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Calls and tadpoles of the species of *Lysapsus* (Anura, Hylidae, Pseudae)

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Abstract. Larval and bioacoustical characters are crucial for anuran systematics. Nevertheless, advertisement calls and larval morphology of most Neotropical anuran species are unknown. *Lysapsus* is a peculiar group of aquatic frogs that have been subject to recurrent taxonomic changes. Recent molecular phylogenetic and external morphology work gave support for some of the recognized species (*L. laevis* and *L. caraya*), but considerable doubt remains over the distinction among *L. limellum* and *L. bolivianus*. We describe the tadpoles of *L. caraya* and *L. bolivianus*, the advertisement call of *L. laevis*, and redescribe the advertisement calls of *L. caraya*, *L. limellum*, and *L. bolivianus*. Our results support the genus based on larval and bioacoustical characters. *Lysapsus caraya* and *L. laevis* have distinct advertisement calls, but no visual or statistically significant differences were observed between advertisement calls of *L. limellum* and *L. bolivianus*. Tadpoles of *L. caraya*, *L. limellum*, and *L. bolivianus* are similar based on external morphology and contrast against the distinct tadpole of *L. laevis*. Considerable doubt is raised on the validity of *L. bolivianus*, which either is a junior synonym of *L. limellum* or constitutes a cryptic species based on currently available data. Data on osteology, musculature, and chondrocranium, allied to robust phylogeographic analyses will help clarify the taxonomic status of these two putative species and the biogeographic relationships between the Amazon and the Paraná River basins.

Keywords: bioacoustics, larvae, paradoxical frogs, South America, taxonomy.

Introduction

The use of non-traditional characters for taxonomy and systematics has long been advocated to complement traditional data sets used to identify species and infer phylogenetic relationships (Blair, 1962; Ford and Cannatella, 1993; Garda et al., 2002). With the recent developments in DNA-based techniques, accumulation of data on DNA sequences is now cheaper, easier, and faster than morphological, ecological, and behavioural characters. Still, on an integrative taxonomy perspective, the use of independent groups of characters to decipher biodiversity is fundamental to help unravel cryptic and sibling species (Padial and De La Riva, 2009).

Frogs are one of the key targets for cryptic species investigations (Bickford et al., 2010) and the use of molecular techniques has incited a rapid increase in the number of recognized species (Fouquet et al., 2007). Despite the undeniable value of molecular data and because species must be treated as working hypotheses (Dayrat, 2005), the use of other supporting lines of evidence is crucial to corroborate possible evolutionary lineages identified through molecular markers. Because most frogs recognize mates through advertisement calls (Ger-

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hardt, 1988) and tadpoles are morphologically very different from adults, anuran species descriptions and taxonomic appraisals can benefit from the systematic use of such characters.

In fact, larval and bioacoustical characters have been extremely valuable in anuran systematics (Blair, 1958, 1962; Cruz, 1982). Heyer et al. (1996), for example, while revising the taxonomic status of the *Leptodactylus mystaceus* species complex recognized a new sibling species differentiated by the advertisement call. Guerra et al. (2011) analysed the advertisement calls of the *Rhinella granulosa* species group and corroborated a recent morphology-based taxonomy (Narvaes and Rodrigues, 2009). Several other similar examples abound in the literature (Pombal-Jr. and Haddad, 1999; Abrunhosa et al., 2005; Canedo and Pombal-Jr., 2007; Glaw et al., 2010). Likewise, tadpole morphology has been crucial to recognize genera (Cruz, 1982) and diagnose species of several Neotropical anuran groups (Kolenc et al., 2008; Weber and Caramaschi, 2008). Despite the obvious importance of such characters, advertisement calls and larvae morphology of most Brazilian anuran species are unknown (Bastos et al., 2011; Provete et al., 2011), and such data paucity limits comprehensive comparisons needed to resolve problems with amphibian anuran taxonomy.

The eleven species of *Pseudis* and *Lysapsus* form a peculiar group of aquatic frogs, known as "paradoxical frogs", closely related to hylid tree frogs (Duellman and Trueb, 1986; da Silva, 1998). They form a clade within Hylidae (Pseudae, *sensu* Garda and Cannatella, 2007), with small tree frogs of the genus *Scarthyla* as a sister group (da Silva, 1998; Darst and Cannatella, 2004), and are widely distributed in river floodplains of South America, east of the Andes (Duellman, 1999; Garda and Cannatella, 2007; Garda et al., 2010). Two recent papers presented identical phylogenetic reconstructions for these species based on DNA sequences and indicated that *Pseudis* was not monophyletic (Aguiar et al., 2007; Garda and Cannatella, 2007). However, distinct taxonomic solutions were chosen: Garda and Cannatella (2007) resurrected the genus *Podonectes* (which included *P. cardosoi* and *P. minuta*) while Aguiar et al. (2007) placed *Lysapsus* as a junior synonym of *Pseudis*. Aguiar et al. (2007) further recommended the recognition of the species status for two subspecies of *L. limellum*, *L. limellum limellum* and *L. limellum bolivianus*, while Garda and Cannatella (2007) suggested both be called *L. limellum*. Aguiar et al. (2007) decision to raise these subspecies was based on the strong support for the groups formed by these populations and slight karyotypic differences among them (Busin et al., 2006). However, neither morphometric differences nor external morphology characters that unequivocally differentiate these species have been identified (Garda et al., 2010).

There are currently four recognized species in *Lysapsus* (Cope, 1862): *L. bolivianus* Gallardo, 1961, *L. caraya* Gallardo, 1964, *L. laevis* (Parker, 1935), and *L. limellum* Cope, 1862. These frogs are distributed east of the Andes, from Guyana and northern Brazil to northern Argentina, but absent in the Caatinga domain (Garda et al., 2010). They are small frogs that reach up to 24 mm in snout-vent length (SVL) and are commonly found in ponds along the Amazonas, Paraná-Paraguay, and Araguaia River floodplains (Garda and Cannatella, 2007; Garda et al., 2010). Only the larvae of *Lysapsus laevis* and *L. limellum* (Kehr and Basso, 1990; Caramaschi and Niemeyer, 2004), and the advertisement calls of *L. bolivianus*, *L. limellum*, and *L. caraya* (Barrio, 1970; Bosch et al., 1996; Bastos et al., 2011) have been described.

Herein, we described the tadpoles of *L. caraya* and *L. bolivianus* and the vocalization of *L. laevis*. Furthermore, we redescribe the advertisement calls of *L. caraya*, *L. limellum*, and *L. bolivianus* based on a large sample of frogs from the Amazon and Paraná River basins. Our goals were to: 1) establish a framework within which advertisement calls of all species can be compared; 2) identify external anatomical larval characters useful to characterize currently recognized species of *Lysapsus*; 3) evaluate the support for currently recognized species of *Lysapsus* based on larval and bioacoustics characters.

Material and methods

Calls

We used calls of *Lysapsus caraya* from Conceição do Araguaia Municipality, Pará State, and Montes Claros de Goiás Municipality and Araguapaz Municipality, both in Goiás State. Calls of *L. laevis* were recorded in Boa Vista Municipality, Roraima State. All recordings (except in Araguapaz and Montes Claros de Goiás) were performed with a Sony TCM-5000EV cassette recorder with a *atus* ATR55 Telemike™ directional microphone. We used calls of *L. bolivianus* from Macapá Municipality, Amapá State, and Humaitá Municipality, Amazonas State. Recordings were performed with a Marantz® PMD 660 digital recorder with a Sennheiser® e614 supercardioid directional microphone in Macapá and a Sony TCM-5000EV cassette recorder with a *atus* ATR55 Telemike™ directional microphone in Humaitá. The recordings of *L. caraya* from Montes Claros de Goiás Municipality and *L. limellum* from Corumbá Municipality were performed with a Marantz® PMD 660 digital recorder with a Sennheiser® ME 66 supercardioid directional microphone. Furthermore, we used calls of *L. limellum* from Corumbá Municipality, Mato Grosso do Sul State, Cáceres Municipality, Mato Grosso State, Departamento de Artigas (Bella Unión), Uruguay, Departamento de Santa Fé, Argentina, and Departamento de San Bernardino, Paraguay.

Analogical recordings were digitized at a sampling rate of 44 kHz in Raven Pro 1.3®. We positioned microphones approximately 1 m away from the calling frogs. Water temperature at 1 cm depth was measured after most recordings in Macapá and Humaitá, and vouchers were collected at these locations. Specimens were collected not only for the present work, but also for morphological (Garda et al., 2010), phylogenetic (Garda and Cannatella, 2007), and natural history manuscripts (Garda et al., 2007). No data on temperature or voucher specimens were available for recordings loaned (table 1), precluding the use of temperature and body size as covariables in statistical analyses.

We analysed calls with Raven Pro 1.3 (Cornell Lab of Ornithology) using the following parameters: FFT 512 (1024 for power spectrums), overlap 50, and DFT 512. We described all call types after carefully searching for acoustic patterns within all recordings. We used number of pulses, length, dominant and fundamental frequencies to differentiate among call types. To characterize each call type we averaged the following variables per individual and for all recordings: 1) number of pulses, 2) pulse dominant frequency, 3) call dominant frequency, and 4) call length. We sampled ten calls for most individuals (and averaged temporal and frequency parameters per individual). Terminology of calls follows Duellman and Trueb (1986).

Because call type A was virtually identical in *Lysapsus bolivianus* and *L. limellum*, we compared three advertisement call parameters statistically among these species: call duration (CD), dominant frequency (DF), and number of pulses (NP). To control possible influences of body size on call parameters being compared, we fitted a linear regression between each variable with snout-vent length in recordings from Amapá (where body sizes of vouchers were available). This procedure helped us select variables that were not subject to body size variation, which could affect comparisons among these species since no data for body size were available for *L. limellum* recordings. Next, we used two sample t tests to compare CD, DF, and NP among species. All variables were $\log_{10}$-transformed before analyses. Call B was present in only a few recordings of *L. limellum*, therefore precluding statistical comparisons of this call type among these species.

Tadpoles

Thirty-eight tadpoles (Gosner stage 25 to 41) and nine metamorphosing specimens (Gosner stage 42 to 46) of *Lysapsus caraya* were collected in Aruanã (Goiás State, Brazil S 14°56′54.3″, W 51°02′29.5″), in two field trips (August 05, 2007 and August 08, 2008). We collected tadpoles of *L. bolivianus* (53 between Gosner stage 25 to 41, and five between Gosner stage 42 to 26) in Tartarugalzinho (Amapá State, Brazil N 1°30′21.4″, W 50°54′41.0″).

All specimens were killed in a 10% ethanol solution, preserved in formalin 10%, and staged following Gosner (1960). Larvae of *L. caraya* are deposited at the Professor Antonio Sebben's collection at the University of Brasilia, and larvae of *L. bolivianus* are deposited at Coleção Herpetológica da Universidade de Brasília (lots CHUNB 43490 and CHUNB 43490). We measured the following variables using Mitutoyo digital callipers (precision 0.1 mm): total length, body length, body height, body width, maximum tail height, tail muscle height, tail muscle width, tail length, eye diameter, and eye-nostril distance. Measurements and terminology follow Altig and McDiarmid (1999).

Results

Calls

We identified two distinct calls for *Lysapsus bolivianus*, *L. limellum*, and *L. laevis* (calls A and B, figs 1 and 2): call A is emitted more frequently, and may be classified as an advertisement call. It was observed in all species (including *L. caraya*) and is composed of several pulses, which may be concatenated or not (fig. 1).

The call A of *L. bolivianus* consists of a short call with duration ranging from 0.15 to 0.27 s

Table 1. Recording localities used in the comparisons among advertisement calls of *Lysapsus bolivianus* and *L. limellum*.

Species	Municipality, State, Country	Locality	N	Latitude, Longitude	Date
L. bolivianus	Macapá, AP, Brazil	APA do Curiaú	23	0°09′04.6″N, 51°02′22.9″W	May 2009 and March 2010
L. bolivianus	Humaitá, AM, Brazil	Transamazônica highway	3	7°32′47.8″S, 63°4′21.8″W	July 2003
L. bolivianus	[1]Silves, AM, Brazil	Aldeia dos Lagos	1	2°50′S, 58°12′W	not available
L. caraya	Conceição do Araguaia, PA, Brazil	–	1	8°15′S, 49°16′W	April 19, 2005
L. caraya	Montes Claros de Goiás, GO, Brazil	–	1	16°00′S, 51°23′W	June 17, 2007
L. caraya	[2]Araguapaz, GO, Brazil	–	1	15°04′S, 50°37′W	October 25, 2001
L. laevis	Boa Vista, RR, Brazil	–	1	02°49′S, 60°40′W	June 9, 2006
L. limellum	[1]Departamento de Artigas, Uruguay	Bella Unión	1	30°15′S, 57°34′W	not available
L. limellum	[3]Corumbá, MS, Brazil	–	10	19°00′S, 57°39′W	May 2010
L. limellum	[5]Cáceres, MT, Brazil	–	1	16°04′S, 57°39′W	August 2010
L. limellum	[5]Cáceres, MT, Brazil	Bay near Cáceres	1	16°04′S, 57°39′W	November 1992
L. limellum	[5]Departamento Santa Fé, Argentina	Helvetia	1	31°37′S, 60°41′W	December 1991
L. limellum	[4]Departamento San Bernardino, Paraguay	Near lake Ypacarai	1	25°13′S, 57°31′W	January 1992

[1] Recording kindly provided by Axel Kwet; [2] Recording kindly provided by Rogério P. Bastos; [3] Part of the recordings kindly provided by Cynthia Prado; [4] Loan from Federal University of Mato Grosso, Brazil; [5] Recording loaned by Unicamp and recorded by Adão Cardoso.

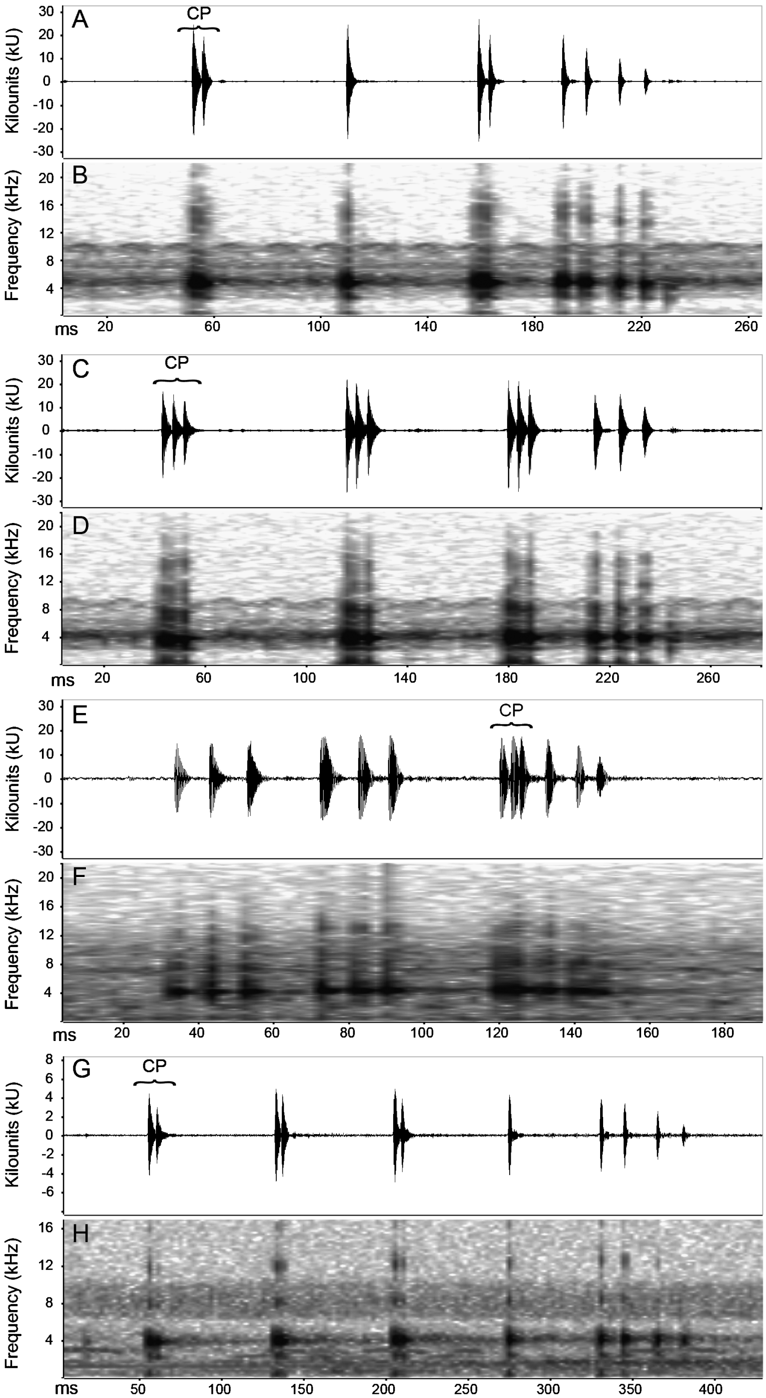

Figure 1. Calls A of *Lysapsus* species: (A) oscillogram and (B) audiospectrogram of *L. bolivianus*; (C) oscillogram and (D) audiospectrogram of *L. caraya*; (E) oscillogram and (F) audiospectrogram of *L. laevis*; (G) oscillogram and (H) audiospectrogram of *L. limellum*. CP indicates an example of concatenate pulse.

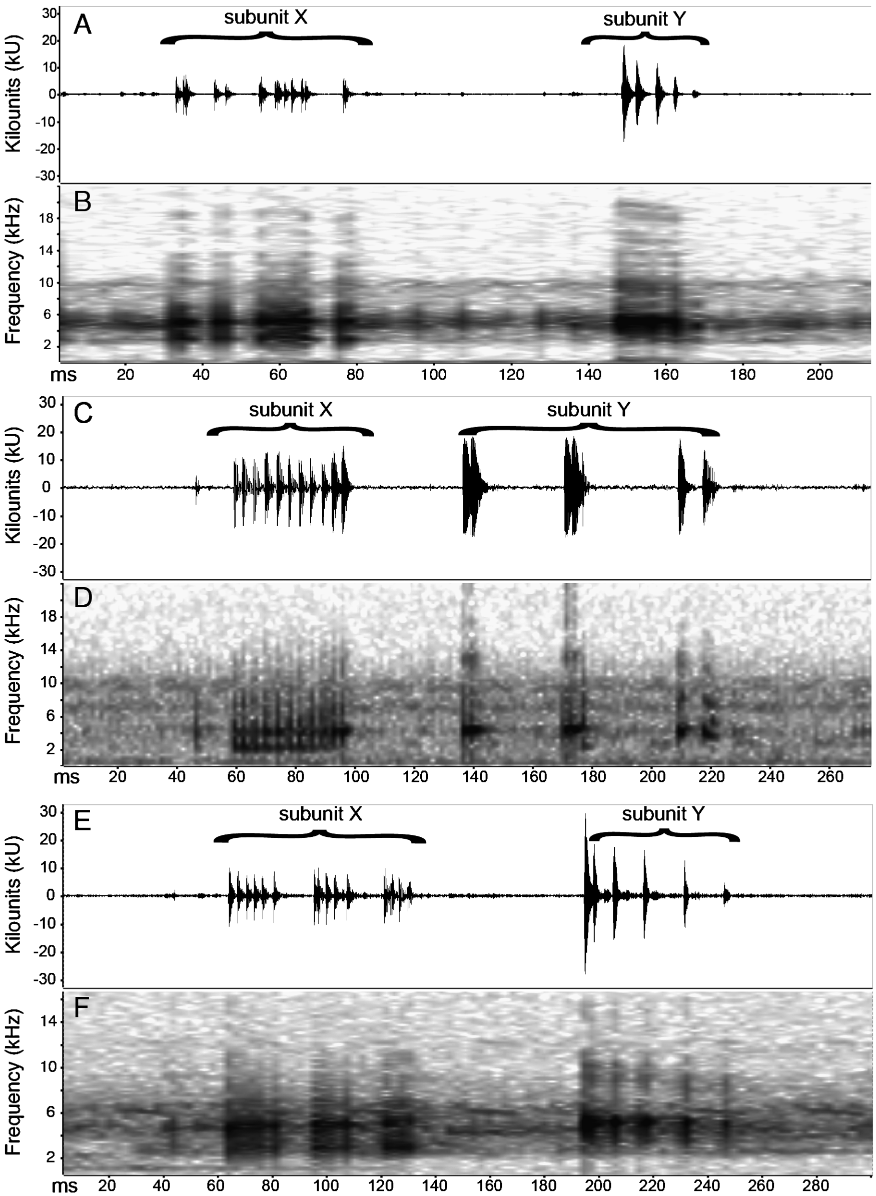

Figure 2. Calls B of *Lysapsus* species: (A) oscillogram and (B) audiospectrogram of *L. bolivianus*; (C) oscillogram and (D) audiospectrogram of *L. laevis*; (E) oscillogram and (F) audiospectrogram of *L. limellum*.

(0.21 ± 0.03 s), dominant frequency ranging from 3617 to 5254 Hz (4624 ± 326 Hz), and is composed of 5 to 12 pulses (8.45 ± 1.46). For *L. caraya*, duration of call A ranges from 0.17 to 0.20 s (0.18 ± 0.01 s), dominant frequency ranges from 3937 to 4823 Hz (4227 ± 303 Hz), and number of pulses ranges from 11 to 15 (12.58 ± 1.39). For *L. laevis*, call A duration ranges from 0.06 to 0.46 s (0.18 ± 0.09 s), dominant frequency ranges from 2067 to 4479 Hz (4033 ± 568 Hz), pulse number ranges from 10 to 20 (15.56 ± 3.18). Duration of call A from *L. limellum* ranges from 0.12 to 0.34 s (0.22 ± 0.05 s), dominant frequency ranges from 3703 to 5599 Hz (4691 ± 473 Hz), and number of pulses from 6 to 12 (8.97 ± 1.38).

Call B is composed of two distinct notes (x and y, fig. 2, Appendix). The first note (x) is longer in both *L. bolivianus* and *L. laevis*, has more pulses with lower amplitude and lower overall dominant frequency. Call B is usually observed in large aggregations and is emitted during intense social interactions. In *L. bolivianus*, the entire duration of call B ranges from 0.11 s to 0.21 s (0.14 ± 0.02 s). The first note duration ranges from 0.02 s to 0.08 s (0.05 ± 0.02 s), with a dominant frequency ranging from 2412 to 5168 Hz (3941 ± 1074 Hz), and possesses from 6 to 22 pulses (11.41 ± 4.32). The second note duration ranges from 0.01 to 0.09 s (0.03 ± 0.02 s) with a dominant frequency ranging from 3876 to 5426 Hz (4812 ± 493 Hz), and possesses from 4 to 6 pulses (5.08 ± 0.59). Call B of *L. laevis* if formed by two distinct notes, with different frequencies. The entire call duration ranges from 0.127 to 0.234 s (0.170 ± 0.040 s). The first note duration ranges from 0.01 to 0.06 s (0.03 ± 0.02 s) with a dominant frequency ranging from 2412 to 5168 Hz (3996 ± 888 Hz), and possesses from 2 to 5 pulses (3.60 ± 1.02). The second note duration ranges from 0.03 to 0.13 s (0.09 ± 0.04) with a dominant frequency ranging from 4134 to 4479 Hz (4341 ± 169), and posses 3 to 9 pulses (5.08 ± 0.59). Average parameters of calls A and B for each individual recorded are provided in Appendix.

We did not find a call B for *L. caraya*. However, this may be the result of the social context of the species when recordings were conducted, because call B was only recorded in large choruses with intense social interactions among frogs (and our calls of *L. caraya* were never recorded in such conditions). During intense choruses with social interactions among calling males, individuals emit several short, random crackling notes. These notes are commonly emitted by individuals that switch from call A to call B. Hence, further recordings of *L. caraya*, preferably during the beginning of the rainy season when large choruses are formed, are needed to confirm if this species emits call type B.

For *L. bolivianus*, snout-vent length was not significantly correlated with dominant frequency ($t_{19} = -0.40$, $P = 0.69$) or call length ($t_{19} = 0.33$, $P = 0.74$) of call type A. However, the number of pulses of call A was significantly correlated with SVL ($t_{19} = 2.14$, $P = 0.046$). Nevertheless, *L. limellum* did not differ significantly from *L. bolivianus* in dominant frequencies ($t_{36} = 1.12$, $P = 0.27$), number of pulses ($t_{36} = 0.65$, $P = 0.52$), or call length ($t_{36} = -0.75$, $P = 0.46$) of call type A.

Tadpoles

Tadpoles of *Lysapsus caraya* and *L. bolivianus* are similar in external morphology and morphometric measurements (table 2, figs 3 and 4).

Table 2. Measurements (mm) of *Lysapsus bolivianus* and *L. caraya* tadpoles, both at Gosner stage 37. Values are average ± standard deviation; ranges are given below respective averages.

Measurements	*L. bolivianus* (n = 9)	*L. caraya* (n = 8)
Total length	35.3 ± 1.5	32.5 ± 2.7
	33.5-36.9	28.9-36.4
Body length	10.9 ± 0.8	10.6 ± 0.7
	9.9-11.9	9.7-11.7
Body height	7.2 ± 0.6	6.4 ± 0.5
	6.5-8.2	5.6-7.1
Body width	7.0 ± 0.8	5.8 ± 0.5
	5.9-8.2	5.2-6.5
Maximum tail height	7.5 ± 0.5	6.5 ± 0.6
	6.8-8.2	5.6-7.0
Tail muscle height	2.8 ± 0.3	2.8 ± 0.3
	2.5-3.3	2.3-3.2

Table 2. (Continued.)

Measurements	*L. bolivianus* (n = 9)	*L. caraya* (n = 8)
Tail muscle width	2.6 ± 0.26 2.3-2.9	2.3 ± 0.3 1.8-2.6
Tail length	24.6 ± 1.3 22.7-26.6	32.5 ± 2.7 28.9-36.4
Eye diameter	1.8 ± 0.25 1.6-2.3	1.7 ± 0.17 1.5-2.0
Interorbital distance	4.3 ± 0.3 3.9-4.6	6.4 ± 0.4 5.8-7.1
Internarial distance	1.9 ± 0.4 1.3-2.3	2.9 ± 1.1 2.2-5.6
Nostril major radius	0.6 ± 0.1 0.4-0.8	0.5 ± 0.1 0.4-0.6
Nostril to tip of the snout	1.9 ± 0.1 1.7-2.1	2.3 ± 0.2 2.1-2.6
Spiracle length	2.3 ± 1.0 1.5-4.1	1.6 ± 0.2 1.3-2.0
Spiracle opening to tip of the snout	7.1 ± 0.7 6.3-8.2	6.8 ± 0.5 6.1-7.8
Oral disc width	1.9 ± 0.1 1.8-2.0	1.6 ± 0.2 1.3-1.9

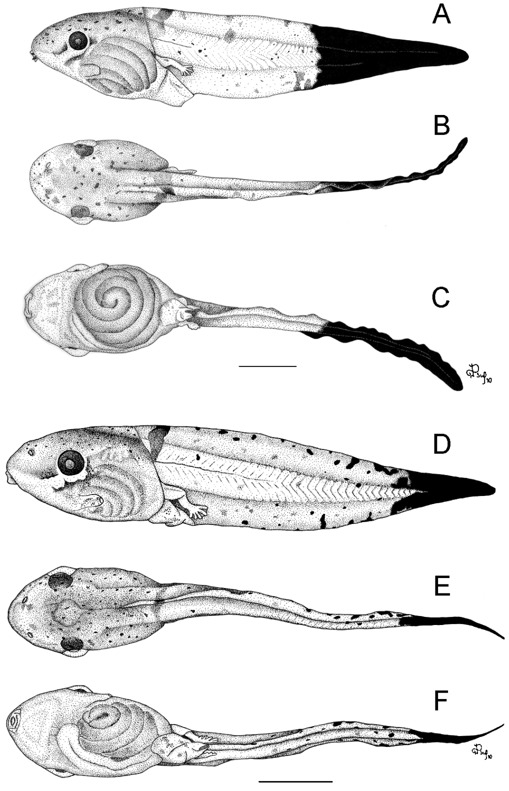

Figure 3. (A) Lateral, (B) dorsal, and (C) ventral views of tadpole of *Lysapsus bolivianus*; and (D) lateral, (E) dorsal, and (F) ventral views of tadpole of *L. caraya*.

Hence, we describe tadpoles of both species together.

External morphology (Stage 37). Tadpoles of *Lysapsus caraya* and *L. bolivianus* are exotrophic, lentic, and nektonic guild members (McDiarmid and Altig, 1999). Body slightly compressed, oval in lateral view, elliptical in dorsal view. Body length 32.7% of total length in *L. caraya*, and 30.6% in *L. bolivianus*. Snout rounded in dorsal view, varying among individuals from rounded to slightly acute in lateral view. External nares elliptic, dorsolaterally positioned, anterodorsally directed, with margins marked by lighter coloration without projections. Eyes rounded, laterally positioned and directed, and visible from dorsal, ventral, and lateral views. Eye diameter represents 26.5% of body height in *L. caraya*, and 24.8% in *L. bolivianus*. Eyes are closer to the spiracle-opening axis than to the snout axis. Eye diameter 26.5% of body height in *L. caraya* and 24.8% in *L. bolivianus*. Oral disc ventral (fig. 3A, C, D, F), not emarginated; two rows of alternated marginal papillae on the anterior portion, two or three rows on its lateral portion, and a single, short

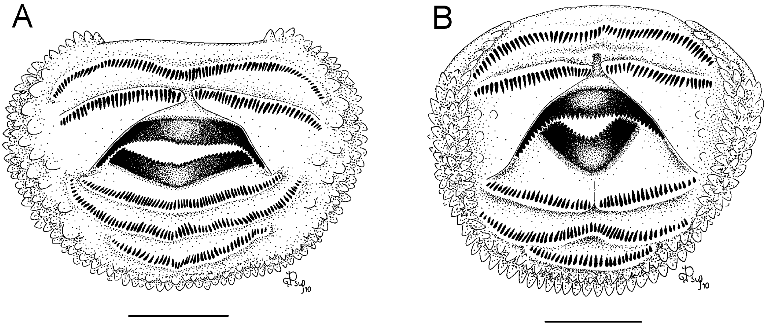

Figure 4. Oral disks of (A) *Lysapsus bolivianus* and (B) *L. caraya*.

row aligned on its posterior portion, with wide anterior gap (fig. 4). Few scattered submarginal papillae on the lateral portions. We identified nine different oral formulas in *L. caraya*, with varying proportions in the samples obtained and with no relationship with larval stage: 47% formula 2(2)/3(1), Gosner stages ranging 25-37; 30% formula 2(2)/3, Gosner stages ranging 25-39; 5% formula 2(2)/3(1, 2) Gosner stages ranging 25-33; 2(2)/3(2); and less then 3% for the other four formulas: 1(1)/3(1), Gosner stage 27; 2(1, 2)/3, Gosner stage 27; 2/3, Gosner stage 40; 2/3(1), Gosner stage 40, and 2(2)/2(2), Gosner stage 37. For *L. bolivianus* the variation of oral formula was similar: 63% formula 2(2)/3, Gosner stages ranging 26-39; 30% formula 2(2)/3(1), Gosner stages ranging 27-39, 7% formula 2/3, Gosner stage 34. Jaw sheaths narrow, with very short and conical serration; upper jaw sheath "arc" shaped and lower jaw sheath "V" shaped; upper jaw sheath wider than the lower one. Spiracle single, sinistral, postero-laterally directed, with its opening midlaterally positioned and elliptically shaped, centripetal wall fused to body; the spiracle opening is midlateral and elliptical. Vent tube and vent tube opening medially positioned relative to the ventral fin, vent tube attached to the ventral fin. Intestinal mass clearly visible in preserved animals, and in *L. caraya* it is positioned in such way that the imaginary line of the intestinal coil is subparallel to the longitudinal body axis (Faivovich, 2002), while in *L. bolivianus* it is centrally positioned in the posterior portion of the body. A big intestinal loop lies at the right side of the abdomen, drawing the intestinal mass to the left side (Caramaschi and Niemeyer, 2004). This intestinal loop is also lighter in coloration.

Tail fins low, dorsal and ventral fins about equal in height and almost parallel to the tail musculature. Dorsal fin originates before the tail and body junction, while the ventral fin originates at the posterior ventral terminus of the body and is hidden by the vent tube. Distance between tail musculature myotomes (visible against a dark background) increases gradually along the tail posterior half.

Overall, coloration of *L. caraya* tadpoles in life is light green, with the ventral surface of the body darker. Body dorsum is brown with spots, which in lateral view form patterns under the eyes and a distinct band at the anterior region of the dorsal fin. In lateral view, tail light green with variable numbers of brown and black spots, sometimes with a black tail tip. Ventral body iridescent white. Iris bright red. *Lysapsus bolivianus* tadpoles in preservative have the dorsal body brown, and the lateral and ventral portions overall dark grey (mainly because of the intestine). Tail light cream, with few irregular brown spots. Dorsal and ventral fins transparent cream. Caudal portion of the tail dark. Iris dark grey.

Discussion

Calls

Lysapsus species frequently call in large choruses, where males engage in an irregular calling behaviour with several individuals calling simultaneously (Hödl, 1977; Bosch et al., 1996), and this has made the individualization of calls and the analysis of their characteristics historically difficult. Nevertheless, we observed one common call in all species analysed. Call A is analogous in structure to the advertisement call of *Pseudis* (Barrio, 1970; Kwet, 2000; Vaz-Silva et al., 2007) and we tentatively classify it as an advertisement call. This advertisement call A is unique in structure in *L. caraya*, with several concatenated pulses (Bastos et al., 2011) and provides, along with molecular (Garda and Cannatella, 2007), morphometric, and distributional data (Garda et al., 2010), unequivocal support for the validity of the species. The advertisement call of *L. laevis* appears the most distinct of the group (with 10-22 pulses, fig. 1E-F), corroborating its position as a sister to other *Lysapsus* (Garda and Cannatella, 2007). However, because recording of only one individual was available to us, we cannot state unequivocally which call parameters characterize this species. Advertisement calls of *L. limellum* and *L. bolivianus* are virtually identical in all parameters compared visually and statistically. We also compared the amplitude modulation patterns in the advertisement call among this species (results not shown) and no differences were observed. Although we did not compare call B statistically (because of low sample size), we could not identify characters that might distinguish these two species.

Call B is unique in structure, has not been reported for other species of Pseudae, and may represent the only known non-molecular synapomorphy for *Lysapsus* (Faivovich et al., 2005). Along with differences in body size of adults and larvae (Garda et al., 2010), behaviour (Garda et al., 2007), and morphometry (Garda et al., 2010), this bioacoustical character endorses furthermore the recognition of two genera within the group, in spite of the validity or not of *Podonectes* (Garda and Cannatella, 2007). *Lysapsus* engage in intense social interactions and can be found calling throughout most of the year (Prado and Uetanabaro, 2000; Garda et al., 2007). Call type B may have one or more functions such as to intensify the attractiveness of calling males in large choruses, or compose aggressive and/or territorial displays.

Tadpoles

Slender bodies and black tail tips (except in *L. laevis*) characterize *Lysapsus* tadpoles (Kehr and Basso, 1990). Black tail tips have been attributed to phenotypic plasticity in response to predator densities in North American *Acris* tadpoles (Caldwell, 1982). These black marks would help deflect attacks of invertebrate predators, preserving vital parts of the tadpole. Kehr and Basso (1990) suggested that a similar mechanism might be working on *Lysapsus* tadpoles. However, because *Pseudis* tadpoles also have black tail tips in early stages (Dixon et al., 1995; Downie et al., 2009a), and variation in tadpole colour among ponds with different predator concentrations has not been reported for *Lysapsus*, this character may be a shared phylogenetic characteristic of the group rather than a predator-induced change.

Black tails tips are absent in *L. laevis* and in *P. minuta* (de Sá and Lavilla, 1997; Caramaschi and Niemeyer, 2004). Tadpoles of *P. minuta* furthermore do not become melanistic in later stages (common in other *Pseudis*), what has been interpreted as a case of heterochrony in larval development (de Sá and Lavilla, 1997). Tadpoles would have a disruptive/deflecting coloration when small to deflect invertebrate predators (Dixon et al., 1995), but would become darker to avoid fish predators as they grew larger and were no longer threatened by small predators. However, because *Pseudis* populations vary in adult sizes (Garda et al., 2010) in response to size at metamorphosis (Downie et al., 2009b; Fabrezi and Goldberg, 2009), tadpoles in some populations may not present pigmented stages because metamorphosis is triggered faster where ponds' permanence is not long enough. In fact, tadpoles of the sister species of *P. minuta* (*P. cardosoi*) are pigmented when large (DJS pers. obs.; Kwet, 2000), and there are populations of *P. paradoxa* where advanced larvae are not melanistic (AAG, unp. data).

Besides advertisement call characteristics cited above, other tentative synapomorphies for *Lysapsus* may include chondrocranial characters that have been shown to differ among *L. limellum* and *P. paradoxa* (Hass, 2003; Candioti, 2004; Alcalde and Barg, 2006). The main difference between *L. caraya* and *L. bolivianus* tadpoles is the intestinal loop, which lies at the right side of the abdomen and draws the intestinal mass to the left side in *L. caraya* (intestinal mass centralized in the posterior portion of the body in *L. bolivianus*). *Lysapsus laevis* tadpoles are easily distinguished from the other three species because of higher tail fins and absence of dark pigmentation at caudal portion of tail (Caramaschi and Niemeyer, 2004).

The oral apparatus can vary interspecifically and ontogenetically in anuran amphibians, and differences in the oral formula have been recurrently used for taxonomic purposes (Altig and McDiarmid, 1999). The most frequent oral formula found in both *L. bolivianus* and *L. caraya* tadpoles described here is 2(2)/3. This is the same formula reported by Kehr and Basso (1990) for *L. bolivianus*. Few studies report on variation in tadpole oral formula. Such variations can be due to physical breaks or ontogeny (Altig and McDiarmid, 1999). However, because the variation we report is found in different larval stages, physical breaks or natural variation are most likely the causes for the diversity of oral formulas found.

The tadpole of *L. bolivianus* described herein is undistinguishable from *L. limellum* tadpoles from Corrientes, Argentina (Kehr and Basso, 1990; Candioti, 2004). Along with similarities in advertisement calls (see above), morphometry (Garda et al., 2010), cytogenetics (Busin et al., 2006), and external morphology (Garda et al., 2010), this sheds considerable doubt on the validity of *L. bolivianus*. However, to avoid taxonomic instability, we refrain from synonymizing these species. Detailed internal anatomic comparisons among larvae and adults are warranted so osteological and/or chondrocranial differences can be investigated. Furthermore, we believe that a phylogeographic study on the species is crucial to identify if one or more lineages exist and if these lineages occupy distinct hydrographic basins (Paraná and Amazon). These two hydrographic basins share several species of frogs, lizards, and other vertebrates, and distinct evolutionary scenarios are possible for the region, including ancient splits, recurrent periods of contact and isolation, and recent dispersal.

At last, detailed analysis of more calls from different individuals of all species is needed in order to: 1) confirm if call B is lacking in *L. caraya*; 2) statistically test for differences among species in call type B; and 3) evaluate the behavioural contexts within which these calls are produced. *Lysapsus* are one of the few species that are found calling throughout most of the year in Brazil (Prado and Uetanabaro, 2000; Garda et al., 2007), and present a unique opportunity to study frog reproductive behaviour with direct observations and playback experiments.

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Appendix

Table A.1. Parameters for call A from several individuals from all species of *Lysapsus*.

Recording collection label	Dominant frequency (Hz)	Call duration (s)	Number of pulses
	Lysapsus caraya		
ASUFURN103	4220.5-4823.4	0.165-0.183	12-15
	4470.7 ± 123.90	0.173 ± 0.006	13.6 ± 1.1
ASUFURN101	3750.0-3937.5	0.005-0.198	11-12
	3918.7 ± 59.29	0.171 ± 0.059	11.4 ± 0.5
	Lysapsus bolivianus		
ASUFRN088	4565.0-4737.3	0.183-0.264	7-8
	4668.4 ± 54.48	0.235 ± 0.024	7.8 ± 0.4

Table A.1. (Continued.)

Recording collection label	Dominant frequency (Hz)	Call duration (s)	Number of pulses
ASUFRN089	4909.6-5081.8	0.152-0.229	7-8
	4952.65 ± 60.88	0.197 ± 0.020	7.1 ± 0.3
ASUFRN091	4651.2-5254.1	0.168-0.272	7-9
	4926.8 ± 221.64	0.205 ± 0.32	7.7 ± 0.7
ASUFRN118	4478.9-4823.4	0.148-0.221	5-9
	4608.1 ± 121.80	0.184 ± 0.027	7.4 ± 1.3
Not collected 1	4565.0-4737.3	0.146-0.228	9-11
	4668.4 ± 67.95	0.181 ± 0.028	9.8 ± 0.6
Not collected 2	4478.9-4651.2	0.145-0.217	6-8
	4565.0 ± 43.07	0.182 ± 0.023	7.1 ± 0.6
Not collected 3	4306.6-4909.6	0.190-0.250	8-11
	4633.9 ± 202.22	0.192 ± 0.020	9.6 ± 1.2
ASUFRN116	4478.9-4651.2	0.164-0.225	9-11
	4586.6 ± 76.36	0.198 ± 0.023	10.3 ± 0.7
KWET 19B9	3617.6-4220.5	0.220-0.263	8-12
	3789.83 ± 182.71	0.218 ± 0.150	10.2 ± 1.2
Lysapsus laevis			
ASUFRN117	2067.2-4478.9	0.056-0.459	10-22
	4134.1 ± 568.30	0.176 ± 0.094	15.9 ± 3.5
Lysapsus limellum			
Kwet 17A04	4909.6-5598.6	0.189-0.265	7-10
	5525.3 ± 219.60	0.219 ± 0.025	7.9 ± 1.3
ASUFRN106	4909.6-5168.0	0.161-0.223	8-10
	5064.6 ± 88.96	0.192 ± 0.019	9.10 ± 0.7
ASUFRN107	4478.9-4995.7	0.189-0.250	7-12
	4720.1 ± 222.96	0.219 ± 0.024	10.0 ± 1.9
ASUFRN108	4651.2-5168.0	0.191-0.260	9-10
	4931.1 ± 215.33	0.216 ± 0.031	9.7 ± 0.5
ASUFRN109	4220.5-4909.6	0.119-0.228	6-8
	4651.2 ± 306.55	0.175 ± 0.048	7.0 ± 0.8
ASUFRN110	4565.0-4737.3	0.138-0.178	7-8
	4633.9 ± 72.08	0.162 ± 0.017	7.2 ± 0.5
ASUFRN111	4392.8-5081.8	0.122-0.205	7-11
	4860.4 ± 247.92	0.169 ± 0.028	8.9 ± 1.2
ASUFRN112	4823.4-5081.8	0.192-0.213	8-10
	4995.7 ± 121.81	0.206 ± 0.010	8.5 ± 1.0
ASUFRN113	4478.9-4909.6	0.209-0.233	9-11
	4622.5 ± 248.66	0.220 ± 0.012	10.0 ± 1.0
ASUFRN114	3703.7-3876.0	0.268-0.336	8-11
	3781.23 ± 85.67	0.313 ± 0.022	9.60 ± 1.2
UFMT 49A02	4306.6-4565.0	0.226-0.257	10-11
	4431.04 ± 87.32	0.243 ± 0.010	10.11 ± 0.3
AJC 120/02	3914.1 ± 221.6	0.129 ± 0022	6.0 ± 1.4
	3750.0-4218.8	0.097-0.147	5.0-8.0
AJC 118/05	4429.7 ± 43.4	0.251 ± 0.081	9.6 ± 0.2
	4406.2-4500.0	0.138-0.426	8.0-11.0
AJC 117/04	3956.2 ± 59.3	0.284 ± 0.051	9.5 ± 1.7
	3843.8-4031.2	0.164-0.328	6.0-11.0

Table A.2. Parameters for calls type B of species of *Lysapsus*.

Species	Call duration (s)	Dominant frequency note X (Hz)	Dominant frequency note Y (Hz)	Note X duration (s)	Note Y duration (s)	Note X pulse number	Note Y pulse number
				Lysapsus bolivianus			
ASUFRN091	0.132-0.149	2670-5168	5081-5426	0.037-0.084	0.019-0.033	6-15	4-6
	0.139 ± 0.005	4556 ± 975	5254 ± 90	0.054 ± 0.015	0.024 ± 0.005	9.3 ± 3.16	5.0 ± 0.47
KWET19B9	0.159-0.205	3531-3876	3876-3962	0.021-0.084	0.037-0.089	8-22	5-6
	0.186 ± 0.016	3689 ± 138	3904 ± 44	0.066 ± 0.024	0.056 ± 0.019	16.3 ± 4.76	5.3 ± 0.52
Not collected	0.130-0.154	2411-4478	4392-4909	0.031-0.071	0.013-0.040	7-14	4-6
	0.138 ± 0.007	3021 ± 952	4657 ± 155	0.042 ± 0.011	0.027 ± 0.009	9.2 ± 1.9	5.3 ± 0.63
ASUFRN082	0.105-0.139	4737-4995	5081-5254	0.032-0.070	0.020-0.029	8-19	4-5
	0.127 ± 0.011	4855 ± 112	5189 ± 60	0.049 ± 0.013	0.025 ± 0.003	14.0 ± 4.07	4.6 ± 0.52
				Lysapsus laevis			
ASUFRN117	0.127-0.234	2411.7-5168.0	4134.4-4478.9	0.012-0.060	0.032-0.132	2-5	3-9
	0.170 ± 0.042	3996.6 ± 992.6	4341.1 ± 188.7	0.032 ± 0.019	0.087 ± 0.044	3.6 ± 1.1	7.2 ± 2.4