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## EDITED BY

Fabio Santos Nascimento,  
University of São Paulo, Brazil

## REVIEWED BY

Eduardo De Farias Geisler,  
National Institute of Amazonian Research  
(INPA), Brazil  
Luiz Carmo,  
Federal University of Rio de Janeiro, Brazil

## \*CORRESPONDENCE

Thomas Eltz

✉ thomas.eltz@rub.de

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# Look up! Stratification and vertical approach to auditory prey cues in frog-biting midges (Corethrellidae)

Thomas Eltz <sup>1,2\*</sup>, Hannah Luisa Kimm <sup>1</sup>, Roger Böddicker <sup>1</sup>,  
Maximilian Sigl <sup>1</sup>, Rudolf Meier <sup>3,4</sup> and Jonas Virgo <sup>1</sup>

<sup>1</sup>Department of Animal Ecology, Evolution and Biodiversity, Ruhr-Universität Bochum, Bochum, Germany, <sup>2</sup>Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador, <sup>3</sup>Center for Integrative Biodiversity Discovery, Leibniz Institute for Evolution and Biodiversity Science, Museum für Naturkunde, Berlin, Germany, <sup>4</sup>Institute for Biology, Humboldt University, Berlin, Germany

Frog-biting midges (Corethrellidae) are tropical, blood-sucking micro-predators of frogs that locate their prey by eavesdropping on anuran mating calls. In the present study we used acoustic traps broadcasting the call of Savage's Thin-toed Frog (*Leptodactylus savagei*) to elucidate frog-biting midge stratification in the rainforest as well as the directionality and spatial scale of their phonotactic approach. In an Amazonian rainforest in Ecuador, midges were attracted to acoustic traps in all forest strata, including the canopy of an emergent tree 38 m above the forest floor, with a significant effect of stratum on midge species composition. Of the eight putative species (MOTUs) of *Corethrella* delimited using COI barcoding (N = 158) four were also found in the highest forest stratum (28.5–38 m), with one of them showing a preference for the middle and top canopy. In both Ecuador and Costa Rica catch numbers of ground-based traps differed strongly depending on the orientation of an attached mesh tunnel (65 cm) that was used to filter approach directions. Very few midges entered traps when mesh tunnels allowed horizontal (0°) or inclined (45°) approach vectors, while the highest catch numbers were recorded when tunnels were oriented vertically (90°). Consistent results were obtained when the length of the attached tunnel was extended to 250 cm, confirming that the primary approach to a calling host follows a vertical trajectory, and demonstrating that frog-biting midges can perceive acoustic stimuli in the acoustical far field. When stimulus intensity was varied, midges showed a significant phonotactic response at sound pressure levels as low as 56 dBA at tunnel entrance (65 cm), equivalent to a male *Leptodactylus savagei* calling from ~30 m distance. Our results emphasize that, despite their minute size, frog-biting midges are highly sensitive to acoustic stimuli. They are also highly mobile, occupy all strata of the neotropical rainforests and preferentially home in on their prey from above.

## KEYWORDS

acoustic ecology, ectoparasites, insect hearing, Nematocera, Culicomorpha

## Introduction

Frog-biting midges (Corethrellidae), the sister group of mosquitoes (Culicidae) and phantom-midges (Chaoboridae), are mostly tropical, tiny (1–2 mm), blood-sucking midges that have an unusual way of locating their blood hosts, frogs. Female midges eavesdrop on the nocturnal mating calls of male frogs, being attracted maximally by certain combinations of frequency and pulse duration/rate (Bernal et al., 2006; Camp, 2006; Grafe et al., 2008; Virgo et al., 2019). Dozens of midges can sometimes be seen imbibing blood from an individual male frog at a tropical pond, and several hundreds of midges may feed on blood from a frog in one night (Bernal et al., 2006). While there are other dipteran species using acoustic stimuli for prey location, e.g. certain frog-biting culicid mosquitoes (Bartlett-Healy et al., 2008) or tachinid parasitoids of crickets (Müller and Robert, 2001), in corethrellids phonotactic predation is characteristic for the entire pan-tropical group (Borkent, 2008). Corethrellid activity is strictly nocturnal and frogs appear to be the only natural blood hosts (Borkent, 2008; Cutajar and Rowley, 2020). While specialized on locating frog calls they seem to have a relatively generalized acoustic template (Legett et al., 2018), with at least the more common species in the neotropics being attracted to a broad range of frog calls and even pure sinusoidal tones (Legett et al., 2018; Virgo et al., 2019; Geisler et al., 2024).

Although frog-biting midges have gained increasing interest for their phonotactic behavior in recent years, many aspects remain unknown. One major area of uncertainty is the spatial range within which frog-biting midges detect and react to auditory cues, which has important implication for the ecology and evolution of interactions with their hosts. Hearing in Nematocera (“midges” within the insect order Diptera) is mediated by flagellar ears, i.e. the plumose antennae that are vibrated in response to particle-velocity changes resulting from sound in air (Göpfert et al., 1999; Pantoja-Sánchez et al., 2023). Traditionally such ears are considered to function only in the near field, i.e. over distances of centimeters to decimeters (Albert and Kozlov, 2016). However, the effective range of a hearing organ depends ultimately on its sensitivity, and more recent research suggests that mosquitoes can detect sounds from distances of > 5 m if these sounds were of sufficient intensity (Bartlett-Healy et al., 2008). In behavioral and physiological experiments sounds broadcast from distances up to 10 m (perceived at a minimum sound level of 31 dB) evoked stimulus-related responses in *Aedes aegypti* (Menda et al., 2019). The numbers of female *Corethrella* attracted to acoustic lures, and the immediacy of their approach, would suggest that they possess comparable capacities, but this has not been investigated in a systematic way.

In the present study we investigated spatial aspects of corethrellid foraging behavior in the field using acoustic traps. Initially, we investigated the directionality of the phonotactic approach to increase our understanding on how these tiny insects locate their blood hosts in a complex three-dimensional environment. These experiments were stimulated by visual observations made in the light cone of a strong LED torch in La

Gamba (R. Böddicker & T. Eltz, pers. obs.). When a torch was pointed to the dark night sky above a sound trap in a forest clearing, midges could be observed “falling down” towards the speaker from >3 m height (the distance at which the torch produced visible reflections from midges) within seconds after playback commenced. In our experiments we therefore tested the hypothesis that midges prefer a vertical approach to an acoustical stimulus over a horizontal or inclined one. Additionally, we measured the minimum stimulus intensity that is sufficient to elicit a phonotactic approach from a certain distance and estimated the distance from which natural frog calls might be approached in the natural habitat. Finally, having obtained evidence for a strong vertical component in the phonotactic approach, we assessed the presence of frog-biting midges in the different vertical strata of the rain forest and examined whether there is stratum-specificity among different species/molecular taxonomic units (MOTUs) of midges. Although vertical stratification has been investigated in several insect taxa, including also hematophagous Diptera in tropical forests (Basset et al., 2008), this aspect has not been investigated in detail in frog-biting midges (Corethrellidae; but see de Souza Amorim et al., 2022).

## Methods

The experiments were done from 2022 to 2024 at two lowland neotropical forest sites: at the La Gamba research station in southern pacific Costa Rica ([www.lagamba.at](http://www.lagamba.at)), and at Tiputini Biodiversity Station (TBS) located in Amazonian Ecuador adjacent to the Yasuní National Park ([www.tiputini.com](http://www.tiputini.com)). Both localities harbor species-rich frog communities (Cisneros-Heredia, 2006; Franzen and Kollarits, 2018). Whereas La Gamba is relatively well studied with regard to frog-biting midges (Sigl et al. 2025, Virgo et al., 2019, 2021, Virgo and Eltz, 2022) very little is known about *Corethrella* in the western Amazon.

In all experiments midges were trapped with custom made, compact (height 17 cm, diameter 13 cm), fan-driven (Biogents 5V) traps broadcasting a call of the widespread Savage’s Thin-toed Frog, *Leptodactylus savagei*, consisting of series of ‘whorups’ of 0.25 s pulse duration and a dominant frequency of 379 Hz (see <https://homepage.ruhr-uni-bochum.de/thomas.eltz/Frogs/Leptodactylidae.html>). Series of *L. savagei*-calls have proven to be highly attractive for *Corethrella* spp. at several neotropical localities (Virgo et al., 2019, Virgo & Eltz, pers. obs.) and for a wide range of *Corethrella* species (Virgo et al., 2021). For example, in La Gamba, traps broadcasting *L. savagei* calls attracted all of the five species of *Corethrella* that are regularly collected directly from frogs, including one species that feeds predominantly on tree frogs (Virgo et al., 2021). While *L. savagei* only ever calls from ground level, or even from holes in the ground, the broad attractiveness of its call led us to use it as standardized attractant also in the vertical stratification assay.

In the present study we played repeated series of 24 *L. savagei*-‘whorups’ (1 per 1.8 s) followed by 15 s intervals of silence for variable times (see below). If not indicated otherwise these series

were played at a standardized intensity using Jay-Tech K10/A100 speakers placed 10 cm above the opening of the actual suction trap where midges were caught in a fine nylon mesh bag (Biogents). At the used speaker level the *L. savagei* call produced an average peak sound pressure level (SPL) of 78.06 dB(Flat) at 65 cm from the source (measured in the lab with an Acoustilizer AL1, NTi Audio AG, Liechtenstein), corresponding to 74.3 dB at 1 m. Midge samples were immobilized in a freezer and counted under a stereomicroscope. Excluded from counts were the occasional culicid and other non-corethrellid midges.

If not indicated otherwise all statistical tests were done with GraphPad Prism 10.

## Vertical stratification assay

In November 2022 we used the Canopy Tower at TBS to assess midge stratification. This tower has been erected next to a massive emergent tree (*Ceiba pentandra*, height ca 50 m [Figure 1](#)) without substantial alterations of the natural forest surroundings, i.e. the tree and tower is embedded in the continuous natural forest matrix with other herbaceous and woody vegetation nearby (see [Figure 1](#)). We trapped midges with three separate trap units at nine evenly spaced heights (platforms) along the stairs of the tower, from 0 m (forest floor) to 38 m (top platform). Traps were operated for ten

minutes to obtain samples, with speakers placed horizontally above the trap (0°) and always aligned in the same compass direction.

Trap units were moved to other heights following a scheme that left at least one platform empty between traps at all times (i.e., maintained > 9.50 m distance in height between them) and avoided an association of night and height. Four rotations were done per night (from 20:30 to 21:30 h), repeated in four nights, resulting in 3 (traps) × 4 (rotations) × 4 (nights) = 48 midge samples, with 5 to 6 replicates per height. These samples were stored in 2 ml Eppendorf tubes filled with EtOH (abs.). Midges were counted per sample and Spearman's rank correlation was used to test for an effect of height on midge number. From 31 samples that contained midges a total of 264 individual midges were selected randomly for DNA barcoding, without prior knowledge of species affiliation and aiming at covering all heights equally. Genomic DNA was isolated following the GeneReleaser protocol (BioVentures Inc.), adapted by ([Weigand, 2013](#)) and optimized for *Corethrella* by [Virgo et al. \(2021\)](#). For barcoding, a 658 bp fragment of the cytochrome oxidase subunit I (COI) gene was amplified using primers HCO1490/LCO02198 ([Folmer et al., 1994](#)), each tagged with a unique 9 bp sequence as described by [Srivathsan et al. \(2023\)](#). Pooled amplicons were sequenced on an Oxford Nanopore MinION at the Center for Integrative Biodiversity Discovery (Museum für Naturkunde Berlin). All barcodes were queried against the NCBI (BLASTN with minimum e-value of 1e-10) and BOLD databases to check for

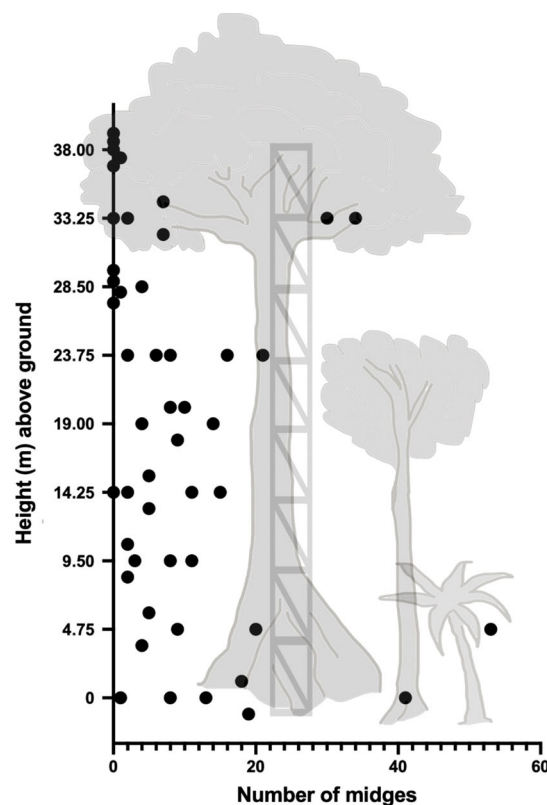


FIGURE 1

Frog-biting midges (*Corethrella* spp.) in acoustic traps broadcasting *Leptodactylus savagei* calls (right) on the canopy tower at Tiputini Biodiversity Station in Amazonian Ecuador (left). Grey shades in the right panel show an approximation of the vegetation structure around the tower.

membership in *Corethrella*. For species delimitation, barcodes confirmed as likely belonging to *Corethrella* spp. were analyzed using ASAP (Assemble Species by Automatic Partitioning; Puillandre et al., 2021). For partitioning we included *Corethrella* sequences of 17 putative species obtained from Genbank, that had been corroborated with a second marker (ITS2; Virgo et al., 2019).

For statistical analysis of MOTU composition the 31 samples from which the sequenced midges originated were grouped in three strata, combining three heights each (low=0–9.5 m, middle=14.25–23.75 m, and high=28.50–38 m). To test for differences in midge MOTU composition a one-way PERMANOVA was calculated using the R package *vegan* (Oksanen et al., 2015), using Bray-Curtis distances as a distance metric, with ‘stratum’ as a grouping variable and 999 permutations.

## Homing-angle experiment

To assess the vertical (meridional) angle of the approach to acoustic stimuli we put trap units in mesh tunnels that were midge-proof and had only one opening. This allowed us to “point” the whole arrangement at variable angles away from the sound source. Two different setups were used, short and long: 90x60x60 cm nylon mesh Life Unearthed Pop-Up-Insect Cages (90 x 60 x 60 cm) were cut open at one small side (60x60 cm) to provide the short setup, and fully collapsible, 250x50 cm cylindrical nylon Mesh Tunnels (Spordas, for children) were closed at one end with a plastic bag, providing the long set-up. In both setups a trap unit was placed at the closed end, resulting in distances of 65 cm (short setup) or 250 cm (long setup) from the opening to the sound source/trap. These distances represent the minimal distances at which trapped midges must have perceived the stimulus to make the decision to orient towards it. We used setups of different length to vary the minimal distance of perception/orientation.

The short setup was operated at horizontal (0°), inclined (45°) and vertical (90°) orientation in two separate experiments at TBS (November 2022) and La Gamba (March 2023). The traps themselves remained upright regardless of angular treatment to allow equal suction efficiency, but the trap speakers were oriented in alignment with the tunnel to maintain sound pressure levels at the tunnel opening. All experiments were conducted within the first 3 hours of the night. The trap setups were always placed at the edge of forest clearings, just outside of overhanging vegetation, at sites that were at least 15 meters apart from each other. Each site was only used once for a given angular treatment and traps were run for ten minutes per replicate. Three (TBS) or six (La Gamba) trap units were rotated systematically through sites and angle treatments. We collected 9 replicate samples per angle treatment in TBS, resulting in 27 samples, and 6 replicate samples in La Gamba, resulting in 18 samples. In TBS we also collected control samples without attached tunnel and with the speaker in vertical orientation (90°; 8 samples).

The long setup was only used in La Gamba (March 2023) with four different angle treatments (0°, 45°, 72.5°, 90°). Only two tunnels were available, so we rotated two trap units/tunnels through treatments and sites. The general procedure was similar, but in this case we used SoundCore Anker Mini 3 loudspeakers, and

trapping duration was 30 min per replicate. At the used sound level, the *L. savagei* call produced an average sound pressure level (SPL) of 68.5 dBA at the opening 250 cm from the source (measured in the field with a Benetech Digital Sound Level Meter GM1351), corresponding to 76.5 dBA at 1 m.

## Behavioral response threshold

To determine the minimal sound pressure level that would trigger phonotactic behavior to *L. savagei* calls in midges we used the short setup (see homing-angle experiment) with vertical orientation of the tunnel and variable speaker level setting (level 5 to 10, Jay-Tech speakers, see Results). For every treatment trial we ran a silent control that except for the lack of the frog-call play-back was identical to the treatment. Individual pairs of treatment and control traps were placed at a distance of ten meters in similar situations and operated for 20 minutes simultaneously. A total of 72 paired trials were run during the first three hours of nine nights in March 2024 in the station garden or near the Laguna in La Gamba, Costa Rica (see Results for number of replicates per speaker-level setting). During each trial we measured SPL of the played *L. savagei* call at the tunnel entrance of the treatment trap using a Benetech Digital Sound Level Meter GM1351. The device was regularly checked for deviations before the trials using a Voltcraft SLC-100 calibrator. For comparison we took a series of controlled measurements in the lab, thereby excluding the partly substantial nocturnal background noise in the field. To test for differences in midges captured between treatment and silent control traps we used Wilcoxon matched-pairs test.

To determine at what distance the SPL of natural calls of *Leptodactylus savagei* would fall below the behavioral response threshold we measured SPL at individual males calling in Costa Rica and calculated threshold distances using the inverse square law. A total of four frog individuals were measured from distances between 3.5 to 8 m, with 1 to 4 replicate measurements each. The frogs were calling from variable sites: one from underneath a pile of fallen leaves, one from a hole in a road embankment, one from within a bush and one openly from the edge of a pond. Distances to frogs (or their hideouts) were measured using a RockSeed laser distance meter. Repeated SPL measurements were taken from different angles. Mean threshold distances as well as SPL at 1 m were calculated for each measurement using the inverse square law, means calculated for individual frogs, and grand means across frog individuals. Although the field conditions certainly violated some of the assumption of the calculations (spherical sound emission, no reflections), we are confident that they allow a crude estimate of threshold distance.

## Results

### Vertical stratification assay

A total of 439 frog-biting midges were trapped at different heights on the TBS canopy tower, with an overall decrease in numbers from bottom to top (Figure 1; Spearman Rank Correlation:  $N = 45$ ;  $R_s = 0.52$ ;



$p < 0.001$ ). However, there were numerous midges active also at higher strata, with the third and fourth highest midge counts at 33.25 m, in the lower canopy of the emergent support tree.

We obtained 158 COI barcodes that matched the genus *Corethrella* in BLAST analyses (average of 97.6% percent pairwise identity; range 86.1–100%).

Based on these sequences, the best-supported ASAP partition, defined by the minimum ASAP score (4.50), was selected for further analysis and yielded 24 MOTUs, with the next two best-ranked partitions suggesting 42 (score = 5.0) or 23 (score = 7.50) MOTUs, respectively. Of these 24 MOTUs, nine derived from the stratification assay, of which one could be assigned to the widely distributed *Corethrella amazonica* (96.5–97.7% pairwise identity;  $N = 31$ ; Borkent, 2008; Geisler et al., 2024). The remaining eight could only be identified to the genus level as *Corethrella* sp. (Figure 2). However, four of these putative species corresponded to *Corethrella* MOTUs delimited in previous studies (96.5–100.0% pairwise identity; see Supplementary Material Table 1). The delimitation of the 17 included reference MOTUs obtained from Genbank was largely recovered, except for two MOTUs (*Corethrella peruviana* and *Corethrella* sp. *peruviana* 1), which were combined into a single MOTU (Supplementary Material 2).

There were 5 (low), 8 (middle) and 5 (high) different MOTUs in the three different strata, with the four most common ones having been trapped in all three strata. Based on PERMANOVA there was a

significant effect of stratum on midge MOTU composition ( $N = 31$  samples;  $F = 2.72$ ;  $p = 0.0105$ ). This effect seems to have been driven mostly by one MOTU, *Corethrella* sp. EC1, which increased in both absolute and relative numbers from low to middle to high stratum.

## Homing-angle experiment

There was a significant effect of angular treatment on midge numbers, a result that was qualitatively identical in TBS and La Gamba (Kruskal-Wallis Test: TBS:  $N = 27$ ;  $p < 0.01$ ; La Gamba:  $N = 18$ ;  $p < 0.001$ ; see Figure 3 for *post-hoc* comparisons). Very few midges entered traps when mesh tunnels were oriented horizontally ( $0^\circ$ ) or were inclined by  $45^\circ$ , while maximal catch numbers were recorded when tunnels were oriented vertically ( $90^\circ$ ; Figure 3). Absolute catch numbers were approximately three times as high in La Gamba compared to TBS, which is in accordance with our impression of outstanding frog-biting midge abundance around the La Gamba research station (Virgo et al., 2019).

Absolute midge numbers were substantially lower with the long setup in La Gamba, reflecting lower sound pressure levels at the tunnel entrance. However, relative proportions of catches corresponded closely to the short setup, with the  $67.5^\circ$  treatment being intermediate between  $45^\circ$  and  $90^\circ$  (Kruskal-Wallis Test:  $N = 48$ ;  $p < 0.001$ ; see Figure 4 for *post-hoc* comparisons).

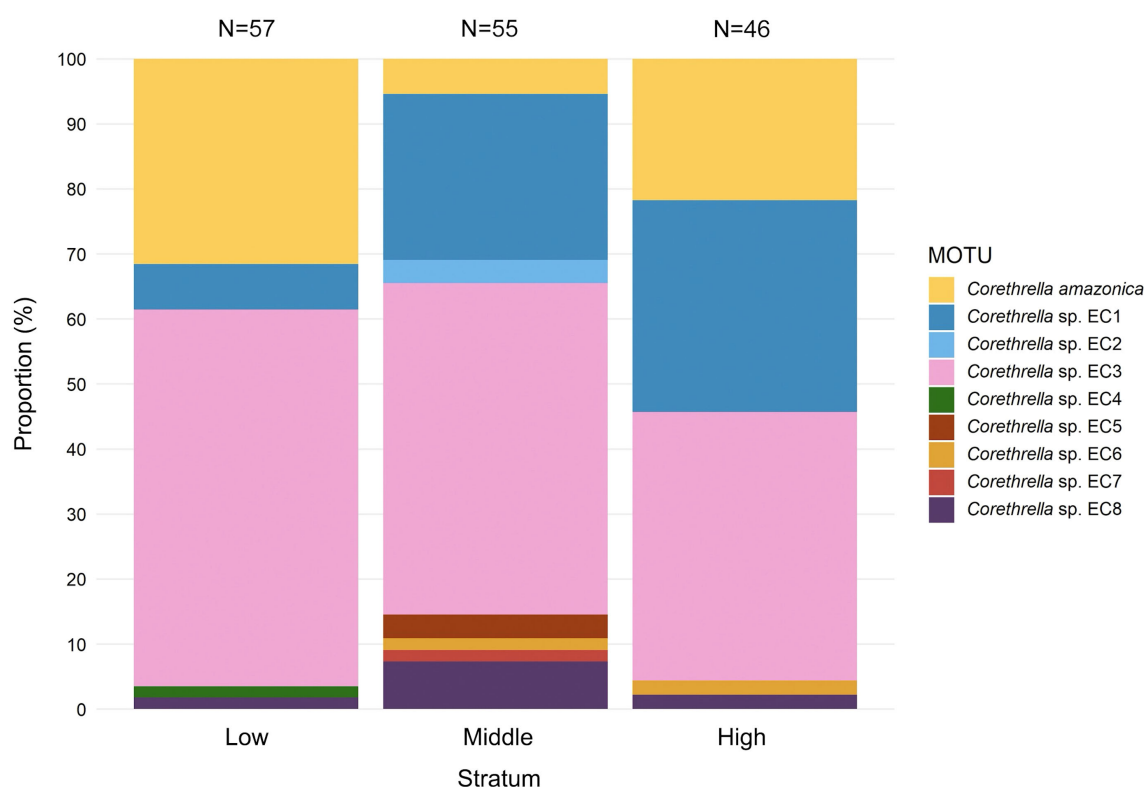
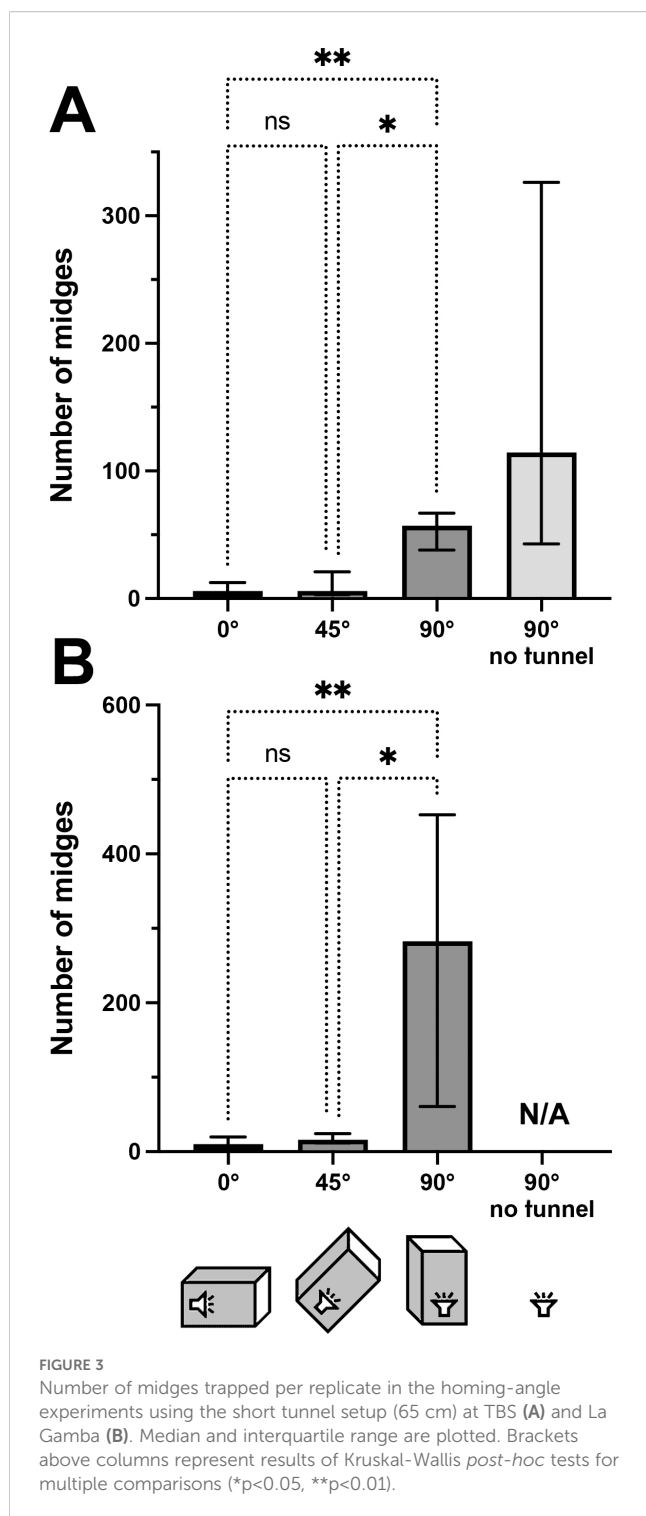


FIGURE 2

MOTU composition of frog-biting midges (*Corethrella* spp.) in acoustic traps broadcasting *Leptodactylus savagei* calls on the canopy tower at Tiputini Biodiversity Station in Amazonian Ecuador. Sequenced midges ( $N = 158$ ) were drawn from samples taken at nine different heights (see Figure 1), which were grouped into three strata for analysis and visualization.



The species composition of midge samples was not investigated in the Homing-angle experiment (see [Discussion](#)).

## Behavioral response threshold

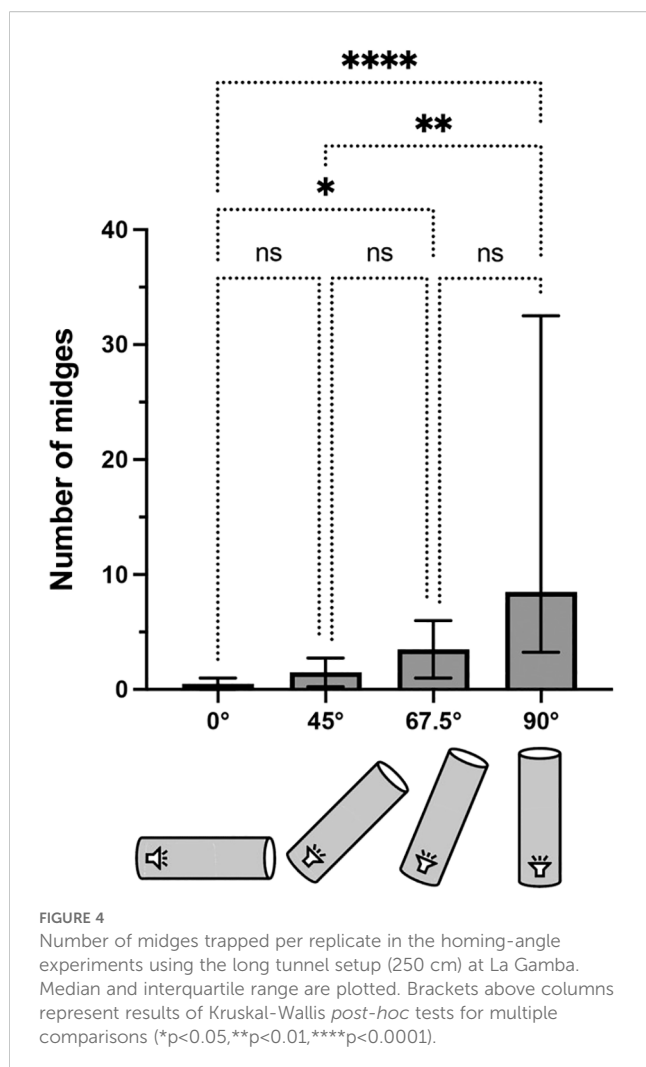
In the experiment to determine the behavioral response threshold the silent control traps were all empty except for a

single trap that had captured 1 midge. Treatment traps broadcasting *L. savagei* calls captured 0 to 369 midges. The percentage of traps with more midges than in the paired control increased from lower to higher speaker levels/sound pressure levels, with a steep increase in trapping success between level 7 and 8 ([Figure 5](#)). However, already at level 7 there were significantly more midges in the treatment traps than in silent controls (Wilcoxon matched-pairs test:  $N = 19$ ;  $p < 0.05$ ). This level corresponded to a measured mean peak SPL of the played-back *L. savagei* calls at the tunnel entrance of 56.5 dBA (field) or 55.8 dBA (lab). Calls of wild male *L. savagei* measured in Costa Rica produced an average SPL at 1m of 84.5 (+/-4.5) dBA ( $N = 4$ ). Assuming unobscured (free field) sound propagation their call SPL would fall below the *Corethrella* behavioral threshold at a distance of 28.7 (+/-11.9) m.

## Discussion

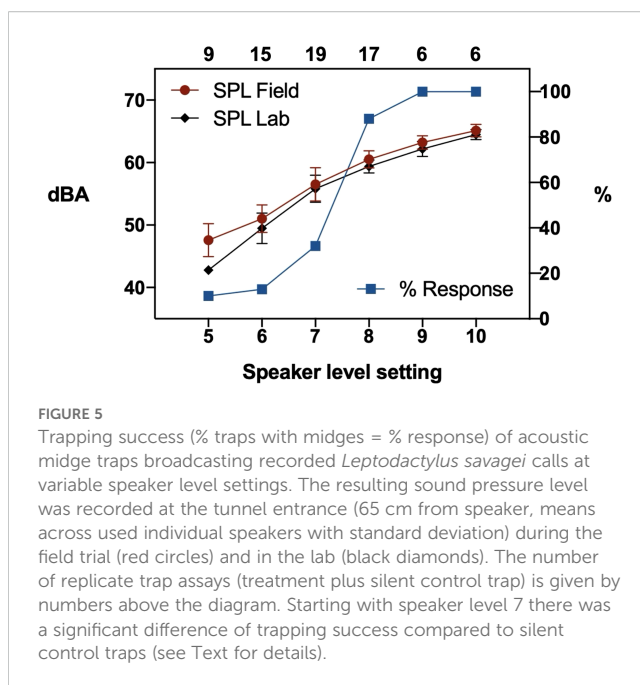
This study expands our understanding of spatial aspects of frog-biting midge foraging and is the first to explore *Corethrella* stratification in a rainforest with complex vertical structure. Our results demonstrate that frog-biting midges are highly mobile and present in all strata of the rainforest, including the top canopy. This corroborates the findings of a previous study using Malaise traps in the Brazilian Amazon, which had found a small number of *Corethrella* sp. at a height of 24 m in the forest canopy ([de Souza Amorim et al., 2022](#)). [McKeever and French \(1991\)](#) sound-trapped corethrellids at 0.5, 1.0, 2.0, and 3.0 m above ground in swampland in Georgia, USA, and found that midge numbers declined sharply with height. They attributed this to humidity being highest near the ground, resting sites most abundant, and air movement slowest, an environment deemed suitable for *Corethrella*. In Amazonian Ecuador rainforest we also found declining numbers of midges with height above ground, but there were still substantial numbers of midges in the canopy. Presumably, low humidity and wind are less of a problem for small midges in a tropical rainforest, and, clearly, resting sites were present in all strata around the canopy tower.

We found a moderate stratification among *Corethrella* MOTUs, with the four most common MOTUs being present in all three strata. For the rarer MOTUs sample size was too small to evaluate their vertical distribution. Of the more common ones, *Corethrella* sp. EC1 showed perhaps the clearest trend towards a canopy preference, but future studies with larger sample sizes will have to confirm this trend. From the literature, the evidence for hematophagous dipterans to stratify vertically in forest environments is mixed, with some species/groups having a predictable ground or canopy preference ([Swanson and Adler, 2010](#)), whereas others show no, weak or seasonally dependent preferences ([McGregor et al., 2018](#)). Generally, it is unclear whether stratum preferences are caused by host preferences or if travelling height is determined by other factors (e.g. search for breeding sites, avoidance of predation) and preferred hosts are simply the ones most commonly encountered at that height. It should be emphasized that most studies on stratification of



hematophagous dipterans were conducted in temperate forests and/or on only moderate vertical scales (e.g., ground vs. 10 m for canopy in (Swanson and Adler, 2010)). In tropical forests the scale and complexity of stratification is more substantial as are the challenges of canopy access and species identification. Here, limited data exists for medically important groups such as Ceratopogonidae and Phlebotominae, suggesting patterns of moderate stratification (Dias-Lima et al., 2002; Basset et al., 2008 and references therein) similar to what we found for Corethrellidae in the present study.

It could be argued that in our acoustical trap-based stratification assay frog-biting midges were lured to canopy traps from lower strata. Based on our own calculations, the SPL of the used calls could have attracted midges from distances of up to 7 m (see also discussion below). While we cannot exclude that inter-stratum attraction has influenced the stratum-specific community composition, frog-biting midges were attracted to canopy traps quickly, i.e. already during the first of a given days' short (10 min) broadcast intervals. Thus, it seems likely that they were not lured to those heights by the traps but were already present in the canopy when traps started to broadcast. Tree frogs (Hylidae), of which at least two species were regularly heard calling from the epiphyte-covered branches at the site, are common targets of *Corethrella* in



the neotropics (Virgo and Eltz, 2022; Virgo et al., 2021). Indeed, one *Corethrella* sp. EC8, captured at a height of 23.75 m during our assay, contained a blood meal later identified by DNA barcoding (Sigl & Eltz, unpublished). It was derived from *Osteocephalus fuscifacies*, a medium-sized tree frog previously recorded in Tiputini (Cisneros-Heredia, 2006) and known to breed in bromeliads (Jungfer et al., 2000). Corethrellids may have also been drawn to the canopy by water-filled bromeliad leaf axils, which we have found to be important breeding habitats in La Gamba (Virgo, Sigl & Eltz, unpublished data). The upper canopy of the canopy tower support tree in Tiputini is densely populated with epiphytes, including many sizable bromeliads from the genus *Aechmea*.

Judging from the homing angle experiments, the primary approach to auditory cues includes a phase of vertical descent. Catch numbers of traps were maximal when mesh tunnels were open to the vertical but very low when mesh tunnels were oriented horizontally. These results were obtained at both rainforest localities (Amazonian Ecuador and Costa Rica), suggesting that a vertical approach may be common among species of *Corethrella*. Although we have not identified the midges in the homing angle experiment to species level, the species composition in Ecuador was probably similar to that of the vertical stratification assay (Figure 3; the two assays were done during the same 10-days visit and at sites only 500 m distant from each other). In La Gamba, Costa Rica, we can refer to previous studies that have found a different set of species dominated by the super-abundant *C. ranapungens*, which represented 65 to 98% of trap catches depending on season (Virgo et al., 2019, 2021). Thus, it seems safe to assume that the vertical approach pattern is not a quirk of just one species.

Vertical descent on acoustic stimuli has also been demonstrated for females of the cricket-parasitizing tachinid fly, *Ormia ochracea* (Müller and Robert, 2001). In *Ormia* the descent is remarkably

accurate in complete darkness: flies are able to land only centimeters away from the loudspeaker after a flight distance of approximately 4 m (Müller and Robert, 2001). While *Ormia ochracea* uses tympanal ears for directional hearing, the vertical approach of *Corethrella* may be related to the mechanism that enables directional hearing with flagellar ears. Behavioral and electrophysiological experiments with *Culex pipiens* suggest that the in-flight position of antennae is optimal for binaural hearing focused in front of, above and below a mosquito (Lapshin and Vorontsov, 2023), resulting in an auditory space of female mosquitoes that has sufficient resolution to estimate the direction of a sound source. Possibly, an even more narrow directional tuning of flagellar responsiveness improves phonotactic targeting in even smaller insects such as frog-biting midges.

Alternatively, the vertical approach to acoustic stimuli may represent an optimal foraging strategy for *Corethrella*. In a dense rainforest environment, where flight paths are obstructed by branches, leaves, and other vegetation, a vertical descent may be energetically more efficient. By leveraging gravity to assist in their downward movement, host-seeking midges can cover greater distances more quickly and with less energy expenditure than in the horizontal plane. Additionally, this approach would minimize the risk of overshooting the target as the frog's call can only originate from as far as ground level, providing a clear and defined location for the midge to target. Finally, sound scattering in forests results mostly from tree trunks which are oriented vertically, resulting in significant reduction of sound intensity along the horizontal plane (Bullen and Fricke, 1982). Propagation in the vertical may be largely unobscured and, using a vertical descent, *Corethrella* may be able to achieve more precise targeting of sound sources.

Our results also have implications regarding the distance that sounds may travel to be perceived by flagellar ears. Traditionally, such ears were considered to function only in the near field, i.e. over distances of centimeters to decimeters (Albert and Kozlov, 2016), in accordance with a function of detecting flight tones produced by conspecifics in mate-seeking mosquitoes (Pantoja-Sánchez et al., 2023). However, in behavioral experiments female *Aedes aegypti* mosquitoes became airborne in response to acoustic stimuli originating 3 m away from their mesh cage while their antennal sensilla were stimulated from even larger distances (10 m), at a sound pressure level of only 31 dB (in an anechoic chamber, Menda et al., 2019). Our field experiments support these results, demonstrating that female *Corethrella* respond to calls played from a distance of at least 2.5 m, the length of the long-tunnel setup, clearly in the far field. It seems that even tiny insects with flagellar ears are capable of exploring an acoustic space of surprisingly large dimensions. Just how large is this space in a 1 mm frog-biting midge encountering natural frog call intensities? We have determined that behavioral responses to *L. savagei* playback calls are elicited at sound pressure levels as low as 56 dBA. Based on averaged SPL measurements of four bull frogs calling in their natural environment in Costa Rica, and assuming zero reflectance from vegetation, we estimated that calls could be detected from a distance of up to 28.7 m. Clearly, there are many potential errors involved in these calculations. However, an perceptual space with a diameter of several dozen meters would fit

well with observations of hundreds of frog-biting midges being attracted quickly and reliably to acoustic traps at neotropical forest sites.

## Data availability statement

Datasets are deposited at Figshare and will publicly available as of the date of publication (<https://doi.org/10.6084/m9.figshare.30539921>). The sequences used in the stratification assay are available in BOLD at [dx.doi.org/10.5883/DS-TIPEC22](https://dx.doi.org/10.5883/DS-TIPEC22) and in GenBank under the accession numbers PX525671–PX525828.

## Ethics statement

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

## Author contributions

TE: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. HK: Investigation, Methodology, Writing – review & editing. RB: Investigation, Methodology, Writing – review & editing. MS: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. RM: Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. JV: Investigation, Methodology, 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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