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## EDITED BY

Filitsa Karamaouna,  
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Greece

## REVIEWED BY

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Ken-Ichi Harano,  
Tamagawa University, Japan

## \*CORRESPONDENCE

H. M. Wallace

✉ [helen.wallace@qut.edu.au](mailto:helen.wallace@qut.edu.au)

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# Tall trees and exotic herbs identified in pollen and nest materials of seven cavity-nesting solitary bee species in Australia

R. S. Wilson<sup>1</sup>, A. Keller<sup>2</sup>, S. D. Leonhardt<sup>3</sup>, T. J. Smith<sup>4</sup>,  
C. J. Burwell<sup>5</sup>, C. Fuller<sup>6</sup>, A. Shapcott<sup>7</sup>, B. F. Kaluza<sup>8</sup>  
and H. M. Wallace<sup>9\*</sup>

<sup>1</sup>School of Environment and Science, Griffith University, Nathan, QLD, Australia, <sup>2</sup>Center for Organismic Adaptation, Cellular and Organismic Networks, Ludwig-Maximilians-Universität München, Munich, Germany, <sup>3</sup>Department of Life Science Systems, Technical University of Munich, Freising, Germany, <sup>4</sup>School of the Environment, University of Queensland, St Lucia, QLD, Australia, <sup>5</sup>Biodiversity and Geosciences, Queensland Museum, Brisbane, QLD, Australia, <sup>6</sup>Kin Kin Native Bees, Kin Kin, QLD, Australia, <sup>7</sup>Centre for Bioinnovation, University of the Sunshine Coast, Sippy Downs, QLD, Australia, <sup>8</sup>Department of Public Technology and Innovation Planning, Fraunhofer Institute for Technological Trend Analysis INT, Euskirchen, Germany, <sup>9</sup>School of Biology and Environmental Science, Queensland University of Technology, Brisbane, QLD, Australia

**Introduction:** Solitary bees are important pollinators yet vulnerable to decline in resource-poor landscapes. Resources for solitary bees may be supplemented by land managers through targeted plantings, however, little is known about the specific plants used by different species. This study identified plants used by seven cavity-nesting solitary bee species in natural and agricultural landscapes over two years by DNA metabarcoding of pollen bread, larvae, frass and nest materials.

**Methods:** We sequenced samples from the bee species: *Megachile mystacaena* (23 nests), *Megachile mackayensis* (2), *Megachile simplex* (11), *Megachile deanii* (3), *Hylaeus ruficeps* (1), *Hylaeus nubilosus* (6) and *Hyleoides concinna* (3). We identified 118 plant taxa from brood provisions (or larvae and frass proxies) and nest materials, however, just 20 taxa accounted for more than 70% of all resources used. Importantly, trees were the most frequently provisioned pollen source for all bees (48 taxa).

**Results:** Introduced plants were used by all bee species studied, even in natural landscapes. Native plants, however, still accounted for more than 50% of relative read abundance for six of seven bee species. Plants identified in nest materials were mostly herbaceous species for leafcutter bees, vines and trees for cellophane bees and parasitic plants (Santalales, “mistletoes”) for resin bees.

**Discussion:** Agri-environment schemes to support pollinators may be benefit from the inclusion of these taxa or plants with similar characteristics, particularly flowering trees.

## KEYWORDS

amplicon sequencing, bee hotels, DNA metabarcoding, *Hylaeus*, *Hyleoides*, *Megachile*, solitary bees, trap nests

## 1 Introduction

Bees are the most economically important pollinators in agroecosystems, yet are especially sensitive to the loss of floral resources (Winfrey et al., 2009; Kennedy et al., 2013; Vanbergen et al., 2013). The majority of the world's 20,500 known bee species are solitary (Michener, 2007; Danforth et al., 2019; Ascher and Pickering, 2020). Solitary bees can have unique adaptations for pollination mutualisms and are equally effective or better pollinators than honey bees for many crops (Minckley and Roulston, 2006; Winfrey, 2010). However, some species are particularly vulnerable to decline in homogenous, resource-poor agroecosystems, and loss of these bees can result in less stable pollination services (Kennedy et al., 2013; Vanbergen et al., 2013; Martínez-Núñez et al., 2019). Pressures on bee communities in agroecosystems may be reduced by retaining natural or non-crop vegetation or increasing resource diversity on farm (Kennedy et al., 2013; Goulson et al., 2015; Mallinger et al., 2019; Rollin et al., 2019). Little is known, however, about the specific resource requirements of many species, aside from common and commercial species.

Plants that provide food and nest materials for solitary bees are critical for bee conservation programs. Characteristics of plants (e.g. plant form) used by bees could indicate which plants are useful to functionally similar bees. For example, several species of leafcutter bees (*Megachile* spp.) prefer leaves from woody trees and shrubs over herbs and vines in India (Kambli et al., 2017) and Canada (MacIvor, 2016), indicating that tree and shrub cover are important to retain for bees that use leaves for nest materials. Other plant characteristics like indigeneity (exotic or native) are also important to consider when selecting forage for bees (Morandin and Kremen, 2013; Pardee and Philpott, 2014). For example, while habitat restoration programs may prefer the use of native plants (Morandin and Kremen, 2013), exotic annual herbs can also provide critical, short-term resources for bees and may be appropriate to plant or retain on farms to supplement crop pollinators (Bretagnolle and Gaba, 2015; Seitz et al., 2020; Jones et al., 2021).

Identifying resources used by solitary bees could help further our understanding of why some species persist in simplified agroecosystems, and which resources could support those that do not, particularly among tropical species. Most research on resource use by bees has been conducted in temperate regions of North America, Europe, and South America (Sobek et al., 2009; Kennedy et al., 2013; Staab et al., 2018; Tonietto and Larkin, 2018). Similar studies in tropical and subtropical agroecosystems are rare (Staab et al., 2018), despite much of the tropics undergoing significant land use change (IPBES, 2019).

In this study, we investigate resource use by seven solitary bee species, including short-tongued Colletidae and long-tongued Megachilidae, in natural and agricultural landscapes in the tropics. We used metabarcoding to examine nest provisions and plant materials used to construct nests, as this technique can enable a comprehensive analysis of plant sources used by bees (Elliott et al., 2021; Wilson et al., 2021). We ask the following research questions: 1) What plants are used by different cavity-nesting solitary bee species in natural and agricultural landscapes for provisioning and

nesting materials?; and 2) What are the characteristics (plant form, indigeneity, life cycle) of plants used by cavity-nesting solitary bees in natural and agricultural landscapes?

## 2 Methods

Plants were identified in the provisions and nest materials of seven bee species: *Megachile mystacaena*, *Megachile mackayensis* and *Megachile deanii* (resin bees); *Hylaeus nubilosus*, *Hylaeus ruficeps* and *Hyleoides concinna* (cellophane bees); and *Megachile simplex* (a leafcutter bee). These species in particular were included due to sample availability from a previous study that measured trap nest occupation (Wilson et al., 2020).

### 2.1 Study sites and field collections

We installed trap nests in three orchards and four forests at northern and southern latitudes ( $-24^{\circ}38'$  to  $-27^{\circ}29'$  S,  $152^{\circ}06'$  to  $153^{\circ}06'$  E) of south-east Queensland, Australia in the spring (October) of 2016 (Figure 1A). Sites were categorized as “forest” or “orchard” if they had  $\geq 75\%$  forest or orchard canopy cover respectively within a 500m radius around trap nests. Orchards were a matrix of commercial macadamia (*Macadamia integrifolia* Maiden and Betche  $\times$  *M. tetraphylla* Johnson) and forest fragments. Other sites were open eucalypt forests and woodlands, dominated by an overstory of *Eucalyptus* and *Corymbia* species (e.g. *Corymbia intermedia*, *C. trachyphloia*, *Angophora leiocarpa*, *Eucalyptus latisinensis*, *E. exserta*, *E. tereticornis*, *E. acmenoides* and/or *E. pilularis*) (Specht, 1970). Plant species identities, abundance and diversity at study sites were estimated from transect surveys (Kaluza et al., 2016; Kaluza et al., 2017).

Four units of timber trap nests were installed per site. Each unit contained five short cavities (100mm long  $\times$  6mm diameter) and five long cavities (150mm long  $\times$  8mm diameter) drilled into timber blocks lined with removable paper tubes (PaperTunnel™ liners, ©Pollinator Paradise). This design was chosen to allow whole nest management and to attract species that nest above-ground in existing hollow cavities. Trap nest units were checked for occupation every three to four months from February 2017 to December 2018. Occupied trap nests were recovered and replaced with fresh paper tubes each sampling period. Recovered nests ( $n=590$ ) were placed in fine mesh (organza) bags for transport until dissection. Paper tubes of the recovered nests were dissected and samples taken of nest materials (entire nest cap) and pollen bread (subsample of all pollen balls per nest) or proxies for pollen bread such as bee larvae or frass (one random larva per nest or all frass remaining in nest) (Figure 1D). Samples were stored in 1.5mL Eppendorf Safe-Lock Tubes® and stored at  $-18^{\circ}\text{C}$  until DNA extraction. Dissected nests with larvae or pupae were resealed, transferred to clean organza bags (one nest per bag) and stored together in emergence boxes outdoors (Steckel et al., 2014). All nests were checked monthly for emergents, which were then frozen at  $-18^{\circ}\text{C}$  and pinned for identification (Figure 1). Emergent bees were identified to species or morphospecies by CJB using available taxonomic literature and reference to collections in the

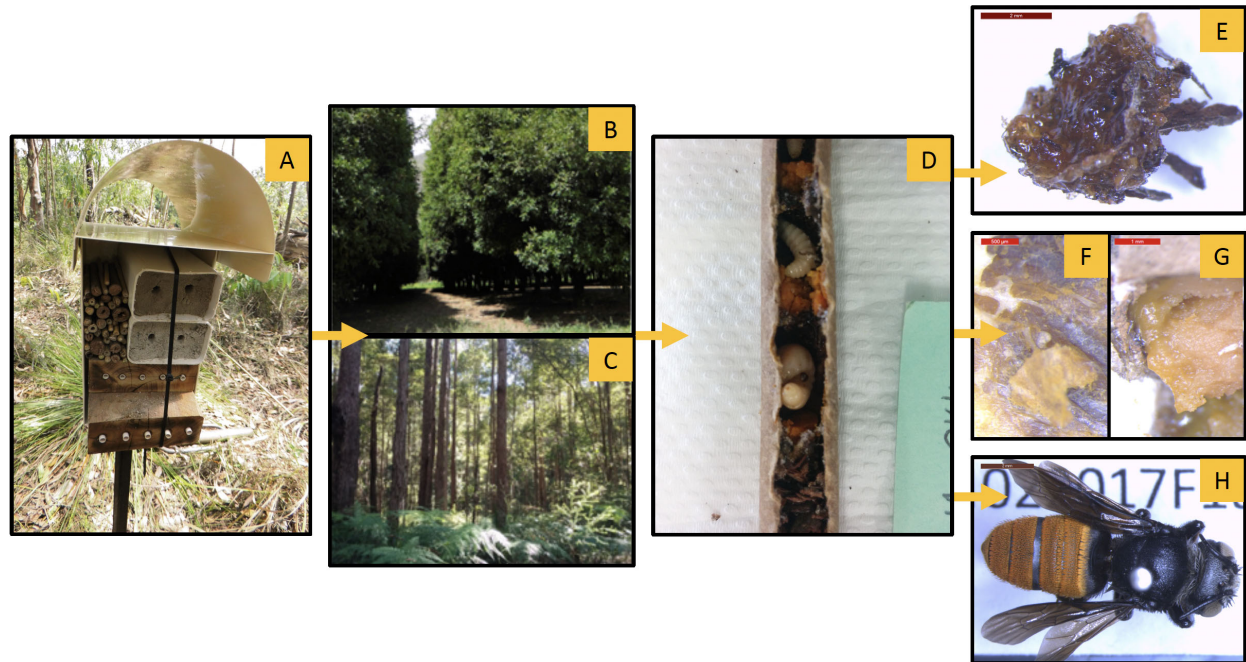


FIGURE 1

Schematic of field sample collections. Trap nests were installed in weather-proof units (A) in orchards (B) and forests (C). Occupied nests were recovered and dissected (D) to obtain nest materials (E), frass/larvae proxies (F) or pollen (G) and then resealed and stored in an emergence box until adults developed (H). Source: Adapted from Wilson et al. (2020), © 2020 by the authors, published under CC BY 4.0.

Queensland Museum. Cavities in trap nests typically attract bee species from the families Megachilidae and Colletidae which typically encapsulate brood cells and close nests with resin and other plant material (resin or leafcutter bees) or glandular secretions that are cellophane-like when dry (hylaeine bees) (Bell and Cardé, 2013). As such, nests without adult emergents (i.e. dead larvae or pupae) were determined to likely host family or subfamily from nest materials or provisions (e.g. “cellophane” to Hylaeinae; “resin” to Megachilidae).

## 2.2 DNA library preparation and sequencing

We used DNA metabarcoding to identify plant sources of nest materials and provisions using protocols described by Wilson et al. (2021). Briefly, dry samples of nest materials ( $n=60$ ), pollen bread ( $n=32$ ) and pollen proxies ( $n=10$ ) were individually weighed and subsampled to  $<0.02\text{g}$  (mean =  $0.014\text{g}$ ), frozen with liquid nitrogen and disrupted on a TissueLyser II (Qiagen, Germany) with silica-zirconia beads. DNA was then isolated using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturers protocol. Positive (known plant DNA) and negative controls (water) were incorporated from DNA extractions through to sequencing. We amplified DNA using polymerase chain reactions (PCR) with the primer sets *rbcL2* (Palmieri et al., 2009) + *rbcLaR* (Kress and Erickson, 2007) and ITS-S2F (Chen et al., 2010) + ITS4R (White et al., 1990). Primers consisted of a forward and reverse adapter, variable index, pad, link and primer sequence (Kozich et al., 2013; Sickel et al., 2015). Triplicate PCR was performed for each sample and marker using the following conditions: heat

activation at  $95^\circ\text{C}$  for 3min; 35 cycles of denaturation at  $95^\circ\text{C}$  for 30sec, annealing at  $55^\circ\text{C}$  ( $58^\circ\text{C}$  for *rbcL*) for 30sec and elongation at  $72^\circ\text{C}$  for 1min; followed by a final extension at  $72^\circ\text{C}$  for 10min, holding at  $10^\circ\text{C}$  until storage. Each reaction contained  $5\mu\text{L}$  KAPA Hifi HotStart ReadyMix (2x) (KAPA Biosystems, MA),  $0.6\mu\text{L}$  each of forward and reverse primer (from  $10\mu\text{M}$  aliquots) and  $3.8\mu\text{L}$  of DNA isolate (or RNase-free water for negative controls) to a total volume of  $10\mu\text{L}$ . Amplicons were pooled by sample and concentrations normalized using the SequalPrep™ Normalization Plate Kit (Invitrogen Corporation, CA). Samples were quantified with a Quantus™ Fluorometer (Promega Corporation, WI) and the average library size was determined with a High Sensitivity DNA assay on an Agilent 2100 Bioanalyzer (Agilent Technologies, CA). Libraries were pooled equimolarly, denatured with  $0.1\text{N}$  NaOH, neutralized with  $200\text{mM}$  Tris (pH 7) (Quail et al., 2008), and diluted to  $4\text{pM}$  with HT1 buffer. Sequencing was performed on a MiSeq® using the FASTQ Workflow and a 5% PhiX control, with the addition of  $3\mu\text{L}$  of Read 1, Index 1 and Read 2  $100\mu\text{M}$  primers to wells 12, 13 and 14 of the MiSeq v2 500-cycle cartridge (Illumina, CA) for ITS2 or a v3 600-cycle cartridge for *rbcL*, respectively.

## 2.3 Data analysis

Raw reads were obtained from the MiSeq and quality-filtered to remove short reads ( $<100\text{bp}$ ) and those with ambiguous base pairs. Forward and reverse reads for ITS2 were joined, however, for *rbcL*, only forward reads were used due to low overall quality of reverse reads. All reads were assigned to amplicon sequence variants (ASVs), filtered for chimeras and denoised (Edgar, 2010). A reference sequence database of local plant species was created for

each marker using the bcdatabaser tool (Keller et al., 2020). Reference sequences were included only for plant species present at study sites and that were between 200bp and 2000bp in length (405 species, 93% of all identified in transects). Each local database consisted of 330 *rbcL* and 213 ITS2 references from Genbank (Benson et al., 1994), plus *rbcL* references for 29 plant species sequenced in-house (Pearl et al., 2022). For each marker, ASVs were classified against the appropriate local reference database using global alignments with a threshold of 97% identity. Those ASVs that did not align with local references were then hierarchically classified against a general reference database of all Viridiplantae for *rbcL* (doi.org/10.5281/zenodo.3339890) or ITS2 (doi.org/10.5281/zenodo.3339029) (Edgar, 2010; Keller et al., 2020). Sample-specific reads were mapped to ASVs to obtain relative read abundance (RRA) estimates (Deagle et al., 2019). All taxonomic assignments were verified by checking their occurrence within the study region using the Atlas of Living Australia (bie.ala.org.au). Assignments to hybrids and taxa not likely to occur in the region ( $n=32$ ) were reassigned to the next likely taxonomic level (e.g. *Crotalaria monophyla* to *Crotalaria* sp.; *Listia* sp. to Fabaceae sp.). All ASVs that were not assigned to families of vascular plants (e.g. algae) were removed. Taxa identified in blank controls that were present in low abundance in real samples (<1%) were assumed to be contaminants and so also removed (McFrederick and Rehan, 2019).

Data were analyzed separately for *rbcL* and ITS2 read-matches to account for differences in hierarchical assignments and availability of reference sequences. All analyses were performed in the statistical software 'R' (v 3.5.2, R Development Core Team, Austria). The mean (or median) relative read abundance of plant taxa per sample was calculated as a proportion (Deagle et al., 2019). The plant traits "life cycle", "form" and "indigeneity" were determined through literature review and assigned to each species manually. Traits for taxa not identified to species were unable to be determined and so excluded from trait analysis. Non-metric Multidimensional Scaling (NMDS) in the *Phyloseq* package was used to compare the composition of plants detected per bee nest with Bray–Curtis distances, two dimensions and up to 999 permutations (McMurdie and Holmes, 2013). We then used permutational multivariate ANOVAs (PERMANOVAs) using the same distance matrices to test the explanatory power of factors on calculated distances (Anderson, 2001). This method is well suited for our data, since it partitions variation in a distribution-free setting within the space of the dissimilarity measure, thus allowing for the non-normal data typical of ecological studies (Anderson, 2014).

## 3 Results

### 3.1 Botanical sources of nest materials and provisions

All bee species examined were floral generalists. We identified 118 plant sources of solitary bee provisions and nest materials from 22 orders, 34 families, 67 genera and 69 species across all bee species

and samples (Supplementary Materials). Of this, just 20 plant species accounted for more than 70% relative abundance of all resources used (Table 1). Bees in forests used ~5 to 13 plant species, while those in orchards used ~4 to 7 plant species (median richness, Table 2).

The composition of plant resources used varied with bee species ( $R^{2rbcL}=0.31$ ,  $P = 0.001$ , stress=0.13;  $R^{2ITS2} = .25$ ,  $P = 0.001$ ; stress=0.18) and land use ( $R^{2rbcL}=0.08$ ,  $P = 0.003$ ; Figure 2), but not season ( $R^{2rbcL}=0.03$ ,  $P = 0.253$ ;  $R^{2ITS2} = .01$ ,  $P = 0.685$ ). Plants used in orchards were generally a subset of those species used in forests (Figure 2).

We identified key plants used for multiple purposes and by multiple bee taxa (Figure 3). For example, *Modiola caroliniana* (Malvaceae) and *Passiflora foetida* (Passifloraceae) were identified in brood provisions and all nest material types (Figure 3). Similarly, *Lophostemon suaveolens*, *L. confertus* and *Eucalyptus racemosa* (Myrtaceae) were identified in pollen, resin and cellophane samples (Figure 3). Importantly, some plants were only used or available in forests but not orchards (e.g. *Melaleuca* spp.) and some only in orchards but not forests (e.g. *Rubus* spp.) (Figure 3). Overlap was observed between plant sources of pollen samples and proxies for pollen samples (larvae, frass), indicating that these sample types may be used in the absence of pollen to ascertain provisions (Figure 3).

Resin bees (*M. mystacaena*, *M. mackayensis* and *M. deanii*) used 93 plant taxa across all specimens and sites (Supplementary Materials), particularly *Amyema* spp. (mean proportion 2–55%, Loranthaceae), *M. caroliniana* (4–18%) and *Passiflora* spp. (1–11%) for nest materials; and *L. confertus* (8–18%) and *Crotalaria* spp. (1–29%, Fabaceae) for pollen. The cellophane bees (*Hylaeus nubilosus*, *H. ruficeps* and *Hyleoides concinna*) frequently provisioned pollen from Myrtaceae (13 out of 54 taxa found for both pollen and nest materials) and in high abundances (2 to 63%) – particularly *Eucalyptus tereticornis*, *E. racemosa*, *E. crebra*, *L. confertus*, *L. suaveolens* and *Backhousia* sp. (Supplementary Materials). Plant materials identified in cellophane bee nest caps were mostly *M. caroliniana* (10–41%) and *Passiflora* spp. (1–20%) (Supplementary Materials). Leafcutter bee (*M. simplex*) nest materials were sourced from 35 plant taxa, particularly *Passiflora* spp. (10–48%), *M. caroliniana* (11–16%), *Sida* spp. (1–10%, Malvaceae) and *Solanum* spp. (Solanaceae, 4–7%); and pollen was sourced from 23 taxa, mostly *Desmodium uncinatum* (Fabaceae, 1–16%), *Eucalyptus* spp. (7–12%, Myrtaceae) and *Macadamia* sp. (Proteaceae, 1–9%) (Supplementary Materials).

### 3.2 Characteristics of botanical resources

Introduced plants were used by all bee species studied. Of the 118 plant taxa identified, 70 were native to the study region, 26 were introduced and 22 were undetermined. Plants with undetermined indigeneity included taxa that were only assigned to genus or higher taxonomic levels which are known to have both native and introduced species in Australia. The median relative read abundance of introduced plants varied greatly between bee species, from very low (e.g. 1–16% for *M. mackayensis*) to very high (39–86% for *H. concinna*) (Table 3). Native plants, however,

TABLE 1 Plant forms and indigeneity of taxa most commonly used by solitary bees in forest and agricultural landscapes in the subtropics.

Form	Indigeneity	Family	Species	RRA rbcl	RRA ITS2	FOO rbcl	FOO ITS2
Herb	Introduced	Fabaceae	<i>Crotalaria lanceolata</i>	0.00	0.11	0%	32%
Herb to shrub	NA	Fabaceae	<i>Crotalaria</i> sp.	0.03	0.00	8%	0%
Herb to shrub	Introduced	Fabaceae	<i>Desmodium</i> sp.	0.02	0.00	6%	0%
NA	NA	Fabaceae	Fabaceae sp.	0.00	0.02	0%	9%
Tree	Native	Himantandraceae	<i>Galbulimima baccata</i>	0.03	0.00	30%	0%
Parasite	Native	Loranthaceae	<i>Amyema cambagei</i>	0.24	0.00	54%	0%
Parasite	Native	Loranthaceae	<i>Amyema</i> sp.	0.00	0.07	0%	24%
Parasite	Native	Loranthaceae	<i>Amylothea dictyophleba</i>	0.03	0.00	36%	0%
Parasite	Native	Loranthaceae	Loranthaceae sp.	0.00	0.03	5%	24%
Herb	Introduced	Malvaceae	<i>Modiola caroliniana</i>	0.04	0.13	29%	38%
Herb	Introduced	Malvaceae	<i>Sida rhombifolia</i>	0.00	0.03	0%	17%
Tree	Native	Myrtaceae	<i>Eucalyptus crebra</i>	0.00	0.03	0%	11%
Tree	Native	Myrtaceae	<i>Eucalyptus racemosa</i>	0.00	0.04	0%	17%
Tree	Native	Myrtaceae	<i>Eucalyptus</i> sp.	0.13	0.00	63%	0%
Tree	Native	Myrtaceae	<i>Lophostemon confertus</i>	0.07	0.06	34%	17%
Tree	Native	Myrtaceae	<i>Lophostemon suaveolens</i>	0.00	0.09	0%	44%
Vine	Introduced	Passifloraceae	<i>Passiflora foetida</i>	0.08	0.00	34%	0%
Vine	NA	Passifloraceae	<i>Passiflora</i> sp.	0.00	0.11	0%	44%
Tree	Native	Proteaceae	<i>Macadamia</i> sp.	0.04	0.02	40%	13%
Herb	Native	Solanaceae	<i>Solanum nodiflorum</i>	0.02	0.01	28%	29%

Taxa shown have the highest relative read abundance (RRA, measured as mean proportion per sample) and the highest frequency of occurrence (FOO, measured as % of samples that the taxon was detected in) for each marker. Taxa with no applicable form or indigeneity (NA) are unknown species that were classified to genera where different forms and indigeneity may be possible.

still accounted for more than 50% of relative read abundance for six of seven bee species.

The proportion of resources used by plant form shows that trees are the most provisioned pollen source for all bees studied, particularly the cellophane bees (Figure 4). Leafcutter bees also provision pollen from shrubs and herbaceous plants, as do resin bees (Figure 4). In contrast to pollen sources, the most common plant form for nest materials was herbaceous plants, particularly for

leafcutter bees (Figure 4). Vines and trees were also commonly used plant forms for cellophane and resin bee nests (Figure 4). Surprisingly, parasitic plants (Santalales, “mistletoes”) were the most frequently used resource to construct nests for resin bees (Figure 4). Most taxa identified were perennials (94 of 118 taxa), except for herbaceous plants, which were mostly annuals (13 taxa) (Supplementary Materials). The introduced annuals *Modiola caroliniana* (Malvaceae) and *Crotalaria lanceolata* (Fabaceae);

TABLE 2 Observed species richness of plants used by solitary bee species.

Bee species (n)	Median plant richness			
	rbcl		ITS2	
	Forests	Orchards	Forests	Orchards
<i>Megachile (Callomegachile) mystacaena</i> (23)	5 (3–7)	–	5 (4–8)	–
<i>Megachile (Eutricharaea) mackayensis</i> (2)	6 (4–11)	–	7 (5–7)	–
<i>Megachile (Eutricharaea) simplex</i> (11)	–	5.5 (4.25–10.5)	–	5.5 (4–7.75)
<i>Megachile (Rhodomegachile) deanii</i> (3)	6 (4–10)	4 (3–10)	8 (6–9.5)	5 (4–5)
<i>Hylaeus (Euprosopoides) ruficeps</i> (1)	7 (7–7)	–	8 (8–8)	–
<i>Hylaeus (Hylaeorhiza) nubilosus</i> (6)	13 (12.5–13.5)	7.5 (6.75–8.25)	6 (4.5–7.5)	5 (5–5)
<i>Hyleoides concinna</i> (3)	8 (5–12)	–	5 (5–5)	–

Values are median (interquartile range) number of plant species identified per sample, per marker, in forests and orchards. Dashes (–) indicate bee species absent from orchards or forests. Values next to species names (n) indicate the number of nests sampled. Further information about the bee species studied (abundance in forests and orchards, number of brood cells per nest and nesting behavior) is provided in Wilson et al. (2020).

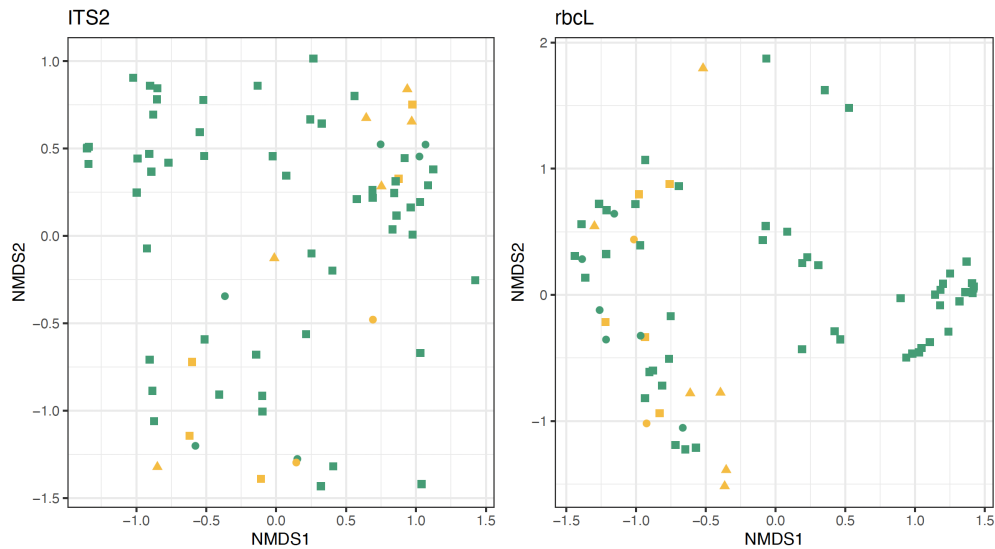


FIGURE 2

Ordination of Bray–Curtis distance showing differences in community composition of plant species used by cellophane bees (circles), leafcutter bees (triangles) and resin bees (squares) in forests (green) and orchards (yellow). Each point represents combined resources for one nest in one season in one year. Plants used in orchards are generally a subset of those used in forests.

Table 1) were used by all bee groups studied. Similarly, the trees *Eucalyptus racemosa*, *Lophostemon confertus* and *L. suaveolens* were also used by all bee groups (Myrtaceae; Table 1).

## 4 Discussion

This study identified key sources and characteristics of plants used by solitary bees for brood provisions and nest materials in natural and agricultural landscapes. Several plants were identified as key sources of both nest provisions and materials for multiple bee species (*Modiola caroliniana*, *Passiflora foetida*, *Lophostemon suaveolens*, *L. confertus* and *Eucalyptus racemosa*). Of the 118 plant taxa identified, most were native, perennial shrubs or trees (55 taxa). Trees were the most common source of pollen, whereas herbaceous plants (mostly annuals) were the most common source of nest materials. Introduced plants (26 taxa) were used by all bee species studied, even in natural landscapes (up to 86% for one bee species).

### 4.1 Botanical sources of nest materials and provisions

The composition of plants used varied significantly with bee species and landscape. Plants used by bees in orchards were mostly a subset of those in forests, suggesting that bees in forests used or had access to a wider range of resources. While there was much overlap, some plants were only identified in nests from one of the two land uses (e.g. *Melaleuca* spp. trees in forests and *Rubus* spp. shrubs in orchards), despite being present in both landscapes. Furthermore, more plant taxa were identified in forest-dwelling bee nests (median ~5 to 13 species) compared to those in orchards (~4 to 7 species), including the two bee species present in both land

uses (*M. deanii* and *H. nubilosus*). As such, at least some of the differences in bee resource-use between landscapes is likely due to the processes of landscape simplification (i.e. reducing the overall number of resources available to and or used by solitary bees) (Vanbergen et al., 2013; Goulson et al., 2015; Wood et al., 2016).

Bee species with high abundance in the region may be less susceptible to changes in resource availability. Records from the *Atlas of Living Australia* (2026) indicate that 164 native bee species (excluding social species) exist in our study region around Bundaberg in the north and the Sunshine Coast in the south. Four of the seven species included in our study are common in the region and across the mainland of Australia (*H. nubilosus*, *H. concinna*, *M. mystaceana* and *M. simplex*). There are no records of *H. ruficeps* in the study region, however, this bee is commonly recorded elsewhere in Queensland and other states. Similarly, records of *M. deanii* are less common in our study region (4 of 189 nationwide), however, it's propensity to steal wax from other species and construct nests in manmade objects indicates that the species is very adaptable (Arcaro and Houston, 2024). The resin bee *M. Mackayensis* is particularly rare. Records show limited but consistent recent detections of *M. Mackayensis*, with four records between 1888 and 1957 then five records between 2016 and 2025, all from Queensland, New South Wales and Western Australia. Other trap-nest studies have only detected *M. Mackayensis* in the understorey and canopy of densely forested habitats, which may be why sightings are particularly rare (Wilson et al., 2020; Morris et al., 2015).

Similarities in the resources used by bees present in both natural and agricultural landscapes may indicate plants important for their persistence. For example, six pollen sources, mostly members of Passifloraceae, were used by *M. deanii* in forests and orchards; and six members of Myrtaceae were used by *H. nubilosus* as major pollen sources in both landscapes. Interestingly, the overlapping plants between landscapes used by these bees are all tree and vine

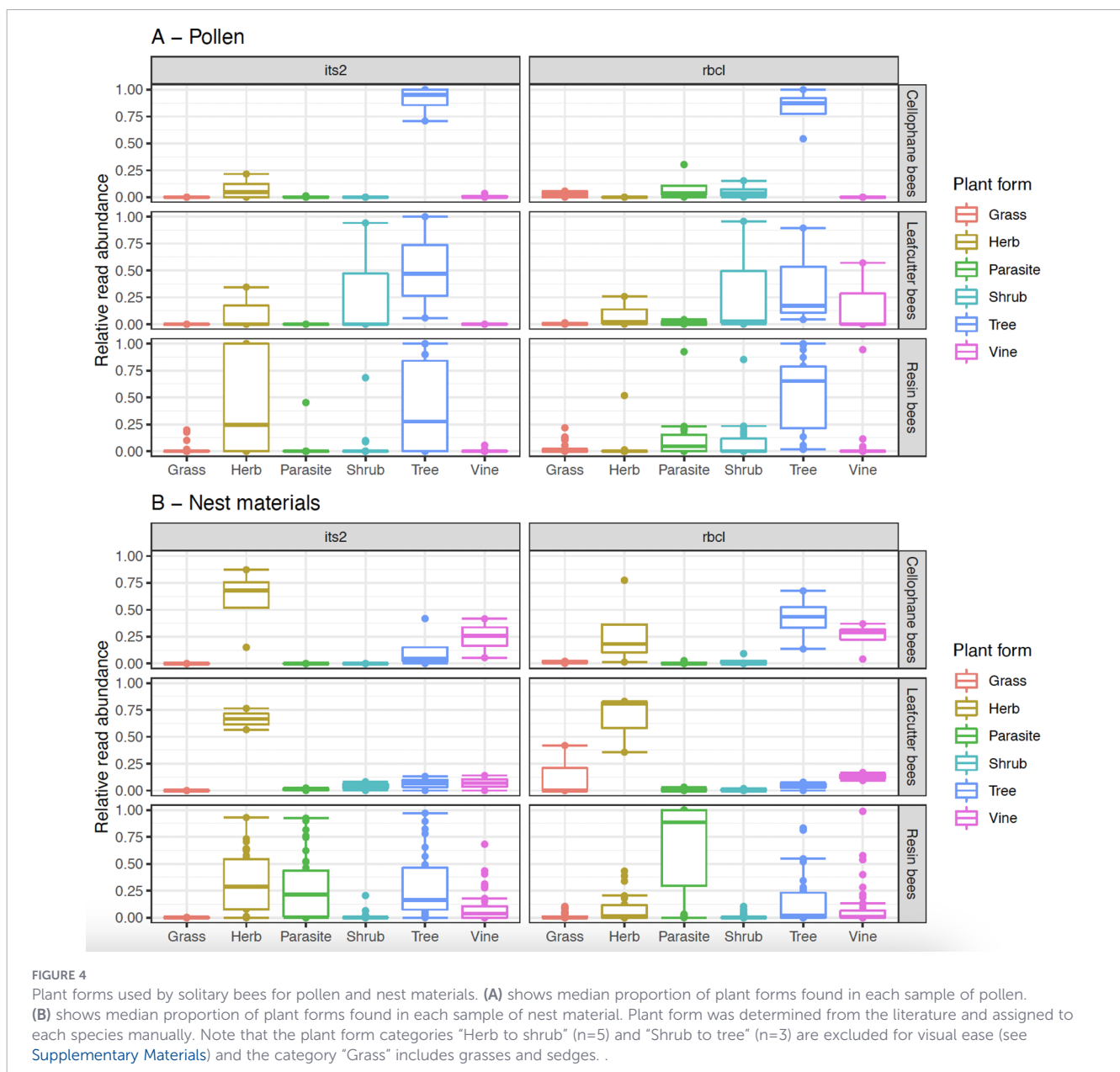


TABLE 3 Median proportion (interquartile range) of introduced plants identified in provisions and nest materials of solitary bees, per marker.

Bee species	Median proportion of introduced plants	
	ITS2	<i>rbcl</i>
<i>Megachile (Callomegachile) mystacaena</i>	0.35 (0.01, 0.73)	0.00 (0.00, 0.03)
<i>Megachile (Eutricharaea) mackayensis</i>	0.16 (0.05, 0.45)	0.01 (0.00, 0.28)
<i>Megachile (Eutricharaea) simplex</i>	0.43 (0.08, 0.55)	0.47 (0.13, 0.75)
<i>Megachile (Rhodomegachile) deanii</i>	0.49 (0.06, 0.57)	0.08 (0.01, 0.22)
<i>Hylaeus (Euprosopoides) ruficeps</i>	0.22 (0.22, 0.22)	0.00 (0.00, 0.00)
<i>Hylaeus (Hylaeorhiza) nubilosus</i>	0.17 (0.07, 0.40)	0.14 (0.00, 0.40)
<i>Hyleoides concinna</i>	0.86 (0.43, 0.88)	0.39 (0.19, 0.44)

main source of nest materials for resin bees. It is unlikely that these are pollen contaminants from provisioning foraging bouts, as the greater median proportion of mistletoes in nest caps (approx. 0.87) compared to pollen bread (0.05) suggests the collection is intentional. The detection of any plant material in hylaeine nest caps has also not been reported elsewhere to our knowledge.

Importantly, we identified the same plants from larvae and frass as those found in pollen bread for each bee species, thus demonstrating that such samples can be used as proxies for diet analyses with DNA metabarcoding. Bee-plant interactions have also been detected via metabarcoding swabs of solitary bee nests (Sickel et al., 2023) and a similar approach has been used on whole bee, pupal and larval specimens to study microbiomes and pathogens (e.g. Erban et al., 2017; Keller et al., 2018; Voulgari-Kokota et al., 2020).



## 4.2 Characteristics of botanical resources

Tree species were the most commonly provisioned pollen source of all bees studied. Traditionally, bee visits to tall trees have been difficult to observe due to the difficulty involved in sampling the canopy (i.e. with climbing ropes or flight-intercept traps) (Ramalho, 2004; Ulyshen et al., 2010). With DNA metabarcoding, however, we were able to quantify bee use of 43 tree species – half of which grow to heights greater than 20m. For example, bees used *Polyscias elegans* (20–30m, Araliaceae), *Gmelina leichhardtii* (15–60m, Lamiaceae), *Syncarpia glomulifera* (25–60m, Myrtaceae) and *Eucalyptus pilularis* (40–70m, Myrtaceae) (Floyd, 1990; Brooker and Kleinig, 1999; Boland et al., 2006). This study adds to emerging molecular evidence that a disproportionate amount of bee-collected pollens come from trees (Somme et al., 2016; Donkersley, 2019; Voulgari-Kokota et al., 2019; Vaudo et al., 2020; Cannizzaro et al., 2022). This information is important for the design of revegetation programs or agri-environment schemes that currently focus on flower-strip plantings as inter-crop forage for bees (Wood et al., 2017; Gresty et al., 2018). Plantings or conservation of established trees as inter-crop forage could focus on species that fulfill multiple resource requirements such as *Lophostemon suaveolens*, *L. confertus* and *Eucalyptus racemosa* (Myrtaceae), which are native to the study region and were identified in pollen, resin and cellophane samples.

Introduced plants (26 taxa) were used by all bee species studied. The proportion of introduced plant use varied greatly between bee species, from very low (e.g. 1–16% for *M. mackayensis*) to very high (39–86% for *H. concinna*). This is the first record to our knowledge of exotic plant use by these species. Native plants (70 taxa), however, still accounted for more than 50% of relative read abundance for six of seven bee species. The most commonly used native plants were all in the family Myrtaceae: *L. suaveolens*, *L. confertus*, *E. racemosa*, *E. tereticornis*, *E. crebra*, *Melaleuca* spp., and *Backhousia* species. Native bee preferences for mostly native plants over co-occurring weedy or exotic plants has also been observed in restored agricultural areas (Morandin and Kremen, 2013) and urban gardens (Pardee and Philpott, 2014). Bee use of introduced plants is known to increase with abundance of individual introduced plants in agricultural and semi-natural landscapes (Williams et al., 2011) and in times when native plants are less likely to be flowering, such as early or late in a bees' flying season (Seitz et al., 2020). As fast-growing, early- or late-blooming, and often nutritious species, introduced annuals may fill temporal gaps in flowering resource availability or reward where mature, native flowers are lacking (Mach and Potter, 2018; Russo et al., 2019). Land managers can thus easily provide forage for solitary bees by either managing existing populations of introduced plants (e.g. by mowing before seed set) or including selected species in strip plantings between crop rows or human-dominated landscapes (Lerman et al., 2018; Seitz et al., 2020). Selection of exotic plant species must exclude those that compete with endemic plants such as supergeneralist perennials (Vila et al., 2009; Stouffer et al., 2014; Jones et al., 2021). As such, two introduced plants that may be of interest to land managers seeking forage for bees are *Modiola*

*caroliniana* and *Crotalaria lanceolata*. These plants were used by all bee groups studied and are present in almost all tropical and subtropical continents (Jacobi et al., 2005; Barkley et al., 2006). Both plants are also annual herbs that typically colonize disturbed sites, and so pose little risk of invading undisturbed areas (Davis and Thompson, 2000).

Herbaceous plants were the most common source of nest materials for leafcutter (*Megachile (Eutricharaea)*) and cellophane (Hylaeinae) bees. Microscopic examination of the cell linings of other species of *Hylaeus* (Hylaeinae) showed the presence of fiber-like strands embedded in a solid matrix, suggesting that their linings are comprised of two different components (Espelie et al., 1992). Many other bees are known to mix waxy secretions (from exocrine glands) with plant materials before using them in nest construction (e.g. stingless bees, Meliponini) (Wille, 1983). Our results suggest that hylaeine bees mix their own exocrine secretions with plant sources. Personal observation of *H. nubilosus* constructing nests suggests this species does add plant material (human-made sawdust or frass from wood-boring insects – a source of cellulose) to their secretions when sealing or “capping” a nest entrance, however, they appear to use only secretions to line or divide brood cells (RW; CF; TS personal observations).

Surprisingly, parasitic plants (Santalales, “mistletoes”) were the most frequently used resource to construct nests by resin bees. Possible source materials may be the glue-like secretions of haustorial hairs, used by all parasitic plants to attach to their host (Yoshida et al., 2016), or wax from the cuticles of laminar surfaces (Wollenweber et al., 2000).

## 5 Conclusion

This study found that native shrubs or trees and introduced herbs are key plant sources of nest provisions and materials for cavity-nesting solitary bees in natural and agricultural landscapes. Plants used in both orchards and forests by the same bee taxa were all tree and vine species, supporting the idea that habitat complexity is important for sustaining bee populations in agricultural landscapes. Furthermore, introduced plants were used for pollen and/or nest materials by all bee species studied, probably due to their ubiquity in both landscapes, and may be crucial for filling temporal/nutritional gaps in resource availability. However native plants accounted for more than 50% of relative read abundance for six of seven bee species. Based on these findings, we recommend providing forage for bees by retaining or planting patches of complex habitat (i.e. with trees, shrubs and vines) and managing herbaceous weeds and mistletoes in ways that reduce spread while retaining forage during periods of resource scarcity.

## Data availability statement

The data presented are deposited in the Zenodo repository, accession number 10.5281/zenodo.18932434.

## Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

## Author contributions

RW: Conceptualization, Formal Analysis, Writing – review & editing, Data curation, Investigation, Methodology, Writing – original draft, Validation, Visualization. AK: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – review & editing. SL: Conceptualization, Formal Analysis, Methodology, Writing – review & editing. TS: Methodology, Writing – review & editing, Data curation, Investigation. CB: Formal Analysis, Investigation, Methodology, Writing – review & editing, Data curation. CF: Data curation, Investigation, Methodology, Writing – review & editing. AS: Data curation, Methodology, Writing – review & editing, Supervision. BK: Methodology, Writing – review & editing, Conceptualization, Resources. HW: Conceptualization, Methodology, Writing – review & editing, Formal Analysis, Funding acquisition, Investigation, Project administration, Supervision.

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## 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/frbee.2026.1744662/full#supplementary-material>

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