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Hematophagous Flies Attracted to Frog Calls in a Preserved Seasonal Forest of the Austral Neotropics, with a Description of a New Species of Corethrella (Diptera: Corethrellidae)

Vinícius Matheus Caldart¹,²*, Maurício Beux dos Santos¹, Samanta Iop¹, Luiz Carlos Pinho³, and Sonia Zanini Cechin¹

¹Programa de Pós Graduação em Biodiversidade Animal, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, RS, Brazil
²Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav. 14, n.° 321, Cidade Universitária, CEP 05508-900, São Paulo, SP, Brazil
³Universidade Federal de Santa Catarina, Departamento de Ecologia e Zoologia, Campus Trindade, CEP 88040-900, Florianópolis, SC, Brazil

The signaler–eavesdropper interaction has been investigated for a wide range of organisms, and although many flies feed on calling frogs, this dynamic has been addressed only poorly in the austral Neotropics. We investigated this interaction in southern Brazil using pairs of suction traps (acoustic + silent) broadcasting frog calls or an artificial white noise in ponds and streams. From 139 sessions, flies of the genera Corethrella (Corethrellidae), Forcipomyia (Ceratopogonidae) and Uranotaenia (Culicidae) were collected, including five Corethrella species, the most abundant of which was previously unknown and is formally described here. Additionally, we present the southernmost records of Corethrella iopesi, C. alticola and C. atricoma. Numbers of Forcipomyia midges and Uranotaenia mosquitoes did not differ between silent traps and traps broadcasting frog calls, and did not differ between white noise traps and adjacent silent traps. However, the number of female Corethrella was significantly higher in traps broadcasting calls of the pond-breeding frog P. aff. gracilis compared to adjacent silent traps; calls of this frog attracted the five Corethrella species and also collected significantly more female Corethrella than the white noise. By evaluating different taxa of flies and broadcasting different sounds, we demonstrated that Corethrella midges were attracted only to the acoustic cue of P. aff. gracilis calls, while Forcipomyia and Uranotaenia were captured in traps by chance. Our results suggest that female Corethrella feed on males of the common pond-breeding frog P. aff. gracilis in southern Brazil, and highlight the utility of frog call traps in revealing the diversity of Corethrella in the austral Neotropics.

Key words: acoustic communication, eavesdroppers, Corethrella, anurans, white noise

INTRODUCTION

Illegitimate receivers, or eavesdroppers, represent a non-target audience that may exploit a signal emitted by a given animal to its intended receivers. The nature of the interaction between the signaler and the eavesdropper influences the costs that the eavesdropper may impose on the signaler (Zuk and Kolluru, 1998; Peake, 2005). The illegitimate receivers of a given acoustic signal, for instance, may be non-target conspecifics (Halfwerk et al., 2014), predators (Tuttle and Ryan, 1981; Halfwerk et al., 2014), parasitoids (Zuk et al., 2006) or hematophagous frog-biting midges (Bernal et al., 2006; Grafe et al., 2008). The selective pressure of eavesdroppers on signalers may thus either constrain the reproductive success of signalers, if their acoustic signals constitute sexually selected traits (Ryan, 1980; Zuk et al., 2006), or impose a direct survival cost due to increased exposure to acoustically-oriented predators or parasitoids (Tuttle and Ryan, 1981; Zuk et al., 2006).

A widespread signaler–eavesdropper interaction occurs between male frogs and acoustically-oriented hematophagous flies, mainly the frog-biting midges of the genus Corethrella. These midges belong to the family Corethrellidae, which includes 107 extant species found primarily in tropical and subtropical areas worldwide (Yu et al., 2013; Borkent, 2014; Amaral and Pinho, 2015). Female Corethrella locate male frogs using their calls as an auditory cue, and feed on their blood to obtain the blood meal necessary for egg production, a process in which midges may transmit trypanosomes to hosts (Borkent, 2008; Ferguson and Smith, 2012). Borkent (2008) suggested that frog-biting midges have likely had an important role in the history of frog evolution, as both groups have been interacting for at least 190 million years and currently live in sympathy in most parts of the globe. Interactions between frog-biting midges and calling frogs...
Corethrella of flies would be attracted to a non-natural abiotic source of frequency range white noise, to test whether different taxa putative unattractive sound in the form of an artificial, wide-calls recorded from three potential frog-host species of the austral Neotropics (Amaral and Pinho, 2015).

Besides Corethrella, some other genera of hematophagous flies are also known to feed on frogs. At least one mosquito species, Uranotaenia lowii (Culicidae), is known to feed selectively on amphibians, and to locate frog-hosts using the acoustic cue of their calls (Borkent and Belton, 2006). This mosquito was attracted in Costa Rica to the call of a tree frog native to the southeastern United States, the barking tree frog *Hyla gratiosa*, which also effectively attracted many female Corethrella (Borkent and Belton, 2006). Likewise, many female Uranotaenia and female Corethrella were attracted to frog calls broadcasted on Iriomote Island, of the Ryukyu Archipelago, in subtropical Japan (Toma et al., 2005). The specific components of frog calls that are attractive to species of Uranotaenia and Corethrella are not well understood, but Borkent and Belton (2006) suggested that the antennae of *U. lowii* and Corethrella species might resonate at the fundamental frequency of *Hyla gratiosa* calls, which is around 400–500 Hz.

While there is compelling evidence indicating the use of auditory cues in host-seeking behavior of Corethrella (McKeever and French, 1991; Toma et al., 2005; Bernal et al., 2006; Grafe et al., 2008; Bernal and Silva, 2015), as well as some evidence suggesting that at least some species of Uranotaenia are also capable of this (Toma et al., 2005; Borkent and Belton, 2006), the specific cue used in host location is not known for other hematophagous flies that also feed on frogs. This may be the case for biting-midges of the genus Forcipomyia (Ceratopogonidae), which have been observed attacking the frogs Leptodactylus chaquensis and *Pseudis paradoxa* in Argentina (Spinelli et al., 2002). In fact, evidence of the interaction between acoustically-oriented hematophagous flies and male frogs from the austral Neotropics is circumstantial. We are aware of only the following three records: the above-mentioned interaction between *Forcipomyia* and *Leptodactylus chaquensis* and *Pseudis paradoxa* (Spinelli et al., 2002), an interaction between unidentified hematophagous flies and male *Crossodactylus schmidti* (Caldart et al., 2014), and that between Corethrella aticola attracted to *Physalaemus cuvieri* calls in southern Brazil (Amaral and Pinho, 2015).

In the present study, we investigated, for the first time in the austral Neotropics, phonotactic responses of different taxa of hematophagous flies to varying sources of sound. During field phonotaxis trials performed in ponds and streams, we broadcasted loops of natural advertisement calls recorded from three potential frog-host species of the Atlantic Forest in southern Brazil. We also broadcasted a putative unattractive sound in the form of an artificial, wide-frequency range white noise, to test whether different taxa of flies would be attracted to a non-natural abiotic source of sound. We predicted that acoustically-oriented hematophagous flies would be attracted to the acoustic stimuli of only the frog calls, especially the frog-biting midges of the genus Corethrella that are well known to use auditory cues in host-seeking behavior (McKeever and French, 1991; Bernal and Silva, 2015). Additionally, female and male adults of a new species of Corethrella belonging to the rotunda group are described, and the interaction of this species with its frog hosts is discussed.

**MATERIALS AND METHODS**

**Ethical approval**

All applicable national and institutional guidelines for the care and use of animals were followed. All procedures performed were in accordance with the ethical standards of the institution or practice where the studies were conducted. Activities at Turvo State Park, including the collections of the Diptera taxa, were performed in accordance with the licenses issued by SEMA-RS (#23/2011) and ICMBio-IBAMA (#29505-4).

**Study Area**

The study was conducted between October 2013 and February 2014 (austral spring and autumn) in three streams and three ponds (Supplementary Figure S1) within Turvo State Park (27°14’34.08”S, 53°57’13.74”W, 376 m.a.s.l.), located in the municipality of Derrubadas, in the northwestern region of Rio Grande do Sul State, Brazil. The park covers an area of 17,491.4 ha (SEMA, 2005) adjacent to the border of the Uruguay River and near the Argentinian forests of Moconá Provincial Park and the Yaboti International Biosphere Reserve, and is one of the last large remnants of mesophytic semideciduous forest (sensu Oliveira-Filho et al., 2006) in southern Brazil. The local climate is characterized as subtropical sub-humid with a dry summer, an annual rainfall of 1787 mm, a mean annual temperature of 18.8°C and a mean temperature of the coldest month of 13.3°C (ST SB v climate type, sensu Maluf, 2000).

**Sound Preparation**

Natural calls of three anuran species commonly found in the study area were broadcasted. These were the stream-breeding frog *Crossodactylus schmidtii* (Hyloidae; Caldart et al., 2010), the pond-breeding frog *Physalaemus aff. gracilis* (Leptodactylidae; Kvet et al., 2010), and the pond-breeding tree-frog *Scinax perereca* (Hylidae; Kvet et al., 2010; Frost, 2015). Males of the stream-breeding frog *C. schmidtii* call upon exposed rocks in the stream bed with little acoustic overlap between nearby males (Caldart et al., 2010, 2011). They are most acoustically active during daylight hours, though may occasionally be heard calling at night (Caldart et al., 2016). The snout–vent length (SVL) of male *C. schmidtii* ranges from 22.14–27.95 mm, and mature individuals of both sexes may occur throughout the year at the study area (Caldart, unpublished data). Males of the pond-breeding frog *P. aff. gracilis* form dense calling choruses while floating in the water, partially hidden within aquatic vegetation (top et al., 2012). Male *P. aff. gracilis* have an SVL ranging from 26–31 mm, are acoustically active during the day and the night, and are reproductively active from September to February (Kvet et al., 2010). Males of the pond-breeding tree-frog *Scinax perereca* also form dense choruses at night and call while perched in small trees or in bushes located within or near ponds (Kvet et al., 2010). Male *Scinax perereca* have an SVL ranging from 36–41 mm, and are acoustically and reproductively active from September to February (Kvet et al., 2010); at the study area *S. perereca* commonly co-occurs in ponds with *P. aff. gracilis* (top et al., 2012). The advertisement calls of six males of *C. schmidtii*, six males of *S. perereca* and five males of the pond-breeding frog *P. aff. gracilis* were recorded at the study area, with the number of recorded calls per male ranging from three to five. The recordings were made using a Marantz® digital recorder (model PMD671) coupled to a Sennheiser® microphone (model ME66) positioned at a distance of 50 cm from the calling male. The sound pressure level of calls (in dB, re 20 μPa
was measured with an Impac® sound level meter (model IP-120C; C weighting curve: 31.5–8000 Hz, dB range: 40–130 dB) at a distance of 50 cm from the calling male. The recorded calls were analyzed with the SoundRuler software v. 0.9.6.0 (http://soundruler.sourceforge.net; (Bee, 2004; Gridi-Papp, 2004) at a sampling frequency of 44,100 Hz and a resolution of 16 bits, in order to obtain measurements of the following call parameters: call duration, call interval, number of notes, note duration, call rate, call duty cycle, and call dominant frequency. Subsequently to the bioacoustics analysis of the recordings we created playback loops of calls, using the software Adobe Audition, to be broadcasted during field trials. Call loops were created to represent average calls of each frog species based on the mean values obtained for the call parameters, including the call duty cycle.

The call loop of C. schmidtii (Supplementary Figure S2) consisted of a long call of 15.4 s duration, comprising 53 notes, emitted at a call rate of two calls per minute, with 15 s of silent interval between calls and a mean call dominant frequency of 3350 Hz; the call loop was equalized at an amplitude of 70 dB with the Impac® sound level meter positioned in front of the loudspeaker prior to each session. Call loop of C. schmidtii was adjusted to maintain the call duty cycle at 15 per cent. The call loop of P. aff. gracilis calls (Supplementary Figure S3) consisted of a 90 s chorus comprising whine calls of approximately 5 s each, emitted at a call rate of 10 calls per minute, with a silent interval of 41 s between choruses and a mean call dominant frequency of 588 Hz; the call loop was equalized at an amplitude of 69 dB and adjusted to maintain the call duty cycle at 80 per cent. Finally, the call loop of S. perereca (Supplementary Figure S4) consisted of a 23 s chorus comprising short calls of 0.5 s, emitted at a call rate of 114 calls per minute, with a silent interval of 29 s between choruses and a mean call dominant frequency of 3129 Hz; the call loop was equalized at an amplitude of 60 dB and adjusted to maintain the call duty cycle at 25 per cent. In addition, we also broadcasted a loop of an artificial white noise equalized at 66 dB, which was created to encompass the frequency range and the mean amplitude of calls of the three anuran species; the noise loop was composed by equal-amplitude frequencies, ranging from 0–10,000 Hz, emitted at a 5 s noise duration and a silent interval of 5 s between noise periods (Supplementary Figure S5). The white noise loop was adjusted to maintain the noise duty cycle at 50 per cent.

Field trials
We collected flies by broadcasting the frog call loops from three pairs of suction traps (modified from Mckeever and Hartberg, 1980), which consisted of a PVC pipe with a suction device in the middle, and a funnel at the top with an attached plastic container filled with 80% alcohol for collecting the sucked flies. The plastic container was attached to the lower end of the funnel to avoid damaging the flies from passing through the suction fan. The funnel was made with a thin net-grid that allowed the passage of the air generated by the fan, thus enabling the suction of all collected flies toward the plastic container. At the base of the trap we placed a small battery that powered the suction device, and near the opening of the funnel a Sony® loudspeaker was attached (model ICD-PX333; overall frequency response: 75–20,000 Hz) that broadcasted the frog call loops equalized at each respective amplitude.

The suction traps were disposed in a paired design in ponds and streams for trapping sessions of 45 minutes of duration during the diurnal period (11:00 to 17:00) and the dusk/nocturnal period (18:00 to 23:00). Each pair consisted of an acoustic trap broadcasting the frog call loop and an adjacent silent control trap placed approximately 5 m from the acoustic trap. To minimize acoustic interference, pairs of traps were placed at least 20 m apart from each other, and between two consecutive trapping sessions we switched the stimulus of the pairs (i.e., the acoustic trap became the silent trap, and vice-versa) to control for the possibility that the equipment would attract flies. We did from 4–7 diurnal trapping sessions per day and 4–10 dusk/nocturnal trapping sessions per day. We waited for at least seven days before running new trapping sessions in a pond or a stream that had been sampled earlier in the same month. Trapping sessions were conducted at air temperature and air humidity values varying from 20–32°C and 56–83%, respectively. We performed a total of 139 trapping sessions, totaling nearly 105 hours of sampling effort.

Statistics
The number of female flies captured in the acoustic and control traps was compared using the Wilcoxon signed-rank test for each type of acoustic stimulus separately (i.e., anuran species and white noise). This paired test allows controlling for non-measured environmental factors that could influence the trapping success at each trap pair position (e.g., local variation in temperature, light conditions). We compared further the number of female flies significantly attracted to a given acoustic stimulus with the number of flies captured in traps broadcasting a white noise, using the Mann-Whitney test. All data used were non-normally distributed (Shapiro-Wilk test; W > 0.55 and P < 0.001, in all cases). Analyses were made in the Past software, version 3 (Hammer et al., 2001).

Taxonomy
To identify the collected flies to the genus level we consulted taxonomic studies that present morphological diagnostic characters of the genera that we captured (Thielmann and Hunter, 2007; Marino and Spinelli, 2008; Borkent, 2008; Brown et al., 2009). Species of Corethrella were identified using a key to the New World species (Borkent, 2008). Corethrella specimens were mounted on slides in Euparal following procedures outlined by Sæther (1969). Terms, measurements, characters analyzed and examination techniques are based on Borkent (2008). The holotype of the new species is deposited at Museu de Zoologia da Universidade de São Paulo (MZUSP), Paratypes are deposited at Instituto Nacional de Pesquisas da Amazônia (INPA), Coleção Entomológica da Universidade Federal de Santa Catarina (UFSC) and MZUSP, Voucher specimens of the remaining species are deposited at UFSC. This article was registered in the Official Register of Zoological Nomenclature (ZooBank) as http://zoobank.org/1B03910D-4038-4A01-B7B3-99399379E064.

RESULTS
We conducted 104 hours and 25 minutes of trapping sessions, totaling 139 sessions, of which 59 (43% of total) collected at least one of the target taxa of hematophagous flies. Most collected specimens were frog-biting midges of the genus Corethrella (n = 188), followed by midges of the genus Forcipomyia (n = 80), and mosquitoes of the genus Uranotaenia (n = 16). Species of Corethrella were captured only at dusk or at night (33 from 99 dusk/nocturnal sessions; 33% of total), as well as specimens of Uranotaenia (10 from 99 dusk/nocturnal sessions; 10% of total). Differently, specimens of Forcipomyia were captured both during the day and at night (30 from 137 diurnal/nocturnal sessions; 22% of total).

The numbers of captured specimens of Forcipomyia midges and Uranotaenia mosquitoes did not differ between silent traps and their adjacent acoustic traps broadcasting calls of Crossodactylus schmidtii, Physalaemus aff. gracilis or Scinax perereca (Table 1). Likewise, the numbers of captured specimens of Forcipomyia and Uranotaenia did not differ between traps broadcasting a white noise and adjacent silent traps (Table 1). These flies were randomly cap-
tured in either acoustic or silent traps, with a mean number of 1.13 (range 0–4) specimens of *Forcipomyia* collected in the acoustic traps (median = 1; interquartile range = 0–1) and 1.5 (range 0–13) specimens collected in the silent traps (median = 1; interquartile range = 0–1.25). The mean number of *Uranotaenia* specimens in the acoustic traps was 1.1 (range 0–4) (median = 1; interquartile range = 0–2), and in silent traps it was 0.7 (range 0–2) (median = 1; interquartile range = 0–1).

The number of specimens of *Corethrella* captured, on the other hand, was significantly higher in traps broadcasting *C. schmidti* or *S. perereca* calls and their adjacent silent traps (Fig. 1). Traps broadcasting *P. aff. gracilis* calls attracted a mean of 6 (range 1–26) female *Corethrella* per trapping session (median = 2; interquartile range = 1–7.25) versus a mean of 1.3 (range 0–6) collected in adjacent silent traps (median = 0; interquartile range = 0–2.25). Furthermore, traps broadcasting *P. aff. gracilis* calls collected significantly more female *Corethrella* (mean of 6, range 1–26) compared to traps broadcasting a white noise (Mann-Whitney test: *U* = 6, *P* < 0.05), which collected an average of just 0.8 (range 0–1) female *Corethrella* (median = 1; interquartile range = 0.5–1).

The advertisement call of *P. aff. gracilis* attracted a total of 60 female *Corethrella*, including the five *Corethrella* species sampled at the study area (Table 2). The most abundant of them was previously unknown and is formally described in the taxonomy section of this work. Four *Corethrella* species were collected in ponds, of which two were exclusive to this type of habitat, and three species were collected in streams, with only *C. yucuman* sp. nov. exclusive to this habitat. Interestingly, along with nearly all females of *C. yucuman* sp. nov caught in the trapping sessions (n = 12), we also collected individual males and male swarms (n = 70).

### Taxonomy

**Corethrella yucuman** Caldart & Pinho sp. n.  
(Figs. 2–3, Table 3)

For LSID, see http://zoobank.org/382AE851-E335-46F8-91CA-FAEF915548BC.

**Type material**  
Holotype: female adult: Brazil, Rio Grande do Sul State, Derrudas, Parque Estadual do Turvo (Turvo State Park), Riacho Salto, 27.13936’S 53.881209’W, 185 m a.s.l., 17.xii.2013, silent frog call trap (PG1N, control), VM Caldart et al. leg. (MZUSP). Paratypes: two females, as holotype (UFSC); one female and two males, as holotype except frog call trap (PG1N, *Physalaemus aff. gracilis* calling) (INPA); two females, as holotype except CS2N, control (MZUSP); one female, as holotype except frog call

### Table 1. Results of the Wilcoxon signed-rank test comparisons between *Forcipomyia* midges and *Uranotaenia* mosquitoes collected in acoustic traps broadcasting *Crossodactylus schmidti*, *Physalaemus aff. gracilis* and *Scinax perereca* advertisement calls or a white noise, and the adjacent control silent traps. The N below each acoustic stimulus refers to the number of sessions with midgets and mosquitoes captured. "NA" refers to a statistical result not available because only a single capture session survived.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><em>C. schmidti</em></td>
<td><em>Z</em> = 0.43</td>
<td><em>Z</em> = 0.45</td>
</tr>
<tr>
<td>(N = 20)</td>
<td><em>P</em> &gt; 0.05</td>
<td><em>P</em> &gt; 0.05</td>
</tr>
<tr>
<td><em>Physalaemus</em> aff. <em>gracilis</em></td>
<td><em>Z</em> = 1</td>
<td><em>Z</em> = 0.28</td>
</tr>
<tr>
<td>(N = 7)</td>
<td><em>P</em> &gt; 0.05</td>
<td><em>P</em> = 0.05</td>
</tr>
<tr>
<td><em>Scinax</em> perereca</td>
<td><em>Z</em> = 0.38</td>
<td><em>Z</em> = 0.00</td>
</tr>
<tr>
<td>(N = 6)</td>
<td><em>P</em> &gt; 0.05</td>
<td><em>P</em> &gt; 0.05</td>
</tr>
<tr>
<td>White noise</td>
<td><em>Z</em> = 0.58</td>
<td><em>Z</em> = 0.05</td>
</tr>
<tr>
<td>(N = 7)</td>
<td><em>P</em> &gt; 0.05</td>
<td><em>P</em> &gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. List of the *Corethrella* species captured in ponds and streams using acoustic traps broadcasting frog calls and adjacent silent control traps in southern Brazil. *Acoustic stimuli (cs = Crossodactylus schmidti; pg = Physalaemus aff. gracilis; sp = Scinax perereca)* arranged in order of highest to lowest number of frog-biting midges collected; **Although these anuran species do not call in streams, some specimens of *Corethrella yucuman* sp. nov. were collected in a few trials with their calls in lotic habitats (three sessions each species). Number of male *Corethrella* captured are given within parenthesis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Acoustic stimuli</th>
<th>Ponds</th>
<th>Streams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acoustic trap</td>
<td>Silent trap</td>
<td>Acoustic trap</td>
</tr>
<tr>
<td><em>Corethrella yucuman</em> sp. nov.</td>
<td>cs, pg**, sp**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Corethrella lopesi</em> Lane</td>
<td>pg, sp, cs</td>
<td>61</td>
<td>5</td>
</tr>
<tr>
<td><em>Corethrella alticola</em> Lane</td>
<td>pg, cs, sp</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td><em>Corethrella atricornis</em> Borkent</td>
<td>pg</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Corethrella aff. wirthi</em> Stone</td>
<td>pg</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>22</td>
<td>61</td>
</tr>
</tbody>
</table>

![Fig. 1. Number of frog-biting midges (*Corethrella*) captured in traps broadcasting *Crossodactylus schmidti*, *Physalaemus aff. gracilis* and *Scinax perereca* advertisement calls or a white noise, compared with adjacent silent traps. Shown here are the mean numbers of captured females with the standard error as whiskers, and the maximum number of midgets captured in a single capture session as black dots. The N below each acoustic stimulus refers to the number of sessions with female midges captured. **Significant difference by the Wilcoxon signed-rank test (**Z* = 2.63, *P* < 0.01); *Significant difference by the Mann-Whitney test (*U* = 6, *P* < 0.05); ns: no significant difference by the Wilcoxon signed-rank test for the calls of *C. schmidti* (**Z* = 0.69, *P* > 0.05), *S. perereca* (**Z* = 0.72, *P* > 0.05) and the white noise (**Z* = 1.34, *P* > 0.05).]
Hematophagous flies and frog calls

trap (CS1N, *Crossodactylus schmidti* calling) (INPA); one female, as holotype except frog call trap (RB2N, white noise) (INPA); one female, as holotype except RB5N, control (INPA); one female and five males, as holotype except 15.ii.2014, frog call trap (SP1N, *Scinax perereca* calling) (MZUSP); two males, as holotype except 14.ii.2014, CS1N control (UFSC); one male, as holotype except Riacho Maria, 27.243489°S, 53.953972°W, 382 m a.s.l., 13.xi.2013, frog call trap (PG1N, *Physalaemus aff. gracilis* calling) (MZUSP); one male, as previous except silent frog call trap (CS1N, MZUSP).

**Fig. 2.** *Corethrella yucuman* Caldart & Pinho sp. n., female adult. (A) Head, anterior view. (B) Coronal suture. (C) Antenna. (D) Clypeus. (E) Right palpus. (F) Thorax and legs, lateral view. (G) Wing.
control) (INPA).

**Etymology**
The specific epithet refers to Yucumã Falls, the world’s widest waterfall (extending about 1800 meters), located on the Uruguay River near the tributary stream where the new species was found. Yucumã Falls and a portion of the riparian forest where the new species was found are currently under the threat of disappearing due to hydroelectric power plants planned for the Uruguay River. The name is to be
Table 3. Number of sensilla coeloconica on adults of Corethrella yucuman

<table>
<thead>
<tr>
<th>Flagellomeres</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
<th>XIII</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td>1–2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td>1–2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1–1</td>
</tr>
</tbody>
</table>

regarded as a noun in apposition.

**Diagnostic characters**

**Adult female**: Only extant species of Corethrella with legs, most of thorax and abdomen uniformly brown, wing uniformly pigmented, R2 basal to apex of M1 and coronal suture absent. **Adult male**: Only extant species of Corethrella with legs, most of thorax and abdomen uniformly brown, wing uniformly pigmented, R2 basal to apex of M1 and R2 shorter than the stem of R2+3.

**Descriptions**

**Female adult** \((n = 8–10)\). Outline in anterior view nearly circular, 1.2–1.5 times wider than long. Two large setae on frons, between ventromedial area of the ommatidia. Coronal suture absent (Fig. 2B). Antenna lightly pigmented; pedicel without distinctive, more elongate, stout, dorsal or dorsolateral setae, flagellomeres as in Fig. 2C, sensilla coeloconica distributed as in Table 3. Flagellomere XIII with well-developed apical bifurcation. Mandible with small, pointed teeth. Clypeus (Fig. 2D) squareish, with 3–5 stout setae. Palpus (Fig. 2E) light brown, segment 3 swollen apically. Thorax (Fig. 2F). Uniformly medium to dark brown, scutellum darker. Posterior portion of dorsocentral row with single elongate setae situated somewhat lateral to one another. Prescutal suture very short, not extending more than halfway to dorsocentral row of setae. Aneponerum uniformly brown with pattern of pigmented veins and/or scales; veins (other than costa and wing margin) with very slender scales, Apex of R2 basal to M1; R2 1.1–1.5 times longer than R0+3; wing length 1.00–1.13 mm. Halter as dark as scutellum.

**Male adult**: \((n = 11)\) as female, except: **Head** (Fig. 3A). 1.3–1.5 times wider than long. Coronal suture long (Fig. 3B). Flagellomeres as in Fig. 3C, sensilla coeloconica distributed as in Table 3. Clypeus (Fig. 3E) with 1–3 stout seta.

**Thorax** (Fig. 3F). Aneponerum with group of 2–3 setae; posterior anepisternals 1–3.

**Wing** (Fig. 3G). R2+3 0.6–0.8 times longer than R2; wing length 0.91–1.01 mm.

**Legs**. Midleg with thick, subapical setae on each of at least tarsomeres 1–3. Claw of foreleg longer than those of mid-, hind leg; each claw without inner tooth; anterior claws of each leg without a basal prong; foreleg claws unequal; midleg claws equal.

**Abdomen** (Fig. 3H). Uniformly brown with 9th segment darker.

**Genitalia** (Figs. 3I–J). Gonocoxite (Fig. 3J) medium brown, about 3.0–3.5 longer than wide (at midlength); gonocoxite with well-defined dorsal row of 5 slender setae plus a more ventral thick one, located near the two basal ones, at 0.41–0.49 gonocoxite’s length. Gonostylus slender, straight for most of its extension, but curved apically; sub-basal seta apparently absent; apex pointed with somewhat stout apical seta, with one tiny seta close to apex. Aedeagus (Fig. 3J) slender, elongate, tapering from base, acuminate apically, with lateral margins fusing subapically.

**Larva and pupa**. Unknown.

**DISCUSSION**

In this study we investigated phonotactic responses of three taxa of hematophagous flies to frog calls in southern Brazil. By broadcasting different acoustic stimuli we found that frog-biting midges (Corethrella) were attracted to the calls of the common pond-breeding frog Physalaemus aff. gracilis, and that when compared to traps broadcasting a white noise, the calls of P. aff. gracilis attracted significantly more frog-biting midges. In contrast, Forcipomyia midges and Uranotaenia mosquitoes were not attracted to any of the tested frog calls. Recent studies in tropical sites also attracted Corethrella species with frog calls (Bernal et al., 2006; Grafe et al., 2008), but similar studies testing the attraction of frog-biting midges to frog calls were lacking in the austral Neotropics. Likewise, the interaction between frog-hosts and hematophagous Forcipomyia (e.g., Spinelli et al., 2002) and Uranotaenia (e.g., Borkent and Belton, 2006) has never been investigated in the austral Neotropics with regards to the cue used in host-seeking behavior. Our study is the first to present evidence that the advertisement calls of three frog species from subtropical Brazil (Physalaemus aff. gracilis, Crossodactylus schmidti and Scinax perereca) were not attractive to Forcipomyia midges and Uranotaenia mosquitoes, while the call of P. aff. gracilis was effective in attracting females of all Corethrella species registered in this study.

The pattern of abundance of Corethrella at the study site was similar to that found in other subtropical sites, but much lower compared to what has been registered for tropical sites. For instance, Grafe et al. (2008) collected an average of 37 frog-biting midges per trapping hour in Borneo, with abundance ranging from 0–268, while on Iriomote Island in subtropical Japan, Toma et al. (2005) collected a total of 131 female Corethrella over 10 sampling nights with frog call traps. We collected an average of six flies per trapping hour broadcasting P. aff. gracilis calls, with abundance ranging from 1–26 frog-biting midges. Amaral and Pinho (2015) recently reported a similar pattern of low abundance of Corethrella in another site from subtropical Brazil, where broadcasted calls of the common frog Leptodactylus latrans captured no more than 20 frog-biting midges during one...
hour of trapping. The patterns of abundance of frog-biting midges in the austral Neotropics are certainly not conclusive given that studies are at incipient stages and have covered just a few locations. However, the low abundance of *Corethrella* in subtropical Brazil is not unexpected, as this taxon is most diverse and abundant in the lowland tropics (Borkent, 2008), while the sampled sites in southern Brazil are located at the latitude 27° S (Amaral and Pinho, 2015; present study), which is near to the austral distribution limit known for *Corethrella* in South America (latitude 30° S in Argentina; Borkent, 2008). Moreover, patterns of richness and abundance of frog-biting midges are expected to vary depending on the frog species and the type of calls used as bait, as well as the type of habitat sampled (Borkent, 2008).

We collected five species of *Corethrella*, most were collected in ponds and all species were captured using the call of *P. aff. gracilis*. Interestingly, the advertisement call of a congeners species, *Physalaemus cuvieri*, also attracted frog-biting midges in southern Brazil (Amaral and Pinho, 2015). *Physalaemus cuvieri* and *P. aff. gracilis* are common pond-breeding frogs in southern Brazil, whose males form choruses and call while floating in the water to attract mates (Kwet et al., 2010). The advertisement calls of both species are spectrally similar, sharing such features as harmonic structure, wide frequency range, dominant frequency located below 1 kHz, and call sound pressure level of around 69 dB (*P. cuvieri*: Gambale and Bastos, 2014; *P. aff. gracilis*: this study). Another common and apparently effective species that attracts *Corethrella* in southern Brazil is the pond-breeding butter-frog, *Leptodactylus latrans* (Amaral and Pinho, 2015), the advertisement call of which also presents a dominant frequency below 1 kHz (Straughan and Heyer, 1976). In contrast, calls of the other two frog species broadcasted for attracting frog-biting midges in our study have dominant frequencies above 3 kHz (*C. schmidti*: Caldart et al., 2011; *S. perereca*: Magrini and Giaretta, 2010) and collected fewer frog-biting midges. Moreover, the duty cycles of the broadcasted calls of *C. schmidti* and *S. perereca* were lower than the duty cycle of *P. aff. gracilis* calls, though according to our recordings, such differences are likely close to what occurs in nature. In the study area at least 15 pond-breeding anuran species were recorded during nocturnal calling activity (Iop et al., 2012), hence it is quite likely that interactions between *Corethrella* and calling frogs of other species occur. Studies broadcasting calls of different frog species simultaneously would be relevant for testing the attraction of female *Corethrella* to different call frequencies and duty cycles, and we suggest that species of *Physalaemus* and *Leptodactylus* be further tested to answer this question for subtropical Brazil.

We collected individual male *Corethrella* and male swarms in nearly all trapping sessions where females of *Corethrella yucuman* sp. n. were caught. Female *Corethrella* have evolved a strategy to feed on nocturnal frogs because most species of anurans vocalize at night and the mating swarms of *Corethrella* are formed near the habitats where anurans aggregate for reproduction (Borkent, 2008; Silva and Bernal, 2013). It has recently been shown that female *Corethrella* strongly depend on the mating calls emitted by male frog hosts for long-distance host location (Bernal and Silva, 2015). However, it is known that male individuals of *Corethrella may also be attracted to calling frogs in order to find conspecific mates (Borkent, 2008). Our collection of male individuals and male swarms along with female *Corethrella yucuman* sp. n. (Table 2) lends additional support to this claim. There is very little information about the mating behavior of frog-biting midges, but for at least one species, *Corethrella appendiculata*, it was reported that mating swarms consisted predominantly of males, which indicates a male-biased lek-like mating system in this species (Silva and Bernal, 2013) that is similar to the one found in other culicomorph species. Thus, this is expected to also occur in *Corethrella yucuman* sp. n.

In contrast to what we found for *Corethrella*, Forcipomyia midges and *Uranotaenia* mosquitoes were not attracted to any of the frog calls used. We did expect to collect many more individuals of *Uranotaenia* in traps broadcasting frog calls, as was reported in Costa Rica (Borkent and Belton, 2006) and on a subtropical island in Japan (Toma et al., 2005). However, it is possible that *Uranotaenia* species respond to call frequencies particularly sensitive to their antennae. For example, the antennae of *Uranotaenia lowii* are thought to resonate at around 400–500 Hz, which is the fundamental harmonic of *Hyla gratiosa* calls (Borkent and Belton, 2006). Alternatively, species of *Uranotaenia* may use non-acoustic cues in frog–host location, including visual stimuli, carbon dioxide, water vapor or other volatile frog skin secretions (Borkent and Belton, 2006). Thus, while *Uranotaenia* mosquitoes were not attracted to the calls of *P. aff. gracilis*, *C. schmidti* and *S. perereca*, our results do not preclude the existence of interactions between *Uranotaenia* and male frogs in southern Brazil. For *Forcipomyia* midges, on the other hand, data from the literature and our results suggest that these midges do not use acoustic cues of frog calls in host-seeking behavior. Although *Forcipomyia* midges have been observed attacking the frogs *Leptodactylus chaquensis* and *Pseudis paradoxa* in Argentina (Spinelli et al., 2002), they may have used non-acoustic cues to find their hosts because most hosts of *Forcipomyia* biting females are invertebrates that do not rely primarily on airborne acoustic signals for communication, such as spiders, dragonflies, damselflies and phasmids (Borkent and Spinelli, 2007), except for katydids (Greenfield, 2016) and some caterpillars (Brown et al., 2007). While the specific cues used for host location by *Forcipomyia* and *Uranotaenia* species remain poorly understood, further conclusion should be avoided until studies about the attraction of these taxa to different types of host cues are performed.

**Taxonomy, distribution and biology of *Corethrella***

This study reports the southernmost records of *C. iopesi*, *C. alticola* and *C. atricornis*, expanding the distribution of these species to the austral portion of the Neotropics, in the southernmost Brazilian state of Rio Grande do Sul. *Corethrella iopesi* was previously known from the states of Bahia, Rio de Janeiro and São Paulo (Borkent, 2008), *C. alticola* was previously known from the states of Goiás, São Paulo (Borkent, 2008), and Santa Catarina (Amaral and Pinho, 2015), and *C. atricornis* was previously known only from the state of São Paulo (Borkent, 2008).

The females of *C. yucuman* sp. n. key out to *C. anniae* in Borkent (2008), but it is easily distinguished from this spe-
cies based on pigmentation of katepisternum, which is completely pale in C. anniae and brown, only slightly paler than the scutellum, in C. yucuman sp. n. Also, the prescutal suture is very short in C. yucuman sp. n. and elongate in C. anniae, and the R2 is basal to the apex of M1 in C. yucuman sp. n., while distal in C. anniae. The males key out to C. brevivena (couplet 26) because the R2 is shorter than the stem of R2-3. The new species clearly belongs to the rotunda group because the ventral portion of posterior anepisternum is continuous with the dorsal portion. Another synapomorphy of the rotunda group, although homoplastic along the genus, is the wing lacking patterned pigmentation (Borkent, 2008).

Corethrella yucuman sp. n. is the first species belonging to the rotunda group found in South America; the eight other species are known only from a few localities in Texas (USA) and Costa Rica, at elevations ranging from sea level to 600 m (Borkent, 2008). The new species was found only along two streams in Turvo State Park (southern Brazil), at elevations between 185–382 m. Immature individuals of rotunda group species are unknown, although unreared larvae from hyporheic zones in small streams in Texas may belong to Corethrella kerrvillensis (as discussed by Borkent, 2008). Such hyporheic zones may be the C. yucuman sp. n. larval habitat, as trapping sessions in ponds were not effective in collecting adults of this species. The serrate mandibles of the female adults and their attraction to Physalaemus aff. gracilis, Crossodactylus schmidti and Scinax perereca calls suggest that they feed on frog blood in nature.

Concluding remarks

In this study we investigated phontactonic responses of three taxa of hematophagous flies to frog calls in subtropical Brazil. While Forcipomyia midges and Uranotaenia mosquitoes were randomly captured in both frog call and silent traps, calls of the common pond-breeding frog Physalaemus aff. gracilis attracted five species of Corethrella, one of which is formally described herein. Traps broadcasting a white noise did not differ from adjacent silent traps in the number of captured flies, regardless of the diptera taxa or the broadcasted frog call. Moreover, white noise traps collected fewer frog-biting midges compared to traps broadcasting P. aff. gracilis calls, which suggests that female Corethrella feed on males of this widely distributed pond-breeding frog in southern Brazil. We emphasize the importance of using frog call traps to reveal the unknown diversity of acoustically-oriented frog-biting midges of the austral Neotropics, especially Corethrella. We also draw attention to the need for studies that test the role of non-acoustic cues of frog hosts in the host-seeking behavior of Forcipomyia midges and Uranotaenia mosquitoes. Studies about the interaction between hematophagous flies and frog hosts are needed to better understand the cues used by these flies to locate hosts, the degree of host specificity, and the evolution of adaptations in frog hosts and hematophagous flies.

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