;

✉ itthayakorn.p@cmu.ac.th

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Morphology and molecular phylogeny of *Dothideomycetes* fungi associated with *Dracaena* plants

Napalai Chaiwan^{1,2*}, Kevin David Hyde^{3,4,5},
Ruvishika Shehali Jayawardena^{3,4,5}, Saowaluck Tibpromma⁶,
Dhanushka N. Wanasinghe⁷, Ishara Sandeepani Manawasinghe⁸,
Dimuthu S. Manamgoda⁹ and Itthayakorn Promputtha^{2*}

¹Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand, ²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ³Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand, ⁴Mushroom Research Foundation, School of Science, Chiang Mai, Thailand, ⁵School of Science, Mae Fah Luang University, Chiang Rai, Thailand, ⁶Center for Yunnan Plateau Biology Resources Protection and Utilization, College of Biology and Food Engineering, Qujing Normal University, Qujing, Yunnan, China, ⁷Department of Soil Science, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, ⁸Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Guangzhou, China, ⁹Department of Botany, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Dracaena species are widely recognized for their exceptional drought tolerance, making them ideal candidates for sustainable landscaping and ecological restoration in arid regions. Limestone outcrops hosting *Dracaena* are unique ecosystems characterized by extreme environmental conditions such as nutrient-poor substrates. Thus, they provide valuable opportunities for studying fungal diversity and their adaptations. Despite their ecological importance, knowledge concerning fungal communities associated with limestone-inhabiting *Dracaena* species remains limited, particularly within the diverse biogeographic contexts of Thailand. Microfungal samples were collected from dead wood and leaves of *Dracaena* species across seven provinces in Thailand (Chiang Rai, Kanchanaburi, Krabi, Nakhon Si Thammarat, Ratchaburi, Songkhla, and Tak). Fungal taxa were identified and characterized through detailed morphological examinations combined with multi-gene phylogenetic analyses using Actin (*act*), Internal transcribed spacer (ITS), the large subunit of nuclear ribosomal RNA (LSU), translation elongation fac-tor 1- α (*tef1- α*), and beta-tubulin (*tub*) gene regions. This study documents eleven fungal taxa isolated from *Dracaena* substrates, belonging to seven families across five fungal orders. Three new species *viz.* *Cladosporium dracaenae*, *C. dracaenicola* and *Torula dracaenae* were described, and eight new host records were established (*Bipolaris coffeana*, *Curvularia lunata*, *Lasiodiplodia bruguierae*, *L. lignicola*, *L. thailandica*, *Longididymella clematidis*, *Ochroconis musae* and *Zasmidium citrigriseum*). Species descriptions, color photographic plates, phylogenetic trees and updated taxonomic notes are provided for all

isolated taxa. The findings advance the current understanding of microfungal diversity associated with limestone outcrop habitats and *Dracaena* species, contributing to broader ecological and conservation efforts. By revealing novel fungal species and previously undocumented host-fungus interactions, this study underscores the rich but underexplored fungal biodiversity of limestone ecosystems in Thailand.

KEYWORDS

fungal biodiversity, Dothideomycetes, drought tolerance plant, multi-loci phylogenetic analysis, new fungal taxa

1 Introduction

Dothideomycetes is the largest and most recognized class of ascomycete fungi, renowned for its ecological diversity and significance in taxonomic and biological research (Hongsanan et al., 2020). This class includes a wide array of taxa such as endophytes, epiphytes, saprobes, pathogens of humans and plants, lichens, lichenicolous fungi, nematode-trapping fungi, and rock-inhabiting fungi (Hongsanan et al., 2020; Li et al., 2023). Dothideomycetes encompasses over 32 orders, 191 families, 1,495 genera, and more than 19,000 species (Pem et al., 2024). Characteristically, members of this class possess bitunicate asci with fissitunicate dehiscence and are found on a variety of hosts in terrestrial habitats (Hongsanan et al., 2020; Pem et al., 2024). Recent discoveries of numerous novel species, genera, families, and orders have significantly expanded the classification and understanding of Dothideomycetes.

Dracaena, a genus within the *Agavaceae* family of monocotyledonous plants, includes 198 species (POWO, 2021; van Kleinwee et al., 2022). These plants are distributed across Africa, Madagascar, the Arabian Peninsula, Indian Ocean islands, South Asia, Southeast Asia, Australia, Micronesia, Hawaii, Mexico, Central America, northwestern South America, Cuba, and Macaronesia (Mabberley, 2008). In Europe and Canada, *Dracaena* species are valued for their ornamental and medicinal uses (Almaghrebi et al., 2024). *Dracaena marginata* is a popular houseplant known for its ability to reduce formaldehyde levels in the air (Jaminson, 2012). Despite the fact that this species is an ecologically important plant species, studies on fungi associated with *Dracaena* species are not well established.

Ascomycetes can be found on *Dracaena* plants as pathogens, endophytes, or saprobes (Thongkantha et al., 2008; Crous et al., 2015; Lin et al., 2016; Chaiwan et al., 2019). Notably, *Dracaena* species exhibit a rich fungal diversity, as illustrated by Thongkantha et al. (2008), who documented 127 saprobic fungal records from *Dracaena* and *Pandanus* in Thailand. These taxa comprised 40 Ascomycota and one Basidiomycota, including 86 asexual morphic taxa. Of these documented fungi, eight sexual species and three asexual morphic taxa were newly described species. However, since

2010, investigations into microfungi associated specifically with *Dracaena* have been limited (Ariyawansa et al., 2015; Lin et al., 2016; Chaiwan et al., 2021). Therefore, this study aims to investigate and identify microfungi associated with *Dracaena* species in Thailand by combining morphological characteristics and molecular phylogenetic analyses, providing detailed descriptions and illustrations.

2 Materials and methods

2.1 Sample collection, morphological studies, and isolation

Specimens were collected from *Dracaena* plants in seven provinces: Chiang Rai, Kanchanaburi, Krabi, Nakhon Si Thammarat, Ratchaburi, Songkhla, and Tak of Thailand. Each sample was placed in a separate envelope or plastic bag and taken to the laboratory for observation and fungal isolation. Samples were examined using a Nikon ECLIPSE 80i compound light microscope, and micromorphological structures (fruiting bodies or mycelium from culture) were photographed with a Canon EOS 600D digital camera attached to the microscope. Measurements were conducted using the Tarosoft® Image Framework program. Figures were processed with Adobe Photoshop CS6 Extended version 10.0 (Adobe Systems, USA). Specimens were isolated using single spore techniques (Senanayake et al., 2020). The sample was isolated on potato dextrose agar (PDA) plates and incubated at 25°C. Germinating spores were aseptically transferred to fresh PDA plates and incubated at 25°C. Cultures were grown for 2–4 weeks, during which morphological characteristics such as color, colony appearance, and texture were recorded. The specimens and living cultures were deposited in the Fungarium of Mae Fah Luang University (MFLU) and the Culture Collection of Mae Fah Luang University (MFLUCC) in Chiang Rai, Thailand. Facesoffungi and Index Fungorum numbers were obtained as described by Jayasiri et al. (2015) and Index Fungorum (Index Fungorum, 2025). New taxa were established based on the recommendations outlined by Jeewon and Hyde (2016); Chethana et al. (2021), and Jayawardena et al. (2021).

2.2 DNA extraction, PCR amplification, and sequencing

Spore mass or fruiting bodies or mycelium were used to extract DNA. The Biospin Fungus Genomic DNA Extraction Kit BioFlux (BioFlux®, Hangzhou, China) was used to extract DNA from fruiting bodies or spore mass. PCR amplification was performed using primer pairs, ACT512F/ACT783R for the actin (*act*) gene following the method described by Carbone and Kohn (1999), ITS4/ITS5 for the internal transcribed spacer region of ribosomal DNA (ITS), LR0R/LR5 for large subunit nuclear ribosomal DNA (LSU), *tef1*- α -983F/EF-2218R for translation elongation factor 1- α gene (*tef1*- α), and Bt2a/Bt2b for β -tubulin (*tub*) following the method described by Dissanayake et al. (2020). The genes were amplified using universal primers (Supplementary Table 1). The PCR mixer comprises 1 μ l forward primer, 1 μ l reverse primer, 9.5 μ l distilled deionized (DD) water, and 12.5 μ l mixer. The PCR conditions for ITS, LSU, and *tef1*- α were 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 50 s at 55°C, and 90 s at 72°C, and a final elongation step at 72°C for 10 min. The conditions for *act* were an initial elongation step of 2 min at 95°C, followed by 35 cycles of 45 s at 95°C, 45 s at 55°C, and 1 min at 72°C, and a final elongation step of 10 min at 72°C. Conditions for *tub* were an initial 8 min of 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 1 min at 72°C, and a final elongation step at 72°C for 5 min. The PCR products were purified and sequenced at Shanghai Sangon Biological Engineering Technology and Service Co. The PCR products were purified and sequenced using the same primers for each gene region. The amplification reactions followed the method described by Chaiwan et al. (2019). The quality of PCR products was assessed using 1% agarose gel electrophoresis stained with ethidium bromide. The PCR products were sent for sequencing at Sangon Biotech in Kunming, China.

2.3 Sequence assembly, alignment, and phylogenetic analyses

All sequences obtained from each strain were used for BLAST searches in the nucleotide database of GenBank (<http://blast.ncbi.nlm.nih.gov/>) to determine their closest taxa. The phylogenetic positions of Dothideomycetes isolates were initially assessed using sequence data from *act*, ITS, LSU, *tef1*- α , and *tub* genes. Species-level identifications within each group were then determined using multigene phylogenetic analysis based on individual and combined gene regions. Reference sequences and outgroups for each fungal group were selected from recent literature and GenBank data [<https://blast.ncbi.nlm.nih.gov>] (NCBI, 2025). Individual genomic region datasets were aligned separately using the MAFFT version 7.221 server (<http://mafft.cbrc.jp/alignment/software/>). Phylogenetic trees were inferred using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Maximum parsimony analyses were performed with PAUP version 4.0 b10 (Swofford, 2003), excluding uninformative characters and treating all informative characters as unordered and of equal weight. Goodness-of-fit values, including

tree length (TL), consistency index (CI), retention index (RI), homoplasy index (HI), and rescaled consistency index (RC), were calculated. Statistical support for branches was assessed using maximum parsimony bootstrap (MPBS) analysis with 1,000 replicates (Felsenstein, 1985). Maximum likelihood analysis was performed online using The CIPRES Science Gateway v. 3.3 (<http://www.phylo.org/portal2/>; Miller et al., 2010) and RAxML v. 7.2.8 (Stamatakis et al., 2008) as part of the BRAXML-HPC BlackBox on TG tool were used. Phylogeny website tool BALTER (<http://sing.ei.uvigo.es/ALTER/>) (Glez-Peña et al., 2010) was used to transfer the alignment file for RAxML analysis. All free model parameters were estimated by RAxML and ML estimated 25 per site rate categories. The model selected for ML was GTRGAMMA. The CIPRES Science Gateway v. 3.3 (<http://www.phylo.org/portal2/>; Miller et al., 2010) was used as part of MrBayes on XSEDE on the TG tool. Phylogeny website tool BALTER (<http://sing.ei.uvigo.es/ALTER/>) (Glez-Peña et al., 2010) was used to transfer the alignment file for MrBayes analysis. Phylogenetic trees were visualized and annotated using TreeView (Page, 1996) and formatted using PowerPoint 2010 (Microsoft Corporation, WA, USA).

2.4 Genealogical concordance phylogenetic species recognition analysis

The related species were analyzed using the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) analysis model. This model assesses significant recombinant events to determine recombination levels within closely related species and supports the significance of new species in a clade. The pairwise homoplasy index (PHI) test, which evaluates the concordance and discordance of multiple gene phylogenies due to recombination and mutations, was performed using SplitsTree4, as described by Quaedvlieg et al. (2014). The analysis, conducted with a locus concatenated dataset, identified significant recombination if the PHI value was below the 0.05 threshold ($\Phi_w < 0.05$), indicating that the recombination levels among related species were not different. Conversely, a PHI value above 0.05 ($\Phi_w > 0.05$) suggested no significant recombination, implying differences among related species in the group. The relationships between closely related species were visualized by constructing a split graph using both the LogDet transformation and split decomposition options.

3 Results

Dothideomycetes O.E. Erikss. & Winka

Botryosphaerales C.L. Schoch, Crous & Shoemaker

Botryosphaerales was originally introduced to include accommodate *Botryosphaeriaceae* (Schoch et al., 2009). Subsequently, the order was expanded to encompass six families: *Aplosporellaceae*, *Botryosphaeriaceae*, *Melanopsaceae*, *Phyllostictaceae*, *Planistromellaceae*, and *Saccharataceae* (Phillips et al., 2019; Hongsanan et al., 2020; Wijayawardene et al., 2022).

Botryosphaeriaceae Theiss. & H. Syd.

Wijayawardene et al. (2022) recognized 22 genera within *Botryosphaeriaceae*. Species in this family are diverse, functioning as endophytes, pathogens, and saprobes across a wide range of hosts, including woody plants (Crous et al., 2004; Slippers and Wingfield, 2007; Mehl et al., 2013; Trakunyingcharoen et al., 2014; Doilom et al., 2016; Manawasinghe et al., 2016). They are reported on numerous hosts and have a broad geographical distribution (Chethana et al., 2016). The family includes over 100 species and 22 genera (Hongsanant et al., 2020), with several species being significant pathogens of economically important crops, including commercial plantation tree species (Jayawardena et al., 2019). Notably, species of *Lasiodiplodia* are well-known dieback pathogens affecting woody hosts globally (Manawasinghe et al., 2016). For example, *Lasiodiplodia theobromae* causes dieback and wood discoloration in various tropical plants, such as *Acacia mangium* in Indonesia (Slippers and Wingfield, 2007; Jayawardena et al., 2020). However, there is currently no information on *Botryosphaeriaceae* affecting *Dracaena* sp. In this study, we introduce three new host records from *Dracaena* sp. from Thailand.

Lasiodiplodia Ellis & Everh.

Lasiodiplodia is a widespread genus belonging to *Botryosphaeriaceae* (Alves et al., 2008; Phillips et al., 2019; Wijayawardene et al., 2022). These species have been recorded from many hosts, reporting as plant pathogens (Abdollahzadeh et al., 2010) causing fruit rot, stem-end rot, and dieback on a wide range of hosts. Species of this genus are reported as endophytes (Slippers and Wingfield, 2007; Chen et al., 2015) and saprobes (Abdollahzadeh et al., 2010; Liu et al., 2012; Dissanayake et al., 2016; Hyde et al., 2019) as well. In the present study, phylogenetic analysis of *Lasiodiplodia* was based on combined ITS and *tef1-α* sequence data of selected *Botryosphaeriaceae* isolates. Related sequences were obtained from GenBank. There were 141 taxa included in the analyses, which comprise 1,691 characters including gaps. The tree is rooted in *Diplodia seriata* and *Diplodia mutila* best-scoring RAxML tree with a final likelihood value of $-5,535.293762$. The matrix had 472 distinct alignment patterns, with 50.17% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.215775, C = 0.287336, G = 0.258654, T = 0.238234; substitution rates AC = 1.014675, AG = 4.025490, AT = 1.480709, CG = 1.199950, CT = 5.465426, GT = 1.000000; gamma distribution shape parameter α = 0.223674. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.02 changes. The isolates obtained in this study are in red, and ex-type taxa are in black bold (Supplementary Figure 1). Three collections from our study clustered with *Lasiodiplodia bruguierae*, *L. lignicola*, and *L. thailandica*.

Lasiodiplodia bruguierae J.A. Osorio, Jol. Roux & Z.W. de Beer, in Osorio, Crous, De Beer, Wingfield & Roux, Fungal Biology 121(4): 374 (2016) (Supplementary Figure 2)

Index Fungorum number: IF188476

Saprobic on dead leaves of *Dracaena fragrans*. Sexual morph: undetermined. Asexual morph: *Conidiomata* 150–200 $\times$ 146–200 μm ($\bar{x}$ = 175 $\times$ 173 μm ; n = 10), pycnidial, semi-immersed, unilocular, solitary, scattered, globose or subglobose, dark brown.

Conidiomatal wall 20–30 μm wide, outer layers dark brown to black, thick-walled, inner layer thin-walled, pale brown to hyaline, comprising two to three layers of dark- brown cells of *textura angularis*. *Paraphyses* up to 40 μm long, 2–3 μm wide, hyaline, septate, cylindrical, occasionally branched, ends rounded. *Conidiogenous cells* 19–21 $\times$ 3–6 μm ($\bar{x}$ = 20 $\times$ 4.5 μm ; n = 15), holoblastic, hyaline, cylindrical. *Conidia* 20–30 $\times$ 12–13 μm ($\bar{x}$ = 25 $\times$ 12.5 μm ; n = 30), initially hyaline and aseptate when immature, becoming 1-septate at the median, dark brown, thin-walled, ellipsoid to obovoid, base truncate or rounded.

Culture characteristics: Conidia germinating on PDA within 4–5 h. Colonies growing on PDA reaching 45 mm diam. After 1 day at 25°C in the dark, initially whitened in a few days, after 7 days becoming gray, fast growing, raised, fluffy, dense, filamentous, reaching the edge of the petri dish after 2 days.

Material examined: Thailand, Chumphon Province, Phato District, Pang Wan, on dead leaves of *Dracaena fragrans*, 2 September 2017, Napalai Chaiwan, NCCP1, (MFLU 22–0183), living culture MFLUCC 18–1117.

Known distribution: *Bruguiera gymnorhiza* (South Africa) (Osorio et al., 2016; Dou et al., 2017), and dead leaves of *Dracaena fragrans* — (This study).

GenBank accession number: ITS: OM919717, *tef1-α*: OP099910.

Notes: Phylogenetic analyses show that *L. bruguierae* (MFLUCC 18–1117) is closely related to *L. bruguierae* (CMW41470, CMW41614, CMW42480). *Lasiodiplodia bruguierae* was first isolated from asymptomatic branches of *Bruguiera gymnorhiza* in South Africa (Osorio et al., 2017). In comparison of the nucleotide variation in ITS of our collection with those of *L. bruguierae*, 1 out of 594 (0.17%) (position 51 our species showed A but *L. bruguierae* showed T) revealed nucleotide differences, whereas the *tef1-α* nucleotides showed no nucleotide differences. Based on these, we introduce our collection as a new host record from *Dracaena fragrans*.

Lasiodiplodia lignicola (Ariyaw., Jian K. Liu & K.D. Hyde) A.J.L. Phillips, A. Alves & Abdollahz., in Phillips, Alves, Abdollahzadeh, Slippers, Wingfield, Groenewald & Crous, Stud. Mycol. 76: 120 (2013). (Supplementary Figure 3)

Index Fungorum number: IF 559796

Saprobic on dead leaves of *Dracaena fragrans*. Sexual morph: undetermined. Asexual morph: *Conidiomata* 200–250 $\times$ 100–130 μm ($\bar{x}$ = 225 $\times$ 115 μm ; n = 10), pycnidial, semi-immersed, unilocular, solitary, scattered, globose or subglobose, dark brown. *Paraphyses* arising from the onidiogenous layer, initially aseptate becoming up to 1-septate when mature, hyaline, tip rounded unbranched. *Conidiomatal wall* 25–40 μm wide, outer layer dark brown to black, thick-walled, inner layer, pale brown to hyaline, thin-walled comprising two to three layers of dark- brown cells of *Textura angularis*. *Conidiogenous cells* 18–22 $\times$ 6–9 μm ($\bar{x}$ = 20 $\times$ 7.5 μm ; n = 15), holoblastic, hyaline, cylindrical. *Conidia* 10–20 $\times$ 5–10 μm ($\bar{x}$ = 17.5 $\times$ 9.5 μm ; n = 30), initially hyaline and aseptate when immature, becoming medianly 1-euseptate, dark brown, thin-walled, ellipsoid to obovoid, base truncate or rounded, with longitudinal striations from apex to base.

Material examined: Thailand, Krabi Province, on dead leaves of *Dracaena fragrans*, 21 December 2018, Napalai Chaiwan, Krabi1, (MFLU 22-0127).

Known distribution: Dead wood (Thailand), Woody branch (China) (Liu et al., 2012; Wang et al., 2019; Wu et al., 2021), and dead leaves of *Dracaena fragrans* — (This study).

GenBank accession number: ITS: OM919716, *tef1*- α : OP169686

Notes: We were unable to obtain a culture because the spores of the species did not germinate. Phylogenetic analyses show that *L. lignicola* (MFLU 22-0127) is closely related to *L. lignicola* (CBS134112) (Supplementary Figure 1). The morphology of this species resembles *L. lignicola* in having thin-walled paraphyses and holoblastic conidiogenous cells (Liu et al., 2012; Wang et al., 2019). However, the conidia of *L. lignicola* (CBS134112) ($15\text{--}17.5 \times 8\text{--}11 \mu\text{m}$) are larger than our new host record ($10\text{--}20 \times 5\text{--}10 \mu\text{m}$). A comparison of the ITS nucleotides of *L. lignicola* (CBS134112) with those of this new host record revealed 6 out of 631 (0.95%) nucleotide differences. This new host record represents the first reported instance on *Dracaena fragrans* in Thailand.

Lasiodiplodia thailandica T. Trakunyingcharoen, L. Lombard & Crous, Persoonia 34: 95 (2015) (Supplementary Figure 4)

Index Fungorum number: IF510941

Saprobic on dead leaves of *Dracaena fragrans*. Sexual morph: undetermined. Asexual morph: *Conidiomata* $200\text{--}300 \times 150\text{--}250 \mu\text{m}$ ($\bar{x} = 250 \times 200 \mu\text{m}$; $n = 10$), pycnidial, semi-immersed, unilocular, solitary, scattered, globose or subglobose, dark brown. *Conidiomata wall* $50\text{--}65 \mu\text{m}$ wide, outer layers dark brown to black, thick-walled, inner layers thin-walled, pale brown to hyaline, comprising two to three layers of dark- brown cells of *textura angularis*. *Paraphyses* up to $40 \mu\text{m}$ long, $1.5\text{--}2.5 \mu\text{m}$ wide, hyaline, septate, cylindrical, occasionally branched, ends rounded. *Conidiogenous cells* $19\text{--}33 \times 5\text{--}10 \mu\text{m}$ ($\bar{x} = 26 \times 7.5 \mu\text{m}$; $n = 10$), holoblastic, hyaline, cylindrical. *Conidia* $30\text{--}50 \times 10\text{--}15 \mu\text{m}$ ($\bar{x} = 40 \times 12.5 \mu\text{m}$; $n = 30$), initially hyaline and aseptate when immature, becoming medianly one septate, dark brown, thick-walled, ellipsoid to obovoid, base truncate or rounded, with longitudinal striations from apex to base.

Material examined: Thailand, Kanchanaburi Province, Sangkhla Buri District, on dead leaves of *Dracaena fragrans*, 24 October 2018, Napalai Chaiwan, KAN3 (MFLU 22-0146), *ibid* KLV2 (MFLU 22-0147).

Known distribution: *Acacia confuse*, *Albizia chinensis*, *Magnolia candollii*, *Podocarpus macrophyllus* (China) (Dou et al., 2017; de Silva et al., 2019), *Mangifera indica* (Thailand) (Trakunyingcharoen et al., 2014) and dead leaves of *Dracaena fragrans* — (This study).

GenBank accession numbers: KAN3; ITS: OM919715. KLV2; ITS: ON000546, *tef1*- α : 269 OP099911.

Notes: The morphology of this collection is similar to *L. pseudotheobromae* (Gomdola et al., 2020), although it features septate paraphyses and slightly larger conidia ($22\text{--}33 \times 13\text{--}15 \mu\text{m}$ vs. $30\text{--}50 \times 10\text{--}15 \mu\text{m}$) (Gomdola et al., 2020; Pipattanakuldee et al., 2019). The phylogenetic analyses (Supplementary Figure 1) show that our isolates cluster with the ex-type strain of

L. thailandica. *Lasiodiplodia thailandica* was first described from symptomless twigs of *Mangifera indica* in Thailand (Trakunyingcharoen et al., 2014) and has also been reported on *Albizia chinensis*, *Podocarpus macrophyllus* (Dou et al., 2017), and *Magnolia candollii* (de Silva et al., 2019). Based on these, we introduce our collection as *L. thailandica* as a new host record from *Dracaena fragrans* from Thailand.

Cladosporiales Abdollahz. & Crous

Cladosporiales was introduced based on phylogenetic analyses by Abdollahzadeh et al. (2020), with *Cladosporiaceae* as the only family in this order. Initially, *Cladosporiaceae* was classified under Capnodiales but was later reassigned to *Cladosporiales* based on subsequent phylogenetic analyses (Abdollahzadeh et al., 2020; Hongsanant et al., 2020; Wijayawardene et al., 2022). Members of this order are commonly found as saprobic, endophytic, fungicolous, lichenicolous, and can act as pathogens in humans and plants (Torres-Cortés et al., 2015; Wijayawardene et al., 2022).

Cladosporiaceae Chalm. & R.G. Archibald

Hyde et al. (2024) accepted eight genera in *Cladosporiaceae*: *Acroconidiella*, *Cladosporium*, *Davidiellomyces*, *Graphiopsis*, *Neocladosporium*, *Rachicladosporium*, *Toxicocladosporium* and *Verrucocladosporium*. They are reported on a wide range of hosts as endophytes, pathogens, and saprobes (Sandoval-Denis et al., 2016; Jayawardena et al., 2020). Members of *Cladosporiaceae* are commonly found on soil, food, paint, textiles, and other organic matters or colonize as secondary invaders leaf lesions caused by plant pathogenic fungi and common fungal components isolated from the air (Bensch et al., 2010).

Cladosporium Link

Cladosporium is a cosmopolitan genus isolated from diverse environments, including soil, food, paint, textiles, and plant pathogenic fungi, and is known to cause allergies and diseases in plants and animals (Bensch et al., 2012). It is the most common fungal component isolated from the air (French, 1989; Flannigan and Wotton, 2001). The small conidia of *Cladosporium* are typically formed in branched chains, facilitating their dispersion through the air. The *Cladosporium* species complex includes three major groups: *C. herbarum*, *C. cladosporioides*, and *C. sphaerospermum* (Bensch et al., 2012). The *C. sphaerospermum* complex is characterized by globose or subglobose, pigmented, almost smooth to verrucose terminal conidia and 0–3-septate, smooth, or verrucose ramoconidia (Ellis and Yates, 1971; Zalar et al., 2007). The *C. cladosporioides* complex exhibits significant variability, with conidia ranging from smooth or nearly so to irregularly verrucose, verrucose, or rough-walled. In contrast, all species within the *C. herbarum* complex have ornamented conidia with features ranging from minutely verrucose to verrucose, echinulate, or spiny (Torres-Cortés et al., 2015). In this study, we introduce one new species and two new host records within the *C. cladosporioides* species complex from Thailand. Phylogenetic analyses of *Cladosporium* are based on combined ITS, *tef1*- α , and *act* sequence data of selected *Cladosporiaceae* isolates. Related sequences were obtained from GenBank. There are 114 taxa included in the analyses, which comprise 2,745 characters including gaps. The tree is rooted in *Cladosporium herbarum*. The best-scoring RAxML tree with a final likelihood value of $-22,003.238457$ is presented. The matrix

had 1,285 distinct alignment patterns, with 60.03% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.233071, C = 0.288405, G = 0.248984, T = 0.229540; substitution rates AC = 1.517403, AG = 3.134825, AT = 1.668455, CG = 1.130508, CT = 4.683471, GT = 1.000000; gamma distribution shape parameter α = 0.426819. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.2 changes. The isolates obtained in this study are in red, and ex-types taxa are in black bold (Supplementary Figure 5). In this analysis, *Cladosporium dracaenae* isolates from our collections clustered with *Cladosporium dracaenicola* and *Cladosporium tenuissimum*. To confirm the results from phylogenetic analyses, the PHI test was done. Results of the pairwise homoplasy index (PHI) test of *Cladosporium dracaenae*, *Cladosporium dracaenicola*, and closely related species were determined using both LogDet transformation and split decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset. The new taxon is in red bold type. P=1 (Supplementary Figure 6). 328

Cladosporium dracaenae Chaiwan & Jayaward, sp. nov. (Supplementary Figure 7)

Index Fungorum number: IF 559789 Facesoffungi number: FoF10748

Etymology: Referring to the host genus on which the fungus was collected, *Dracaena* (Asparagaceae).

Holotype: MFLU 22-0185

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: undetermined. Asexual morph: on PDA. *Mycelium* 2–4 μm wide ($\bar{x}$ = 3.2 μm ; n = 30), hyphae branched, septate, often appearing somewhat darkened and not constrictions, irregular, hyaline to pale brown or pale olivaceous-brown, smooth, minutely verruculose, thin walls. *Conidiophores* 20–70 $\times$ 2–3 μm ($\bar{x}$ = 45 $\times$ 2.5 μm ; n = 30), macronematous, arising laterally or terminally from hyphae, erect, straight to slightly flexuous, filiform to narrowly cylindrical 0–3-septate, septa often appearing darkened, sometimes pluriseptate with septa in short succession, especially toward the apex, septa not constricted, pale olivaceous-brown, smooth to minutely verruculose, thin walls or almost so, sometimes forming ramoconidia and fragments., unbranched or branched, brown occasionally slightly geniculate, non-nodulose. *Conidiogenous cells* undifferentiated or conidiophores reduced to conidiogenous cells, cylindrical, 4–18 μm long, usually neither geniculate nor nodulose, with a single or up to three protuberant, subdenticulate, thickened, and darkened. *Conidiogenous scars* thickened and conspicuous, protuberant. *Ramoconidia* rarely formed. A branch of a conidiophore at the base of branches having scar, having a truncate or slightly convex, to detached conidiogenous cells or short, fertile, terminal branches, and reclassified branched conidia. *Conidia* 12.5–16.5 $\times$ 2.5–3.0 μm ($\bar{x}$ = 20 $\times$ 2.5 μm ; n = 30), catenate, in branched chains, conidial chains branching in all directions, terminal chains with up to nine conidia, small terminal conidia globose or subglobose, hyaline to dark brown, narrower at both ends, straight, guttulate.

Culture characteristics: Colonies on PDA reaching 20–40 mm diam after 2 weeks, olivaceous- gray brown to black, reverse olivaceous- gray to leaden- gray or olivaceous-black, velvety, powdery to felty-woolly, regular, glabrous or feathery, aerial

mycelium absent or sparse, growth flat with a somewhat elevated colony center, without prominent exudates, sporulation profuse.

Material examined: Thailand, Chiang Rai Province, dead leaves of *Dracaena* sp. (Asparagaceae), 17 November 2017, Napalai Chaiwan, NCCR2, (MFLU 22-0185, holotype), ex-type living culture MFLUCC 18-0919.

GenBank accession numbers: NCCR2; ITS: OM908927, *tef1*- α : OP099913

Notes: In the phylogenetic analysis, our isolates form a monophyletic clade that is a sister clade to *Cladosporium tenuissimum* (Supplementary Figure 5). Morphologically, this collection is similar to *Cladosporium tenuissimum* (in *Cladosporium cladosporioides* complex) (Bensch et al., 2010). Base pair comparison of ITS with *C. tenuissimum* isolate XCWN2) shows 90 out of 515 (17.47%) differences, and that in *tef1*- α (with *Cladosporium* sp. isolate CW_T3L3C12.1) shows 8 out of 1230 (0.65%) differences, indicating that this is a distinct taxon (Jeewon and Hyde, 2016; Chethana et al., 2021; Jayawardena et al., 2021). Consequently, we identify this new species as *C. dracaenae*, reported from *Dracaena* spp. in Thailand. 367

Cladosporium dracaenicola Chaiwan & Jayaward, sp. nov. (Supplementary Figure 8)

Index Fungorum number: IF559789 Facesoffungi number: FoF10748

Etymology: Referring to the host genus on which the fungus was collected, *Dracaena* (Asparagaceae).

Holotype: MFLU 22-0186

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: undetermined. Asexual morph: on PDA. *Mycelium* 2–4 μm wide ($\bar{x}$ = 3.2 μm ; n = 30), hyphae branched, septate, often appearing somewhat darkened and not constrictions, irregular, hyaline to pale brown or pale olivaceous-brown, smooth, minutely verruculose, thin walls. *Conidiophores* 50–80 $\times$ 2–3 μm ($\bar{x}$ = 65 $\times$ 2.5 μm ; n = 30), macronematous, arising laterally or terminally from hyphae, erect, straight to slightly flexuous, filiform to narrowly cylindrical 0–3-septate, septa often appearing darkened, sometimes pluriseptate with septa in short succession, especially toward the apex, septa not constricted, pale olivaceous-brown, smooth to minutely verruculose, thin walls or almost so, sometimes forming ramoconidia and fragments., unbranched or branched, brown occasionally slightly geniculate, non-nodulose. *Conidiogenous cells* undifferentiated or conidiophores reduced to conidiogenous cells, cylindrical, 4–18 μm long, usually neither geniculate nor nodulose, with a single or up to three protuberant, subdenticulate, thickened, and darkened. *Conidiogenous scars* thickened and conspicuous, protuberant. *Ramoconidia* rarely formed. *Ramoconidia* 6–12 $\times$ 2–3 μm ($\bar{x}$ = 8.5 $\times$ 2.5 μm ; n = 30), a branch of a conidiophore at the base of branches having scar, having a truncate or slightly convex, to detached conidiogenous cells or short, fertile, terminal branches, and reclassified branched conidia. *Conidia* 5–6 $\times$ 2.5–3 μm ($\bar{x}$ = 5.5 $\times$ 2.5 μm ; n = 30), catenate, in branched chains, conidial chains branching in all directions, terminal chains with up to nine conidia, small terminal conidia globose or subglobose, hyaline to dark brown, narrower at both ends, straight, guttulate.

Culture characteristics: Colonies on PDA reaching 20–40 mm diam after 2 weeks, olivaceous, gray olivaceous or olivaceous-gray, reverse olivaceous- gray to leaden- gray or olivaceous-black, velvety, powdery to felty-wooly, margins white, regular, glabrous or feathery, aerial mycelium absent or sparse, growth flat with a somewhat elevated colony center, without prominent exudates, sporulation profuse.

Material examined: Thailand, Songkhla Province, Hat Yai District, dead leaves of *Dracaena* sp. (*Asparagaceae*), 5 September 2018, Napalai Chaiwan, TNC6, (MFLU 22–0186, holotype), ex-type living culture MFLUCC 18–0915.

GenBank accession numbers: TNC6; ITS: OM908928.

Notes: In the phylogenetic analysis, our isolates formed a monophyletic clade and are a sister clade to *Cladosporium tenuissimum* (Supplementary Figure 5). Morphologically, this collection resembles *Cladosporium cladosporioides* (within the *Cladosporium cladosporioides* complex) (Bensch et al., 2010). Comparison of ITS nucleotides with *C. halotolerans* (CBS127371) reveals 8 out of 594 (1.3%) nucleotide differences, whereas comparison with *C. dracaenae* (MFLU 22-0185) shows 18 out of 594 (3%) nucleotide differences, indicating that they are distinct taxa. Our species showed conidiophores ($65 \times 2.5 \mu\text{m}$) smaller than *Cladosporium tenuissimum* up to ($310 \times 460 \mu\text{m}$). Also, *Ramoconidia* (8.5×2.5) our strain smaller than *Cladosporium tenuissimum* ($22 \times 41 \mu\text{m}$). Consequently, we identify the new species as *C. dracaenicola*, which has been reported from *Dracaena* sp. in Thailand.

Mycosphaerellales (Nannf.) P.F. Cannon.

Mycosphaerellales was introduced based on phylogenetic analyses by Schoch et al. (2006), Hyde et al. (2024) recognized 11 families within Mycosphaerellales (*Aeminiaceae*, *Cystocoleaceae*, *Dissoconiaceae*, *Extremaceae*, *Mycosphaerellaceae*, *Neodevriesiaceae*, *Phaeothecoidiaceae*, *Phillipsiaceae*, *Schizothyriaceae*, *Teratosphaeriaceae*, and *Xenodevriesiaceae*) (Norimova et al., 2024; Piątek et al., 2024; Hyde et al., 2024). Mostly plant-associated fungi, with a significant number being plant pathogens, endophytes, or saprobes (Norimova et al., 2024; Piątek et al., 2024).

Mycosphaerellaceae Lindau

Wijayawardene et al. (2022) recognized 119 genera within *Mycosphaerellaceae*. Hongsanan et al. (2020) listed 106 genera as doubtful within *Mycosphaerellaceae*, based on the work of Videira et al. (2017).

Zasmidium Fr.

Zasmidium is a genus within *Mycosphaerellaceae*, established by Fries in (1849). *Zasmidium cellare* is the type species of this genus (Zhao et al., 2016; Arzanlou et al., 2007). Species of *Zasmidium* have been reported from various plant hosts (Shivas et al., 2010; Crous et al., 2014; Quaedylied et al., 2014). In this study, we report a new record of *Zasmidium citrigriseum* from Thailand. For *Zasmidium*, the phylogram was generated from RAXML analysis based on combined LSU, ITS, and *act* sequence data of selected *Zasmidium* isolates. Related sequences were obtained from GenBank. There are 27 taxa included in the analyses, which comprise 2,044 characters including gaps. The tree is rooted in *Pseudozasmidium eucalypti* (CBS121101). The best-scoring RAXML tree with a final likelihood

value of $-6,614.766180$ is presented. The matrix had 454 distinct alignment patterns, with 33.32% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.232177, C = 0.263299, G = 0.287808, T = 0.216717; substitution rates AC = 1.575049, AG = 2.045497, AT = 1.643817, CG = 1.149239, CT = 5.459635, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.144082$. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.04 changes. The isolates obtained in this study are in red, and ex-types taxa are in black bold (Supplementary Figure 9). In this analysis, our collection clustered with 83% ML and 0.93 PP of *Zasmidium citrigriseum*.

Zasmidium citrigriseum (F.E. Fisher) U. Braun & Crous [as “citri-griseum”], IMA Fungus 5(2): 337–442 (2014) (Supplementary Figure 10)

Index Fungorum number: IF 622149

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: see Crous et al. (2014). Asexual morph: *Mycelium* external, smooth, septate, branched, with wide hyphae. Conidiophores micronematous, arising from superficial mycelium, solitary, erect, straight to slightly curved, branched laterally or unbranched, medium to dark brown, slightly wider at the base. *Conidiogenous cells* terminal, cylindrical to subcylindrical, tapering to a flattened apical region, smooth to finely verruculose, medium brown, measuring $20 \times 60 \mu\text{m}$; scars thickened, somewhat darkened, 2–2.5 μm wide. *Conidia* broadly fusiform to obovoid, 0–1-septate, pale olivaceous, paler toward the apex; terminal conidia ovoid, 0-septate, pale olivaceous to hyaline, paler toward the apex, with a truncate base extending up to 5 μm wide on each side, tapering toward the polar ends.

Culture characteristics: Colonies on PDA reaching 2–4 cm diameter after 2 weeks. Young colonies are olivaceous-brown, darkening with age. The reverse is olivaceous-brown to leaden-gray or olivaceous-black. Texture ranges from velvety and powdery to felty-wooly.

Material examined: Thailand, Nakhon Si Thammarat Province, Cha-uat District, on dead leaves of *Dracaena* sp., 19 December 2018, Napalai Chaiwan, specimen NSW1 (MFLU 22-0189); living culture MFLUCC 18–0903.

Known distribution: Vietnam, Thailand, USA. Also reported from *Acacia confusa*, *Albizia chinensis*, *Magnolia candollii*, *Podocarpus macrophyllus* (China) (Dou et al., 2017; de Silva et al., 2019), *Mangifera indica* (Thailand) (Trakunyingcharoen et al., 2014), and dead leaves of *Dracaena fragrans* (this study).

GenBank accession numbers: ITS: OM919719, LSU: OM919720, *act*: OP099914

Notes: *Zasmidium citrigriseum* has been reported from various hosts in different countries, including *Musa* in America, *Acacia* in Thailand, *Eucalyptus* in Vietnam, and *Aeglopsis*, *Citrus*, *Fortunella*, *Murraya*, and *Poncirus* in North and South America (Crous et al., 2019; Pretorius et al., 2003). In our study, we identified the same fungus in Thailand on *Dracaena* sp. A BLASTn search of GenBank showed that the LSU sequence had 99.42% similarity with *Zasmidium anthuriicola* (no nucleotide differences in the aligned region), confirming our strain as a new host record of *Zasmidium citrigriseum* from *Dracaena* in Thailand.

Pleosporales Luttr. ex M.E. Barr

Pleosporales is one of the most species-rich orders within the class *Dothideomycetes*, encompassing species that inhabit a wide range of ecosystems, including terrestrial, freshwater, and marine environments (Phookamsak et al., 2014, 2017; Bakhshi et al., 2019; Jones et al., 2019a, b; Luo et al., 2019; Tennakoon et al., 2019). Hongsanan et al. (2020) and Wijayawardene et al. (2022) recognized 91 genera within *Pleosporales*. The family *Paralophiostomataceae* was introduced into this order by Hongsanan et al. (2020).

Didymellaceae Gruyter, Aveskamp & Verkley

Hyde et al. (2024) recognized 44 genera within *Didymellaceae*. Taxa in this family are known to cause plant diseases, including leaf and stem blight, which can lead to plant death (Wanasinghe et al., 2018; Phukhamsakda et al., 2020; Wijayawardene et al., 2022).

Longididymella L.W. Hou, L. Cai & Crou

The type species of *Longididymella*, originally described as *Phoma clematidina* on *Clematis ligusticifolia* (causing necrotic leaf spot), was characterized by both its sexual and asexual morphs (Woudenberg et al., 2009). The new combination *Longididymella clematidis* was synonymized by Hou et al. (2020) from *Anthodidymella clematidis* and proposed as *Didymella clematidis* (CBS 123705) (Phukhamsakda et al., 2020). *Longididymella* species are saprobic or necrotic and commonly occur on flowering and herbaceous plants. Previously, this species was introduced from *Clematis* plants in the USA (Woudenberg et al., 2009; Phukhamsakda et al., 2020). In this study, we isolated the asexual morph of *Longididymella clematidis* from *Dracaena* sp. in Thailand. Phylogenetic analysis of *Longididymella* was generated from RAxML analysis based on combined LSU sequence data of selected *Didymellaceae* isolates. Related sequences were obtained from GenBank. There are 28 taxa are included in the analyses, which comprise 1,354 characters including gaps. The tree is rooted to *Calophoma complanate* (CBS 268.92). The best-scoring RAxML tree with a final likelihood value of $-1,470.814319$ is presented. The matrix had 42 distinct alignment patterns, with 4.19% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.254580, C = 0.209195, G = 0.302369, T = 0.233856; substitution rates AC = 2,406.556015, AG = 21,439.684114, AT = 2,181.215287, CG = 2,016.393030, CT = 45,321.905016, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.020000$. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.002 changes. The isolates obtained in this study are in red and ex-types taxa are in black bold (Supplementary Figure 11). In this analysis, our collections clustered with *Longididymella clematidis* CBS123705.

Longididymella clematidis (Woudenb., Spiers & Gruyter) L.W. Hou, L. Cai & Crous, in Hou, Groenewald, Pfenning, Yarden, Crous & Cai, Stud. Mycol. 96: 339 (2020) (Supplementary Figure 12)

Index Fungorum number: IF833503

Basionym: *Didymella clematidis* Woudenb., Spiers & Gruyter in Woudenberg et al., Persoonia 22:60 (2009)

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 50–400 $\times$ 80–350

μm ($\bar{x} = 225 \times 215 \mu\text{m}$; $n = 5$), pycnidial, solitary, sometimes aggregated, uniloculate, immersed under epidermal layer, subglobose to depressed, coriaceous thin-walled, brown to dark brown, with ostiolate. *Ostioles* not observe. *Conidiomatal wall* 10–30 μm wide, of two to five layers, each cell-layer 10 μm wide, light-brown to brown cells of *textura globulosa*, heavily pigmented in the outer layers, lined with a hyaline innermost layer bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2.5–4.5 $\times$ 1.5–3.5 μm ($\bar{x} = 3.5 \times 2.5 \mu\text{m}$; $n = 20$), phialidic, determinate, discrete, ampulliform, cylindrical to sub-cylindrical, smooth-walled, hyaline, arising from the inner layer of conidioma. *Conidia* 2–3 $\times$ 4–8 μm ($\bar{x} = 2.5 \times 6 \mu\text{m}$; $n = 50$), oblong or oval, slightly curved toward the ends, rounded ends, with 1(–2) guttules in each cell, hyaline, aseptate, smooth-walled.

Culture characteristics: Colonies on PDA reaching 2–4 cm diam after 7 days dark-brown color, reverse dark-brown to black, margins white, regular aerial mycelium, growth flat with a somewhat elevated colony center, without prominent exudates, sporulation profuse.

Material examined: Thailand, Tak Province, Umphang District, on dead leaves of *Dracaena* sp., 21 August 2019, Napalai Chaiwan, Umpangsoil2 (MFLU 22-0187), living culture MFLUCC 22-0099. **Host and Distribution:** *Clematis ligusticifolia* (Woudenberg et al., 2009), *Dracaena* sp. (This study). USA (Woudenberg et al., 2009), Thailand (This study).

GenBank accession numbers: LSU: ON000548.

Notes: *Longididymella clematidis* (MFLU 22-0187) has been previously reported causing leaf spots on *Clematis ligusticifolia* (Woudenberg et al., 2009). Our strain represents a new host record (Phukhamsakda et al., 2020; Woudenberg et al., 2009). A BLASTn search of GenBank revealed that the LSU sequence of our strain has 99.54% similarity ($5/908 = 0.55\%$ nucleotide differences) to *Longididymella clematidis* (CBS123705). Our species is characterized by pseudothecial, globose, subglobose to pyriform ascomata, cylindrical asci with club-shaped bases and hyaline, septate, ovate to obpyriform ascospores. *Conidiomata* pycnidial, solitary, sometimes aggregated, uniloculate, immersed under the epidermal layer, brown to dark brown. Based on these, we identify this strain as a new host record for *Longididymella clematidis*.

Pleosporaceae Nitschke

Hyde et al. (2024) accepted 23 genera in *Pleosporaceae* comprising *Allonecta*, *Alternaria*, *Bipolaris*, *Clathrospora*, *Comoclathris*, *Curvularia*, *Decorospora*, *Diademosia*, *Dichotomophthora*, *Exserohilum*, *Extrawettsteinina*, *Gibbago*, *Johncornia*, *Paradendryphiella*, *Platysporoides*, *Pleoseptum*, *Porocercospora*, *Prathoda*, *Pseudoyuconia*, *Pyrenophora* (= *Mariellottia*), *Tamaricicola*, and *Typhicola*. *Pleosporaceae* taxa occur as saprobes and plant pathogens.

Bipolaris Shoemaker

Bipolaris species are plant pathogens with a global distribution (Hongsanan et al., 2020; Fernandez et al., 2022, 2024). They are commonly reported on a variety of plant diseases, including leaf spots, leaf blights, root rots, and other symptoms in field crops (Manamgoda et al., 2014). *Bipolaris* species are characterized by dematiaceous hyphomycetous fungi that produce pale- brown to

dark- brown asexual conidia and conidiophores. In this study, we present a new record of *Bipolaris* from a different host in Thailand. Phylogenetic analyses of *Bipolaris* were done using RAXML analysis based on combined ITS and *tef1*- α sequence data of selected *Pleosporaceae* isolates. Related sequences were obtained from GenBank. There are 23 taxa included in the analyses, which comprise 1,537 characters including gaps. The tree is rooted to *Curvularia lunata*. The best-scoring RAXML tree with a final likelihood value of $-2,674.255709$ is presented. The matrix had 139 distinct alignment patterns, with 15.20% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.218186, C = 0.246306, G = 0.288802, T = 0.246706; substitution rates AC = 0.763615, AG = 5.761060, AT = 2.722841, CG = 0.695515, CT = 2.038141, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.020000$. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.02 changes. The isolates obtained in this study are in red and ex-type taxa are in black bold (Supplementary Figure 13). In this analysis, our collections clustered with *Bipolaris coffeana* (BRIP14845).

Bipolaris coffeana Sivan., Trans. Br. mycol. Soc. 84(3): 404 (1985) (Supplementary Figure 14)

Index Fungorum number: IF105089

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: Undetermined. Asexual morph: *Vegetative hyphae* septate, subhyaline to brown, branched, smooth, 3–4 μm in width. Colonies on PDA reaching approximately 4–6 cm in diameter after 7 days at 25°C, surface funiculose, margin fimbriate, olivaceous black to olivaceous grey, velvety with sparse aerial mycelium. *Conidiophores* erect, often branched, in most cases uniformly brown, sometimes pale brown at apex, arising singly, septate, flexuous, in most cases geniculate toward the apex, up to 210 μm long, 3–4 μm wide, generally wide in basal parts, tapering in median and upper parts, straight or flexuous, geniculate in apex, frequently unbranched, cells walls thicker than those of the vegetative hyphae, pale brown to brown, basal cells sometimes swollen. *Conidiogenous cells* mostly integrated, terminal, or intercalary with sympodial proliferation, smooth, brown, mono- or polytretic. *Conidia* with bipolar germination, four-celled, smooth-walled or slightly verruculose, asymmetrically swollen and curved at the third cell from base, rarely symmetric swelling and straight, pale to dark brown, end cells paler and thin-walled than central cells, ellipsoidal to clavate to obovoid, asymmetrical with paler end cells, usually curved at the third cell from the base, (80–110 $\times$ 10–18 μm , 3-distoseptate, hila slightly protuberant, thickened and darkened. *Chlamydospores* with verruculose nodes, terminal or intercalary, proliferating sympodially, with circular and thickened scars, brown, cylindrical to swollen.

Culture characteristics: Colonies growing on PDA 4–6 cm diam after 8 d days of incubation at 25°C, circular with filiform margin, dark green to greenish-black, aerial mycelium sparse to moderate, floccose with age; reverse greyish green to brownish black.

Material examined: Thailand, Chiang Rai Province, Mea Lao district, on dead leaves of *Dracaena* sp., 17 November 2017, Napalai Chaiwan, DNC1.1 (living culture MFLUCC 17–2600).

Host and distribution: *Bouteloua gracilis*, *Coffea arabica* (Kenya) (Tan et al., 2014), *Cynodon dactylon* (New Zealand) (Manamgoda et al., 2012), *Digitaria* sp. (Poaceae) (USDA fungal host data base) —*Dracaena fragrans* Thailand, USA (Jeon et al., 2015, This study).

GenBank accession numbers: ITS: OP090572, *tef1*- α : OP099915.

Notes: *Bipolaris coffeana* has been reported from *Coffea arabica* in Kenya (Tan et al., 2014) and also from *Bouteloua gracilis* and *Cynodon dactylon*. The combined phylogenetic analysis of the *Bipolaris* generic complex in this study shows that our strain has high bootstrap support with *Bipolaris coffeana*. This section includes descriptions and illustrations of the new host record based on molecular and morphological data (Ferdinandez et al., 2022). This species is characterized by fusiform shaped conidia, central cells not much darker but broader than the distal ones. This is the first report of *Bipolaris coffeana* isolated from *Dracaena fragrans* in Thailand.

Curvularia Boedijn

Curvularia species are significant pathogens reported globally. They have been identified as plant pathogenic fungi and have also been associated with animal and human diseases (Kusai et al., 2016). Previously, the classification of *Curvularia* species was unclear due to a lack of molecular data, and the genus exhibits overlapping morphological characteristics with *Bipolaris* (Manamgoda et al., 2012). In this study, we present a new host record for *Curvularia lunata* from Thailand. Phylogenetic analyses of *Curvularia* were based on ITS sequence data of selected *Pleosporaceae* isolates. Related sequences were obtained from GenBank. There are 92 taxa included in the analyses, which comprise 694 characters including gaps. The tree is rooted with *Cochliobolus nisikadoi*. The best-scoring RAXML tree with a final likelihood value of $-4,650.452400$ is presented. The matrix had 358 distinct alignment patterns, with 26.96% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.236630, C = 0.245701, G = 0.229962, T = 0.287707; substitution rates AC = 1.587196, AG = 2.075234, AT = 1.262201, CG = 1.663658, CT = 3.666320, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.245992$. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.03 changes. The isolates obtained in this study are in red and ex-type taxa are in black bold (Supplementary Figure 15). In this analysis, our collections clustered with 88% ML and 1 PP with *Curvularia lunata* (CBS730.96).

Curvularia lunata (Wakker) Boedijn, Bull. Jard. bot. Buitenz, 3 Sér. 13(1): 127 (1933) (Supplementary Figure 16)

Index Fungorum number: IF269889

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: Undetermined. Asexual morph: *Conidiophores* 155–160 $\times$ 60–70 μm ($\bar{x}$ = 157 $\times$ 65 μm ; n = 10), arising occasionally singly or usually in groups, branched, septate, straight, geniculate at upper part, brown to pale reddish, lighter toward apex. *Conidiogenous nodes* dark brown, smooth. *Conidia* 159–165 $\times$ 70–75 μm ($\bar{x}$ = 162 $\times$ 72 μm ; n = 30), smooth-walled, straight or curved, subcylindrical to

fusoid, sometimes narrowly clavate, tapering toward rounded apex, basal cell obconic, pale brown to mid reddish brown, 3–4-distoseptate. *Hilum* inconspicuous, germination bipolar.

Culture characteristics: Colonies growing on PDA 4–6 cm diam after 8 d days of incubation at 25°C, circular with filiform margin, white or cream, aerial mycelium sparse to moderate, floccose with age; reverse brownish to brown.

Material examined: Thailand, Kanchanaburi Province, on dead leaves of *Dracaena* sp., 27 June 2019, Napalai Chaiwan, KAN20 (living culture MFLUCC 22-0076).

Host and Distribution: *Adhatoda* sp., *Agave sisalana*, *Agrostis palustris*, *Allium cepa*, *Allium sativum*, *Alnus rubra*, *Aloe vera*, *Amaranthus spinosus*, *Anacardium occidentale*, *Ananas comosus*, *Andropogon sorghum* var. *sudanensis*, *Antheophora hermaphrodita*, *Arachis hypogaea*, *Areca catechu*, *Artocarpus integra*, *Axonopus affinis*, *Bambusa vulgaris*, *Boehmeria nivea*, *Brachiaria mutica*, *Brassica rapa* subsp. *pekinensis*, *Cajanus cajan*, *Capsicum annuum* var. *annuum*, *Capsicum frutescens*, *Citrus reticulata*, *Dactyloctenium aegyptium*, *Dendrocalamus strictus*, *Digitaria ischaemum*, *Echinochloa colona*, *Eleusine indica*, *Eucalyptus globulus*, *Fragaria ananassa*, *Gladiolus hortulanus*, *Halophila ovalis*, *Helianthus annuus*, *Impatiens sultanii*, *Ipomoea batatas*, *Legenaria vulgaris*, *Linum usitatissimum*, *Lolium multiflorum*, *Mangifera indica*, *Rosa* sp., *Zea mays* (Farr and Rossman, 2025) —of *Dracaena* sp., (This study). Australia, Brazil, California, China, Cuba, Hong Kong, India, Indonesia, Malaysia, Mauritius, Myanmar, Thailand, USA, and Virginia (Farr and Rossman, 2024).

GenBank accession numbers: ITS: OP090556.

Notes: *Curvularia lunata* is the type species of the genus (Boedijn, 1933). It was originally reported from *Saccharum officinarum* (sugar cane) in Java and is also known to cause leaf spots on *Clerodendrum indicum*, an important medicinal plant (Mukherjee et al., 2013), as well as on *Zea mays*, leading to significant yield losses (Li et al., 2006; Manamgoda et al., 2012). There have been reports of *Curvularia lunata* from unknown grasses in Thailand (Manamgoda et al., 2014; Tan et al., 2016). In this study, we isolated *Curvularia lunata* (KAN20) from *Dracaena* sp., a plant substrate in Thailand in 2018. This culture is undergoing sporulation. Many strains of *C. lunata* are misidentified in GenBank (Cai et al., 2011), and accurate identification of the type sequences for *Curvularia* species has been recently reported by Bhunjun et al. (2020).

Torulaceae Nitschke

In 1794, Persoon introduced the genus *Torula* with *T. herbarum* as the type species (Persoon, 1794). Members of *Torulaceae* are commonly found as saprobes on various terrestrial plants and submerged decaying wood in rivers or streams (Su et al., 2018). *Torulaceae* species reported only in their asexual morphs, characterized by micro- or macronematous conidiophores that are erect from the host or aerial, and connected to doliform or ellipsoid, brown, smooth to verruculose conidia (Yang et al., 2022). There are seven genera associated with this family (Wijayawardene et al., 2022).

Torula Pers.

The type species of this genus is *Torula herbarum* was designated from a neotype (CPC 24114) (Crous et al., 2015).

The members of *Torula* are mainly characterized by terminal or lateral, monoblastic, or polyblastic conidiogenous cells which have a basally thickened and heavily melanized wall, with the apex thin-walled and frequently collapsing and becoming coronate (Crane and Miller, 2016). *Torula* has over 500 epithets in Index Fungorum (2025). In the present study, we introduce a new species of *Torula* from dead leaves of *Dracaena* sp. from Thailand. Phylogenetic analyses of *Torulaceae* was based on combined ITS, LSU, SSU, and *rpb2* sequence data of selected *Torulaceae* isolates. Related sequences were obtained from GenBank. There are 44 taxa included in the analyses, which comprise 864 characters including gaps. The tree is rooted to *Dendryphion europaeum* (CPC23231). The best-scoring RAXML tree with a final likelihood value of $-7,716.685771$ is presented. The matrix had 517 distinct alignment patterns, with 31.01% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.249849, C = 0.244700, G = 0.278439, T = 0.227012; substitution rates AC = 2.770901, AG = 3.096398, AT = 2.134703, CG = 1.838546, CT = 7.075441, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.312119$. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.03 changes. The isolates obtained in this study are in red and ex-type taxa are in black bold (Supplementary Figure 17).

In this analysis, our collections clustered with 100% ML and 1 PP of *T. breviconidiophora* KUMCC 18–0130 and 70% ML and 1.00 PP of *T. chromolaenae* (KUMCC 16-0036). This analysis was further confirmed with PHI test. Results of the pairwise homoplasy index (PHI) test of *Torula dracaenae* and closely related species using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset. The new taxon is in red bold type. $P=0.1959$ (Supplementary Figure 18). Based on these, we introduce our collection as a new species *Dracaena* sp.

Torula dracaenae Chaiwan & Jayaward, sp. nov. (Supplementary Figure 19).

Index Fungorum number: IF559437 Facesoffungi number: FoF10752

Etymology: Referring to the host genus from which the fungus was collected: *Dracaena* (Asparagaceae).

Holotype: MFLU 22-0135

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: undetermined. Asexual morph: Colonies effuse on host, black, powdery. Mycelium partly immersed, composed of septate, branched, smooth, pale- brown hyphae. Conidiophores (2.8–)3–4.3 $\times$ 2.5–3 μm ($\bar{x} = 3.8 \times 2.8 \mu\text{m}$; $n = 10$), macronematous, mononematous, solitary, erect, pale brown, verruculose, thick-walled, consisting of one to two cells or reduced to conidiogenous cells, subcylindrical to subglobose, arising from prostrate hypha. Conidiogenous cells (3–)3.2–3.5 $\times$ 3.8–4.6 μm ($\bar{x} = 3.4 \times 4.1 \mu\text{m}$; $n = 10$), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, doliform to ellipsoid. Conidia 10–15 $\times$ 3.0–4.5 μm ($\bar{x} = 13 \times 3 \mu\text{m}$; $n = 20$) solitary to catenate, acrogenous, simple, phragmosporous, dark brown, with apical cell pale brown, minutely verruculose, 3–10-septate, rounded at both ends,

composed of subglobose cells, slightly constricted at some septa, chiefly subcylindrical, rough-walled.

Material examined: Thailand, Kanchanaburi Province, on dead stems of *Dracaena* sp., 20 March 2017, Napalai Chaiwan, KAN2, (MFLU 22-0135, holotype).

GenBank accession numbers: LSU: OM911934; ITS: OM911931.

Notes: Phylogenetic analysis of our collection grouped it with *Torula chromolaenae* and *T. mackenziei*, showing a close relationship to *T. breviconiophora* (Supplementary Figure 17). Our strain clustered with *T. breviconiophora* with strong bootstrap support (100% ML, 1 PP, Supplementary Figure 17). Morphologically, our collection differs from *T. mackenziei* and *T. breviconiophora* but is similar to *T. chromolaenae*. Its conidiophores ($3.8 \times 2.8 \mu\text{m}$) and conidiogenous cells ($3.4 \times 4.1 \mu\text{m}$) are smaller than those of *T. chromolaenae* ($5.8 \times 4 \mu\text{m}$ and $4.7 \times 5.4 \mu\text{m}$, respectively). Additionally, its conidia ($13 \times 3 \mu\text{m}$) are smaller than those of *T. chromolaenae* ($14.5 \times 4.3 \mu\text{m}$). We were unable to obtain a culture as the spores did not germinate. A BLASTn search of GenBank showed that the ITS sequence of our strain has 98.45% similarity to *T. chromolaenae*. The ITS nucleotides reveal 12/729 (1.64%) differences, LSU nucleotides show 1/978 (0.1%) differences, and the SSU nucleotides show 64/1090 (5.87%) differences indicating that they are distinct taxa (Jeewon and Hyde, 2016; Pem et al., 2024). The PHI test for closely related species showed a P-value of 0.2078, indicating no significant evidence of recombination. Based on the phylogenetic support, we introduce our isolate as a new species from *Dracaena* sp. in Thailand.

Venturiales Y. Zhang ter, C.L. Schoch & K.D. Hyde

Wijayawardene et al. (2022) recognized three families within the order Venturiales: *Cylindrosympodiaceae*, *Sympoventuriaceae*, and *Venturiaceae*. Shen et al. (2020) introduced *Cylindrosympodiaceae* with eight genera: *Bellamyces*, *Fagicola*, *Fraxinicola*, *Neofusicladium*, *Parafusicladium*, *Fuscohilum*, *Pinaceicola*, and *Sterila*. However, Wijayawardene et al. (2022) included only *Cylindrosympodium*, *Pseudoanungitea*, *Septonema*, *Sympodiella*, and *Tothia* in this family.

Sympoventuriaceae Y. Zhang ter, C.L. Schoch & K.D. Hyde

Wijayawardene et al. (2022) recognized 17 genera in *Sympoventuriaceae*. Members of *Sympoventuriaceae* are often opportunistic pathogens of vertebrates in natural habitats (Dwivedi, 1959; Barron and Busch, 1962; Hoog et al., 2000) and have also been implicated in human infections (Salkin et al., 1994; Padhye et al., 1994; Singh et al., 2006; Yarita et al., 2007; Ge et al., 2009).

Ochroconis de Hoog & Arx

Wijayawardene et al. (2022) recognized 28 species in the genus *Ochroconis*, with *Ochroconis constricta* serving as the type species. These species are characterized by sympodial conidiogenesis and septate, ellipsoidal conidia that are released rhexolytically. In this study, we report a new record of *Ochroconis musae* from Thailand. Phylogenetic analysis of *Ochroconis* was based on combined LSU, ITS, and *tub* sequence data of selected *Didymellaceae* isolates. Related sequences were obtained from GenBank. There are 28 taxa included in the analyses, which comprise 2,385 characters including gaps. The tree is rooted to *Verruconis calidifluminalis* (CBS125818). The best-scoring RAXML tree with a final likelihood

value of $-17,388.241509$ is presented. The matrix had 1,158 distinct alignment patterns, with 21.98% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.232186, C = 0.241611, G = 0.316968, T = 0.209236; substitution rates AC = 0.942091, AG = 1.876611, AT = 1.006528, CG = 1.020611, CT = 3.796142, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.257007$. Maximum likelihood bootstrap support values $\geq 60\%$ (ML) and Bayesian posterior probabilities (PP) ≥ 0.90 are given in the nodes. The scale bar indicates 0.08 changes. The isolates obtained in this study are in red and ex- type taxa are in black bold (Supplementary Figure 20). In this analysis, our collections clustered with *Ochroconis musae* CBS729.95 and (MFLU 17-2598).

Ochroconis musae (G.Y. Sun & Lu Hao) Samerp. & de Hoog, Mycol. Progr. 14(no. 6): 8 (2015) (Supplementary Figure 21)

Index Fungorum number: IF808843

Saprobic on dead leaves of *Dracaena* sp. Sexual morph: Undetermined. Asexual morph: *Hyphae* effusae, pallide brunneae vel brunneae. *Mycelium* mostly superficiale, immersed, branched septate, pale olive, $1.0\text{--}1.5 \mu\text{m}$ wide. *Conidiophores* 0–3-septate, arising directly from aerial hyphae, straight or slightly flexuous, pale olivaceous brown, smooth, denticulate, solitary. *Conidiogenous cells* sympodial, denticulate, denticles thread-like, $0.5 \times 1.0 \mu\text{m}$, breaking between conidia and conidiogenous cells. *Conidia* $9\text{--}13 \times 2\text{--}3 \mu\text{m}$ ($\bar{x} = 11 \times 2.5 \mu\text{m}$; $n = 20$) solitary, subcylindrical, pale brown, smooth, with the distal end obtuse and proximal end slightly tapered, bearing a minute denticle, clearly 1-septate in the middle, guttulate, conspicuously constricted at the septum.

Culture characteristics: On PDA, colonies 25–30 mm in diameter after 2 weeks, flat, moderately expanding, smooth, dry, flat. The center is white or beige, the margin is grayish brown to dark brown, submerged colony margin, reverse as dark brown in the central portion, not producing pigmentation in agar, hyphae sub-hyaline to pale brown, smooth- and thick-walled, sporulated after 3 weeks.

Material examined: Thailand, Tak Province, Umphang District, on dead leaves of *Dracaena* sp., 23 August 2019, Napalai Chaiwan, poppra5.1 (MFLU 22-0188), living culture MFLUCC 22-0105.

Known distribution: *Musa basjoo* (Samerpitak et al., 2015), *Persea americana* (Crous et al., 2019), *Dracaena* sp (Hyde et al., 2020). China (Samerpitak et al., 2015), Thailand (Crous et al., 2019; Hyde et al., 2020, This study).

GenBank accession numbers: ITS: ON159737; LSU: ON159739; *tub*: ON184274.

Notes: *Ochroconis musae* has previously been recorded from *Musa basjoo* (Samerpitak et al., 2015). Our isolate, collected from *Dracaena* sp. in Thailand, grouped with other *Ochroconis musae* strains in a monophyletic clade with 88% ML support in the phylogenetic analysis (Supplementary Figure 20). Thus, we identify this collection as a new host record of *O. musae* from *Dracaena* sp. in Thailand.

4 Discussion

This study provides morphological descriptions and phylogenetic analyses of 11 fungal taxa isolated from *Dracaena*

species associated with limestone outcrops in Thailand. We describe three new species (*Cladosporium dracaenae*, *C. dracaenicola*, and *Torula dracaenae*), identify seven new host records (*Bipolaris coffeana*, *Curvularia lunata*, *Lasiodiplodia bruguierae*, *L. lignicola*, *L. thailandica*, *Longididymella clematidis*, and *Zasmidium citrigriseum*), and report a new collection of *Ochroconis musae*. These findings considerably expand current knowledge on microfungi associated with *Dracaena* in Thailand.

Our collection represents five fungal orders and among these, Botryosphaerales and Cladosporiales were predominant, aligning with previous research highlighting their common association with woody substrates and their prevalence in tropical and subtropical habitats (Hyde et al., 2005; Marques et al., 2013; Gomdola et al., 2024; Pereira et al., 2024; Slippers et al., 2024). Members of Botryosphaeriaceae, in particular, are frequently recognized as pathogens, endophytes, or saprobes on woody plants and have been recently reported from limestone-rich habitats (Dissanayake et al., 2021; Zhang et al., 2023). For instance, Botryosphaeria guttulata was first described from limestone geology in Guizhou Province, China (Chen et al., 2020), suggesting potential ecological adaptations of Botryosphaeriaceae to extreme environments. It is noteworthy to further explore these ecological roles and their adaptation mechanisms within limestone habitats. In the current study, we introduced two new *Cladosporium* species viz. *C. dracaenae* and *C. dracaenicola*. *Cladosporium* species are ubiquitous fungi commonly associated with biodeterioration processes of limestone substrates, particularly in humid and temperate regions (Nováková, 2009; Trovão et al., 2020). Notably, species such as *Cladosporium cladosporioides* and *C. tenuissimum* have been documented to produce calcium oxalate crystals and acidic metabolites, respectively (Burford et al., 2003; Waqas et al., 2013), facilitating mineral transformations and potentially compromising structural integrity of limestone substrates. Thus, future studies should investigate the potential industrial and ecological impacts of these newly described *Cladosporium* species isolated from limestone environments.

Comparing our findings with earlier records, Thongkantha et al. (2008) documented fungal saprobes and pathogens associated with *Dracaena lourieri* in Thailand, largely relying on phylogenetic analyses from baited and natural substrates, with limited morphological integration. Our investigation aligns with several previous reports while providing clearer taxonomic identification and integrating comprehensive morphological and molecular approaches. For instance, *Ochroconis musae*, previously reported from bananas in China and *Dracaena* in Thailand (Hyde et al., 2020), was also isolated herein, confirming its broader host range. Similarly, although *Torula herbarum* has been recorded on *Dracaena* (Li et al., 2017), we distinguish and introduce *Torula dracaenae* as a new species. Additionally, we reconfirmed *Curvularia lunata* (previously reported by Peregrine and Ahmed, 1982) and reported *Bipolaris coffeana* as a novel association with *Dracaena*. Despite Braun et al. (2014) reporting *Zasmidium dracaenae* on *Dracaena*, we document *Z. citrigriseum* as a new

host record in Thailand. *Lasiodiplodia thailandica*, previously reported from *Mangifera indica* in Thailand, is herein reported along with new host records of *L. bruguierae* and *L. lignicola* from *Dracaena*. *Longididymella clematidis*, earlier known only from *Clematis ligusticifolia* in the USA, represents both a new host and geographical record in this study.

Our results demonstrate significant diversity within the fungal community associated with *Dracaena* species in limestone outcrops in Thailand. Earlier studies often lacked precise identification due to insufficient molecular data. Therefore, future recollections and detailed phylogenetic reassessments are crucial. Biochemical characterization of fungal taxa from limestone habitats would be valuable, given their potential ecological roles and biotechnological applications. Future research should prioritize understanding the ecological contributions and adaptation strategies of fungi colonizing limestone ecosystems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

NC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. ST: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. RJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. DW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. IM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. DM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. IP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review &

editing. KH: Supervision, Conceptualization, Validation, Investigation, Funding acquisition, Resources, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer WD declared a shared affiliation with the author ISM to the handling editor at the time of review.

Correction note

A correction has been made to this article. Details can be found at: [10.3389/fcimb.2025.1686965](https://doi.org/10.3389/fcimb.2025.1686965).

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2025.1550824/full#supplementary-material>

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