

Evidence of full species status of the neotropical leaf-frog *Phyllomedusa burmeisteri bahiana* (A. Lutz, 1925) (Amphibia, Anura, Hylidae)

IVAN SERGIO N. SILVA-FILHO¹ & FLORA A. JUNCÁ^{1,2}

¹*Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Universitária s/nº, BR116, Km 03, 44031-460, Feira de Santana-BA, Brazil.*

²*Corresponding author. E-mail: fjunca@uefs.br.*

Abstract

The full species status of *Phyllomedusa burmeisteri bahiana* was investigated based on tadpoles and vocalizations characteristics. We found distinct characteristics between the tadpoles and advertisement and territorial calls of the former and that of *Phyllomedusa burmeisteri burmeisteri*. The main tadpole differences are related with a skin projection protecting the spiracle, origin of dorsal fin, morphology of ventral fin and distribution of papillae on the oral disc. The advertisement call and territorial call of *Phyllomedusa b. bahiana* were different from the *P. b. burmeisteri* calls especially for the structure and temporal characteristics (pulses/second principally). The color of newly metamorphosed *Phyllomedusa b. bahiana* suggests that the color pattern of the internal sides of the thigh of *P. b. burmeisteri* can be related to a neotenic pattern of development. We elevate *Phyllomedusa b. bahiana* to species level based on morphological and acoustical traits described here.

Key words: *Phyllomedusa bahiana*, *Phyllomedusa burmeisteri*, tadpole, advertisement call, territorial call, taxonomic status

Introduction

Pombal Jr. and Haddad (1992) in a taxonomic revision of *Phyllomedusa burmeisteri* group, considered *Phyllomedusa bahiana* A. Lutz (Figure 1) as a subspecies of *Phyllomedusa burmeisteri* Boulenger. According to Frost (2004), the *Phyllomedusa burmeisteri* group (*sensu* Lutz 1950) includes five species (*P. burmeisteri bahiana* A. Lutz, 1925; *P. burmeisteri burmeisteri* Boulenger, 1881; *P. distincta* B. Lutz, 1950; *P. iheringii* Boulenger, 1885; and *P. tetraploidea* Pombal & Haddad, 1992), all of them distributed in eastern Brazil.



FIGURE 1. Adult specimen of *Phyllomedusa burmeisteri bahiana*, photographed in life by F. A. Juncá.

Phyllomedusa b. bahiana and *Phyllomedusa b. burmeisteri* are very similar in morphology and are differentiated by the color pattern of the inner sides of the thighs. In preserved specimens, the inner sides of the thighs of *P. b. bahiana* are uniform violet and *P. b. burmeisteri* have whitish rounded spots over a blue background. Pombal Jr. and Haddad (1992) described an intermediate form between *P. b. burmeisteri* and *P. b. bahiana* diagnosed by the presence of whitish rounded spots but smaller in number and size than those *P. b. burmeisteri*. This intermediate form was interpreted as a gradient between the color patterns of the inner part of the thighs of *P. b. bahiana* and *P. b. burmeisteri* (Pombal Jr. & Haddad 1992). *Phyllomedusa b. burmeisteri* is distributed from the states of Minas Gerais and Espírito Santo to the State of São Paulo, while *P. b. bahiana* occurs in the states of Bahia and Espírito Santo. The form considered intermediate to these two subspecies occurs in the overlapping area of distribution (Pombal Jr. & Haddad 1992).

The present study contains the description of the tadpole and vocalizations of *Phyllomedusa b. bahiana*, compares the results with the *Phyllomedusa b. burmeisteri* tadpole and vocalizations, and discusses the taxonomic status of the former.

Materials and Methods

Calls of *Phyllomedusa b. bahiana* were recorded and collected in permanent ponds located in the Serra São José (12°06' S 39°02' W; 400 m of altitude), Municipality of Feira de Santana and in a permanent pond located in Lages, Municipality of Morro do Chapéu (11°29' S 41°20' W; 900 m of altitude), Bahia State, Brazil. Both localities occur in the Caatinga Domain (AbSaber 1974), with presence of seasonal forest. The landscape is constituted of emergent rock, intermittent streams, permanent and temporary ponds. The climate of the region is semi-arid with two seasons dry and wet (AbSaber 1974; Cei 1994; Brasil 1981). The wet season at Serra São José is in March, April and May, and the lowest pluviometric indices occur in August, September, and October (Almeida 1992). At Lages, the wet season is from November to January, and the lowest pluviometric readings are registered from August to October (Jesus *et al.* 1985). Serra São José and Lages are approximately 120 and 330 km (respectively) from the type locality (Salvador) of *Phyllomedusa b. bahiana*.

The voucher specimens were deposited in the Coleção Herpetológica do Museu de Zoologia da Universidade Estadual de Feira de Santana (MZUEFS) and the recorded calls were deposited in Sonoteca do Laboratório de Animais Peçonhentos e Herpetologia da Universidade Estadual de Feira de Santana (SUEFS).

Advertisement calls of two males were recorded at Serra São José (SUEFS 6.11, voucher specimen MZUEFS 1512, 21 July 2003, 19.5° C and an SUEFS 7.6, uncaptured specimen, 13 November 2003, 21.5° C air temperature) and territorial calls of one male were recorded at Lages (SUEFS 1.2, voucher specimen MZUEFS 1809, 20 February 2004, 22° C air temperature). The vocalizations were recorded with a SONY WM-D8 Digital Audio Tape (DAT) and a SONY ECM-MS907 Electret Condenser Microphone. Calls were analyzed using Canary 1.2.4 software (Charif *et al.* 1995). The calls were digitized at a sample rate of 44.1 kHz, sample size of 16 bits. Temporal parameters were measured from the waveform. Dominant frequency was analyzed using the spectrum analyzer (in Canary with settings of spectrum analysis resolution filter band-width 174.85 Hz, grid resolution time 1.451 ms, grid resolution frequency 43.07 Hz, window function hamming, amplitude logarithmic) and audiospectrogram display (using the same settings of spectrum analyzer). Call classification and call component terminology follow Wells (1977), Cocroft and Ryan (1995), and Littlejohn (2001).

The advertisement call of one individual of *Phyllomedusa b. burmeisteri* from Palmital, Municipality of Saquarema, Rio de Janeiro, Brazil, was used in our analyses and compared with *P. b. bahiana* advertisement call. This vocalization was recorded with an UHER tape recorder and an UHER microphone at a tape speed of 19 cm/s (Abrunhosa & Vogel 2004).

Tadpoles were collected from Serra São José, preserved in 5 % formalin and deposited in the Coleção Herpetológica do Museu de Zoologia da Universidade Estadual de Feira de Santana (Lots MZUEFS 387, 388 and 389). Species identification was based on part of a

lot of the tadpoles collected (MZUEFS Lot 388), which was maintained in captivity in a plastic box with about 3 l of water, where they were fed on until metamorphosis was completed. Other two lots of tadpoles (MZUEFS 387, 389) were preserved in the field. The development stage of the tadpoles was determined according to Gosner (1960). The determination of the dental formula and description of the oral disc follow Altig and McDiarmid (1999).

Measurements of the tadpoles and terminology follow Wogel *et al.* (2000) and Altig and McDiarmid (1999). The abbreviations follow Wogel *et al.* (2000) (except TMW, ND and SDFD): TL total length, BL body length, TaL tail length, TMH tail muscle height, TMW tail muscle width, MTH maximum tail height, InD internostril distance, IoD interorbital distance, ED eye diameter, SED snout-eye distance, NED nostril-eye distance, ND nostril diameter, SDFD snout-dorsal fin distance, BH body height, BW body width, ODW oral disc width, DFH dorsal fin height, VFH ventral fin height, SND lateral snout-nostril distance laterally. All the measurements involving the eye or nostril were made from the mid-point of these structures. Measures BL, SDFD and TL were taken with caliper (precision 0.1 mm). For all the other measurements a stereomicroscope ZEISS MC 80 DX Stemi SV6 with micrometric ocular lens was used. All the measurements are expressed in millimeters. The drawing was made using a ZEISS stereomicroscope with a camera lucida.

Results

Description of the tadpole of *Phyllomedusa b. bahiana*

External morphology. Measurements of the tadpoles of *Phyllomedusa b. bahiana* are presented in Table 1. Tadpole body oboval in dorsal view and slightly triangular in profile, deeper than wide (Figure 2A and 2C); body length approximately 32% of the total length; maximum width of the body same as interorbital distance; snout not prominent, sub-elliptical in lateral view and semi-circular in dorsal view; eyes dorso-lateral and located slightly above the median line of the body, interorbital distance approximately three times the eye diameter; nostrils small, elliptical, directed and located antero-laterally on tip of snout, nearer to the tip of snout than the eyes; spiracle single (Figure 2B), short, wide, slightly sinistral, located at the end of the first half of the body; centripetal wall of the spiracle tube fused to body wall, opening wide with or without skin projection directed backwards; vent tube short, dextral, attached to ventral fin immediately below the musculature of the tail; tail approximately 68 % of the total body length, distal portion arched dorsal-ventrally; tail musculature thin, tapering gradually to the tip; dorsal fin low, continuous, with arched contour arising posterior to the body; ventral fin origin at the middle of posterior third of the body, much deeper than dorsal fin; oral disc antero-ventral, approximately 0.5 times less than the body width (figure 2D); tooth row formula 2(2)/3(1);

A-2 row interrupted medially by a wide gap, P-1 row interrupted medially by a narrow gap, P-3 three times shorter than P-2, A-2 and P-2 slightly shorter than P-1, P-2 slightly shorter than A-1; marginal and sub-marginal papillae disposed regularly on the lateral sides and dorsolateral portions of the oral disc, in double rows on the lateral portions; upper lip with a wide gap in the median region at the same level as the end of the upper tooth row extremities; lower lip with a narrow gap immediately below the third posterior tooth row; beak black and finely serrated; upper jaw with a medial projection and lower jaw in "V" form.

Color in life. Body and musculature of the tail pale beige. Dorsal and lateral surfaces with very dark brown punctuations, giving a dark appearance. Belly clearer than other regions, without presence of punctuations. Gills reddish. Intestines gray, visible laterally and ventrally through transparent body wall. Tail more pigmented in the upper regions. Beak black.

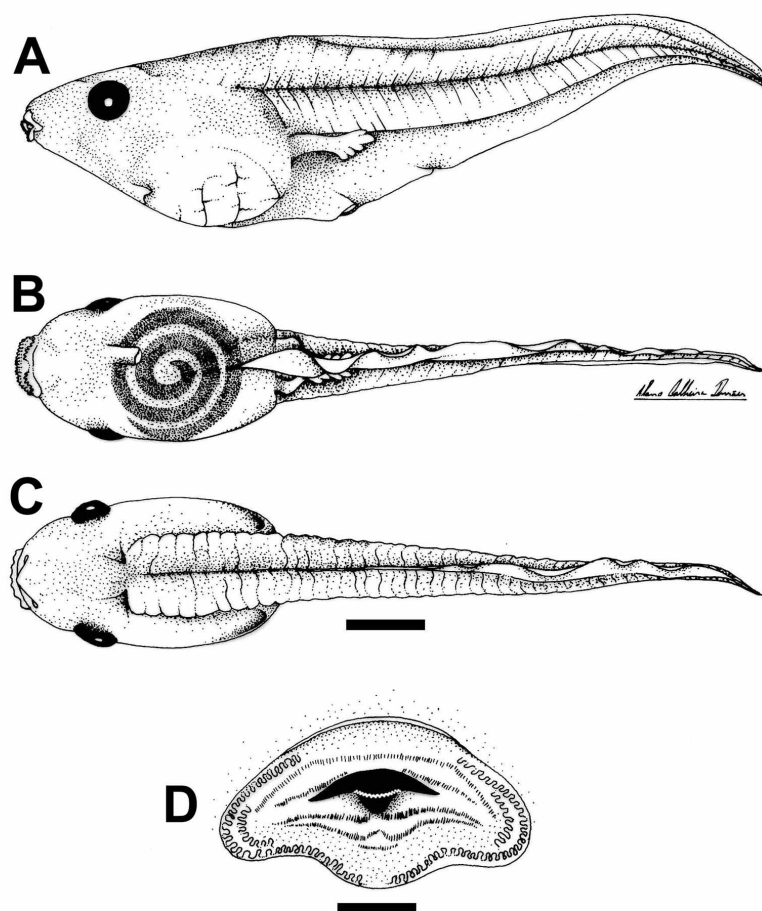


FIGURE 2. Tadpole of *Phyllomedusa burmeisteri bahiana* (MZUEFS 388) stage 36: (A) lateral view; (B) ventral view; (C) dorsal view (scale = 5 mm); (D) oral disc (scale = 1 mm).

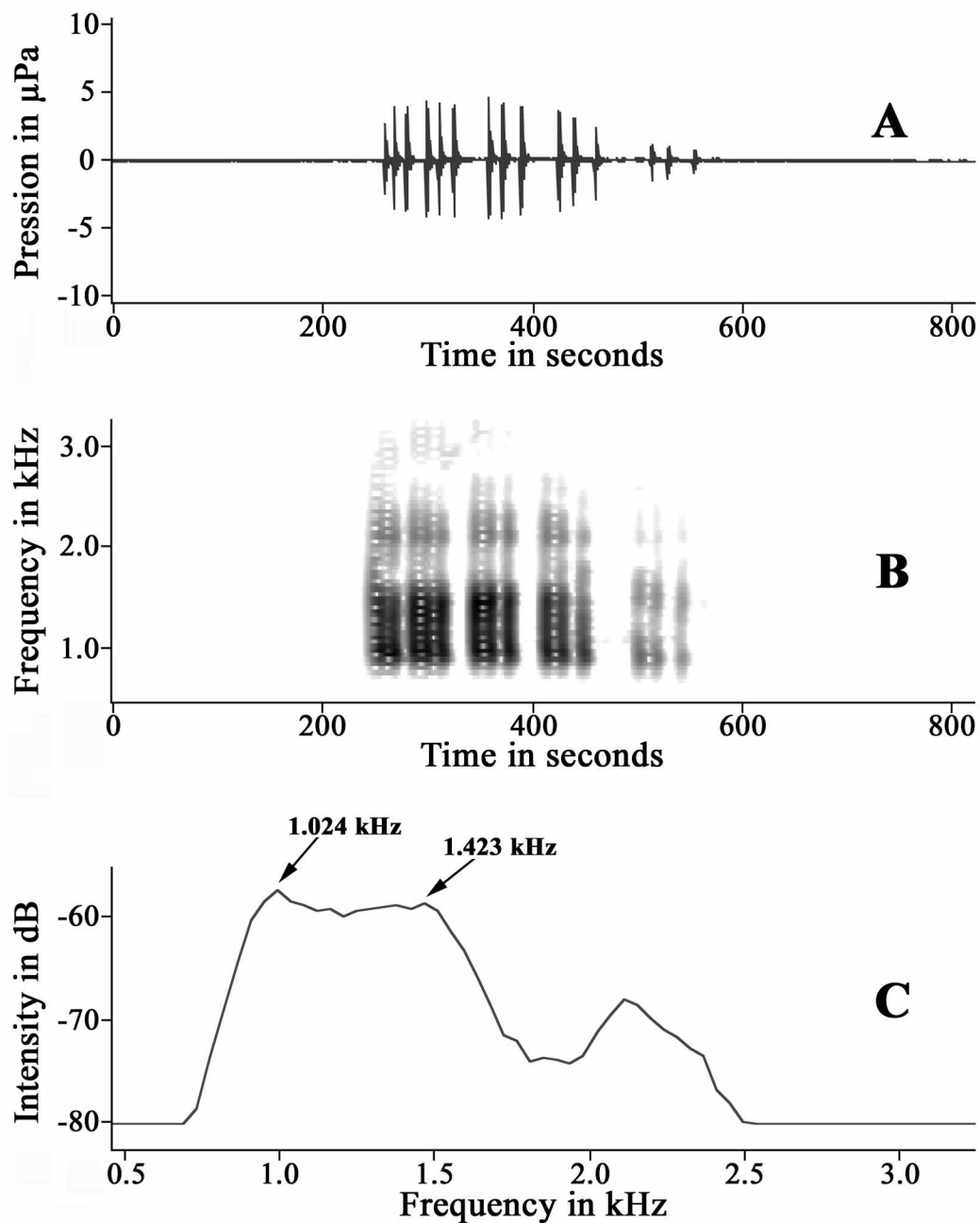


FIGURE 3. Advertisement call of *Phyllomedusa burmeisteri bahiana*: (A) Waveform; (B) Audiospectrogram; (C) Power spectrum.

Color in 5% formalin. Body and musculature of the tail beige to pale brown. Dorsal and lateral surfaces with black punctuations, conferring a darker appearance. Belly dark,

without presence of punctuations. Intestines brownish with dark gray, not very visible laterally and ventrally. Musculature of the tail more pigmented than the fins. Dorsal fin more pigmented than the ventral fin. Beak black.

Newly metamorphosed *Phyllomedusa b. bahiana* (n = 3) had the same color as adults but the color pattern of the internal side of the thigh was equal to the adult individuals of *P. b. burmeisteri* or of the intermediate form cited by Pombal Jr. and Haddad (1992).

TABLE 1. Average (\bar{x}), standard-deviation (SD) and amplitude of variation of the measures (mm) of tadpoles of *Phyllomedusa burmeisteri bahiana* (n = 17; stages 34–36) (abbreviations follow Wogel *et al.* 2000).

Characters	\bar{x}	SD	Amplitude
TL	45.15	4.34	37.6 55.0
BL	14.27	1.3	12.0 16.0
TaL	30.87	3.36	25.5 39.0
TMH	5.52	0.95	4.0 7.5
TMW	4.26	0.79	3.12 5.93
MTH	9.13	1.14	6.87 11.25
InD	3.75	0.23	3.4 4.25
IoD	8.18	0.83	6.1 9.2
ED	2.52	0.18	2.18 2.9
SED	6.52	0.6	5.41 7.5
NED	4.71	0.63	3.06 5.43
ND	0.44	0.04	0.36 0.52
SDFD	17.92	2.19	14.3 21.5
BH	9.95	1.62	7.37 13.25
BW	8.7	1.38	6.62 11.62
ODW	3.41	0.32	2.96 4.0
DFH	0.77	0.11	0.44 0.9
VFH	3.82	0.88	2.62 5.03
SND lateral	1.35	0.24	0.96 1.7

Description of *Phyllomedusa b. bahiana* vocalizations

Two different types of vocalizations were recorded during the fieldwork: advertisement call (Figure 3) and territorial call (Figures 4 and 5). The acoustic parameters are presented in Table 2.

TABLE 2. Acoustical parameters of *Phyllomedusa burmeisteri bahiana* advertisement and territorial calls. Values are presented as mean \pm standard deviation (amplitude). Components = triads, double pulses and single pulses.

Acoustic parameters	Advertisement call	Territorial call	
		Diphasic	Monophasic
<i>n</i> (calls/males)	23 / 2	7 / 1	23 / 1
Call duration (s)	0.28 \pm 0.003 (0.21 0.31)	1.69 \pm 0.28 (1.30 1.91)	0.41 \pm 0.04 (0.32 0.49)
Interval between calls (s)	12.45 \pm 6.47 (7.61 28.38)	7.26 \pm 1.24 (5.11 9.09)	
Interval between call components (s)	0.027 \pm 0.011 (0.010 0.047)	0.029 \pm 0.016 (0.012 0.058)	0.027 \pm 0.009 (0.013 0.051)
Number of notes	1	2	1
Note duration (s)	-	0.39 \pm 0.03 0.41 \pm 0.04 (0.33 0.50)	-
Interval between notes (s)	-	0.822 \pm 0.391 (0.335 1.304)	-
Number of pulses	14.17 \pm 1.92 (12 - 16)	15.75 \pm 0.50 15.25 \pm 0.96 (14 - 16)	16.21 \pm 0.02 (15 - 17)
Pulses/second	54.22 \pm 2.28 (49.24 - 57.98)	37.96 \pm 1.16 39.81 \pm 1.38 (36.30 - 41.54)	40.18 \pm 0.70 (39.49 - 41.09)
Number of triads/call	4.94 \pm 0.20 (4 - 5)	1.44 \pm 0.53 3.50 \pm 0.53 (1 4)	3.50 \pm 0.51 (3 4)
Triad duration (s)	0,040 \pm 0,008 (0,026 0,053)	0,039 \pm 0,003 (0,035 0,041)	0,050 \pm 0,004 (0,043 0,058)
Number of double pulses/call	-	1.27 \pm 0.47 4.29 \pm 0.49 (1 5)	1.11 \pm 0.32 (1 2)
Double pulse duration (s)	-	0.025 \pm 0.001 (0.024 0.028)	0.029 \pm 0.003 (0.26 0.032)
Number of single pulses/call	0.55 \pm 0.67 (0 - 2)	3.00 \pm 0.82 2.17 \pm 1.17 (1 4)	1.95 \pm 0.71 (1 4)
Single pulse duration (s)	0.005 \pm 0.001 (0.002 0.007)	0.008 \pm 0.001 (0.007 0.009)	0.009 \pm 0.001 (0.007 0.011)
Interval between components of pulses (s)	0.009 \pm 0.006 (0.001 0.024)	0.009 \pm 0.003 (0.002 0.013)	0.009 \pm 0.003 (0.004 0.018)
Dominant frequency (kHz)	0.96 \pm 0.04 (0.90 1.03)	1.16 \pm 0.03 (1.12 1.206)	1.20 \pm 0.02 (1.16 1.249)

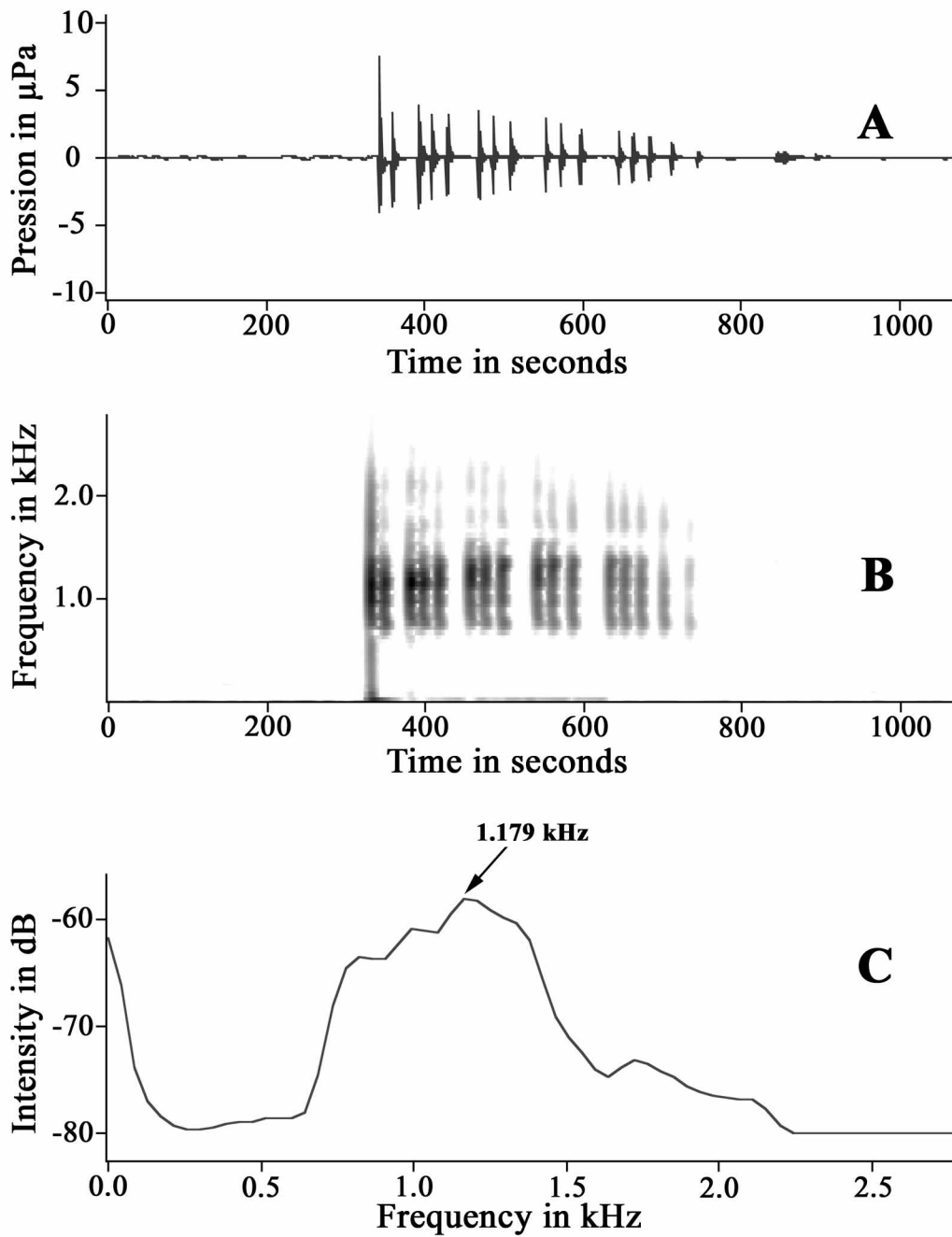


FIGURE 4. Monophasic territorial call of *Phyllomedusa burmeisteri bahiana*: (A) Waveform; (B) Audiospectrogram; (C) Power spectrum.

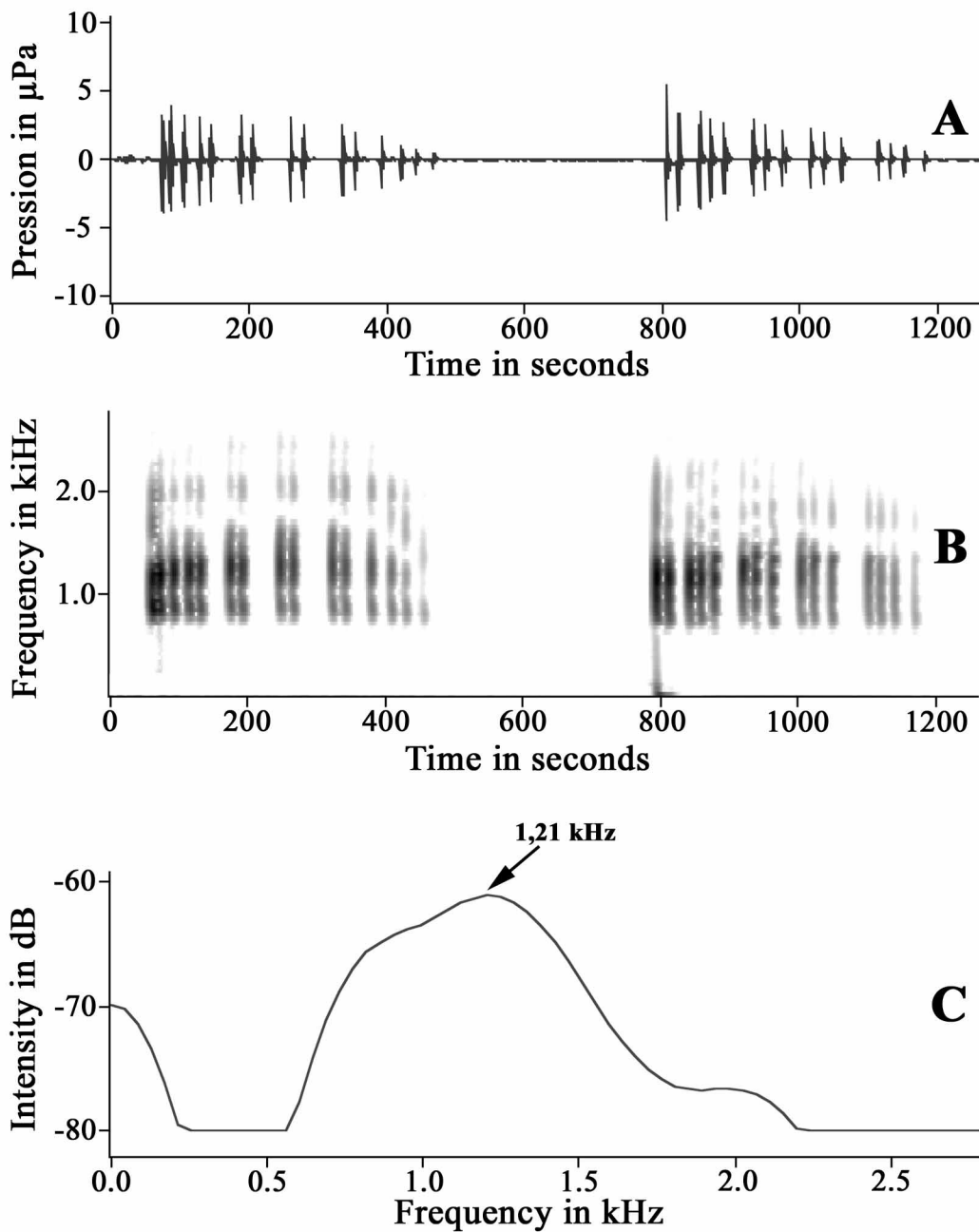


FIGURE 5. Diphasic territorial call of *Phyllomedusa burmeisteri bahiana*: (A) Waveform; (B) Audiospectrogram; (C) Power spectrum.

Advertisement call. The two males (MZUEFS 1512, SVL = 69.15 mm, and other male not captured) recorded for this part of the study were not visibly interacting with

other males. The advertisement call was a monophasic call formed by one note with 12-16 pulses arranged in 4-5 groups of three pulses, called here as triads, and with or without one single pulse interspersed between triads or at the end of the call (Table 2, Figure 3). The interval between calls was very irregular (Table 2). All calls presented two peaks of intensity (Figure 3C) and the dominant frequency was about 0.96 kHz (Table 2, Figure 3b and c) that correspond to the first peak. However, in 27,3% of calls of the SUEFS 7.6 the dominant frequency changed to the second peak of intensity, showing values between 1.412 to 1.938 kHz (1.54 ± 0.20 ; $n=6$).

Territorial call. This call was recorded from a single specimen (MZUEFS 1809, SVL = 68.5 mm) calling in duet with another male on a neighboring tree, and a couple in amplexus was nearby on the ground. The territorial call presented two types: monophasic (Figure 4) and diphasic (Figure 5). The monophasic type is formed by one note with 14-16 pulses arranged in one double pulse, four triads and one or two single pulses at the end (Figure 4A). The diphasic type is formed by two notes (Figure 5). The first note is shorter than the second. The first note is comprised of 15-17 pulses arranged in one double pulse, one triad, 3-4 double pulses, and 3-4 single pulses at the end. The first double pulse and triad have a tendency to form a five-part pulse. The second note is formed by 15-16 pulses arranged in a double pulse, four triads and one or two single pulses and emitted faster than the first note. The second note of the diphasic call is very similar to the note of the monophasic call, but the triads of the latter have a tendency to be separated in double pulses plus a single pulse. The monophasic type is more frequently emitted than diphasic.

***Phyllomedusa b. burmeisteri* - advertisement call from RJ.** The call duration, interval between calls, number of notes, pulse duration, interval between pulses and dominant frequency of advertisement call of one male from Rio de Janeiro, analyzed by Abrunhosa & Wogel (2004) in Avisoft-Sonograph Light I demonstrate the same results when analyzed in Canary 1.2.4 (present work).

In addition, we present the pulse rate (pulses/second). The short call has 45.12 to 49.08 pulses/s (47.26 ± 1.07) and the long call 45.29 to 47.64 pulses/s (46.09 ± 1.34).

Discussion

In the adults, the only morphological characteristic that distinguished *P. b. burmeisteri* and *P. b. bahiana* is the internal coloration of the thigh (Pombal Jr. & Haddad 1992). However, the larval morphology and vocalizations are also diagnostic characters indicating evolutionary divergence. We observe consistent differences between the tadpoles and calls of *P. b. burmeisteri* and *P. b. bahiana*. When we compare tadpoles of *P. b. bahiana* with those described in the literature for *P. b. burmeisteri* (Cruz 1982), we find that the distribution of papilla, the form of spiracle and the form of the ventral fin consistently differentiate the two species.

Phyllomedusa b. bahiana has a spiracle with or without a skin projection protecting the opening, while *P. b. burmeisteri* always has a spiracle with a projection of the skin protecting the opening. The dorsal fin in *P. b. bahiana* originates posterior to the body, while the *P. b. burmeisteri* dorsal fin originates on the beginning of posterior third of the body. The ventral fin is continuous and with an arched contour in *P. b. bahiana*, while *P. b. burmeisteri* has a strongly reduced ventral fin on the second half, and (after that) gradually narrowing until the distal extremity. *Phyllomedusa b. bahiana* has marginal and sub-marginal papilla arranged regularly on the lateral and dorsolateral portions of the oral disc, a double series on the lateral portion and irregularly in one series on the lower portion, while *P. b. burmeisteri* has marginal and sub-marginal papilla arranged irregularly on the lateral portions of the oral disc, in three or four series on the lateral portion and a double series on the lower and dorsolateral portion. The lower lip of *P. b. bahiana* has a small interruption immediately below of the third lower tooth row, while *P. b. burmeisteri* has a continuous lower lip. The upper jaw in the *P. b. bahiana* has a slight medial projection that does not occur in *P. b. burmeisteri*.

The major differences between the advertisement call of *Phyllomedusa b. bahiana* and that of *P. b. burmeisteri* is a monophasic multipulsed note with very well defined triads in *P. b. bahiana*, while the *P. b. burmeisteri* advertisement call is formed by two calls (first shorter than the second) with both having multipulsed notes with pulses tending to be arranged in triads, but almost spaced regularly (Abrunhosa & Wogel, 2004) or pulses generally isolated and spaced regularly (Pombal & Haddad 1992). In addition, the number of pulses and pulse rate of *P. b. bahiana* is respectively lower and faster than for *P. b. burmeisteri* and the pulse duration of the *P. b. bahiana* call is half that of *P. b. burmeisteri* (Abrunhosa & Wogel, 2004).

The territorial call of *Phyllomedusa b. bahiana* is totally distinct when compared with the *P. b. burmeisteri* territorial call (Abrunhosa & Wogel, 2004). *Phyllomedusa b. bahiana* has two call types: a monophasic and a diphasic with two different notes, while *P. b. burmeisteri* has only a monophasic call.

All specimens of *P. b. burmeisteri*, *P. b. bahiana* and intermediate form analyzed by Pombal and Haddad (1992) were adult specimens, which the measurements of SVL were similar to the adult specimens here studied. The internal side of the thighs with spots in newly metamorphosed *P. b. bahiana* (of similar pattern to adult individuals of *P. b. burmeisteri* or the intermediate form) suggests that the occurrence of this pattern of coloration in adult individuals of *P. b. burmeisteri* and the intermediate form could be related to a neotenic pattern of development. This color characteristic in newly metamorphosed of *P. b. bahiana* shows how difficult it is to recognize taxa in this group based on color patterns.

The lack of information about karyotype, tadpoles and advertisement calls of the "intermediate form" also contributes to the imprecision of the relationships involved. However, the divergences found in the larvae morphology and advertisement call of

Phyllomedusa b. bahiana and *P. b. burmeisteri* studied here are consistent with the establishment of *P. b. bahiana* as a different species from *P. b. burmeisteri*.

The results presented above clearly show that *Phyllomedusa burmeisteri bahiana* deserves full species status (*Phyllomedusa bahiana* **stat. nov.**).

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