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An acoustic and morphometric evaluation of the geographic distribution of *Phyllomedusa burmeisteri* (Anura: Phyllomedusidae), with comments on *P. bahiana*

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Abstract

An acoustic and morphometric evaluation of the geographic distribution of *Phyllomedusa burmeisteri* (Anura: Phyllomedusidae), with comments on *P. bahiana*. A recent study, based on phylogenetic and phylogeographic multilocus approaches, detected two evolutionary units (BUR and BUR-RJ) within the range of *P. burmeisteri*. BUR has a wide distribution, whereas BUR-RJ is restricted to a small area coincident with the Serra do Mar mountain range in the Brazilian state of Rio de Janeiro. We evaluate if acoustic and morphometric data support these two major clades within *P. burmeisteri* that were proposed using molecular evidence. We also provide for the first time detailed morphometric data for adult males of *P. burmeisteri* (including topotypes) and the holotype of *P. bahiana,* and we revisit the comparative acoustic diagnosis between *P. burmeisteri* and *P. bahiana.* We were unable to distinguish BUR and BUR-RJ evolutionary units based on morphometric, acoustic or any other feature of external morphology or coloration. Given the high levels of similarity in morphometric and acoustic traits between *P. burmeisteri* and *P. bahiana,* these features appear not to be informative in the differentiation of the two sister species.

Keywords: amphibians, bioacoustics, Leaf Frogs, taxonomy.

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Resumo

Uma avaliação acústica e morfométrica da distribuição geográfica de *Phyllomedusa burmeisteri* (Anura: Phyllomedusidae), com comentários sobre *P. bahiana*. Um estudo recente, baseado em abordagens filogenéticas e filogeográficas a partir de genes mitocondriais e nucleares, detectou duas unidades evolutivas (BUR e BUR-RJ) dentro da distribuição geográfica conhecida de *P. burmeisteri.* O estudo apontou que a linhagem BUR apresenta ampla distribuição, enquanto a BUR-RJ está restrita a uma pequena área coincidente com a Serra do Mar no estado do Rio de Janeiro. Neste estudo, nós avaliamos se os dados acústicos e morfométricos suportam esses dois clados de *P. burmeisteri* propostos com base em evidências moleculares. Também fornecemos dados morfométricos detalhados inéditos de machos adultos de *P. burmeisteri* (incluindo os topótipos), bem como do holótipo de *P. bahiana.* Além disso, revisitamos a diagnose acústica comparativa entre *P. burmeisteri* e *P. bahiana* proposta anteriormente na literatura. Não foi possível distinguir as unidades evolutivas (BUR e BUR-RJ) de *P. burmeisteri* com base em morfometria, bioacústica ou qualquer outro caráter da morfologia externa ou da coloração dos espécimes amostrados. Do mesmo modo, os caracteres morfométricos e acústicos parecem não ser informativos na diagnose comparativa entre *P. burmeisteri* e *P. bahiana,* uma vez que encontramos aqui altos níveis de similaridade entre essas duas espécies-irmãs.

Palavras-chave: anfíbios, bioacústica, pererecas-folha, taxonomia.

Introduction

The genus *Phyllomedusa* Wagler, 1830 comprises 16 species that occur from Panama to the Pacific slopes of Colombia, and South America east of the Andes, including Trinidad, southward to northern Argentina and Uruguay (Duellman *et al.* 2016, Frost 2018). Faivovich *et al.* (2010) found support for a clade within *Phyllomedusa,* the *P. burmeisteri* species group, which currently includes *P. burmeisteri* Boulenger, 1882, *P. iheringii* Boulenger, 1885, *P. bahiana* A. Lutz, 1925, *P. distincta* B. Lutz, 1950, and *P. tetraploidea* Pombal and Haddad, 1992 (Pombal and Haddad 1992, Brunes *et al.* 2010, Duellman *et al.* 2016).

Phyllomedusa burmeisteri was described based on syntypes from “Rio de Janeiro”, “Brazil”, and “Oran Salta, Buenos Ayres” (Boulenger 1882). Funkhouser (1957) suggested “Tijuca”, municipality of Rio de Janeiro, state of Rio de Janeiro, as the type locality of this species, and that the specimen from Buenos Aires, Argentina is *P. sauvagii* Boulenger, 1882. *Phyllomedusa burmeisteri* is associated with lentic water bodies around clearings and forest borders throughout the Brazilian states of Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo (Pombal and Haddad 1992, Brunes *et al.* 2010, 2014). The advertisement call of the species was described based on two males from the municipality of Saquarema (RJ) (Abrunhosa and Wogel 2004). This species is acoustically distinguished from its sister species, *P. bahiana,* by distinct arrangements of the pulses along the call (presence of triads in *P. bahiana*), and tadpole morphology (Cruz 1982, Silva-Filho and Juncá 2006), as well as by molecular information (Brunes *et al.* 2010, 2014).

Based on phylogenetic and phylogeographic multilocus approaches Brunes *et al.* (2014) detected two evolutionary units (BUR and BUR-RJ) within the range of *P. burmeisteri*. According to this study, BUR is widely distributed, occurring in four Brazilian states: Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo, whereas BUR-RJ is restricted to a small area within the Serra do Mar mountain range in the state of Rio de Janeiro. The range of BUR-RJ is partly delimited by the Paraíba do Sul River. The BUR lineage was also highly supported as an unconfirmed species by Brunes *et al.* (2014).

Here we evaluate whether phenotypic information supports the two major clades within *P. burmeisteri* proposed by Brunes *et al.* (2014), based on morphometric and/or acoustic data for 33 localities from four Brazilian states. We present detailed morphometric data for adult males of *P. burmeisteri* (including topotypes) and the holotype of *P. bahiana.* In addition, we revisit the comparative acoustic diagnosis between *P. burmeisteri* and *P. bahiana* proposed by Silva-Filho and Juncá (2006).

Materials and Methods

Morphometry

We collected the specimens under permit #30059 issued by SISBio / ICMBio. Individuals were euthanized by applying 5% lidocaine on their skin. We fixed specimens in 10% formalin and transferred them to 70% ethanol for permanent storage. These specimens are deposited in the collection of frogs at the Museu de Biodiversidade do Cerrado (AAG-UFU), Universidade Federal de Uberlândia, Uberlândia, Minas Gerais state, Brazil. We also analyzed specimens deposited in the following Brazilian collections: Adolpho Lutz Collection (AL-MN), housed in Museu Nacional, Rio de Janeiro, Rio de Janeiro state; amphibian collection of the Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso" (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo state; Célio F. B. Haddad Collection (CFBH), Universidade Estadual Paulista (UNESP), Rio Claro, São Paulo state; and Museu Nacional (MNRJ), Rio de Janeiro, Rio de Janeiro state.

Morphometric features were measured using Mitutoyo digitimatic calipers CD-6" CSX to the nearest 0.1 mm. Eleven traits were measured following Watters *et al.* (2016): snout–vent length (SVL), thigh length (THL), foot length (FL), head length (HL), head width (HW), eye diameter (ED), tibia length (TL) (= shank length), tympanum diameter (TD), eye–nostril distance (END), and disc diameters of third finger (3FD) and fourth toe (4TD).

The morphometric data for adult males of *P. burmeisteri* were classified according to the two evolutionary units proposed by Brunes *et al.* (2014): BUR and BUR-RJ. The municipalities classified as BUR were: Alpinópolis, Carangola, Chiador, Conceição do Mato Dentro (Serra do Cipó), Juiz de Fora, Matutina, São José da Barra, and Viçosa (all in Minas Gerais state); Linhares (Espírito Santo state); Atibaia, Campinas, Corumbataí, Itatiba, Jundiaí, Nazaré Paulista, Rio Claro, and Santo André (all in São Paulo state). Of the 33 males measured from BUR, 10 are molecular vouchers in the genetic analysis by Brunes *et al.* (2014). The municipalities classified as BUR-RJ were: Cachoeiras de Macacu, Campos dos Goytacazes, Duque de Caxias, Macaé, Santa Maria Madalena, Nova Iguaçu, Engenheiro Paulo de Frontin, Rio de Janeiro, Petrópolis, São Gonçalo, and São Pedro da Aldeia (all in state of Rio de Janeiro). Of the 38 males measured from BUR-RJ, 11 are molecular vouchers in the genetic analysis by Brunes *et al.* (2014). Three specimens are topotypes from "Tijuca", Rio de Janeiro, and three other specimens are from other localities near the type locality within the municipality of Rio de Janeiro (RJ). Additionally, we also measured four adult females of BUR-RJ.

We measured the holotype of *P. bahiana* (AL-MNRJ 768). For comparison, we measured 24 adult males of *P. bahiana:* nine from Alagoinhas, two from Gandu, three from Ilhéus, two from Itagibá, two from Jequié, three from Maracás, and three from Piatã (Chapada Diamantina); all these municipalities are in the state of Bahia (Figure 1). Further details on all specimens are given in Appendix I.

Bioacoustics

In the field, we recorded calls with the digital recorders Marantz PMD 670, Marantz PMD 671, Boss 864 (all three with Sennheiser ME67/

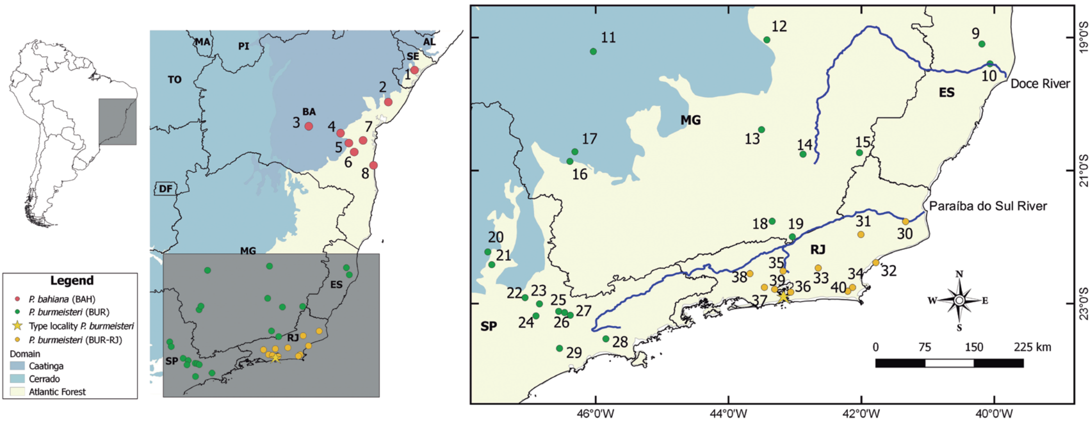

Figure 1. Map of the eastern portion of Brazil showing Brazilian domains and samples included in our morphometric and acoustic analyses. The type locality of *Phyllomedusa burmeisteri* is indicated with a yellow star. The inset shows in detail the sampled localities for the two evolutionary units of *P. burmeisteri* (BUR and BUR-RJ) proposed by Brunes *et al.* (2014). Municipalities: *P. bahiana*: (1) Areia Branca; (2) Alagoinhas; (3) Piatã; (4) Maracás; (5) Jequié; (6) Itagibá; (7) Gandu; (8) Ilhéus; (9) Sooretama; (10) Linhares; (11) Matutina; (12) Conceição do Mato Dentro; (13) Ouro Preto; (14) Viçosa; (15) Carangola; (16) Alpinópolis; (17) São José da Barra; (18) Juiz de Fora; (19) Chiador; (20) Corumbataí; (21) Rio Claro; (22) Campinas; (23) Itatiba; (24) Jundiaí; (25) Atibaia; (26) Bom Jesus dos Perdões; (27) Nazaré Paulista; (28) Salesópolis; (29) Santo André; (30) Campos dos Goytacazes; (31) Santa Maria Madalena; (32) Macaé; (33) Cachoeiras de Macacu; (34) São Pedro da Aldeia; (35) Petrópolis; (36) São Gonçalo; (37) Nova Iguaçu; (38) Engenheiro Paulo de Frontin; (39) Duque de Caxias; (40) Iguaba Grande. Brazilian states: Alagoas = AL; Bahia = BA; Espírito Santo = ES; Minas Gerais = MG; Rio de Janeiro = RJ; São Paulo = SP; Sergipe = SE.

K6 directional microphone), Marantz PMD 661MKII, and M-audio Microtrack II (both with Sennheiser ME66/K6 directional microphones); sampling rates were 44.1 or 48.0 kHz with a resolution of 16 bits. Acoustic terminology follows Köhler *et al.* (2017). The presence (state "0") or absence (state "1") of pulse groups along the call was evaluated for each male recorded. We calculated grand means and standard deviations from mean values for each male recorded, whereas the range (variation) encompassed the absolute minimum and maximum values among the samples. Air temperature was measured using a handheld digital thermometer to the nearest 0.1°C. Calls were analyzed using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program 2014) with the following settings: window type = Hann, window size = 256 samples, 3 dB filter bandwidth = 248 or 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked), grid spacing (spectral resolution) = 43.1 or 46.9 Hz, color map = Cool. Temporal traits were measured in the oscillogram and spectral traits were measured in the spectrogram. Raven Pro 1.5 obtained the dominant frequency and other frequency bands automatically through the "Peak Frequency (Hz)" measurement function by the manual selection of call units. The values of the dominant frequency trait refer to the peak reached in the call. We generated call figures using the package 'seewave' version 2.0.5 (Sueur *et al.* 2008) in R version 3.4.1 (R Core Team 2017). Seewave settings were Hanning window, 90% overlap and 512 points resolution (FFT).

We recorded and analyzed calls from 35 adult males of *P. burmeisteri,* which were also classified according to the two evolutionary units proposed by Brunes *et al.* (2014). Thirty-one males of the following municipalities were classified as BUR: Alpinópolis, Chiador, and Ouro Preto (state of Minas Gerais); Atibaia, Bom Jesus dos Perdões, Campinas, Itatiba, and Salesópolis (state of São Paulo); and Sooretama (state of Espírito Santo). Four males from municipality of Macaé (state of Rio de Janeiro) were classified as BUR-RJ. For acoustic comparisons between *P. burmeisteri* and *P. bahiana,* we recorded and analyzed six adult males of *P. bahiana* from the municipalities of Piatã and Ilhéus (state of Bahia), and Areia Branca (state of Sergipe).

Sound files were deposited in the Arquivo Sonoro da Coleção de Anuros da Universidade Federal de Uberlândia, Uberlândia (state of Minas Gerais), Brazil. Voucher specimens for call recordings of *P. burmeisteri* are: Macaé (state of Rio de Janeiro): AAG-UFU 0530–0531, 0751; Atibaia: AAG-UFU 0444, 0949; Campinas: AAG-UFU 1886–1888 (both in the state of São Paulo); Alpinópolis: AAG-UFU 0958, 4860–4862; Chiador: AAG-UFU 0678 (both in the state of Minas Gerais); Sooretama (state of Espírito Santo): AAG-UFU 6210, 6212. Voucher specimens of *P. bahiana* are: Ilhéus: AAG-UFU 0228; Piatã: AAG-UFU 1677–1679 (both in the state of Bahia) (Figure 2). Further details of the analyzed sound files are in Appendix II.

Statistical Analysis

The multivariate normality assumption was verified through the “mardiaTest” function in R (package ‘MVN’ version 4.0.2; Korkmaz *et al.* 2014), and it was applied to both the morphometric and acoustic data (results = data are not multivariate normal). Considering the (multivariate) acoustic and morphometric data, we searched for discrimination among the two evolutionary units of *P. burmeisteri* (BUR and BUR-RJ) and *P. bahiana* by applying the Random Forests (RF) model (Breiman 2001) using the package ‘randomForest’ version 4.6-12 in R (further details in Liaw and Wiener 2002). The RF results include an estimate of distances among the objects and a Multidimensional Scaling Analysis (MDS). This analisys can be plotted with the “proximityPlot” function of the package ‘rfPermute’ version 2.1.5 (Archer 2016), which also allows the results to be displayed graphically. Analyses were conducted in R.

For the multivariate, multidimensional analysis/plots and statistical tests, we used all the morphometric variables detailed earlier and the following acoustic traits: call duration; number of pulses per call; pulse duration; interpulse interval; pulses per second; presence or absence of pulse groups; and dominant frequency. Acoustic and morphometric traits indicated as important in the multivariate analysis were then tested for statistical significance of differences among the evolutionary units of *P. burmeisteri* and *P. bahiana* using the Exact Wilcoxon Mann-Whitney Rank Sum Test, function wilcox_test of the package ‘coin’ version 1.2-0 (Resampling Statistics model; Hothorn *et al.* 2008) in R. Significance was considered when $p < 0.05$. The significance levels (“p”) of the pairwise comparisons were adjusted considering the number of pairings using the method of Holm (p.adust function in R).

Results

Morphometry

Morphometric features of *P. burmeisteri* (BUR and BUR-RJ) are summarized in Table 1. The adult male SVL of BUR ranged from 53–70 mm and that of BUR-RJ ranged from 54–69 mm. Overlap occurred between the other morphometric features (Table 1). The RandomForest multivariate approach applied to morphometric data indicated a broad overlap among all three partitions (Figure 3A). We found no statistical significance in any trait.

Measurements of the holotype of *P. bahiana* (AL-MN 768, collected in February 1924 in

Figure 2. Adult males of *Phyllomedusa burmeisteri* in life from Brazilian municipalities of Atibaia (**A**), state of São Paulo (AAG-UFU 0958); Macaé (**B**), state of Rio de Janeiro (AAG-UFU 0530); and Alpinópolis (**C**), state of Minas Gerais (AAG-UFU 4860); and an adult male of *P. bahiana* from municipality of Ilhéus (**D**), state of Bahia (AAG-UFU 0229). All these males are call vouchers.

Salvador, state of Bahia) are in Table 1. The holotype is currently somewhat fragile; the head is slightly arched ventrally; a cross section is present on the skin of the chest; the mouth is open, with tongue visible and has a brown-ferruginous faded coloration.

Advertisement Call of P. burmeisteri*—BUR and BUR-RJ Evolutionary Units*

The advertisement call of *P. burmeisteri* (BUR-RJ) consisted of a multi-pulsed note emitted sporadically (Figure 4A, B), lasting 186–409 ms, separated by intervals of 6–44 s. Calls had 9–15 pulses arranged in 4–6 pulse groups per call, containing 2–5 pulses per group (Figure 4A), or regularly spaced (*N* = 1 male, Figure 4B). When present, pulse groups lasted 23–122 ms, separated by intervals of 10–41 ms. The pulse duration varied from 2–15 ms, separated by intervals of 2–35 ms. Pulses were released at a rate of 37–53 pulses per second. The dominant frequency (= fundamental) varied from 1031–1219 Hz.

The advertisement call of *P. burmeisteri* (BUR) also consisted of a multi-pulsed note

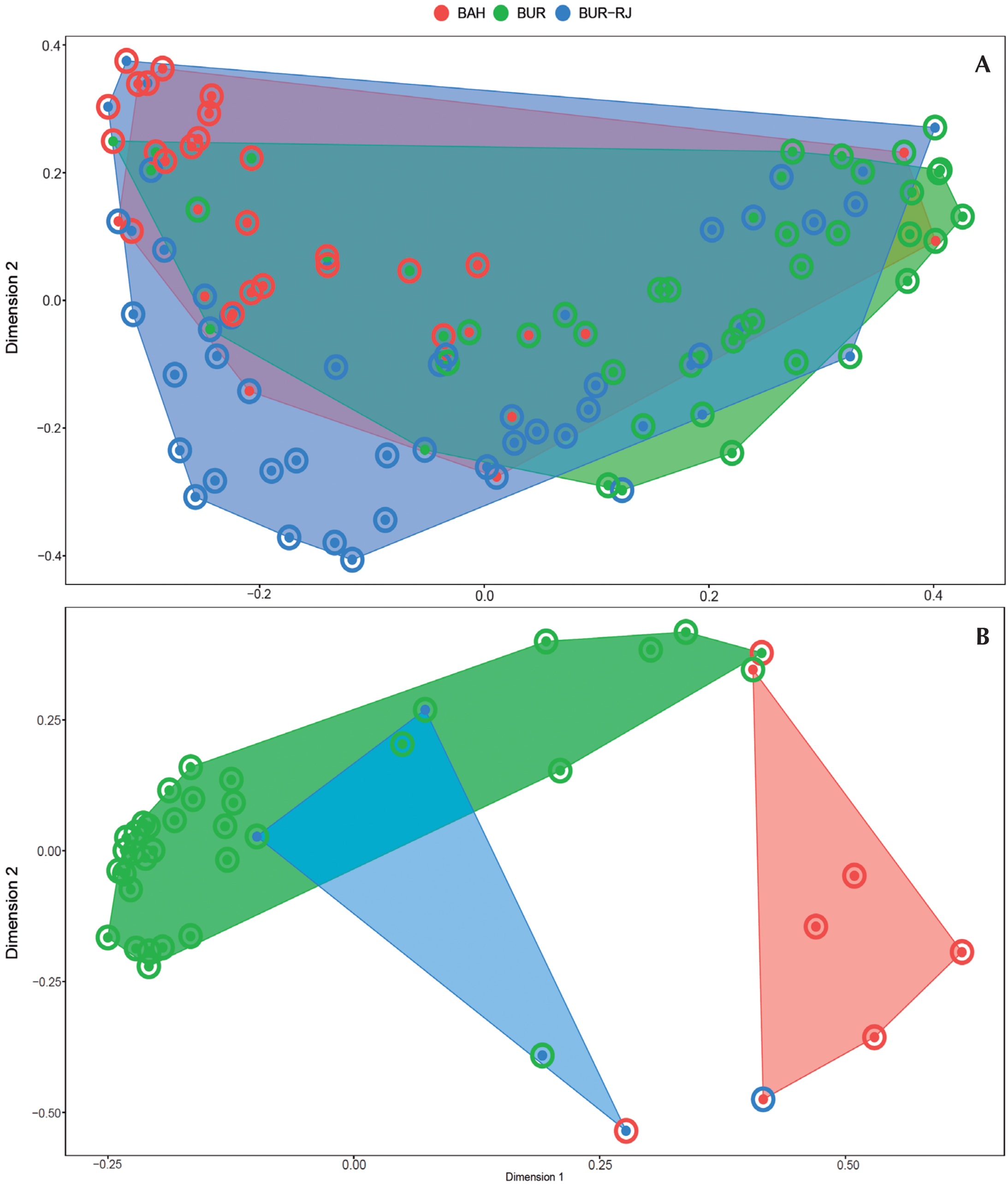

Figure 3. First and second dimensions of the Multidimensional scaling on the proximity scores from the Random Forest analysis considering morphometric (**A**) and acoustic (**B**) traits of adult males belonging to the BUR (green dots) and BUR-RJ (blue dots) evolutionary units of *P. burmeisteri* (according to Brunes *et al.* 2014), and adult males of *P. bahiana* (red dots).

Table 1. Morphometry of adult specimens of the two evolutionary units of *Phyllomedusa burmeisteri* (BUR and BUR-RJ) (according to Brunes *et al.* 2014) from 27 municipalities of four Brazilian states, and holotype and adult males of *P. bahiana* from seven municipalities of the state of Bahia. Values in mm as mean ± SD (minimum–maximum). *N* = number of measured specimens. See Appendix I.

	***P. burmeisteri* (BUR)**	***P. burmeisteri* (BUR-RJ)**		***P. bahiana***	
Traits	Males (*N* = 33)	Males (*N* = 38)	Females (*N* = 4)	Holotype "unsexed"	Males (*N* = 25)
Snout–vent length	61.5 ± 4.0 (52.7–70.4)	62.4 ± 3.6 (53.8–69.2)	74.8 ± 5.6 (69.0–81.6)	70.4	65.8 ± 4.0 (56.4–73.9)
Head length	14.8 ± 1.3 (12.2–17.5)	14.7 ± 1.4 (12.0–19.2)	16.3 ± 0.7 (15.9–17.4)	12.7	15.8 ± 1.5 (12.7–18.3)
Head width	20.1 ± 1.6 (17.4–23.7)	21.2 ± 1.2 (18.2–23.4)	25.9 ± 0.9 (24.8–26.9)	23.0	21.5 ± 1.5 (17.6–24.2)
Eye diameter	6.3 ± 0.5 (5.3–7.7)	6.8 ± 0.5 (5.7–8.0)	7.4 ± 0.5 (6.9–8.0)	6.6	7.0 ± 0.7 (6.3–8.9)
Eye–nostril distance	5.3 ± 0.4 (4.4–6.1)	5.4 ± 0.3 (4.8–6.2)	6.3 ± 0.3 (6.0–6.5)	5.8	5.5 ± 0.5 (4.5–6.3)
Tympanum diameter	3.7 ± 0.4 (3.0–4.5)	3.5 ± 0.4 (2.2–4.5)	4.1 ± 0.5 (3.4–4.5)	2.9	4.0 ± 0.5 (2.9–5.1)
Thigh length	26.6 ± 1.7 (23.1–30.8)	27.4 ± 1.7 (23.3–30.9)	31.8 ± 2.0 (30.3–34.8)	26.9	28.6 ± 1.5 (24.8–31.3)
Tibia length	26.1 ± 1.9 (22.6–30.6)	27.2 ± 1.7 (23.9–30.9)	32.5 ± 2.3 (30.5–35.3)	26.6	27.6 ± 1.7 (22.7–29.8)
Foot length	21.8 ± 1.7 (18.9–26.5)	22.4 ± 1.5 (18.3–25.1)	26.8 ± 2.1 (25.0–29.9)	20.5	22.1 ± 1.7 (18.3–24.7)
Third finger disc diameter	2.0 ± 0.2 (1.6–2.5)	1.9 ± 0.4 (1.3–2.6)	2.2 ± 0.9 (1.6–3.5)	1.1	2.1 ± 0.3 (1.1–2.9)
Fourth toe disc diameter	2.0 ± 0.2 (1.6–2.5)	1.9 ± 0.4 (1.1–2.8)	2.1 ± 0.9 (1.4–3.5)	1.4	2.1 ± 0.3 (1.4–2.9)

emitted sporadically (Figure 4C), lasting 130–881 ms, separated by intervals of 1–89 s. Calls had 7–31 regularly spaced pulses (pulse groups absent). The pulse duration varied from 2–22 ms, separated by intervals of 1–70 ms. Pulses were released at a rate of 26–67 pulses per second. The dominant frequency (= fundamental) varied from 937–1636 Hz. All quantitative traits for both evolutionary units are summarized in Table 2.

Acoustic Comparisons

Regarding calls, the RandomForest multivariate approach revealed no discrimination between the three partitions (Table 3, Figure

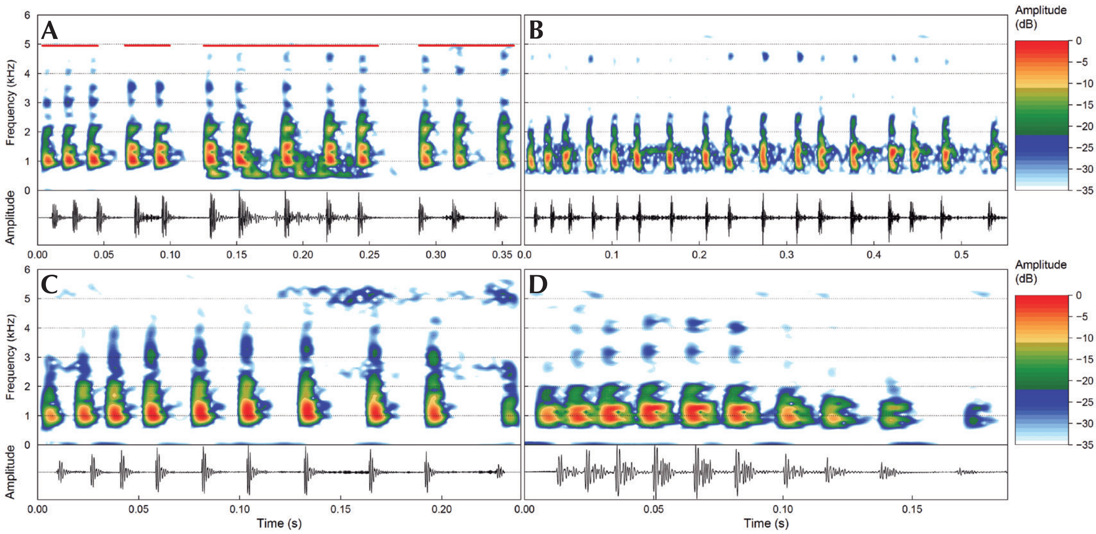

Figure 4. Audiospectogram (above) and corresponding oscillogram (below) of advertisement calls of *Phyllomedusa burmeisteri* (A–C) and *P. bahiana* (D). (**A**) Call with four pulse groups (red lines highlight them). (**B**) Call without pulse groups (pulses regularly spaced). These calls represent two males of *P. burmeisteri* (BUR-RJ) from Macaé, state of Rio de Janeiro. (**C**) Call of a male of *P. burmeisteri* (BUR) from Atibaia, state of São Paulo. (**D**) Call without pulse groups of a male of *P. bahiana* from Ilhéus, state of Bahia. Sound files: (A) Phyllom_burmeisMacaeRJ2bLBM_AAGmt; (B) Phyllom_burmeisMacaeRJ3cTRC_AAGmt; (C) Phyllom_burmeistAtibaiaSP5aAAGm671; and (D) Phyllom_bahianaIlheusBA2aTRC_AAGmt. Further details of these analyzed sound files are in Appendix II.

3B). The values of all traits broadly overlapped (Table 2). The only noticeable difference was the presence of pulse groups (Figure 4A) in three males from Macaé (BUR-RJ). However, we analyzed a male from this same locality without these groups (Figure 4B), which emitted calls similar to those of males from the BUR lineage (Figure 4C).

The RandomForest analysis revealed no discrimination between males of *P. bahiana* and males from either lineages of *P. burmeisteri* (Table 3, Figure 3B). We found a significant difference between BUR and *P. bahiana* in the interpulse interval; however, considerable overlap occurred between these traits (Table 2). Five of the six males of *P. bahiana* analyzed emitted calls with pulses arranged in 2–7 groups, which had 3–6 pulses per group. One male from Ilhéus emitted calls without pulse groups, but with pulses regularly spaced (Figure 4D).

Discussion

Is There Phenotypic Divergence Between the BUR and BUR-RJ Evolutionary Units?

Regarding morphometry, values of snout–vent length (SVL) for *Phyllomedusa burmeisteri* (both BUR and BUR-RJ) are scattered in the literature. Morphometric data on the holotype of *P. bahiana* is provided here for the first time. Pombal and Haddad (1992) provided SVL for *P. burmeisteri,* which ranged from 55.0 to 75.8 mm (mean = 61.9 mm, SD = 4.1, N = 75), based on adult males from a broad area within the distribution of BUR and BUR-RJ. Abrunhosa and Wogel (2004) provided a mean SVL of 63.4

Table 2. Advertisement call traits for the two evolutionary units of *Phyllomedusa burmeisteri* (BUR and BUR-RJ) (according to Brunes *et al.* 2014) from nine municipalities of three Brazilian states, and for *P. bahiana* from three municipalities of two Brazilian states. Mean ± standard deviation (minimum–maximum). *N* = number of males analyzed (number of calls analyzed).

Traits	***Phyllomedusa burmeisteri***		***Phyllomedusa bahiana***
	BUR *N* = 31 (321)	BUR-RJ *N* = 4 (30)	*N* = 6 (91)
Call duration (s)	0.313 ± 0.120 (0.130–0.881)	0.281 ± 0.094 (0.186–0.409)	0.219 ± 0.074 (0.099–0.579)
Intercall interval (s)	13.6 ± 8.7 (1.4–88.9)	13.3 ± 5.6 (6.1–44.5)	15.9 ± 9.9 (1.0–58.5)
Number of pulses per call	13.1 ± 3.7 (7.0–31.0)	11.8 ± 2.3 (9.0–15.0)	10.7 ± 2.5 (6.0–19.0)
Pulse duration (s)	0.008 ± 0.002 (0.002–0.022)	0.007 ± 0.001 (0.002–0.015)	0.007 ± 0.002 (0.002–0.035)
Interpulse interval (s)	0.017 ± 0.005 (0.001–0.070)	0.016 ± 0.003 (0.002–0.035)	0.010 ± 0.003 (0.002–0.039)
Pulses per second	44.1 ± 8.6 (26.2–66.7)	43.6 ± 6.6 (36.7–52.9)	51.2 ± 9.4 (28.6–68.7)
Number of pulse groups per call	-	4.9 ± 0.2 (4.0–6.0)	3.1 ± 1.8 (2.0–7.0)
Number of pulses per group	-	2–5	3–6
Duration of pulse groups (s)	-	0.051 ± 0.002 (0.023–0.122)	0.031 ± 0.016 (0.017–0.068)
Intergroup of pulses interval (s)	-	0.030 ± 0.001 (0.010–0.041)	0.022 ± 0.014 (0.009–0.067)
Dominant frequency (Hz)	1180.9 ± 106.1 (937.5–1636.5)	1117.2 ± 51.8 (1031.2–1218.8)	1128.8 ± 65.1 (937.0–1359.4)
Air temperature (°C)	16.0–25.5	21.0–24.1	23.0–25.0
Water temperature (°C)	18.0–28.0	22.0–30.3	22.0–27.0

Table 3. Confusion matrix for the two evolutionary units (BUR and BUR-RJ) of *Phyllomedusa burmeisteri* and *P. bahiana* based on morphometric and acoustic (values in bold) datasets through a Random Forests model. Settings: number of tree permutations = 1000; number of variables tried at each split = 3.0 | **2.0**; error rates = 40.62 % | **17.07 %**.

	***P. burmeisteri* (BUR)**	***P. burmeisteri* (BUR-RJ)**	***P. bahiana***	**class.error**
P. burmeisteri (BUR)	20 | **30**	7 | **0**	6 | **1**	0.39 | **0.03**
P. burmeisteri (BUR-RJ)	8 | **3**	25 | **0**	5 | **1**	0.34 | **1.00**
P. bahiana	6 | **1**	7 | **1**	12 | **4**	0.52 | **0.33**

± 4.5 (*N* = 13) for specimens from Saquarema (BUR-RJ). The SVL values of *P. burmesiteri* (BUR and BUR-RJ) provided here broadly overlap with the values of both studies cited above. The values of all other morphometric features of the two evolutionary units presented here overlapped, and the multivariate classification method (RandomForest) did not allow discrimination between either evolutionary units of *P. burmeisteri,* or between the two units and *P. bahiana.* Therefore, we were unable to distinguish BUR and BUR-RJ based on morphometric features or any other feature of external morphology or coloration, e.g., color of the hidden areas of the thighs, as already pointed out by Brunes *et al.* (2014).

Brunes *et al.* (2014) suggested that the taxonomic status of the novel evolutionary unit (BUR) would benefit from an integrative approach, including other sources of information, such as reproductive aspects related to prezygotic isolation. In addition, they noted that delimitation of the BUR evolutionary unit is not fully congruent across different types of markers and methods, presenting several challenges for the taxonomic significance of this novel evolutionary unit. We were also unable to distinguish BUR and BUR-RJ based on acoustic evidence. Indeed, further fine-scale studies to evaluate other sources of information for these two lineages, specifically cytogenetic and larval data, may shed further light on the possible differences between them.

Advertisement Call of Phyllomedusa burmeisteri

Abrunhosa and Wogel (2004) recognized two types of advertisement calls of *P. burmeisteri,* the short call (duration: 330–450 ms) and the long call (duration: 560–600 ms). Abrunhosa and Wogel (2004) also reported that pulses tended to be arranged in groups of three and the first triads of the call were closer to one another than the others, independently of the number of pulses per call. These observations were based on two males, each one displaying only one type of call (short or long). Intermediate call duration was found in 10% of the calls of six males from BUR analyzed by us, with values that do not fit the values of minimum and maximum of the short or long calls in Abrunhosa and Wogel (2004). In addition, during our field recordings, we did not record long calls of males from BUR-RJ, as defined by Abrunhosa and Wogel (2004). We believe dividing the advertisement call of this species into these two categories as proposed by Abrunhosa and Wogel (2004) is unwarrarited until additional data on acoustic variation of the species are obtained.

Three males of BUR-RJ had pulse groups (2–5 pulses per group) in their calls, and another individual had regularly spaced pulses (no pulse groups), similar to all males of BUR. Pulse groups may have more than three pulses, as mentioned in Abrunhosa and Wogel (2004), and their presence is variable enough that this acoustic trait is not useful in determining taxonomy of these species or for differentiating the two evolutionary units of *P. burmeisteri.*

Do P. burmeisteri *and* P. bahiana *Differ Acoustically?*

Pombal and Haddad (1992) stated that *P. burmeisteri* and *P. bahiana* had advertisement calls composed of isolated and regularly spaced pulses. Later, Silva-Filho and Juncá (2006) distinguished the advertisement calls of *P. bahiana* and *P. burmeisteri.* They reported that *P. bahiana* had well-defined pulse triads (three-pulse groups), whereas *P. burmeisteri* had an advertisement call formed by two types of calls, both having pulses tending to be arranged in triads, but spaced almost regularly (Abrunhosa and Wogel 2004) or pulses generally isolated and spaced regularly (Pombal and Haddad 1992). Additionally, Silva-Filho and Juncá (2006) found that the number of pulses and pulse rate of *P. bahiana* were, respectively, lower and higher than in *P. burmeisteri,* and the pulse duration of *P. bahiana* was half the duration of that of *P. burmeisteri* (Abrunhosa and Wogel 2004).

As stated above, we did not identify the two types of calls reported by Abrunhosa and Wogel (2004) among the calls recorded by us for *P. burmeisteri.* Based on our larger sample, pulses in the calls of these two species are not only arranged in triads. Our results indicate that the pulse groups of *P. burmeisteri* (BUR and BUR-RJ) can be composed of 2 to 5 pulses, whereas they are composed of 3 to 6 pulses in *P. bahiana.* Thus, the term "triad" cannot be applied to all pulse groups, so we referred herein to all pulse group arrangements simply as pulse groups.

Besides being quite variable, the presence of pulse groups is clearly not a reliable diagnostic character for these two species. This trait was lacking in one male of *P. bahiana* from llhéus, and males of *P. burmeisteri* (BUR and BUR-RJ) had calls with (or without) groups of pulses. "Pulse groups" seems to be a more frequent trait in *P. bahiana* and BUR-RJ than it is in BUR. We were unable to distinguish *P. bahiana* and *P. burmeisteri* based on their number of pulses, pulse duration, and pulse rate, as previously suggested by Silva-Filho and Juncá (2006). We found overlap in all values of the acoustic traits presented here for these two species (Table 2). Therefore, no qualitative or quantitative acoustic trait can be used to distinguish *P. burmeisteri* from *P. bahiana.*

Silva-Filho and Juncá (2006) pointed out differences between these species in larval morphology and territorial calls. Future fine-scale studies are needed to better understand the relevance of these differences. The results of Brunes *et al.* (2014) may reflect a combination of distinct episodes of secondary contact between *P. bahiana* and *P. burmeisteri,* possibly an older hybridization event in the Espírito Santo region, and a more recent secondary contact in southern Bahia. Studies are needed to better understand whether effective reproductive barriers exist between these leaf frog species, because their advertisement calls do not seem to be a good prezygotic barrier of reproductive isolation.

It is well known that rivers serve as geographical barriers for terrestrial vertebrates such as reptiles, birds, and mammals (Wallace 1852, Gascon *et al.* 1996, Patton *et al.* 2000, Hayes and Sewlal 2004), and several case studies have dealt with the influence of rivers on the distribution and genetic structure for amphibians, a semiaquatic group (see Gascon *et al.* 1998, 2000, Lougheed *et al.* 1999, Gehring *et al.* 2012, Yuan *et al.* 2016). Future studies should examine the role of major rivers of the Brazilian east Atlantic basin in the evolutionary history of *P. burmeisteri* and *P. bahiana.* Plio-Pleistocene climatic oscillations induced sea level fluctuations along the Brazilian coast, contributing to changes in the coastal plains of rivers across time. These changes were likely responsible for recurrent episodes of isolation and secondary contact between populations (Dominguez 2009, Brunes *et al.* 2014).

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Appendix I. *Specimens examined.*
(= voucher specimen of the genetic analyses by Brunes* et al. *2014.)*

Phyllomedusa burmeisteri: adult males of the BUR-RJ evolutionary unit: BRAZIL. Rio de Janeiro: Cachoeiras de Macacu: CFBH 30766–30768*; Campos dos Goytacazes: CFBH 27386–27388*, 27390*; Duque de Caxias: MNRJ 3079, 10070, 54770, 67493; Engenheiro Paulo de Frontin (Sacra Família do Tinguá): ZUEC 8089; Macaé: AAG-UFU 0530–0531, 0751; Nova Iguaçu: ZUEC 2804–2805, MNRJ 67422–67423, 67665–67669; Petrópolis: ZUEC 10917; Rio de Janeiro: MNRJ 67308 (Estrada dos Teixeiras, Jacarepaguá), MNRJ 67310 (Jacarepaguá), MNRJ 60678 (Serra do Mendanha), ZUEC 6521 (Campo Grande); Tijuca (Rio de Janeiro; type locality): MNRJ 11286, 67494 (both from estrada velha, Tijuca), 67496 (Tijuca, Guanabara); Santa Maria Madalena: CFBH 27326–27329*; São Gonçalo: MNRJ 67495; São Pedro da Aldeia: MNRJ 21730; adult females of the BUR-RJ: BRAZIL. Rio de Janeiro: Campos dos Goytacazes: CFBH 27389*; Iguaba Grande: MNRJ 57805; Rio de Janeiro: MNRJ 251; São Pedro da Aldeia: MNRJ 21729; adult males of the BUR evolutionary unit: BRAZIL. Minas Gerais: Alpinópolis: AAG-UFU 0958, 4860–4862; Carangola: CFBH 27299*, 27301*; Chiador: AAG-UFU 0678; Conceição do Mato Dentro (Serra do Cipó): ZUEC 16402; Juiz de Fora: CFBH 27257*; Matutina: ZUEC 1797; São José da Barra: ZUEC 1835; Viçosa: CFBH 27579*, 27583*; Espírito Santo: Linhares: ZUEC 3726; CFBH 18084*, CFBH 24841*; São Paulo: Atibaia: AAG-UFU 0444, 0585, 0949, 1288; Campinas: AAG-UFU 1886–1888; Corumbataí: ZUEC 8518; Itatiba: AAG 0049, 0195–0196, Jundiaí: ZUEC 4538; Nazaré Paulista: CFBH 30573–30574*; Rio Claro: CFBH 14428*; Santo André (Paranapiacaba): ZUEC 4494, 6047.

Phyllomedusa bahiana (adult males): BRAZIL. Bahia: Alagoinhas: ZUEC 19952–19960; Gandu: ZUEC 8706–8707; Ilhéus: AAG-UFU 0228–0230; Itagibá: ZUEC 3760–3761; Jequié: ZUEC 3251, 8319; Maracás: ZUEC 0976, 7590–7591; Piatã: AAG-UFU 1677–1679.

Appendix II. *Analyzed sound files (*.wav format) of* Phyllomedusa burmeisteri *and* P. bahiana. *Abbreviations to Brazilian states: ES = Espírito Santo; MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo.*

Label	Date	Municipality (state)	Time	Air (°C)	Water (°C)	Recorder - microphone	Voucher
Phyllom_burmeisAlpinopMG1aAAGm.wav	19 Dec 2009	Alpinópolis (MG)		21	23	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 4860
Phyllom_burmeisAlpinopMG1bAAGm.wav	19 Dec 2009	Alpinópolis (MG)		21	23	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 4860
Phyllom_burmeisAlpinopMG2aAAGm.wav	18/dez/2009	Alpinópolis (MG)	20:20	21	23	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisAlpinopMG3aAAGm.wav	18 Dec 2009	Alpinópolis (MG)	20:25	21	23	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisAlpinopMG4aAAGm671.wav	27 Dec 2011	Alpinópolis (MG)	20:39	21	27	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAlpinopMG4bAAGm671.wav	27 Dec 2011	Alpinópolis (MG)	20:42	21	27	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAlpinopMG4cAAGm671.wav	27 Dec 2011	Alpinópolis (MG)	20:45	21	27	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAlpinopMG5aAAGm671.wav	27 Dec 2011	Alpinópolis (MG)	21:53	21	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 0958
Phyllom_burmeisAlpinopMG5bAAGm671.wav	27 Dec 2011	Alpinópolis (MG)		21	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 0958
Phyllom_burmeisAlpinopMG5cAAGm671.wav	27 Dec 2011	Alpinópolis (MG)	20:54	21	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 0958
Phyllom_burmeisAlpinopMG5dAAGm671.wav	27 Dec 2011	Alpinópolis (MG)		21	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 0958
Phyllom_burmeisAlpinopMG5eAAGm671.wav	27 Dec 2011	Alpinópolis (MG)		21	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 0958
Phyllom_burmeisAlpinopMG5fAAGm671.wav	27 Dec 2011	Alpinópolis (MG)	21:02	21	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 0958
Phyllom_burmeisAtibaiaSP1aAAGb.wav	23 Dec 2003	Atibaia (SP)	19:45	19		Boss 864 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP2aAAGmt.wav	29 Dec 2009	Atibaia (SP)	21:10	24	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0444
Phyllom_burmeisAtibaiaSP2bAAGmt.wav	29 Dec 2009	Atibaia (SP)	21:13	24	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0444
Phyllom_burmeisAtibaiaSP2cAAGmt.wav	29 Dec 2009	Atibaia (SP)	21:15	24	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0444

Appendix II. *Continued.*

Label	Date	Municipality (state)	Time	Air (°C)	Water (°C)	Recorder - microphone	Voucher
Phyllom_burmeisAtibaiaSP3aAAGm671.wav	23 Dec 2011	Atibaia (SP)	19:53	20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP3bAAGm671.wav	23 Dec 2011	Atibaia (SP)	19:53	20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP3cAAGm671.wav	23 Dec 2011	Atibaia (SP)	19:57	20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP3dAAGm671.wav	23 Dec 2011	Atibaia (SP)		20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP3eAAGm671.wav	23 Dec 2011	Atibaia (SP)		20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP3fAAGm671.wav	23 Dec 2011	Atibaia (SP)	20:00	20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP4aAAGm671.wav	23 Dec 2011	Atibaia (SP)	20:20	20	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeistAtibaiaSP5aAAGm671.wav	26 Sep 2014	Atibaia (SP)	20:10	20	19	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeistAtibaiaSP5bAAGm671.wav	26 Sep 2014	Atibaia (SP)	20:51	20	19	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeistAtibaiaSP5cAAGm671.wav	26 Sep 2014	Atibaia (SP)	20:52	20	19	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeistAtibaiaSP6aAAGm671.wav	26 Sep 2014	Atibaia (SP)	20:40	20	19	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeistAtibaiaSP7aAAGm671.wav	26 Sep 2014	Atibaia (SP)	20:43	20	19	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP8aAAGm671.wav	02 Feb 2016	Atibaia (SP)	19:50	21	28	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP9aAAGm661MK2.wav	18 Nov 2017	Atibaia (SP)	21:23	19		Marantz PMD 661MKII - ME66/K6 Sennheiser	
Phyllom_burmeisAtibaiaSP9bAAGm661MK2.wav	18 Nov 2017	Atibaia (SP)	21:25	21		Marantz PMD 661MKII - ME66/K6 Sennheiser	
Phyllom_burmeisBJPerdoesSP1aAAGm661MK2.wav	16 Nov 2017	Bom Jesus dos Perdões (SP)	20:35	18	19	Marantz PMD 661MKII - ME66/K6 Sennheiser	
Phyllom_burmeisBJPerdoesSP1bAAGm661MK2.wav	16 Nov 2017	Bom Jesus dos Perdões (SP)	20:36	18	19	Marantz PMD 661MKII - ME66/K6 Sennheiser	
Phyllom_burmeisBJPerdoesSP2aAAGm661MK2.wav	16 Nov 2017	Bom Jesus dos Perdões (SP)	20:45	18	19	Marantz PMD 661MKII - ME66/K6 Sennheiser	

Appendix II. *Continued.*

Label	Date	Municipality (state)	Time	Air (°C)	Water (°C)	Recorder - microphone	Voucher
Phyllom_burmeisBJPerdoesSP3aAAGm661MK2.wav	16 Nov 2017	Bom Jesus dos Perdões (SP)	21:02	18	19	Marantz PMD 661MKII - ME66/K6 Sennheiser	
Phyllom_burmeisCampinSP1aAAGm671.wav	06 Nov 2011	Campinas (SP)	21:30	18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1bAAGm671.wav	06 Nov 2011	Campinas (SP)	21:30	18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1cAAGm671.wav	06 Nov 2011	Campinas (SP)	21:40	18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1dAAGm671.wav	06 Nov 2011	Campinas (SP)	21:50	18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1eAAGm671.wav	06 Nov 2011	Campinas (SP)		18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1fAAGm671.wav	06 Nov 2011	Campinas (SP)		18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1gAAGm671.wav	06 Nov 2011	Campinas (SP)		18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1hAAGm671.wav	06 Nov 2011	Campinas (SP)		18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinSP1iAAGm671.wav	06 Nov 2011	Campinas (SP)	22:05	18	18	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeistSousasSP2aAAGm671.wav	02 Nov 2013	Campinas (SP)	20:05	19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1886
Phyllom_burmeistSousasSP2bAAGm671.wav	02 Nov 2013	Campinas (SP)		19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1886
Phyllom_burmeistSousasSP2cAAGm671.wav	02 Nov 2013	Campinas (SP)	20:19	19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1886
Phyllom_burmeistSousasSP2dAAGm671.wav	02 Nov 2013	Campinas (SP)	20:25	19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1886
Phyllom_burmeistSousasSP2eAAGm671.wav	02 Nov 2013	Campinas (SP)	20:25	19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1886
Phyllom_burmeistSousasSP2fAAGm671.wav	02 Nov 2013	Campinas (SP)	20:27	19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1886
Phyllom_burmeistSousasSP3aAAGm671.wav	02 Nov 2013	Campinas (SP)	20:12	19	18	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1887
Phyllom_burmeisCampinasSP4aIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:02	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	

Appendix II. *Continued.*

Label	Date	Municipality (state)	Time	Air (°C)	Water (°C)	Recorder - microphone	Voucher
Phyllom_burmeisCampinasSP4bIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:30	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinasSP4cIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:30	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinasSP4dIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:30	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinasSP4eIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:30	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisCampinasSP5aIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:00	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 1888
Phyllom_burmeisCampinasSP5bIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:00	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 1888
Phyllom_burmeisCampinasSP5cIAH_AAGm670.wav	02 Nov 2013	Campinas (SP)	20:00	19	18	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 1888
Phyllom_burmeisChiadorMG1aLBM_AAGmt.wav	02 Dec 2011	Chiador (MG)	23:40	16		M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0678
Phyllom_burmeisChiadorMG1bLBM_AAGmt.wav	02 Dec 2011	Chiador (MG)	23:40	16		M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0679
Phyllom_burmeisChiadorMG1cLBM_AAGmt.wav	02 Dec 2011	Chiador (MG)	23:40	16		M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0680
Phyllom_burmeisChiadorMG1dLBM_AAGmt.wav	02 Dec 2011	Chiador (MG)	23:40	16		M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0681
Phyllom_bahianaIheusBA1aTRC_AAGmt.wav	06 Jan 2011	Ilhéus (BA)	20:12	25	27	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0228
Phyllom_bahianaIheusBA2aTRC_AAGmt.wav	07 Jan 2011	Ilhéus (BA)	22:24	25	27	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0229
Phyllom_burmeisItatibaSP1aAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:50	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisItatibaSP1bAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:55	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisItatibaSP1cAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:02	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisItatibaSP1dAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:15	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisItatibaSP1eAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:35	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	

Appendix II. *Continued.*

Label	Date	Municipality (state)	Time	Air (°C)	Water (°C)	Recorder - microphone	Voucher
Phyllom_burmeisItatibaSP1fAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:35	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisItatibaSP1gAAGmt.wav	25 Nov 2009	Itatiba (SP)	20:36	22	29	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisMacaeRJ1aLBM_AAGmt.wav	24 Sep 2011	Macaé (RJ)	20:00	21	22	M-audio Microtrack II -ME66/ K6 Sennheiser	
Phyllom_burmeisMacaeRJ2aLBM_AAGmt.wav	24 Sep 2011	Macaé (RJ)	20:45	21	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0530
Phyllom_burmeisMacaeRJ2bLBM_AAGmt.wav	24 Sep 2011	Macaé (RJ)	20:45	21	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0530
Phyllom_burmeisMacaeRJ3aTRC_AAGmt.wav	24 Sep 2011	Macaé (RJ)	21:18	21	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0531
Phyllom_burmeisMacaeRJ3bTRC_AAGmt.wav	24 Sep 2011	Macaé (RJ)	21:19	21	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0531
Phyllom_burmeisMacaeRJ3cTRC_AAGmt.wav	24 Sep 2011	Macaé (RJ)	21:20	21	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0531
Phyllom_burmeistMacaeRJ4aTRC_AAGmt.wav	17 Dec 2011	Macaé (RJ)	20:45	24.1	30.3	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 0751
Phyllom_burmeisOuroPretoMG1aAAGm671.wav	05 Jan 2017	Ouro Preto (MG)	23:09	20		Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisOuroPretoMG1bAAGm671.wav	05 Jan 2017	Ouro Preto (MG)	23:10	20		Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisOuroPretoMG1cAAGm671.wav	05 Jan 2017	Ouro Preto (MG)	23:15	20		Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_bahiaPiataBA1aAAGm671.wav	05 Jan 2013	Piatã (BA)	19:11	23	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1678
Phyllom_bahiaPiataBA1bAAGm671.wav	05 Jan 2013	Piatã (BA)	19:12	23	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1678
Phyllom_bahiaPiataBA1cAAGm671.wav	05 Jan 2013	Piatã (BA)	19:14	23	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1678
Phyllom_bahiaPiataBA1dAAGm671.wav	05 Jan 2013	Piatã (BA)	19:17	23	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1678
Phyllom_bahiaPiataBA1eAAGm671.wav	05 Jan 2013	Piatã (BA)	19:21	23	27	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1678
Phyllom_bahiaPiataBA2aLBM_AAGmt.wav	04 Jan 2013	Piatã (BA)	19:40	23	22	M-audio Microtrack II -ME66/ K6 Sennheiser	AAG-UFU 1679

Appendix II. *Continued.*

Label	Date	Municipality (state)	Time	Air (°C)	Water (°C)	Recorder - microphone	Voucher
Phyllom_bahiaPiataBA3aTRC_AAGm671.wav	04 Jan 2013	Piatã (BA)	19:29	23	22	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1677
Phyllom_bahiaPiataBA3bTRC_AAGm671.wav	04 Jan 2013	Piatã (BA)	19:33	23	22	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1677
Phyllom_bahiaPiataBA3cTRC_AAGm671.wav	04 Jan 2013	Piatã (BA)	19:35	23	22	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1677
Phyllom_bahiaPiataBA3dTRC_AAGm671.wav	04 Jan 2013	Piatã (BA)	19:38	23	22	Marantz PMD 671 - ME67/K6 Sennheiser	AAG-UFU 1677
Phyllom_burmeisSalesopolisSP1aAAGm671.wav	28 Dec 2015	Salesópolis (SP)	00:30	23	23	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisSalesopolisSP1bAAGm671.wav	28 Dec 2015	Salesópolis (SP)	00:36	23	23	Marantz PMD 671 - ME67/K6 Sennheiser	
Phyllom_burmeisteriSooretamaES1aTRC_AAGm670.wav	08 Dec 2017	Sooretama (ES)	21:39	25	24	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 6212
Phyllom_burmeisteriSooretamaES2aTRC_AAGm670.wav	08 Dec 2017	Sooretama (ES)	21:41	25	24	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisteriSooretamaES2bTRC_AAGm670.wav	08 Dec 2017	Sooretama (ES)	21:57	25	24	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllom_burmeisteriSooretamaES3TRC_AAGm670.wav	08 Dec 2017	Sooretama (ES)	22:13	25	24	Marantz PMD 670 - ME67/K6 Sennheiser	AAG-UFU 6213
Phyllom_burmeisteriSooretamaES4TRC_AAGm670.wav	08 Dec 2017	Sooretama (ES)	22:20	25	24	Marantz PMD 670 - ME67/K6 Sennheiser	
Phyllomedusa_burmeisteri_Sooretama-ES_2_BFVT_AAGm.wav	07 Dec 2017	Sooretama (ES)	19:00	24		Marantz PMD 670 - ME66/K6 Sennheiser	AAG-UFU 6210
Phyllomedusa_burmeisteri_Sooretama-ES_3_BFVT_AAGm.wav	08 Dec 2017	Sooretama (ES)	22:16	24		Marantz PMD 670 - ME66/K6 Sennheiser	
FNJV12243_AreiaBranca_SE.wav	05 May 2011	Areia Branca (SE)	21:20			Marantz PMD 222 - Audiotechnica AT 835b	
FNJV12244_AreiaBranca_SE.wav	05 May 2011	Areia Branca (SE)	21:22			Marantz PMD 222 - Audiotechnica AT 835b	
FNJV12245_AreiaBranca_SE.wav	05 May 2011	Areia Branca (SE)	21:22			Marantz PMD 222 - Audiotechnica AT 835b	