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Specialisation in frog-biting midges (Diptera: Corethrellidae): A landscape perspective

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Abstract

1. Generally, competition excludes pairs of phylogenetically close species across the landscape. However, habitat, food and behaviour differentiation facilitate co-occurrence of syntopic species. The taxa of frog-biting midges *Corethrella* are assumed to be specialists on calling male frogs, but little is known about how habitat gradients and frog-species composition influence their distribution and permit their coexistence in continuous landscapes.

2. Our aims were to understand how environmental gradients in habitat, sound frequencies and frog-species composition influence *Corethrella* spp. distributions and if habitat and sound frequencies segregate their occurrence across a landscape. We also investigated if habitat selection reduces co-occurrence of species that share similar acoustic niches. The study was conducted in 20 permanent plots in Central Amazonia using different sound attractants as baits to capture *Corethrella* species.

3. Habitat gradients, sound frequencies and frog-species composition all influenced distributions of *Corethrella* spp. However, these factors do little to avoid *Corethrella* co-occurrence, and habitat selection did not reduce co-occurrence of species attracted to the same sound frequencies. These results suggest that competition is unlikely to affect the distribution of adults and that other aspects, such as competition in the larval phase, may be more important to permit their coexistence.

4. The two most abundant species showed strong negative correlations in abundances, potentially indicating competition among these species, but larval habitat specialisation is likely to be the most important cause of this pattern.

KEYWORDS
co-occurrence, habitat selection, host-specificity, micropredator, prey

INTRODUCTION

It is generally assumed that phylogenetically close species need to segregate their niches, or one species will be eliminated by competitive exclusion (Schoener, 1974). Habitat can therefore select species distributions across a landscape (Dunson & Travis, 1967), reducing contact between similar species that share similar behaviour or food resources, mitigating the chances of competitive exclusion.

This is a common assumption for a broad range of vertebrate and invertebrate taxa, where species can share similar resources and behaviour due to spatial segregation (Schoener, 1974). However, when species overlap in habitat, specialisation on different niche axes may enable species coexistence in the landscape (Sandercock, 1967).

Habitat components, as such water availability, soil granulometry and vegetation structure, modulate the distribution of many taxa in tropical landscapes (Oliveira et al., 2009; Peixoto et al., 2023; Rabelo et al., 2021; Torralvo et al., 2021). Particularly for aquatic insects, these factors may be associated with the distinct habitats, such as phytotelmata, ponds and streams, which are used by the immature stages of many species (Gray, 1981; Nakanishi et al., 2013). For taxa like mosquitos and midges, whose aquatic larvae and adult females are both predatory (Lyimo & Ferguson, 2009), species distribution and co-occurrence are further expected to be driven by resource availability for adults across the landscape, and less by habitat gradient changes (Edman, 1979).

However, another critical factor that limits co-occurrence of closely related consumers is the detection of resources (Schoener, 1974). A growing number of studies have explored eavesdropping behaviour, whereby predators intercept the intraspecific signals of prey species to locate them (Bernal & Page, 2022). Eavesdroppers vary in a continuum from totally opportunistic to highly specialist strategies in locating their prey in the landscape (Bernal & Page, 2023). Host specialists usually have sensory adaptations to locate their victims and tight co-evolutionary history due to the high costs of detecting the wrong target (Bernal & Page, 2023). Eavesdropping micropredator flies usually fall in this category of specialists, but some show more flexibility in their host-seeking behaviour to cope with spatiotemporal changes in host abundances (Legett et al., 2017; Lyimo & Ferguson, 2009). Exploitation of different cues may reduce the use of similar resources and permit closely related species to coexist.

Some frog-biting flies are eavesdropping micropredators that are attracted by frog calls. These include many taxa from the infraorder Psychodomorpha and Culicomorpha, such as species in the genera *Sycorax*, *Uranotaenia* and *Corethrella* (Borkent, 2008; Caldart et al., 2016; Cutajar & Rowley, 2020). Sound traps with frog-call playback developed by McKeever and Hartberg (1980) are highly effective in capturing frog-biting fly females (Bernal & Silva, 2015), and facilitate investigations of their acoustic cue exploitation. Among these taxa, such studies have been most intensive for the genus *Corethrella*, which has 126 described extant species (Amaral et al., 2023) and probably many cryptic species yet to be described (Virgo et al., 2021). Some sound features, such as call rate and frequency, can be used to predict which frog species will be most frequently attacked (Meuche et al., 2016), suggesting that *Corethrella* spp. adult females could segregate in host-use due to acoustic niche differentiation.

It is not clear, however, whether acoustic preferences are sufficient to segregate *Corethrella* spp., since multiple species are often captured by the same synthetic and frog-call acoustic lures (e.g. Borkent, 2008; Meuche et al., 2016; Virgo et al., 2019, 2021). Moreover, direct observation of frog-*Corethrella* interactions and the inconsistency in prey specificity also suggest that different prey use would have little influence in reducing co-occurrence of *Corethrella* spp. (Borkent, 2008). These results suggest that syntopic *Corethrella* spp. might not compete in prey use or might be segregating in other niche dimensions, such as habitat, which could lead to them exploiting similar frog species.

It has been suggested that the high species spatial turnover of *Corethrella* spp. in Neotropical forests could be related to variation in habitat gradients, particularly in relation to oviposition sites where competition among larvae would play a key role (Borkent, 2008). However, to the best of our knowledge, only one study evaluated the relationship between habitat types and its effect on *Corethrella* species. The authors found that the peat-swamp habitats of Borneo (Brunei) had less frog specialist *Corethrella* spp. than rainforest areas, suggesting that the observed segregation in *Corethrella* spp. could be mediated by habitat specialisation (Grafe et al., 2018). However, Neotropical *Corethrella* spp. seem to be less specialised than those found in other tropical regions (Legett et al., 2017; Virgo et al., 2019; Virgo et al., 2021), so *Corethrella* spp. there might be more sensitive to spatial changes than to sound or frog species. In that case, if competition for prey is a primary driver of *Corethrella* habitat selection, it is to be expected that the species that most overlap in attraction to sound frequencies, which can be a proxy for prey use, should overlap least in their positions along habitat gradients, resulting in a negative correlation between similarity among species along the sound and habitat gradients.

Another aspect which could indicate competition among *Corethrella* spp. is their abundance correlations. Negative correlation between abundances of syntopic species could indicate that these species are competing directly or indirectly (Lawlor, 1979). Larvae of similar species can exclude each other by resource competition (Juliano, 1998) or by direct antagonistic interactions, such as wasteful killing (Blosser et al., 2013). Consequently, these interactions in the larval stage could influence the abundance of future adults and may reduce co-occurrence among species pairs. Immature stages of two or more *Corethrella* species are rarely found co-occurring in the same habitat, such as tree-holes, leaf axils or phytotelma (Amaral et al., 2023; Borkent, 2008). Exact causes cannot be attributed without further experiments, but if competition among pairs of *Corethrella* spp. larvae exist, strong negative correlations may arise in their adult abundances.

In this study, we investigated how *Corethrella* spp. distributions and co-occurrence vary in relation to habitat characteristics and frog species composition across a tropical landscape. Specifically, we evaluated the possible effects of distance from streams, vegetation structure, soil clay content and frog species composition on the distributions of adults of *Corethrella* species. Additionally, we also evaluated if level of attraction of *Corethrella* spp. changes in relation to sound frequencies. We also evaluated the degree of specialisation of *Corethrella* spp. on the habitat and sound-frequency gradients, allowing us to investigate to what extent habitat selection reduces species co-occurrence for species attracted to the same sound frequencies and if syntopic *Corethrella* spp. show negative correlations in their abundances.
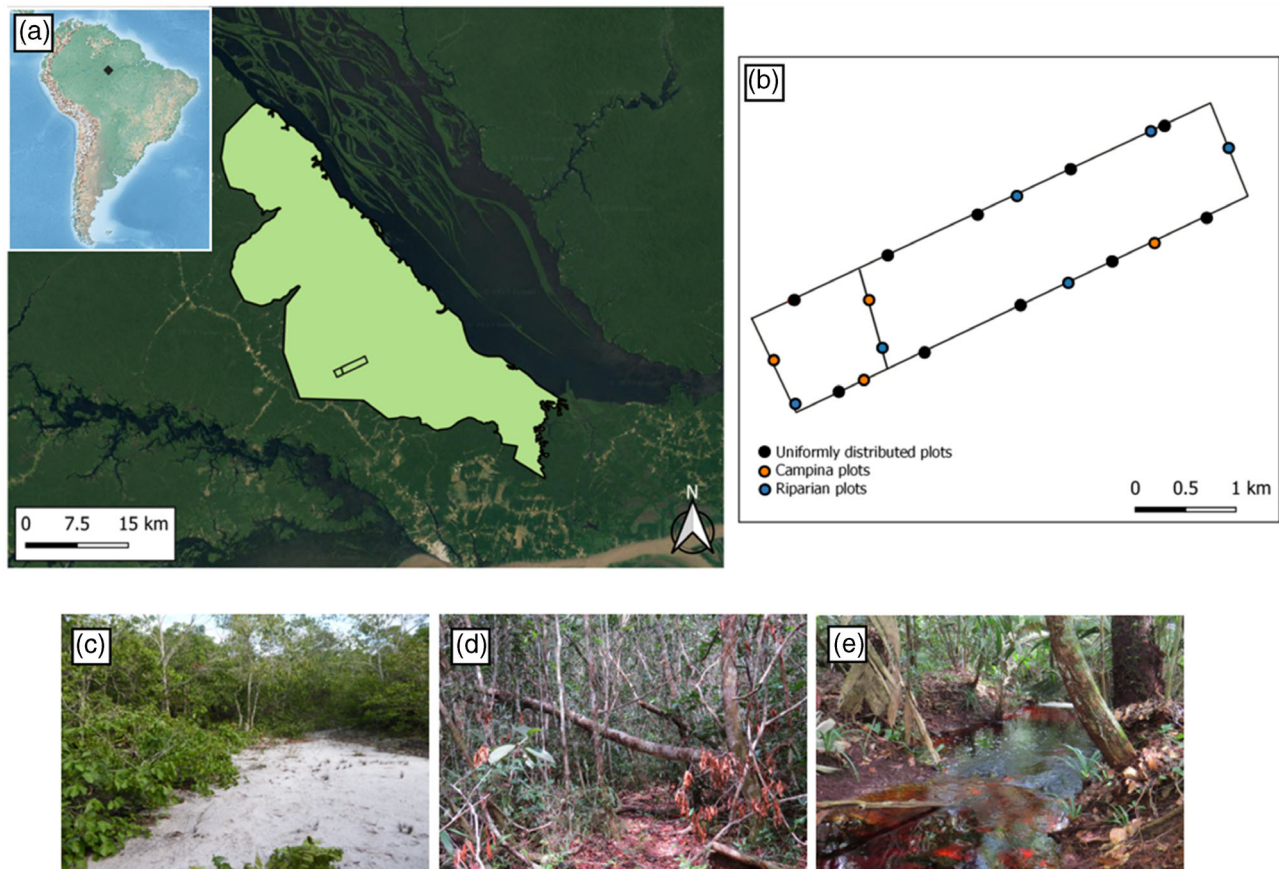
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FIGURE 1 Location of the study site, spatial configuration of plots, and the *campina* and *campinarana* formations where *Corethrella* were sampled in the Rio-Negro Sustainable Development Reserve (RDS—Rio Negro). (a) Map showing RDS—Rio Negro in northern Brazilian Amazon, the boundaries of the RDS—Rio Negro and the RAPELD module. (b) RAPELD module depicting the trail system and distribution of surveyed plots. (c–e) *Campina*, *campinarana* and Riparian *campinarana* formations, respectively.

MATERIALS AND METHODS

Study site

Surveys were conducted in 20 plots of a long-term ecological research (LTER) plot system (module) in the Rio Negro Sustainable Development Reserve (RDS—Rio Negro), Amazonas, Brazil. The sampling module follows the RAPELD (Brazilian acronym for Rapid Survey and Long-term Ecological Research) system (Magnusson et al., 2005, 2013). The area is characterised by the presence of white-sand formations, which are found in scattered patches throughout the Amazon region, but predominantly in the upper Rio Negro basin (Daly et al., 2016). Vegetation formations in the RDS-Rio Negro are dominated by *campinas* and *campinaranas*, with only a few fragments of taller forest on yellow latosols (SEMA, 2016). Water-table fluctuations dictate short-term flooding in this area, and acidic blackwater streams predominate (SEMA, 2016) (Figure 1e). Nutrient-poor podzols occur in both *campina* and *campinarana* formations. Trees with thin trunks and a thick layer of fine roots above soil predominate in the *campinarana* (Figure 1d) (Adeney et al., 2016). However, in some uplands, drier formations of *campina* with permeable exposed white-sand soil and short (~2 m) xeromorphic heath vegetation prevail (Figure 1c) (Daly et al., 2016).

Sampling design

We surveyed *Corethrella* in all 20 plots twice, once at the end of the dry season (October to December 2022) and once at the beginning of the rainy season (January to February 2023). There was a mean of 94 days between surveys of individual plots (min = 61 days; max = 126 days), which would result in at least two generations of *Corethrella* between these periods (McKeever & French, 1991). Although the two periods we surveyed probably reflect extremes in seasonality, a previous study found that *Corethrella* species composition did not change between seasons (Legett et al., 2017) and preliminary analyses indicated no differences in composition in our study (see section *Corethrella* sampling and identification). Frog species were surveyed between October 2022 and January 2023, covering the period of *Corethrella* sampling. Most frogs were detected from their calls, so the frog sampling reflects availability of frogs to *Corethrella* in this period. Few frogs call during the dry season, so wet-season sampling is the most appropriate to evaluate frog availability for *Corethrella*. The RAPELD module sampled consists of two parallel 5-km trails and their associated plots separated by 1 km (Figure 1b). Within the plot system, both riparian and non-riparian 250 m-plots were

established along each trail (Figure 1b). The starting coordinates of the trails were 3°3.746′ S, 60°45.604′ W and 3°3.241′ S, 60°45.843′ W (datum WGS-84). Specific coordinates for individual plots can be accessed in https://ppbio.inpa.gov.br/sitios/RDS_Rio_Negro. The RDS Rio-Negro module has 10 uniformly distributed plots spaced systematically at 1-km intervals that follow elevational contours and six riparian plots that follow stream courses (Figure 1b; Magnusson et al., 2005, 2013). Four additional plots following elevational contours were also installed in patches of *campina* vegetation (Figure 1b,c).

Corethrella sampling and identification

We surveyed each plot using Modified Pan Traps (PTM traps), which use speakers broadcasting sounds to lure *Corethrella* spp. females (Amaral & Pinho, 2015; Geisler et al., 2022). We modified the design of the PTM traps by adding a polystyrene plate above the speakers as protection from heavy rain. One PTM trap was placed every 50 m along the 250-m plot centre lines, for a total of six traps per plot. Six sound files were generated, each with a different artificial sinusoidal tone frequency (500, 1500, 2500, 3500, 4500, and 5500 Hz) and with standardised interpulse interval (0.85 s), pulse duration (0.25 s) and decibels (67–72 dB). We used this standardisation based on the known most attractive sound features for Neotropical *Corethrella* spp. (Virgo et al., 2019). Although call rate is also an important feature to lure *Corethrella* (Meuche et al., 2016; Virgo et al., 2019), we varied only sound frequencies to reduce the number of possible combinations. Theory suggests that *Corethrella* spp. instraspecific communication is based on flight tones that were co-opted to detect frog-calls (Silva et al., 2015). Thus, we assumed that sound frequencies might be the best proxy to evaluate prey exploitation. All sound files were generated using Audacity® software (Audacity team, 2021) and trap positions in plots are given in Supplementary Material 1. Traps were set at least 30 min after sunset, between 19:00 h and 23:00 h, the peak activity period for foraging *Corethrella* spp. females (Borkent, 2008). The traps were active for one hour in each plot and only on nights without rain (except for one night on which it rained suddenly when the traps were active). We used JBL Wind 2® speakers and sound levels were measured with a Voltcraft SL-100® decibel meter, before and after the campaign, at 0.5 m distance. No alteration in decibels greater than 10% was detected.

Adult female *Corethrella* captured in PTM traps were stored in 92% ethanol. All specimens from most samples were morphotyped based on a set of key characters (Supplementary Material 1). Only fifteen samples with approximately more than150 specimens were subsampled. To subsample, we used a petri dish divided in four quadrants and we mixed the material in the petri dish. In order to reduce sampling bias, we used as a randomizer the second hands of a clock. If the seconds at the moment of the sample was between 0–14 s—we analysed the first quadrant of the petri dish. Between 15–29 s, the second quadrant, 30–44 s the third and 45–59 s, the fourth. Only one quadrant was sampled and the proportional species abundance was

estimated by multiplying the subsample by four. Preliminary analysis indicated no changes in *Corethrella* species composition among seasons (Supplementary Material 1). Therefore, we used the sum of each *Corethrella* spp. abundance from both surveys in our datasets.

Specimens were mounted on slides in Hoyer's medium. We chose the most morphologically divergent specimens in each morphotype to mount, because they were more likely to be different species. Except for singletons and doubletons, we mounted slides of at least five individuals from each morphotype. We identified the species using the key to the New World Corethrellidae (Amaral et al., 2023) and comparison with descriptions (Almeida et al., 2021; Amaral et al., 2019; Amaral et al., 2021; Amaral et al., 2023; Amaral & Pinho, 2015; Borkent, 2008; Caldart et al., 2016). Voucher specimens were deposited in the *Coleção Entomológica Mítia Heusi Silveira* at Santa Catarina Federal University (CE-MHS UFSC), Florianópolis, Santa Catarina, Brazil, and the *Entomological Collection* of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. All specimens were collected under ICMBio licence n° 84311-2, which can be accessed at www.icmbio.gov.br/sisbio and DEMUC/SEMA n° 01/2021 process n° 01.01.030101.003046/2021–07.

Frog species composition

Frog species composition for each plot was based on visual and auditory surveys. Two surveys were conducted in all plots during the same period that *Corethrella* spp. were sampled. All plots were surveyed only by one specialist at night (19:00–22:00 h) and surveys lasted about 40 min. To quantify species composition in each plot, we utilised the presence or absence of a species in each segment, giving a relative-abundance index ranging from 0 to 25 per species per plot, following the PPBio Protocol for frog-abundance estimates (https://ppbio.inpa.gov.br/manuais). The highest value registered for each species in each plot in all surveys was used as an index of the relative abundance of that species. Frog-species nomenclature was based on Frost (2023).

Habitat-variable collection

We collected all habitat variables for the same plots that *Corethrella* and frog species were sampled. Stream distance was measured as the linear-distance from each plot to the nearest stream based on field observations or drainage shapefiles derived from QGis 3.16.2 software (Qgis Development Team, 2023). Vegetation structure and soil clay content were measured in a previous study (Pereira et al., in press). Vegetation-structure data were collected using a portable ground LiDAR (Light Detection and Ranging—Riegl LD90-3100VHS-FLP system). In each segment, thirteen variables were measured in relation to canopy height and understory density (Pereira et al. under review). To diminish sampling-error effects, we used only mean values of height, canopy-opening and understory-density values for each plot, which were summarised by Principal Component analysis (PCA)

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and the first ordination axis was used as a proxy for vegetation structure. Soil samples were collected at six points, 50 m apart, in each plot. Each sample was 10-cm deep and the six subsamples were pooled in a single composite sample following the Biodiversity Research Program (PPBio) protocol (http://ppbio.inpa. gov.br). Granulometric analyses were conducted by the Soils Laboratory of the National Institute of Amazonian Research (INPA) Agronomy Department, following the total dispersion protocol of the Brazilian Agricultural Research Corporation (Embrapa) (Donagemma et al., 2017). Soil components were highly correlated and, since clay is often associated with changes in invertebrate distributions (Nielsen et al., 2010) and pond formations, we used clay proportion for further analyses.

Data analysis

Influence of habitat, frog species composition and sound frequencies on *Corethrella* spp.

To investigate the influence of habitat variables, frog species composition and sound frequencies on *Corethrella* spp. we used multivariate generalised linear models from the MVABUND R package (Wang et al., 2012). First, we checked for multicollinearity between the three habitat variables using Variation Inflation Factor (VIF) tests. All VIF values were less than 1.5, indicating little collinearity among habitat variables. We created a MVABUND object using the matrices of the abundances of *Corethrella* species captured in each trap, the habitat variables, sound frequency, the first ordination axis from Principal Coordinates Analysis (PCoA) of frog species composition and site identity, as input. We used the first ordination axis of frog species composition in order to avoid overdispersion. We constructed a *manyglm* model using *Corethrella* species abundances in each trap as response variables, while habitat variables, sound frequencies and the first PCoA ordination axis from frog-species composition were used as predictors. We plotted the Dunn-Smyth residuals vs fits (Linear predictor value) to visually assess patterns in data in order to choose which distribution family would be used. As no pattern was found for residuals of the negative binomial distribution, we used this distribution family in our model. We also used the R function *anova.manyglm*, using block resampling (sites as blocks to resample, instead of rows), to account for the correlation between species and maintain all observations together in resampling, and P-values were calculated using 999 bootstraps. In one campaign, three traps (1500, 3500 and 5500 Hz), in three different plots (one riparian and two uniformly distributed plots), were opened accidentally for approximately 90 min and, in one riparian plot, it suddenly started to rain, which may have affected sampling. Due to that, we generated the same model excluding those four plots from analysis. For completeness, we included these traps and plots in the analyses reported, but analyses excluding them gave qualitatively similar results to the abundance data using all plots (Supplementary Material 1). We also analysed the relationships between *Corethrella* spp. using presence-absence data of all plots and the same predictor variables. However, for the presence-absence

model, we used the binomial family distribution. The MVABUND package also has an option for univariate tests to check how each species responds to the predictors. In this case, we used the function *p.uni*, with block resampling, in *anova.manyglm* for univariate tests. We only discuss results of the univariate tests of the species that occurred in more than two sites.

Association of frog species composition and *Corethrella*-species abundances

Dimensional reduction of data, such as Principal Coordinates Analysis (PCoA), can result in the loss of important information if there are non-linear relationships among species abundances. To complement this approach, we used Mantel tests to compare the association between frog and *Corethrella* spp. composition. However, in our study area, frog-species composition is also related to vegetation structure and soil clay content (Pereira et al., in press). To avoid spurious correlations between *Corethrella* and frog-species composition due to similar effect of habitat on both taxa we used partial Mantel tests to control for possible habitat effects. For partial Mantel tests, we used the total abundance of each *Corethrella* spp. per plot and the highest value of each species abundance per plot for frog species, excluding *Allobates femoralis*, which is a diurnal species and was probably found by chance at night. We generated dissimilarity matrices for each – dataset using the Bray–Curtis transformation derived from the function *vegdist* from the R package *vegan* (Oksanen et al., 2022). The habitat matrix was composed by stream distance, soil clay content and the first PCA ordination axis of vegetation structure. The dissimilarity matrix for this dataset was calculated using Euclidean distances, also derived from the function *vegdist*. We permuted the *Corethrella* dissimilarity matrix in relation to the frog-species dissimilarity matrix, controlling for the habitat dissimilarity matrix. Partial Mantel tests used the product moment correlation (Pearson's correlation) with 999 permutations.

Habitat and sound specialisation of *Corethrella* spp.

Univariate tests on MVABUND can indicate which taxa contributed the most to the multivariate results, but cannot give detailed information about how specialised each species is along of the ecological gradients evaluated (Warton, 2022). Therefore, to address the influence of habitat gradients and sound frequencies on each *Corethrella* spp., we used the *standardise effect size* (SES; Gotelli & McCabe, 2002) to evaluate specialisation on habitat gradients and sound frequencies. We used the abundances for each *Corethrella* spp., per plot, as input matrix to evaluate habitat specialisation. For sound frequencies, we used the abundances of each species in each trap as a sampling unit. The index (I) used for these analyses was the standard deviation for each species in relation to each gradient. We used the observed standard deviation (I_{obs}) minus the mean standard deviations of 999 randomised gradients (I_{sim}) divided by the standard deviation from the

999 randomised standard deviations of the habitat and sound gradients (σsim).

$$SES = Iobs - Isim/\sigma sim$$

Randomised gradients used were based on the normal distribution, as suggested by (Botta-Dukát, 2018). Values above 2 or below −2 indicate that species are specialised in the gradient evaluated. As in the MVABUND analyses, we only interpreted SES for species that were present in more than two plots or traps.

Habitat selection by *Corethrella* spp. with similar sound attractiveness

To investigate how much habitat selection reduces species co-occurrence of *Corethrella* spp. attracted to the same sound frequencies, we also utilised Mantel tests from the R package *vegan* (Oksanen et al., 2022). Mantel tests can be used to address the correlation between dissimilarity (or similarity) matrices of different datasets (Mantel, 1967), generating a Mantel statistic r, which ranges from −1 to 1 (negative or positive correlation), and a p-value. For this analysis, we used total abundance of each species per plot as the species-use matrix, and total abundance of each species per trap as the sound-attraction matrix. We also used only species that occurred in more than two plots for this analysis. Dissimilarities for each dataset were calculated using a Bray–Curtis transformation derived from the function *vegdist*. Then, we permuted the species-use dissimilarity matrix in relation to the sound-attraction dissimilarity matrix. Mantel tests used the product moment correlation (Pearson's correlation) with 999 permutations. If species that occur together tend to have less overlap in sound preference, a significant negative Mantel correlation is expected.

Correlation between syntopic *Corethrella* spp. abundances

To investigate whether syntopic *Corethrella* spp. show negative correlations patterns in their abundances, we used generalised mixed models using the R default function *glm*. We used proportional abundances per plot from the three most abundant and widely distributed species, *C. menini*, *C. unifasciata* and *C. amazonica*. We compared the correlation between species-abundance pair by pair (Supplementary Material 1). We used the Gaussian distribution family for the response variables. We estimated pseudo R^2 for each pairwise comparison using the function r^2 from the package *performance* (Lüdecke et al., 2021). We used Bonferroni corrections for the significance values since we tested the same hypothesis with the three tests.

Graphs of species distributions along habitat gradients were generated with the R script poncho (available at https://figshare.com/articles/code/poncho_R/753347; Dambros, 2020). All analyses were carried out using the R version 4.2.2 (R Core Team, 2022) statistical software.

RESULTS

We captured 6930 *Corethrella* females, belonging to eleven species (Table 1), with a sampling effort of 12 h per site (240 h total). The most abundant species captured were *Corethrella menini*, followed by *C. amazonica*, *C. unifasciata*, *C.* aff. *anniae*, *C. bifida/davisi* and *C. quadrivittata* (Table 1). Singletons and doubletons were represented by five species, *C. edwardsi*, *C. yanomami*, *C.* aff. *brandiae*, *C. appendiculata* and *C. orthicola*. Except for *C. menini C. yanomami*, *C. appendiculata* and *C. quadrivittata*, all other species were new records for Amazonas State, Brazil. *Corethrella* aff. *brandiae* and *C.* aff. *anniae* are candidate species to be described. *C. bifida/davisi* probably represents a species complex yet to be evaluated.

TABLE 1 Total abundances and abiotic variables for the eleven species found in this study, and the mean values of stream distances, vegetation structure, soil clay content and sound frequencies for each species.

| Species | Abundance | MEAN | | | |
		Stream distance (m)	Vegetation structure	Clay (%)	Sound frequency (Hz)
C. menini Feijó et al. 2021	3921	124.2	−0.6	2.4	949
C. amazonica Lane, 1939	2398	49.9	−1.6	2.8	506
C. unifasciata Amaral et al. 2023	459	68.4	−1.14	3.35	861
C. aff. *Anniae*	99	24.2	−1.4	1.53	540
C. bifida/davisi	39	6.47	−1.94	1.55	833
C. quadrivittata Shannon & Del Ponte, 1928	8	13.6	−2	2.1	1875
C. appendiculata Grabham, 1906	2	417.9	−0.7	0.05	500
C. orthicola Borkent, 2008	1	1.5	−2.2	1.05	500
C. edwardsi Lane, 1942	1	289.6	7.08	0.2	1500
C. aff. *Brandiae*	1	1.5	−1.1	1	1500
C. yanomami Amaral et al. 2019	1	50	−2.8	0.5	1500

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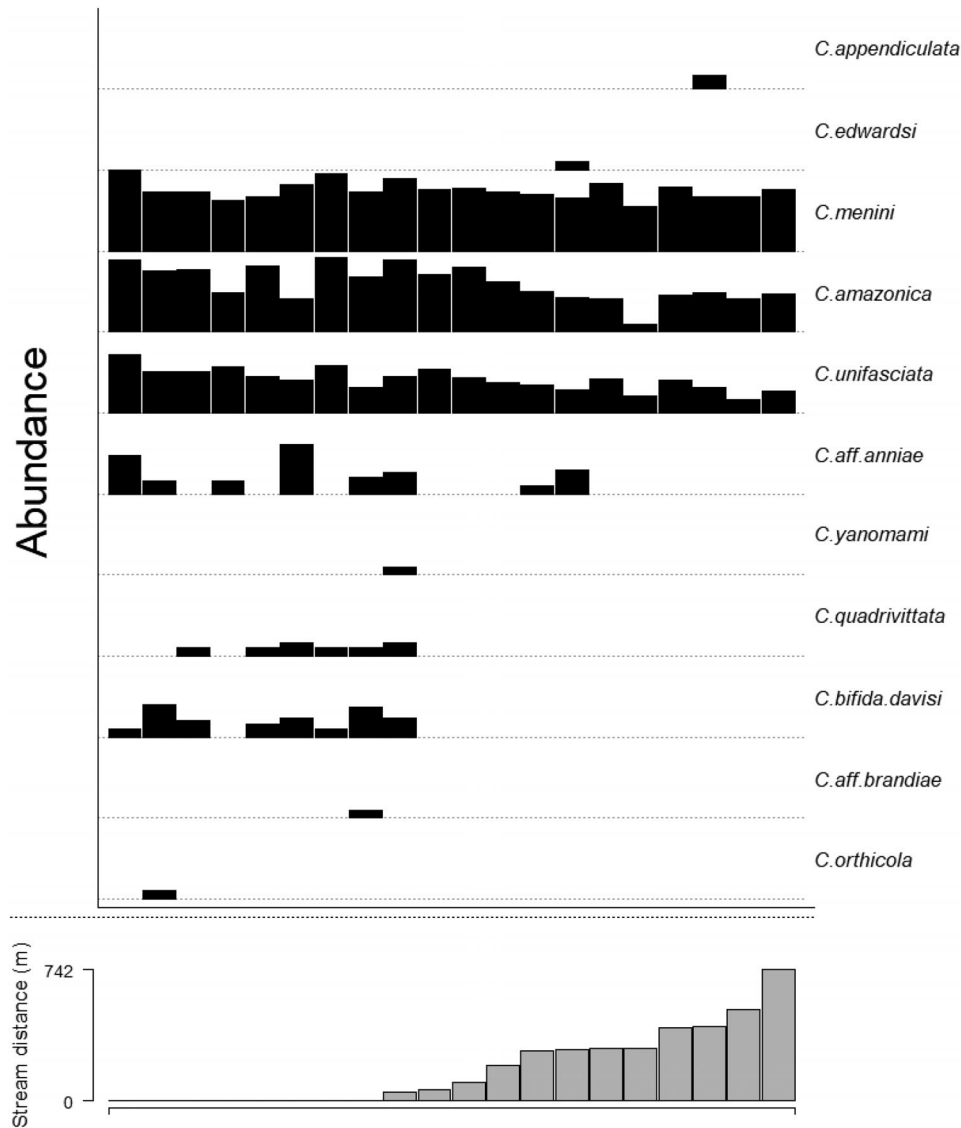Entomology

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FIGURE 2 *Corethrella* species composition ordered by stream distance. Relative abundances of *Corethrella* spp. are log-transformed in $\log_{10}(x + 1)$.

Based on abundance data, MVABUND analyses indicated that *Corethrella* spp. abundances differed in relation to sound frequency (DEV = 322.1, $p = 0.001$) (Figure 5), stream distance (DEV = 45.9, $p = 0.001$) (Figure 2), and a trend for vegetation structure (DEV = 26.6, $p = 0.069$) (Figure 3). Using presence-absence data and excluding plots with sampling errors, the same effects were found (Supplementary Material 1). Partial Mantel tests indicated a significant correlation between frog- and *Corethrella*-species composition (Mantel statistic = 0.22; $p = 0.039$) (see Figure 4).

The patterns shown in species-gradient graphs indicate that most *Corethrella* spp. were more abundant, and some were restricted to, areas close to streams with denser vegetation (Figures 2 and 3). Excluding singletons and doubletons, MVABUND univariate tests indicated that *C. bifida/davisi* is a specialist on riparian areas, with most individuals found within a ~100 m from streams (Table 2). SES indicated *C. quadrivittata* is a specialist on riparian areas and *C. unifasciata*, *C. amazonica* and *C. bifida/davisi* are specialists on

riparian and densely vegetated areas of *campinarana*. Two species, *C. menini* and *C.* aff. *anniae*, were considered generalists on all habitat gradients in both analyses. No species was specialised on the clay gradient (Table 2).

Traps broadcasting at 500 and 1500 Hz captured 97% of the total *Corethrella* individuals and all species found in our study (Figure 5). Traps broadcasting at 500 Hz captured 76% of total individuals and seven species, whereas 1500 Hz traps captured 21% of individuals and six species. Only *C. quadrivittata* showed some trend to be attracted to higher frequencies (1500–3500 Hz) (Figure 5) and *C. amazonica* was restricted to 500 Hz frequencies (99%). Based on SES results, all species were specialists on low-pitch frequencies, where most were attracted mainly by frequencies below 1500 Hz (Table 2). The only exception was that MVABUND showed no specialisation on sound-frequency gradients for *C. quadrivittata*.

Mantel tests indicated that similarity in habitat selection was highly positively related to similarity in attractiveness to sound
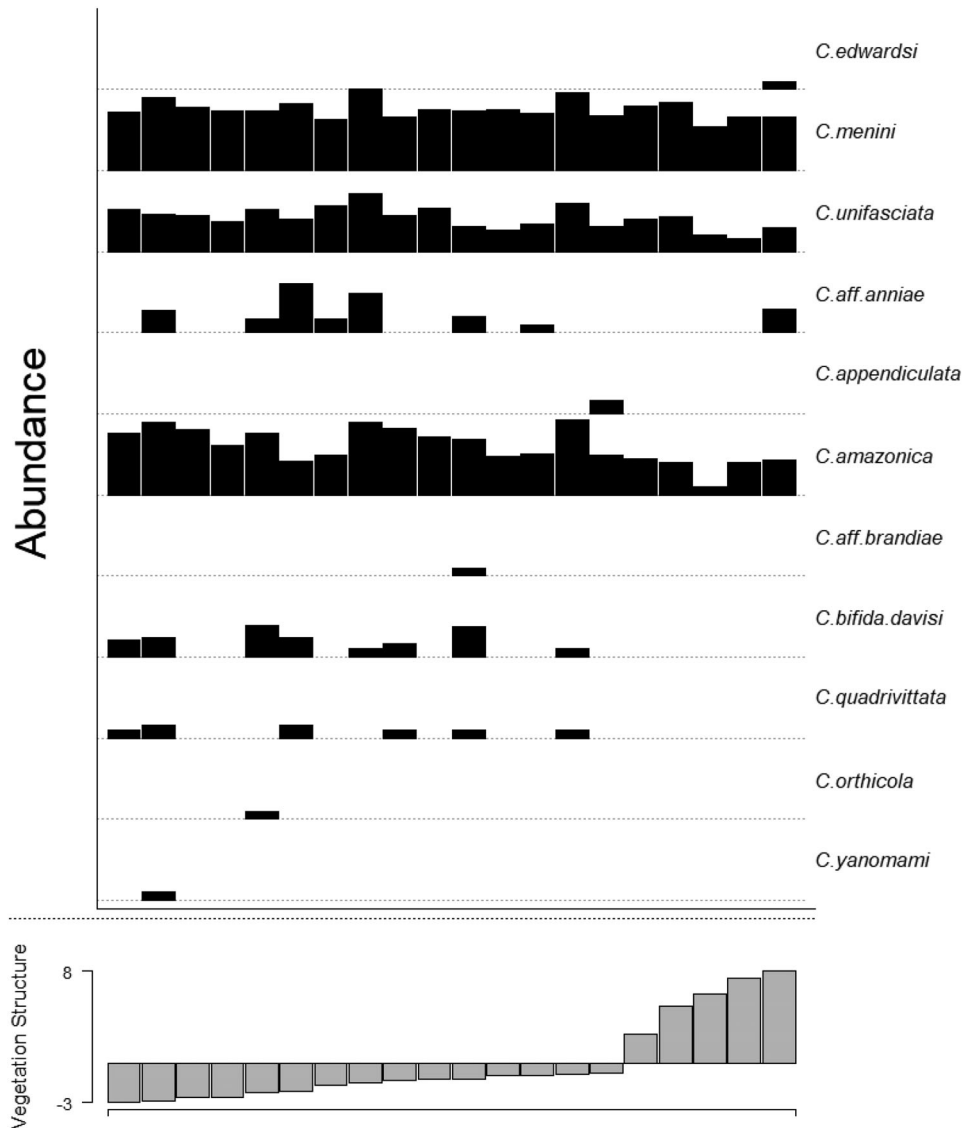
FIGURE 3 *Corethrella* species composition ordered by vegetation structure. Relative abundances of *Corethrella* spp. are log-transformed in $log_{10}(x + 1)$. Positive values in the vegetation-structure gradient indicate areas of *campina* and negative values indicate forested areas of *campinarana*.

frequencies (Mantel statistic $r = 0.9284$, $p = 0.001$) so habitat selection does not result in reduction of overlap by species attracted to similar frequencies.

Generalised mixed models comparing species abundances indicated a strong negative correlation between *C. amazonica* and *C. menini* (pseudo $R^2 = 0.857$, $p = 0.001$, Figure 6), and no correlation between *C. unifasciata* with *C. menini* (pseudo $R^2 = 0.072$, $p = 0.08$) or *C. unifasciata* with *C. amazonica* (pseudo $R^2 = 0.001$, $p = 0.29$), respectively.

DISCUSSION

We found compelling evidence indicating that habitat gradients and frog-species composition influence *Corethrella* spp. distributions. Habitat and frog-species composition, however, do not appear to segregate species in the landscape. The *Corethrella* spp. showed habitat specialisation, particularly in relation to distance from streams and more densely vegetated areas. Most *Corethrella* spp. were attracted by low-pitch frequencies, and all species, except *C. quadrivittata*, showed sound specialisation. However, the sound-frequency attractiveness did not lead to segregation among *Corethrella* spp. Moreover, we found no evidence that habitat selection reduces co-occurrence among *Corethrella* spp. that are attracted to similar sounds. We also found a strong negative relation between abundances for the two most abundant and widely distributed species, *C. menini* and *C. amazonica*.

Most studies with *Corethrella* have been conducted at few sites and usually associated with ponds, with no consideration of the effects of distinct habitats on *Corethrella* species. Grafe et al. (2018) argued that habitat could be an important variable to be considered in studying *Corethrella* ecology, but they did not test this directly. Our results indicate that *Corethrella* spp. distribution is associated with
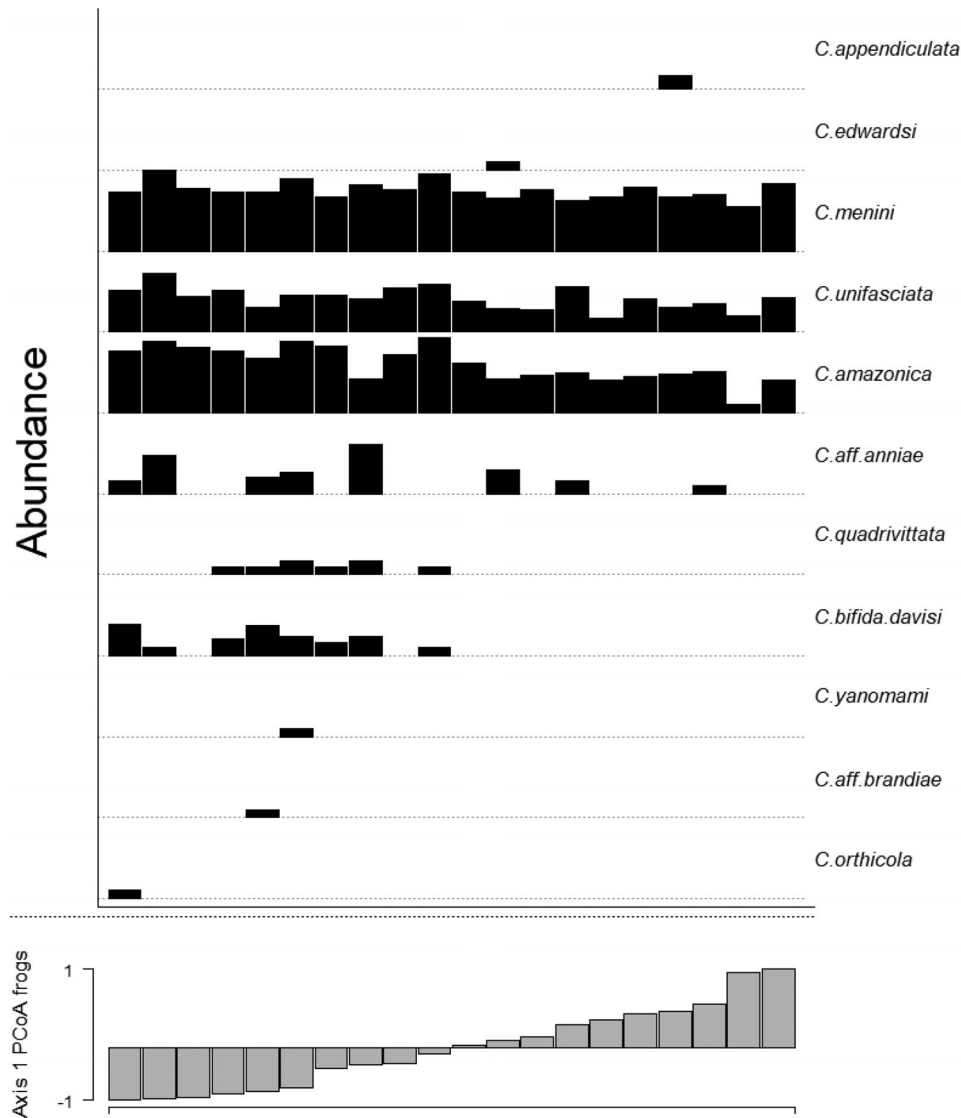
FIGURE 4 *Corethrella* species composition ordered by frog-species composition. Relative abundances of *Corethrella* spp. are log-transformed in $\log_{10}(x + 1)$. Frog-species composition is represented by the first ordination axis from Principal Coordinates Analysis.

environmental gradients and almost all species show habitat speciali-sation. However, habitat selection had little effect on co-occurrence. Few individuals were found in open and drier areas of *campina* (Figure 2) and no species was associated with the gradient in soil clay content. Many other taxa in this region, including frogs, show strong relationships with the soil clay gradient, which is assumed to be related to soil moisture and pond formation (Bueno et al., 2012; Menin et al., 2007; Oliveira et al., 2009; Pereira et al., in press). Perhaps, soil moisture and pond distributions, are related more to water-table fluctuation, and less to the short gradient in soil clay content in our area. Nevertheless, previous studies have suggested that some *Corethrella* spp. are habitat specialists in relation to their oviposition sites, which leads to a division between phytotelmata- and ground-dwelling species (Borkent, 2008). The association of *Core-thrella* adults with more densely vegetated areas was expected, since immature stages are commonly found in tree-holes, bromeliads and

leaf axils (Amaral et al., 2023; Borkent, 2008). However, immature stages are rarely found in streams, even though there are many records of adults occupying these habitats (Borkent, 2008; Amaral et a., 2023). Sampling bias might be one cause of those observations. For example, Dorff et al. (2022) conducted the first study that investi-gated more profoundly immature stages of *Corethrella* in stream-associated habitats. Their findings showed that *Corethrella kipferi* is likely to be an obligate hyporheic dweller and the only member of the superfamily Culicoidea that can be found in this biotope. We found seven other species in riparian areas and we suspect that many more species may be using streams and hyporheic zones to oviposit than previously reported. However, adults may occupy these habitats due to more prey availability or other microhabitats that covary with streams, such as isolated pools. Both hypotheses will remain specula-tive until more information is available in relation to *Corethrella* imma-ture stages.
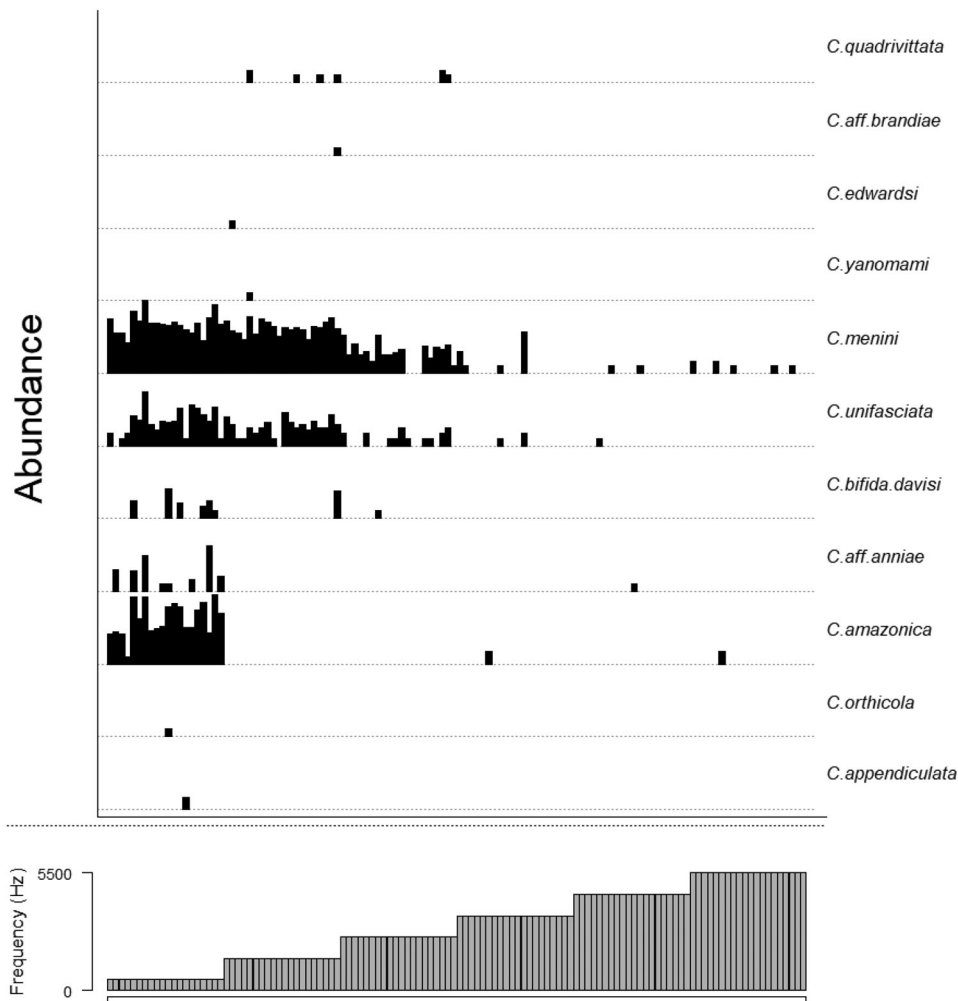
FIGURE 5 Species composition ordered by sound frequencies in $\log_{10}(x + 1)$.

TABLE 2 Comparison between results of each environmental predictor and sound frequencies from MVABUND and Standardised effect size (SES).

Specialisation analysis	Species	Stream distance	Vegetation structure	Soil clay content	Sound frequency
MVABUND (*p*-values)	*C. menini* Feijó et al. 2021	0.898	0.808	0.957	**0.001**
	C. amazonica Lane, 1939	0.657	0.372	0.945	**0.003**
	C. unifasciata Amaral et al. 2023	0.097	0.859	0.945	**0.001**
	C. aff. *anniae*	0.097	0.859	0.445	**0.069**
	C. bifida/davisi	**0.008**	0.859	0.274	**0.069**
	C. quadrivittata Shannon & Del Ponte, 1928	0.092	0.859	0.798	0.469
Standardise effect size	*C. menini* Feijó et al. 2021	−0.5	−1.4	−0.15	**−6.5**
	C. amazonica Lane, 1939	**−2.2**	**−2.56**	−0.06	**−5.6**
	C. unifasciata Amaral et al. 2023	**−2**	**−2.2**	0.64	**−5.2**
	C. aff. *anniae*	−1.3	−0.11	−0.12	**−2.1**
	C. bifida/davisi	**−2.9**	**−2.09**	−0.46	**−2.5**
	C. quadrivittata Shannon & Del Ponte, 1928	**−2.8**	−1.83	0.3	**−2.8**

Note: In bold, significant *p-values* ($p \geq 0.05$) of MVABUND univariate tests and SES for each species.

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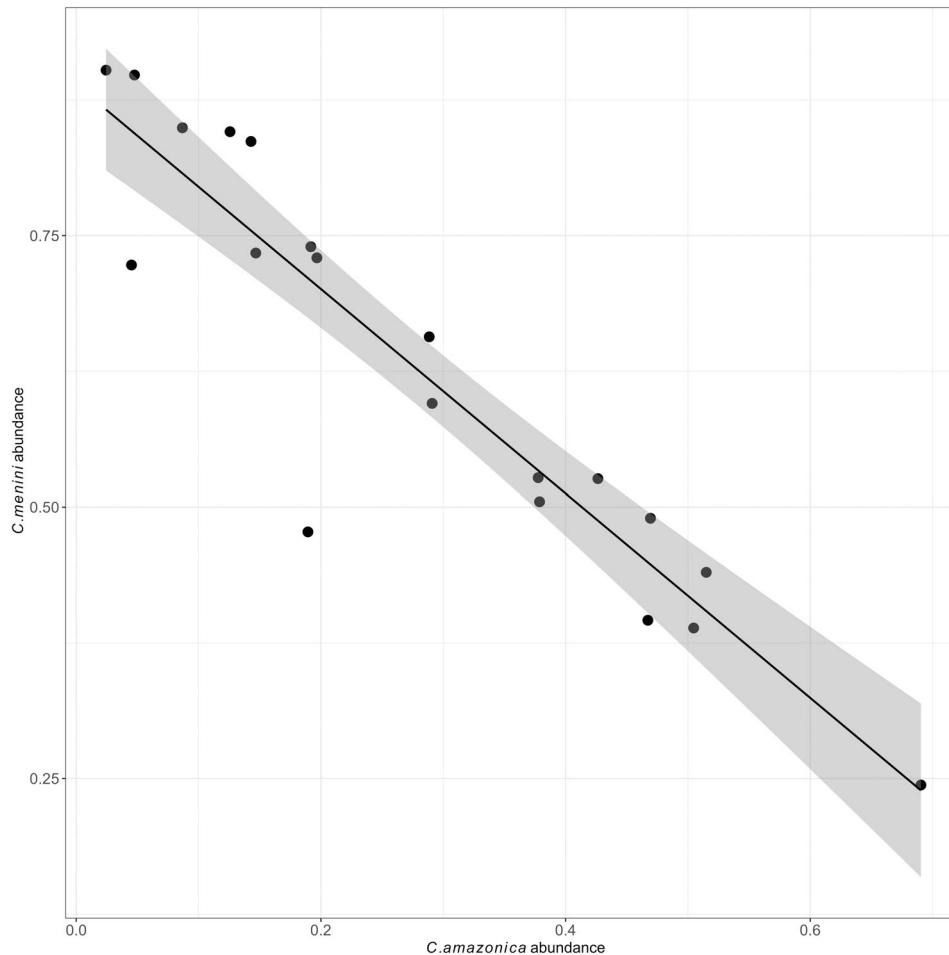Entomological
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FIGURE 6 Generalised mixed model comparing *C. menini* and *C. amazonica* abundance proportions per plot.

On a broader scale, Borkent (2008) suggested that it was unlikely frog-species composition would modulate *Corethrella* distributions. Since many species have distributional ranges wider than their hosts, historical environmental changes likely influenced frog and *Corethrella* distribution in different ways (Borkent, 2008). Our results indicate an influence of frog-species composition on *Corethrella* spp. distribution, at least on a local spatial scale, even after controlling the influence of habitat on both taxa. However, similarly to habitat, frog-species composition minimally reduces co-occurrence of *Corethrella* species. Neotropical *Corethrella* switch host-seeking behaviour across seasons and have low to moderate specialisation on frog species (Legett et al., 2017; Virgo et al., 2021), indicating that they might exploit frogs opportunistically depending on local context and, potentially, do not compete among themselves for resources. Some frogs call seasonally and only during specific climatic conditions (Heard et al., 2015), which makes them an unreliable source for highly specialised species on calling-males. Autogeny in *Corethrella*, and hence independence of blood meals, has only been reported for *C. appendiculata* (but potentially for *C. quadrivittata* and *C. ranapungens*) (Blosser et al., 2013; Borkent, 2008). However, the high rates of autogeny found in field collections of *C. appendiculata* by Blosser et al. (2013) in all months of the year suggest that this trait could be common, even in natural

conditions where frogs are abundant. Perhaps, autogeny and the apparent lack of specialisation on frog assemblages by *Corethrella* adults is due to the inconsistency of calling-male-frog resources across space and time.

Although sound frequencies also influence *Corethrella* spp. abundances in the RDS-Rio Negro, they are also not sufficient to segregate *Corethrella* spp. Almost all species were attracted by low-pitch frequencies, with some being more restricted than others (Figure 5), which is a pattern similar to that shown by other Neotropical *Corethrella* spp. (Virgo et al., 2019). Since there is little published evidence of competition between adults and most studies were conducted in Central America or southern Brazil (Amaral et al., 2021; Ambrozio-Assis et al., 2018; Legett et al., 2017; Virgo et al., 2019), it appears that Neotropical species are little specialised on sound frequencies, and acoustic niches are unlikely to be sufficient to segregate *Corethrella* species. Furthermore, contrary to what has been reported in other tropical regions with direct observations on frog-*Corethrella* interactions (Grafe et al., 2018), habitat selection does not appear to be an important feature to reduce overlap by species along acoustic dimensions. As neither sound, frogs, nor habitat appear to segregate adults of *Corethrella* spp., we suggest that *Corethrella* coexistence is mediated along other niche axes, probably in the larval phase.

In taxa with aquatic larvae and terrestrial adults, different filters can act in each life stage to promote coexistence between closely related taxa. For example, frog assemblages from the Central Amazon can be influenced both by habitat variables when adults and by predation during the aquatic phase (Hero et al., 1998; Rojas-Ahumada et al., 2012). Larvae of *Corethrella* are ambush predators that can be found in a wide range of aquatic habitats (Borkent, 2008; Boulton et al., 1992; Dorff et al., 2022). However, reports of coexistence between immature stages are rare, even with the extensive sampling effort conducted in other studies (Amaral et al., 2023; Borkent, 2008). As a result, it has been suggested that specialisation of immature stages in different aquatic habitats could be segregating *Corethrella* spp. (Amaral et al., 2023). *Corethrella appendiculata* has been reported to undertake wasteful killing of other midges, indicating that surplus killing may be adaptive to eliminate competitors (Lounibos et al., 2008). Furthermore, *Corethrella* larvae use their antennae to capture prey, a mechanism known only to be shared with Chaoboridae (Förster & Schneeberg, 2016). If that feeding strategy is widespread among *Corethrella* spp., antenna size may lead to different prey exploitation among *Corethrella* spp. and result in coexistence mediated by trait differences. Our results indicated that *C. amazonica* and *C. menini* abundances are strongly negatively correlated (Figure 6), which could be an indication of competition between larvae of these species. Since there is little evidence of competition between adults, at least in the niche axes evaluated in our study, larval competitive exclusion or differential prey selection may be the cause of their negatively correlated abundances. However, immature stages are not known for either *C. amazonica*, or *C. menini*. For these reasons, we recommend future studies of the role of larval interactions in structuring *Corethrella* assemblages.

We conclude that, although habitat, frog-species composition and sound frequencies influence the distribution and relative abundances of adult female *Corethrella*, most species overlap on these gradients. Moreover, overlap in sound specialisation is not related to overlap in landscape occupancy. We suggest that the potential for competition among larvae is much greater than the potential for competition among adults and this should be a focus of future studies of *Corethrella* spp. assemblages.

AUTHOR CONTRIBUTIONS

Eduardo de Farias Geisler: Conceptualization; investigation; writing – original draft; formal analysis; methodology; data curation; visualization; software. **Leonardo Leite Ferraz de Campos:** Conceptualization; writing – review and editing; formal analysis. **Vítor Carvalho-Rocha:** Conceptualization; writing – review and editing; formal analysis. **Anthony Santana Ferreira:** Investigation; writing – review and editing. **Jussara Santos Dayrell:** Investigation; writing – review and editing. **Rafaela Caroline dos Santos Pereira:** Writing – review and editing; investigation. **Silionamã Pereira Dantas:** Investigation; writing – review and editing. **Ramiro Dario Melinski:** Investigation; writing – review and editing. **Albertina Pimentel Lima:** Investigation; writing – review and editing. **Luiz Carlos de Pinho:** Conceptualization; investigation; writing – review and editing; methodology; visualization; supervision; resources. **William Ernest Magnusson:** Conceptualization; methodology; formal analysis; project administration; supervision; writing – review and editing; visualization; funding acquisition; validation; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest that might rise bias towards the conclusions and opinions presented in the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.