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Specialization in frog-biting-midges (Diptera: Corethrellidae): a landscape perspective

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Manaus, Amazonas

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SPECIALIZATION IN FROG-BITING-MIDGES (DIPTERA: CORETHRELLIDAE): A LANDSCAPE PERSPECTIVE

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ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 09 dias do mês de novembro do ano de 2023, às 09:h00min, via videoconferência, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: a Dr. Fabricio Beggiato Baccaro, da Universidade Federal do Amazonas – UFAM, o Dr. Mauricio de Almeida Gomes, da Universidade Federal de Mato Grosso do Sul – UFMS e o Dr. **Matheus Mickael Mota Soares**, do Instituto Nacional de Pesquisas da Amazônia – INPA, tendo como suplentes o Dr. Bruno Renaly Souza Figueiredo, da Universidade Federal de Santa Catarina – UFSC e o Dr. Domingos de Jesus Rodrigues, da Universidade Federal de Mato Grosso – UFMT, sob a presidência do orientador, a fim de proceder a arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **EDUARDO DE FARIAS GEISLER**, intitulado: **"SPECIALIZATION IN FROG-BITING-MIDGES (DIPTERA: CORETHRELLIDAE): A LANDSCAPE PERSPECTIVE"**, orientado pelo Dr. William Ernest Magnusson, do Instituto Nacional de Pesquisas da Amazônia – INPA e coorientado pelo Dr. Luiz Carlos de Pinho, da Universidade Federal de Santa Catarina – UFSC.

Após a exposição, o(a) discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

[X]	APROVADO (A)	[ ]	REPROVADO (A)
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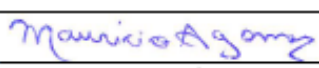Nada mais havendo, a presente ata foi lida, lavrada e assinada pelos membros da Comissão Examinadora.
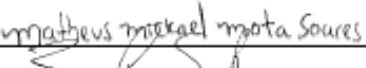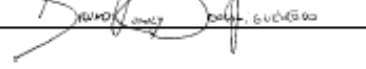
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(Coordenação PPG-ECO/INPA)

Sinopse: Nessa dissertação, buscou-se entender como a distribuição de mosquitos-picadores-de-sapos (Diptera: *Corethrella*) varia ao longo de gradientes ambientais e da composição de anfíbios anuros. Também, avaliamos se a mudança de composição destes mosquitos-picadores-de-sapos varia em função do tipo de frequência sonora utilizada para sua captura. Além disso, investigamos se espécies de *Corethrella* são especialistas em habitat e em frequências sonoras. Também, investigamos se a seleção em habitat reduz a coocorrência das espécies que compartilham o mesmo nicho acústico. Por fim, analisamos correlações entre as abundâncias de três espécies de *Corethrella*.

Palavras-chave: Seleção de habitat; paisagem; coexistência; fase larval; tipo de armadilha; Diptera; Culicomorpha.

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Deixo também registrado que a ciência na Amazônia só avança com o importante trabalho dos auxiliares de campo e os moradores locais. Seus ensinamentos são imprescindíveis para a boa formação dos alunos e para o desenvolvimento da ciência. Assim, finalizo essa sucinta brecha para meu mundo sentimental agradecendo imensamente ao Esaú, Dona Alindomar e Seu Jânio, os quais trabalharam na coleta de dados e me acolheram em todos os difíceis momentos de campo.

RESUMO

1. Geralmente, a competição exclui pares de espécies filogeneticamente próximas em paisagens contínuas. A diferenciação de habitat, alimentação e comportamento são alguns dos fatores que facilitam a coocorrência entre espécies sintópicas. Assume-se que o táxon de mosquitos-picadores de sapos do gênero *Corethrella* sejam especialistas em sapos e atraídos por suas vocalizações. Contudo, pouco se sabe sobre como gradientes ambientais e a composição de seus hospedeiros influenciam sua distribuição e permitem sua coexistência em ao longo da paisagem.

2. Os nossos objetivos foram compreender como os gradientes ambientais de habitat, frequências sonoras e a composição das espécies de sapos influenciam na distribuição das espécies de *Corethrella* e se o habitat e as frequências sonoras segregam a sua ocorrência. Também investigamos se a seleção de habitat reduz a coocorrência entre espécies que compartilham nichos acústicos semelhantes. O estudo foi realizado em 20 parcelas permanentes na Amazônia Central utilizando diferentes atrativos sonoros como iscas para capturar espécies de *Corethrella*.

3. Gradientes de habitat, frequências sonoras e composição de espécies de sapos influenciaram as distribuições de *Corethrella*. No entanto, estes fatores pouco contribuem para evitar a coocorrência e, a seleção de habitat, também não reduziu a coocorrência de espécies atraídas pelas mesmas frequências sonoras. Estes resultados sugerem que é pouco provável que a competição afete a distribuição dos adultos e que outros aspectos, como a competição na fase larval, podem ser mais importantes para permitir a sua coexistência.

4. As duas espécies mais abundantes apresentaram fortes correlações negativas em abundância, indicando competição entre estas espécies, mas a especialização do habitat larval é provavelmente a causa mais importante deste padrão.

Palavras-chave: Seleção de habitat; parasitas; Anuros; interação; hospedeiros; ectoparasitas.

ABSTRACT

1. Generally, competition excludes pairs of phylogenetically close species across the landscape. In another hand, habitat, food and behavior differentiation are some of the factors which facilitate co-occurence between 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 permits 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 occurence across a landscape. We also investigated if habitat selection reduces co-occurence between species which 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-occurence and habitat selection did not reduce co-occurence of species attracted by 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 specialization is likely to be the most important cause of this pattern.

Keywords: Habitat selection; micropredator; host-specificity; co-occurence; prey

SUMÁRIO

Specialization in frog-biting midges (Diptera: Corethrellidae): a landscape perspective

Eduardo F. Geisler, Leonardo L. F. Campos, Vítor Carvalho-Rocha, Anthony S. Ferreira, Jussara S. Dayrell, Rafaela C. S. Pereira, Ramiro Dario Melinski, Albertina P. Lima, Luiz C. Pinho, William E. Magnusson.

ABSTRACT

1. Generally, competition excludes pairs of phylogenetically close species across the landscape. Habitat, food and behavior differentiation are some of the factors which facilitate co-occurence between syntopic species. The taxa of frog-biting midges *Corethrella* are assumed to be specialists on calling male frogs, but little is known about how other ecological gradients and hosts composition influence their distribution and permit their coexistence in continuous landscapes.

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INTRODUÇÃO GERAL

De uma forma geral, assume-se que as espécies filogeneticamente próximas precisam segregar os seus nichos, ou uma espécie será eliminada por exclusão competitiva (Schoenner, 1974). O habitat (ou gradientes ambientais) pode selecionar a distribuição das espécies em paisagens contínuas (Dunson & Travis, 1991), reduzindo o contato e a competição entre espécies que partilham comportamentos ou recursos alimentares semelhantes. Este é um pressuposto comum para inúmeros táxons de vertebrados e invertebrados, em que as espécies podem partilhar recursos e comportamentos semelhantes devido à segregação espacial (Schoenner, 1974). Contudo, quando as espécies se sobrepõem no habitat, a especialização em diferentes eixos de nicho pode permitir a coexistência de espécies na paisagem (Sandercock, 1967).

A disponibilidade de água, granulometria do solo e a estrutura da vegetação modulam a distribuição de diversos táxons em paisagens tropicais (Rabelo et al., 2020; Torralvo et al., 2021; Peixoto et al., 2023; Oliveira et al., 2009). Particularmente para insetos aquáticos, estes fatores podem estar associados aos habitats, como fitotelmatas, lagoas e riachos, que são utilizados pelos estágios imaturos de muitas espécies (Gray, 1981; Nakanishi et al., 2013). No entanto, para alguns dípteros com larvas aquáticas predadoras, como mosquitos e maruins, há a necessidade de realizar repastos sanguíneos em seus hospedeiros para completar seu ciclo de vida. (Lyimo & Ferguson, 2009). Espera-se que. nestes táxons, a distribuição das espécies e a coocorrência sejam mediadas por alterações na disponibilidade de recursos para os adultos ao longo da paisagem, e menos por variações no habitat (Edman, 1979).

Outro fator crítico que limita a coocorrência de consumidores filogeneticamente próximos é como estes recursos são detectados no ambiente (Schoener, 1974). Um número crescente de estudos tem explorado o comportamento de *eavesdropping* (espionagem, tradução livre), o qual consiste na exploração dos sinais intraespecíficos emitidos por suas presas para localizá-las ao longo da paisagem (Bernal & Page, 2022). Os *eavesdroppers* (espiões, tradução livre) variam num contínuo de estratégias oportunistas à especialistas para a localização das suas presas (Bernal & Page, 2023). Em particular, especialistas têm geralmente adaptações sensoriais para localizar os seus hospedeiros e, também, uma história co-evolutiva estreita devido aos elevados custos de detectá-los erroneamente (Bernal & Page, 2023). Mosquitos e

maruins geralmente se enquadram nessa categoria de especialistas, mas algumas espécies mostram mais flexibilidade em seu comportamento de busca para lidar com as mudanças espaço-temporais na abundância de hospedeiros (Lyimo & Ferguson, 2009; Legget et al., 2017). Espera-se que essa exploração de diferentes sinais possa reduzir o uso de recursos semelhantes e permitir a coexistência de espécies proximamente relacionadas. No entanto, não se sabe até que ponto a exploração de diferentes sinais pode segregar as espécies para a maioria dos táxons de micropredadores.

Mosquitos-picadores de sapos são micropredadores que exploram os sinais acústicos de sapos para localizá-los. Este grupo de mosquitos incluem muitos táxons da infraordem Culicomorpha, como as espécies dos gêneros *Uranotaenia*, *Sycorax* e *Corethrella* (Borkent, 2008; Caldart et al., 2016; Cutajar & Howley, 2020). O gênero *Corethrella* Coquillet 1902 possui 126 espécies existentes (Amaral et al. 2023), mas provavelmente ainda há muitas espécies crípticas para serem descritas (Virgo et al., 2021). Uma das características do comportamento de *Corethrella* para buscar hospedeiros que os distinguem da maioria das outras moscas micropredadoras é que, as fêmeas, detectam sapos através das vocalizações emitidas ao defenderem seus territórios e atraírem fêmeas (Mckeever & Hartberg, 1980; Borkent, 2008; Bernal et al., 2015). Os sinais acústicos utilizados pelas espécies de *Corethrella* têm sido intensamente investigadas desde o desenvolvimento de armadilhas sonoras com vocalizações de sapos por McKeever & Hartberg (1980), consideradas atualmente como o método mais eficaz para capturar fêmeas de *Corethrella* (Bernal et al., 2015). Características, como taxa de canto e a frequência sonora, podem ser usadas para prever quais espécies de sapos serão atacadas em maior frequência (Meuche et al., 2017), sugerindo que fêmeas adultas de *Corethrella* species podem segregar no uso do hospedeiro devido à diferenciação do nicho acústico.

No entanto, não está claro se as preferências acústicas são suficientes para segregar *Corethrella* species no uso do hospedeiro, uma vez que várias espécies são frequentemente capturadas nas mesmas iscas acústicas sintéticas e vocalizações de sapos (por exemplo, Borkent, 2008; Meuche et al., 2017; Virgo et al. 2019; Virgo et al. 2021). Além disso, observações em campo de interações sapos-*Corethrella* e a inconsistência na especificidade do hospedeiro e nas faixas de distribuição de ambos os grupos também sugerem que o uso de diferentes hospedeiros teria pouca influência para reduzir a coocorrência de *Corethrella* species (Borkent, 2008). Estes resultados sugerem que as *Corethrella* species sintópicas podem não

competir na utilização de hospedeiros e podem estar segregando em outras dimensões de nicho, como o habitat, o que poderia possibilitar a exploração das espécies de sapos semelhantes.

Foi sugerido que a abrupta mudança na composição de espécies de *Corethrella* em florestas Neotropicais poderia estar relacionada à variação nos gradientes de habitat, particularmente em relação aos locais de oviposição, onde a competição na fase larval teria um papel fundamental (Borkent, 2008; Amaral et al., 2023). No entanto, apenas um estudo avaliou a relação entre os tipos de habitat e seu efeito nas espécies de *Corethrella*. Os autores descobriram que os habitats de turfeiras da ilha de Bornéu (Brunei) tinham menos espécies de *Corethrella* especialistas em sapos do que as áreas de floresta da mesma região (Grafe et al., 2018), sugerindo que a segregação observada nas espécies de *Corethrella* poderia ser mediada pela seleção de habitat (Grafe et al., 2018). No entanto, as espécies Neotropicais de *Corethrella* parecem ser menos especializadas do que aquelas encontradas em outras regiões tropicais (Legget et al., 2017; Virgo et al., 2019; Virgo et al., 2021), portanto, estas espécies podem ser mais sensíveis a mudanças espaciais do que a espécies sonoras ou de sapos. Nesse caso, se a competição por hospedeiros for um fator primário de seleção de habitat de *Corethrella*, é também de se esperar que, as espécies que mais se sobrepõem na atração por determinadas frequências sonoras, se sobreponham menos em suas posições ao longo dos gradientes de paisagem, resultando em uma correlação negativa entre a similaridade das espécies ao longo dos gradientes de som e paisagem.

Outro aspecto que pode indicar se existe competição entre as espécies de *Corethrella* é a correlação entre suas abundâncias. A correlação negativa entre as abundâncias de espécies sintópicas pode indicar competição direta ou indireta (Lawlor, 1979). As larvas de espécies semelhantes podem excluir-se por competição de recursos (Juliano, 1998) ou por interações antagônicas diretas, como matar presas apenas para não serem consumidas por outros indivíduos (Blosser et al., 2013). Consequentemente, estas interações na fase larval podem influenciar a abundância de futuros adultos e reduzir a coocorrência entre certos pares de espécies. Os estágios imaturos de duas ou mais espécies de *Corethrella* são raramente encontrados nos mesmos habitats, tais como buracos de árvores, axilas de folhas ou fitotelmas (Borkent, 2008; Amaral et al., 2023), indicando uma forte exclusão competitiva entre algumas espécies. As causas exatas não podem ser atribuídas sem experimentos direcionados, mas se existir competição entre pares de larvas de *Corethrella* species, podem surgir fortes padrões de correlações negativas nas suas abundâncias adultas.

Neste estudo, investigamos como a distribuição e coocorrência das espécies de *Corethrella* variam em relação às características do habitat e à composição das espécies de sapos ao longo de uma paisagem tropical. Além disso, também avaliamos se a composição das espécies de *Corethrella* muda em relação às frequências sonoras. Especificamente, avaliamos os possíveis efeitos da distância dos igarapés, estrutura da vegetação, teor de argila do solo e composição de espécies de sapos na distribuição de adultos de *Corethrella*. Também avaliamos o grau de especialização das espécies de *Corethrella* nos gradientes de habitat e frequências sonoras, permitindo-nos investigar até que ponto a seleção do habitat reduz a coocorrência de espécies atraídas pelas mesmas frequências sonoras. Por fim, investigamos se as espécies sintópicas de *Corethrella* apresentam correlações negativas nas suas abundâncias.

INTRODUCTION

It is generally assumed that phylogenetically close species need to segregate their niches, or one species will be eliminated by competitive exclusion (Schoenner, 1974). Habitat,

therefore, can select species distributions across a landscape (Dunson & Travis, 1991), reducing contact between similar species that share similar behavior 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 behavior due to spatial segregation (Schoenner, 1974). When species overlap in habitat, however, specialization on different niche axes may enable species coexistence in the landscape (Sandercock, 1967).

Habitat compounds, as such water availability, soil granulometry and vegetation structure, modulate the distribution of many taxa in tropical landscapes (Rabelo et al., 2020; Torralvo et al., 2021; Peixoto et al., 2023; Oliveira et al., 2009). 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 some flies with predatory aquatic larvae (e.g., midges and mosquitoes), adult females' species may also be highly dependent on finding suitable prey to provide blood meals to complete their life cycle (Lyimo & Ferguson, 2009). In these taxa, the species distribution and co-occurence is further expected to be generated by changes in resource availability for adults across the landscape, and less by habitat-gradient changes (Edman, 1979).

However, another critical factor that limits co-occurence of closely related consumers is the detection of resources (Schoener, 1974). A growing number of studies have explored eavesdropping behavior, which is the use of signals by predators to locate prey (Bernal & Page, 2022). Eavesdroppers vary in a continuum from totally opportunistic to closely specialist strategies in locating their prey in the landscape (Bernal & Page, 2023). In particular, 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). Micropredator flies usually fall in this category of specialists, but some show more flexibility in their host-seeking behavior to cope with spatiotemporal changes in host abundances (Lyimo & Ferguson, 2009; Legget et al., 2017). Exploitation of different cues may reduce the use of similar resources and permit closely related species to coexist.

Some frog-biting midges and mosquitoes are eavesdropping micropredators which are attracted by frog calls. These include many taxa from the infraorder Culicomorpha, such as species in genus *Uranotaenia*, *Sycorax* and *Corethrella* (Borkent, 2008; Caldart et al., 2016; Cutajar & Howley, 2020). The genus *Corethrella* has 126 extant species (Amaral et al. 2023),

but there are probably many cryptic species yet to be described (Virgo et al., 2021). One of the features of *Corethrella* host-seeking behavior that distinguishes it from most other micropredator families is that females use calls to locate male frogs (Mckeever & Hartberg, 1980; Borkent, 2008; Bernal & Silva, 2015). Acoustic cues used by *Corethrella* spp. have been intensively investigated since the development of sound traps with frog playbacks by McKeever & Hartberg (1980), which are currently the most effective method to capture *Corethrella* females (Bernal & Silva, 2015). 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 synthetic and frog-call acoustic lures (e.g. Borkent, 2008; Meuche et al., 2016; Virgo et al. 2019; Virgo et al. 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-occurence 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 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 island (Brunei) had less frog-specialist *Corethrella* spp. than rainforest areas, suggesting that the observed segregation in *Corethrella* spp. could be mediated by habitat specialization (Grafe et al., 2018). However, Neotropical *Corethrella* spp. seem to be less specialized than those found in other tropical regions (Legget 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-occurence among species pairs. Two or more *Corethrella* species immature stages are rarely found co-occurring in the same habitat, such as tree-holes, leaf axils or phytotelma (Borkent, 2008; Amaral et al., 2023). Exact causes cannot be attributed without further experiments, but if competition among pairs of *Corethrella* spp. larvae exist, strong negative correlations patterns may arise in their adult abundances.

In this study, we investigated how *Corethrella* spp. distributions and co-occurrence varies in relation to habitat characteristics and frog-species composition across a tropical landscape. Specifically, we evaluated the possible effects of stream distance, vegetation structure, soil clay content and frog-species composition on the distributions of adults of *Corethrella* species. Additionally, we also evaluated if *Corethrella* spp. attractiveness changes in relation to sound frequencies. We also evaluated the degree of specialization 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.

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), Iranduba Municipality, Amazonas, Brazil. The sampling module follows the RAPELD (Brazilian acronym for *Rapid Survey Long-term Ecological Research*) system (Magnusson et al., 2005; Magnusson et al., 2013). The area is characterized 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) (Fig. 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* (Fig. 1D) (Adeney, 2016). However, in some uplands, drier formations of *campina* with permeable exposed white-sand soil and short (~2m) xeromorphic heath vegetation prevail (Fig. 1C) (Daly et al., 2016).

Sampling design

We surveyed *Corethrella* and frog-species in all plots twice, once at the end of the dry season (October to December 2022) and once in the beginning rainy season (January to February, 2023). The RAPELD module consists of two 5 x 1 km trail systems (Fig. 1B). Within the plot system, both riparian and non-riparian plots spanning 250 meters in length were established along each trail (Fig. 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 have 10 uniformly distributed (plots spaced sistematically at 1 km intervals) plots which follows elevational contours and six riparian plots which follows 1,5m aside stream courses (Fig. 1B; Magnusson et al., 2005; Magnusson et al., 2013). Four additional plots following elevational contours were also installed in patches of *campina* vegetation (Fig. 1B, C).
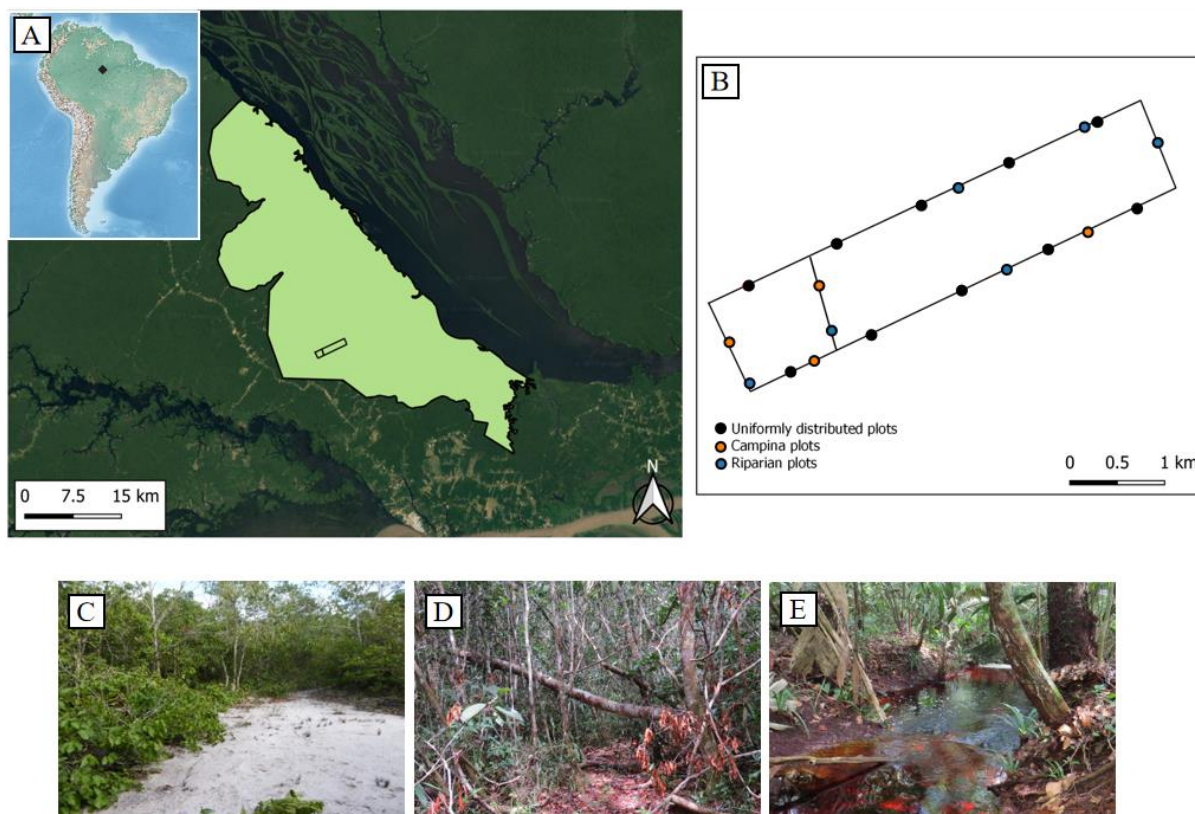
Figure 1. Localization of the studied region, spatial configuration of plots, and the *campina* and *campinarana* formations where *Corethrella* were sampled at the Sustainable Development Reserve of Rio-Negro (RDS - Rio Negro). A) Map showing RDS - Rio Negro localization in northern Brazilian Amazonia, 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.

Corethrella sampling and identification

We surveyed each plot using Modified Pan Traps (PTM traps), which use speakers displaying sounds to lure *Corethrella* spp. females (Amaral et al., 2015; Geisler et al., 2022). In complement to the original PTM traps, we added a polystyrene plate above the speakers as protection from heavy rain. One PTM trap was placed every 50m in the 250m plot trails, for a total of six traps per plot. Six recordings were generated, each with a different artificial sinusoidal tone frequency (500 Hz; 1500 Hz; 2500 Hz; 3500 Hz; 4500 Hz; and 5500 Hz) and with standardized interpulse interval (0.85s), pulse duration (0.25s) and decibels (67-72dB).We used this standardization 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* (Virgo et al., 2019; Meuche et al., 2016), we choose to use sound frequencies as sound baits. Theory suggests that *Corethrella* spp. instraspecific communication are based on fight tones and then potentially 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 recordings were generated using Audacity® software and traps positions in plots are given in Supplementary Material 1. Traps were set after sunset, between 19:00h and 23:00h, the peak activity period for *Corethrella* spp. females foraging (Borkent, 2008). The traps stayed 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 decibels were measured with a Voltcraft SL-100® decibel meter, before and after the campaign, at 0.5m distance. No alterations in decibels greater than 10% were detected.

Female adult *Corethrella* captured in PTM traps were stored in 92% ethanol. All specimens from samples were morphotyped based on a set of key characters (Supplementary material 1). Only fifteen samples with approximately more than150 specimens were subsampled. To avoid sampling bias in those subsamples, we used a petri dish divided in four quadrants and, to choose which quadrant would be sampled, we mixed the material in the petri dish and used clock seconds to decide which quadrant would be sampled. 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). Due to that, we used the sum of each *Corethrella* spp. abundances 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 (Borkent, 2008; Amaral & Pinho 2015; Caldart et al. 2016; Amaral et al. 2019; Amaral et al. 2021; Almeida et al., 2021; Amaral et al., 2023). 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 license nº 84311-2, which can be accessed at www.icmbio.gov.br/sisbio and DEMUC/SEMA nº 01/2021 process nº 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 seasons that *Corethrella* spp. were sampled. All plots were surveyed only by one specialist at night (19:00-22:00h) and lasted about 40min. To quantify species composition in each plot, we utilized 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 variables collection

We collected all habitat variables on 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, 2020). 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. *in press*). To diminish sampling error effects, we used only mean values of height, canopy-opening and understory-density values for each plot. Then, we summarized by Principal Component analysis (PCA) and the first ordination axis was used as a proxy for vegetation structure. Soil samples were collected at six points, 50m apart, in each plot. Each sample was 10cm 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) (Donnagemma et al., 2017). Soil components were highly correlated and, since clay can be associated with changes in invertebrates distribution (Nielsen et al., 2010) and pond formations, we choose clay proportion for further analyses.

Data Analysis

Habitat, frog-species composition and sound frequencies influence on Corethrella spp.

To investigate the influence of habitat variables, frog-species composition and sound frequencies on *Corethrella* spp. we used multivariate generalized linear models from the MVABUND R package (Wang et al., 2012). First, we checked for multicollinearity between all 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-pecies composition and site identity, as input. We choose the first ordination axis of frog-species composition in order to avoid overdispersion. We set a *manyglm* model using *Corethrella* species abundances in each trap as response variables, while habitat variables, sound frequencies and the first ordination axis of PCoA 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 mantain all observations together in resampling, and P-values were calculated using 999 bootstraps. In one campaign, three traps (1500Hz, 3500Hz and 5500Hz), in three different plots (one riparian and two uniformily distributed plots), were opened accidentally for more or less than 90 min and it suddenly started to rain in one of the riparian plots, which may have affected sampling. Due to that, we generated the same previous 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 analyzed the relationships between *Corethrella* spp. using presence-absence data of all plots and the same predictors variables. However, for the presence-absence model, we used 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 considered as valid results the univariate tests of the species that occurred in more than two sites.

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 distances matrices for each dataset using the Bray-Curtis transformation derived from the function *vegdist* from 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-specialization 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 much specialized are each species 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 *standardize effect size* (SES; Gotelli & McCabe, 2002) to evaluate specialization on habitat-gradients and sound frequencies. We used the abundances for each *Corethrella* spp., per plot, as input matrix to evaluate habitat specialization. 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 randomized gradients (I_{sim}) divided by the standard deviation from the 999 randomized standard deviations of the habitat and sound gradients (σ_{sim}).

$$SES = \mathbf{\textit{I}}obs - \mathbf{\textit{I}}sim/\mathbf{\sigma}sim$$

Randomized gradients used were based on the normal distribution, as suggested by (Botta-Dukát, 2018). Values above 2 or below -2 indicate that species are specialized 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 utilized Mantel tests from 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. Dissimilarity distances 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 generalized 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*. Then, we used the function *decostand* (*vegan* package) (Oksanen et al., 2022) to apply a logarithmic transformation (*log(x)+1*) to the proportional abundances in order to reduce the effect of outliers. We compared the correlation between species-abundance pair by pair (Supplementary Material 1). We used the Gaussian distribution family for the response variables. We estimate pseudo R² 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, 2013). 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 hours 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. *brandia*e and *C.* aff. *anniae* are candidate species to be described. *C. bifida/davisi* probably represents a species complex yet to be evaluated.

| 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

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

Based on abundance data, MVABUND analyses indicated that *Corethrella* spp. abundances differed in relation to stream distance (DEV = 45.9, P = 0.001) (Fig. 2), a trend for vegetation structure (DEV = 26.6, P = 0.069) (Fig. 3) and sound frequency (DEV = 322.1, P = 0.001) (Fig. 5). Using presence-absence data and excluding plots with sampling errors, the same effects were found (Supplementary Material 1). Partial Mantel tests indicated a significant

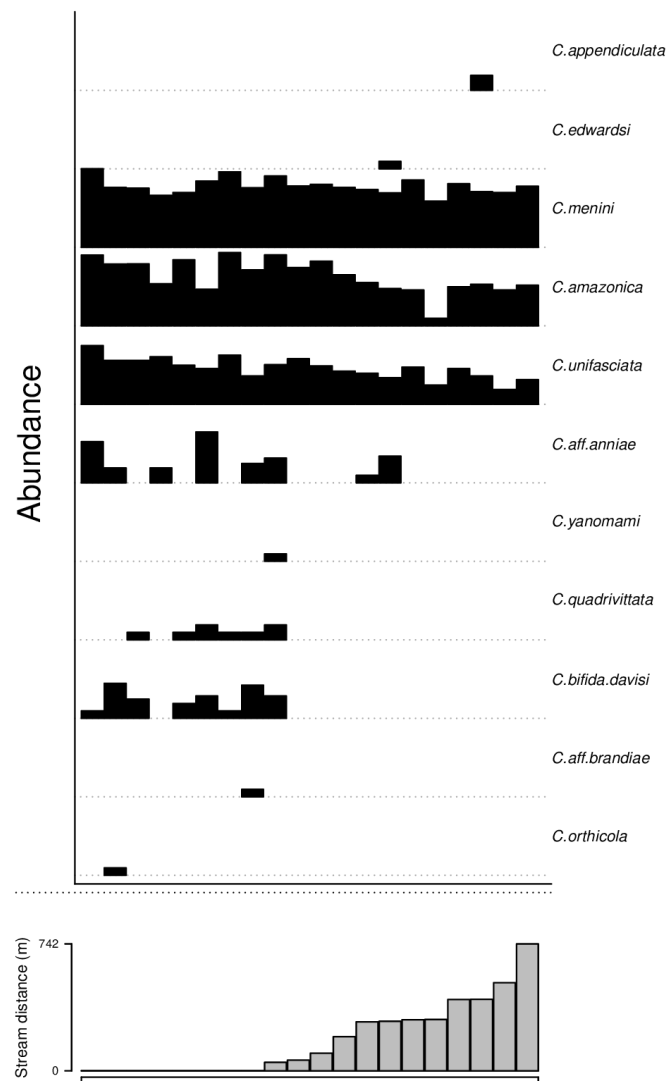correlation between frog- and *Corethrella*-species composition (Mantel statistic= 0.22; *P* = 0.039)

Figure 2. *Corethrella* species composition ordered by stream distance. Relative abundances of *Corethrella* spp. are log-transformed in *log(x+1)*.
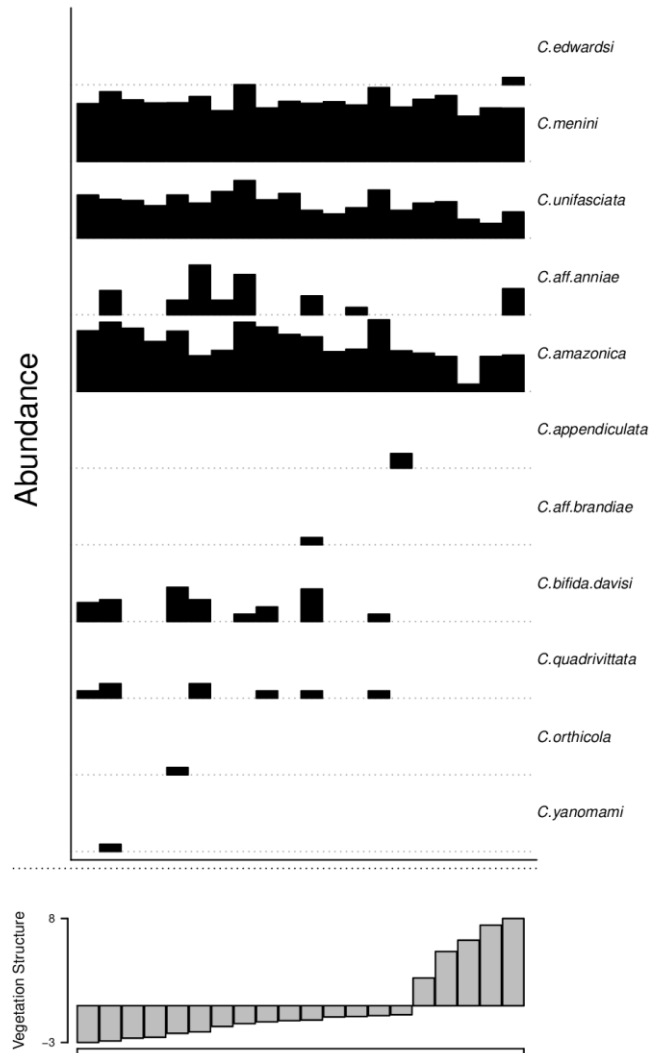
Figure 3. *Corethrella* species composition ordered by vegetation structure. Relative abundances of *Corethrella* spp. are log-transformed in *log(x+1)*. Positive values in vegetation-structure gradient indicate areas of campina and negative values indicate forested areas of *campinarana*.
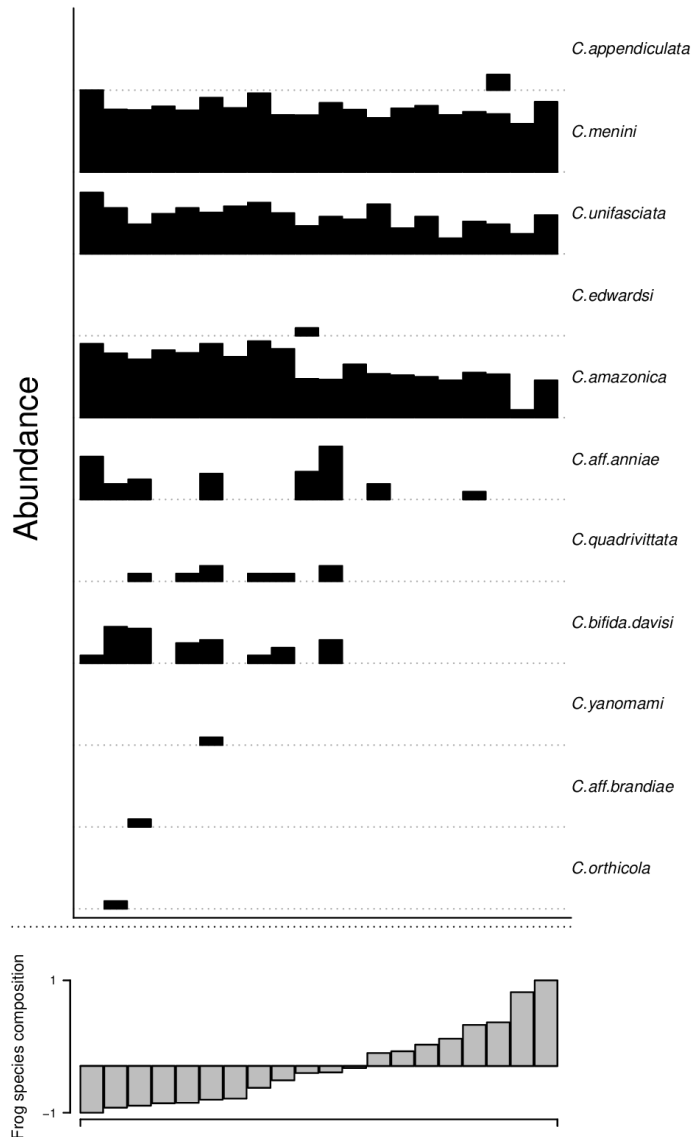
Figure 4. *Corethrella* species composition ordered by frog-species composition. Relative abundances of *Corethrella* spp. are log-transformed in *log(x+1)*. Frog species composition is represented by the first ordination axis from Principal Coordinates Analysis.

The patterns shown in species-gradient graphs indicate that most *Corethrella* spp. were more abundant and some were restricted to areas close to streams and more densely vegetated (Fig. 2 and 3). Excluding singletons and doubletons, MVABUND univariate tests confirmed that *C. bifida/davisi* as a specialists on riparian areas, with most individuals found within a ~100m 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

generalist on all habitat-gradients in both analyses. No species was specialized on the clay gradient (Table 2)

Specialization 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
Standardize 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**

Table 2 - Comparison between results of each environmental predictor and sound frequencies from MVABUND and Standardized effect size (SES). In bold, significant *P-values* of MVABUND univariate tests and SES for each species.

Traps broadcasting at 500 Hz and 1500 Hz frequencies captured 97% of the total *Corethrella* individuals and all species found in our study (Fig 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 Hz - 3500 Hz) (Fig. 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 specialization on sound frequency gradients for C. *quadrivittata*.
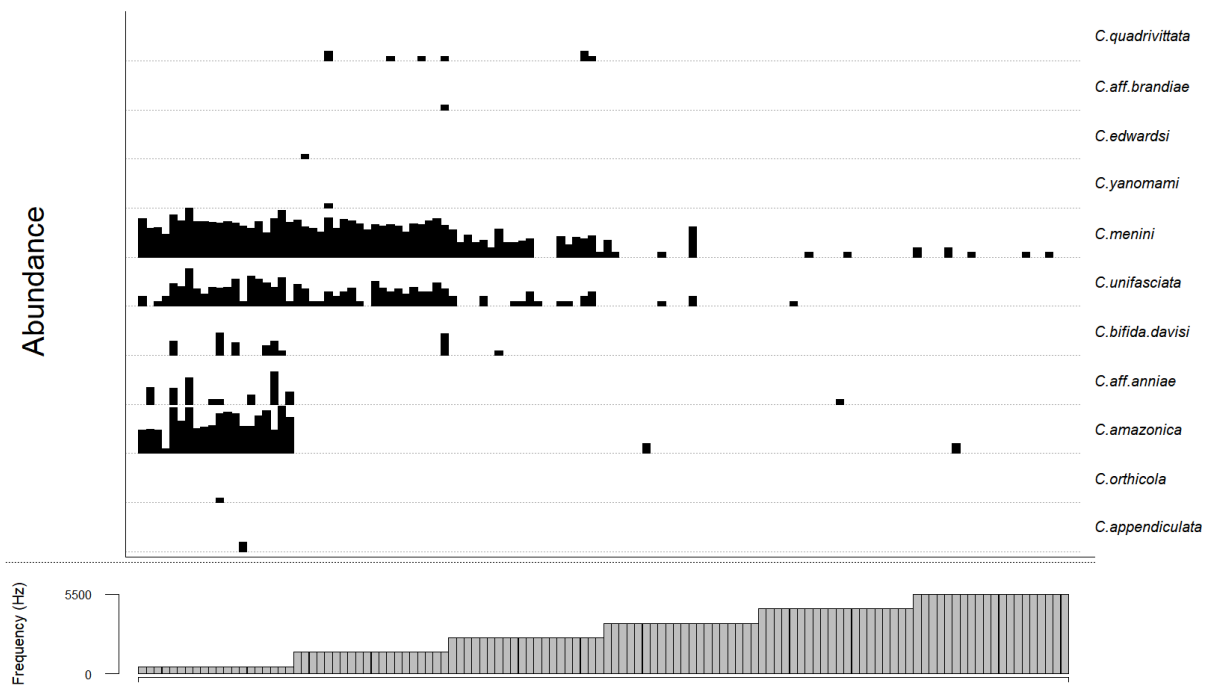
Figure 5. Species composition ordered by sound frequencies in *log (x+1)*.

Mantel tests indicated that similarity in habitat selection was highly positively related to similarity in attractiveness to sound 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.

Generalized mixed models comparing species abundances indicated a strong negative correlation between *C. amazonica* and *C. menini* (pseudo $R^2 = 0.662$, $P = 0.001$, Fig 6), and no correlation between *C. unifasciata* with *C. menini* (pseudo $R^2 = 0.071$, $P = 0.085$) or *C. unifasciata* with *C. amazonica* (pseudo $R^2 = 0.002$, $P = 0.28$), respectively.
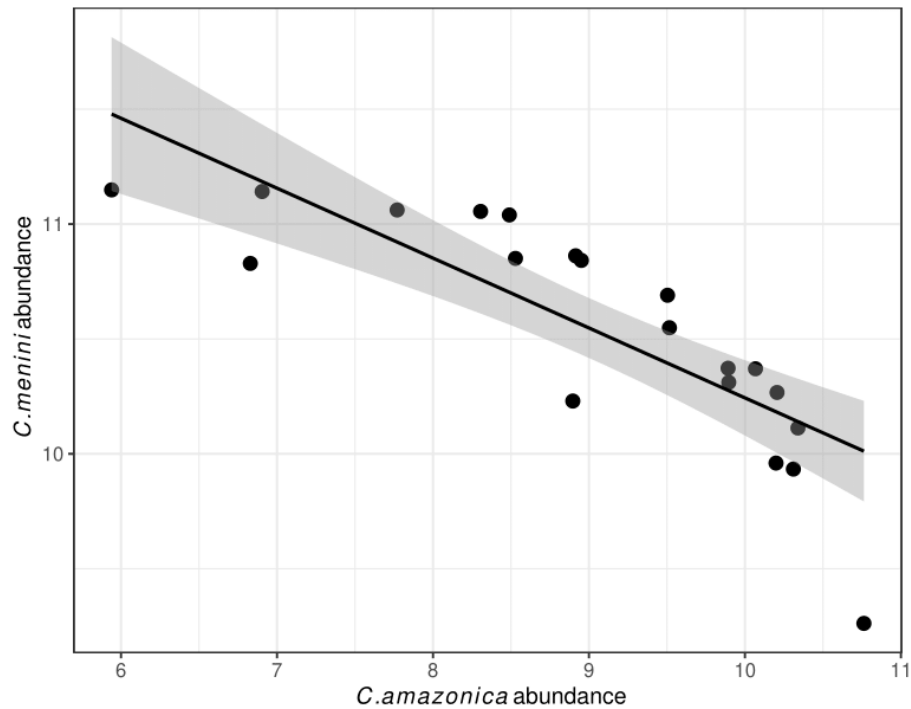
21

Figure 6. Generalized mixed model comparing *C. menini* and *C. amazonica* abundance proportions, in *log(x)+1* , per plot.

DISCUSSION

In our study, we have found compelling evidence indicating that the habitat-gradients and frog-species composition influence *Corethrella* spp. distribution. Habitat and frog-species composition, however, does not appear to segregate species among the landscape. The *Corethrella* spp. showed habitat specialization, 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. quadrivittat*a, showed sound specialization. However, the sound frequency attractiveness do not lead to segregation among *Corethrella* spp. Moreover, we found no evidence that habitat selection reduces co-occurrence among *Corethrella* spp. that share similar attractivennes. We also found a strong negative relation between abudances for the two most 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), however, argue that habitat could be an important variable to be considered in studying *Corethrella* ecology, but didn't tested this directly. Our results indicate that

Corethrella spp. distribution is associated with environmental gradients and almost all species show habitat specialization. However, our results show that habitat-selection have little effect on co-occurrence changes. Few individuals were found in open and drier areas of *campina* (Fig 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 a factor related with soil moisture and pond formations (Menin et al., 2007; Oliveira et al., 2009; Bueno et al., 2012; Pereira et al., *in press*). Perhaps, soil moisture and ponds distribution, are possibly related to the water table fluctuation, and less by the short 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 *Corethrella* adults with more densely vegetated areas was expected, since immature stages are commonly found in tree-holes, bromeliads and leaf axils (Borkent, 2008; Amaral et al., 2023). However, records for stream-associated *Corethrella* were relatively uncommon until recent studies. Before Borkent (2008), only adults of eight species of *Corethrella* were captured next to stream courses and immature stages of only one species were assumed to be found on hyporheic zones (Boulton et al. 1992). However, Subsequent studies found immature stages and adults of at least another 19 *Corethrella* spp. in streams (Caldart et al., 2016; Amaral et al., 2019; Amaral et al., 2023). Moreover, studies also confirmed *Corethrella kipferi* as the only member of the superfamily Culicoidea which can be found in the hyporheic biotope (Dorff et al., 2022). We further found seven other species in riparian areas, and we suspect that many more species may be using streams or hyporheic zones to oviposit than previously reported.

Borkent (2008) suggested that it was unlikely frog species composition would modulate *Corethrella* distributions. Since many species have a distributional ranges wider than their hosts, historical environmental changes likely influenced frog and *Corethrella* distribution in different ways (Borkent, 2008). In contrast, our results indicate an influence of frog species composition on *Corethrella* spp. distribution, 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 behavior across seasons and have low to moderate specialization on frog species (Legget 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 specialized 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*) (Borkent 2008; Blosser et al., 2013). 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 specialization 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, it is not sufficient to segregate *Corethrella* spp., as it was found for habitat and frog species composition. Almost all species were attracted by low-pitch frequencies, with some being more restricted than others (Fig 5), which is a pattern similar to that shown by other Neotropical *Corethrella* spp. (Virgo et al., 2019). Although there is still a huge gap in the areas covered by studies of *Corethrella*, with most studies conducted in Central America or southern Brazil (Legget et al., 2017; Ambrozio-Assiz et al., 2018; Virgo et al., 2019; Amaral et al., 2021), it appears that Neotropical species are little specialized 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 are mediate along other niche axes, particularly in 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 which can be found in a wide range of aquatic habitats (Boulton et al., 1992; Borkent, 2008; Dorff et al., 2022. However, reports of coexistence between immature stages are rare, even with the extensive sampling effort conducted in other studies (Borkent, 2008; Amaral et al., 2023). As a result, it has been suggested that specialization 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 et al., 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 (Fig. 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 competition 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 adults of female *Corethrella*, most species overlap on these gradients. Moreover, overlap in sound specialization 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.

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CONCLUSÃO

   Neste trabalho detectamos fortes evidências que gradientes ambientais e a composição de sapos influenciam na distribuição de *Corethrella*. Também, demonstramos que a composição de *Corethrella* varia em função do tipo de frequência sonora utilizada para sua captura. Contudo, todos estes fatores diminuiram pouco a coocorrência entre espécies de *Corethrella*. Também, demonstramos que a seleção de habitat não diminuiu a coocorrência de espécies que utilizam o mesmo nicho acústico. Por fim, encontramos uma forte correlação negativa entre as abundâncias das espécies *Corethrella menini* e *Corethrella amazonica*, um padrão potencialmente associado à competição entre estas espécies. Conclui-se que, fatores ambientais e associados à localização e uso de recursos, influenciam na distribuição de *Corethrella*. Contudo, competição entre adultos pode não ser um fator determinante para explicar a distribuição e coocorrência de *Corethrella*. Sugerimos que o potencial de competição na fase larval de *Corethrella* pode ser mais relevante do que entre os adultos. Desta forma, indicamos que estudos futuros relacionados à distribuição e coocorrência de *Corethrella* devem focar na fase larval.