



Description of a new species of *Pseudopaludicola* Miranda-Ribeiro, 1926 from the state of São Paulo, Southeastern Brazil (Anura, Leiuperidae)

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Abstract

I describe here a new species of *Pseudopaludicola* (*P. serrana*) found in the southern limits of the Espinhaço mountain range in the state of Minas Gerais, Brazil. This new species is related to *P. saltica* and *P. murundu* and can be diagnosed by the following combination of characters: (1) long hindlimbs and absence of T-shaped terminal phalanges, (2) single, dark, and subgular vocal sac with dark longitudinal folds in males; (3) immaculate to light brown, nuptial pads in males that cover the external part of finger I; (4) SVL/HL greater than 2.7; and (5) advertisement call with dominant frequency above 5 kHz, pulse duration range between 13 and 23 ms, and mean interval between notes of 177 ms. Tadpoles and the advertisement call are also described.

Key words: Anura, species description, *Pseudopaludicola*, tadpole, advertisement call

Resumo

No presente estudo é descrita uma nova espécie de *Pseudopaludicola* (*P. serrana*), encontrada limite sul da cadeia do Espinhaço, no estado de Minas Gerais, Brasil. Esta nova espécie é relacionada com *P. saltica* e *P. murundu* e é diagnosticada pela combinação de caracteres morfológicos, e bioacústicos: (1) presença de pernas longas e ausência de falanges terminais em formato de T, (2) saco vocal subgular simples, escuro e com pregas longitudinais nos machos; (3) calos sexuais de imaculados a marrom claro nos machos, os quais cobrem a porção externa do dedo I; (4) comprimento rostro-cloacal/comprimento da cabeça maior do que 2,7; e (5) canto de anúncio com frequência dominante maior que 5 kHz, duração do pulso variando entre 13 e 23 ms, e intervalo médio entre notas de cerca de 177 ms. Os girinos e o canto de anúncio são descritos.

Palavras chave: Descrição de espécies, Anura, *Pseudopaludicola*, girino, canto de anúncio

Introduction

The genus *Pseudopaludicola* Miranda-Ribeiro, 1926 has currently 12 species (Frost 2009; Cardozo & Lobo 2009; Toledo *et al.* 2010). The genus is distributed over South America and its species inhabit tropical rainforests (Amazon and Atlantic Forest) and open grasslands (Caatinga, Cerrado, Chaco, and Pantanal). Lynch (1989) proposed two sub-groups for the genus: *P. falcipes* (Hensel, 1867) and *P. pusilla* (Ruthven, 1916) groups. Lynch (1989) suggested that the group of *P. pusilla* (which includes *P. boliviana* Parker, 1927, *P. canga* Giaretta and Kokubum, 2003, *P. pusilla*, and probably *P. ceratophryes* Rivero and Serna, 1985) is monophyletic (based on osteological characters: mainly by the presence of T-shaped terminal phalanges) and the group of *P. falcipes* (which includes the remaining species) would be paraphyletic. To corroborate this hypothesis it would be recommended to have a molecular phylogeny, which is lacking by the present moment. However, it is possible to recognize (based on external morphology: extremely long legs; and bioacoustics: multipulsed long advertisement calls) a sub-group inside the group of *P. falcipes*: the group of *P. saltica* (Cope, 1887), including *P. saltica* and *P. murundu* Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-

Pimentel and Haddad, 2010, which are species with proportionally long legs among the *P. falcipes* group (Lobo, 1994; Toledo *et al.* 2010).

Lack of published phylogenies limits our understanding on the evolution and historical relationships of the genus. Besides this, within this genus there should be several undescribed species, masking the understanding of the relationships among the living species. Therefore, I here describe a new species, closely related to *P. murundu* and *P. saltica* from the southern mountain ranges of the state of Minas Gerais, Brazil.

Material and methods

Voucher specimens and description. Adults and tadpoles are deposited in the Museu de Zoologia “Prof. Adão José Cardoso” (ZUEC) at the Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. The terminology used for the adult measurements follows Cei (1980), Heyer *et al.* (1990), and Duellman (2001): snout-vent length, head length, head width, eye diameter, interorbital distance, internarial distance, thigh length, tibia length, and foot length. Description of snout shape in dorsal view follows Heyer *et al.* (1990) and in lateral view follows Cei (1980). Webbing formula follows Myers and Duellman (1982). Measurements were made with a digital caliper to the nearest 0.1 mm. Line drawings of the adult were made in a Zeiss stereomicroscope SV11 with a drawing tube.

Male vocalizations were recorded with a Marantz digital recorder (PMD660), equipped with an external directional microphone (Sennheiser ME 66) positioned ca. 50 cm from the calling male. It was established 16 bits of resolution and 44 kHz of frequency sampling. We analyzed the calls using Raven Pro 1.3 software, where the parameters were set as: FFT and frame length of 512 samples, brightness at 80 %, contrast at 70 %, and the frequencies below 2 kHz and above 10 kHz were filtered. The terminology for the bioacoustical analysis follows that presented in Toledo and Haddad (2005).

General external morphology terminology for the description of tadpoles follows Altig and McDiarmid (1999). The following measurements of tadpoles were taken according to Altig and McDiarmid (1999): total length (TL), body length (BL), tail length (TAL = TL–BL), interorbital distance (IOD), internarial distance (IND), tail muscle width (TMW = maximum tail width), maximum tail height (TMH). The following additional measurements were taken according to Eterovick and Brandão (2001): maximum body height (MBH), maximum body width (MBW), and eye diameter (ED). Measurements were taken in millimeters using a digital caliper to the nearest 0.01 mm under a Zeiss stereomicroscope SV11. Larvae were obtained after egg deposition of a collected couple in a plastic bag. The tadpoles were fed on fish food in the laboratory. Developmental stages of tadpoles were determined according to Gosner (1960). Tooth row formula notation of tadpoles follows Altig (1970).

Statistics. For statistical analyses I adopted a non-parametric test based on the normality and independence of the data. Therefore, I applied Kruskal-Wallis tests, followed by Mann-Whitney comparisons. Significance was considered when $P < 0.05$.

Results

Pseudopaludicola serrana sp. nov.

Holotype. ZUEC 16442, an adult male collected in a temporary pond in the Condomínio Retiro das Pedras (private area), Serra da Moeda, municipality of Brumadinho, state of Minas Gerais, southeastern Brazil (20°06'06.7" S and 43°59'20.8" W; approximately 1.450 m above sea level), by J. Toledo, N. R. Silva, P. G. Taucce, and P. Pinheiro on 01 December 2009 (Figure 1A).

Paratopotypes. Four adult males (ZUEC 16396-98; 16443), one female (ZUEC 16399), and two juveniles (ZUEC 16394-5) all collected with the type.

Diagnosis. *Pseudopaludicola serrana* is assigned to the genus *Pseudopaludicola* due to the presence of hypertrophied antebrachial tubercle (see Lynch, 1989). *Pseudopaludicola serrana* is an intermediate size

species for the genus and is characterized by the following combination of characters: (1) long hindlimbs and absence of T-shaped terminal phalanges, (2) single, dark, and subgular vocal sac with dark longitudinal folds in males; (3) immaculate to light brown, nuptial pads in males that cover the external part of finger I; (4) SVL/HL greater than 2.7; and (5) advertisement call with dominant frequency above 5 kHz, pulse duration range between 13 and 23 ms, and mean interval between notes of 177 ms.

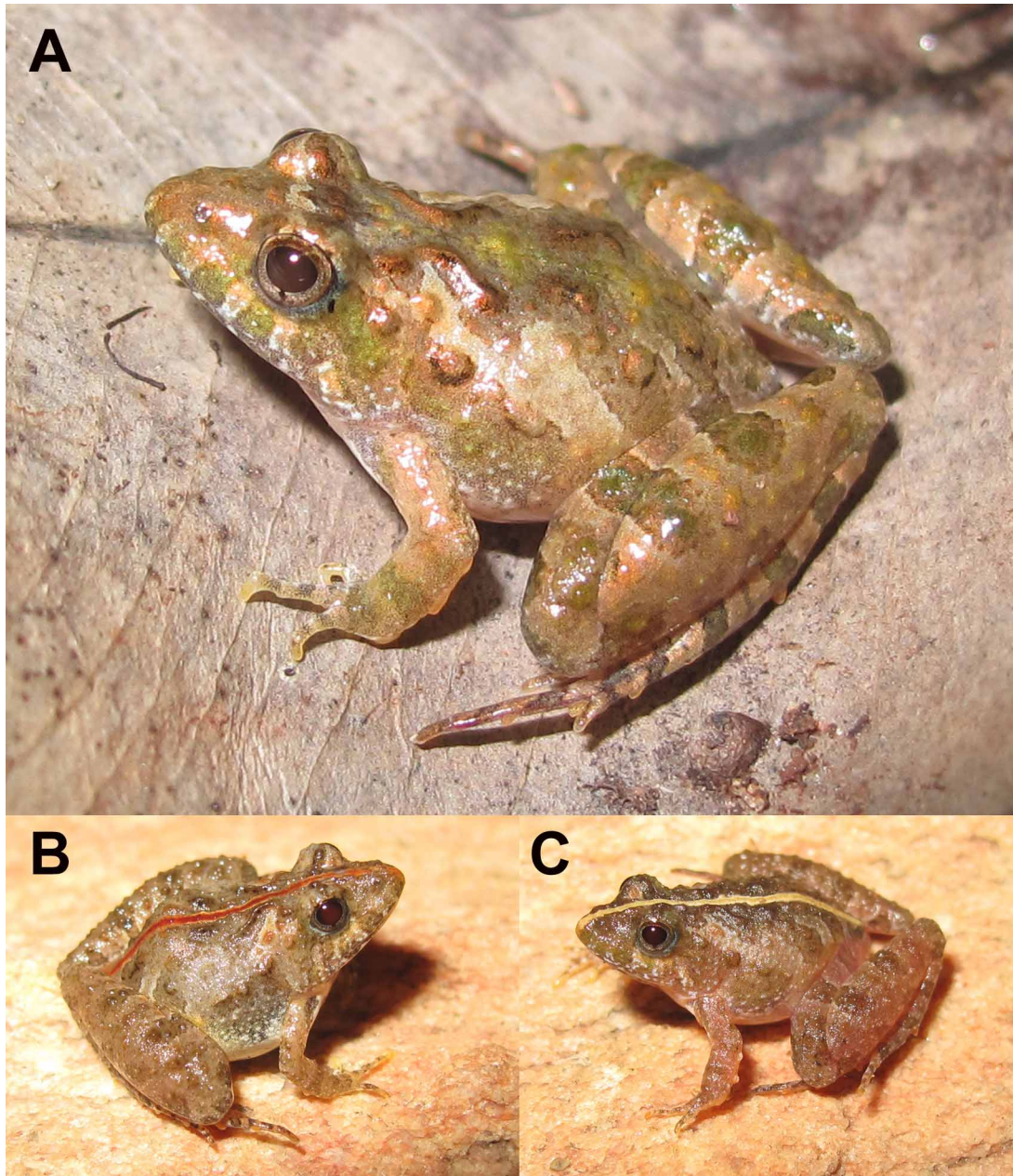


FIGURE 1. Live specimens of *Pseudopaludicola serrana* sp. nov.: the holotype (adult male ZUEC 16442) (A), an adult red-striped female (ZUEC 16455) (B), and an adult white-striped male (ZUEC 16456) (C).

Comparison with other species. *Pseudopaludicola serrana* is distinguished from the species of the *P. pusilla* group (*P. canga*, *P. ceratophryes*, *P. llanera*, and *P. pusilla*) by the absence of T-shaped terminal phalanges (the paratype ZUEC 16396 had its fingers dissected). From the species of the *P. falcipes* group, except from *P. murundu* and *P. saltica*, it distinguishes by having very long hindlimbs, with tibio-tarsal articulation reaching beyond the end of the snout. From *P. saltica* it is distinguished by having larger and clearer nuptial pads (smaller and darker in *P. saltica*), longitudinal folds in the vocal sac (absent in *P. saltica*), and a darker vocal sac (immaculate in *P. saltica*). From *P. saltica* and *P. murundu* it distinguishes by having

the head not as long (SVL/HL = 2.99 ± 0.30 ; 2.71–3.34 / HL/HW = 0.97 ± 0.06 ; 0.86–1.02) as in *P. saltica* (SVL/HL = 2.28 ± 0.11 ; 2.00–2.44 / HL/HW = 1.15 ± 0.08 ; 1.01–1.33) and *P. murundu* (SVL/HL = 2.42 ± 0.12 ; 2.25–2.62 / HL/HW = 1.11 ± 0.07 ; 1.01–1.24) (Table 1). The physical characteristics of the advertisement calls also distinguish *P. serrana* from *P. saltica* and *P. murundu*. Significant differences can be observed in pulse duration (between the three species), interval between pulses (between the three species, although the ranges of variation are overlapped), interval between notes (significant difference between *P. serrana* and the two other species), and dominant frequency (significant difference between *P. saltica* and the two other species) (see Tables 2, 3, and 4).

Description of the holotype. Body elliptic and broad. Head triangular, slightly longer than wider. Snout sub-elliptical in dorsal view and rounded in profile (Figure 2A, 2B). Nostrils slightly protuberant, directed anterolaterally. Mouth opening ventral. *Canthus rostralis* rounded. Loreal region slightly concave. Choanae rounded. Eye protuberant, its diameter almost the same size of the interorbital distance. Interorbital area flat. Tympanum indistinct. Vocal sac single, externally expanded, large, and with longitudinal folds; vocal slits present. Vomerine teeth absent. Tongue elliptical, longer than wide. Finger length I<IV<II<III. Toe length I<II<III=V<IV. Finger and toe tips without disks (Figure 2C, 2D). Thumb with keratinized pale beige nuptial pad. Finger webbing absent and toe webbing reduced I-II 2-3 III 3-4 IV-V. Finger and toe subarticular tubercles conical and single. A large subconical ulnar tubercle. Few rounded supernumerary tubercles in the hand in the area delimited by the first subarticular tubercles, the elliptical internal metacarpal tubercle, and the ovoid external metacarpal tubercle. Hindlimbs robust and long. Thigh shorter than tibia; foot longer than thigh and slightly shorter than tibia. Supernumerary tubercles absent in the foot. Metatarsal tubercles present, elliptical; internal larger than the external; external more protuberant than the internal. A well developed fold from the internal metatarsal tubercle to the mid-ventral tarsus. Skin of belly smooth; ventral surface of thigh granular. Dorsum of head, body, and limbs smooth with scattered tubercles; the skin on the scapula region has two arc shaped granular folds. Flanks without tubercles. Cloacal region not granular. Measurements of the holotype are presented in table 1.

TABLE 1. Measurements of the type series (holotype, males including the holotype, and females) of *Pseudopaludicola serrana* **sp. nov.** Values presented in millimeters as mean \pm standard deviation (range).

Measurement	Holotype	Paratypes	
	Male	Males (N = 5)	Female
Snout-vent length	15.2	15.22 ± 0.31 (15.0–15.8)	18.9
Axial-groin length	4.8	5.07 ± 0.36 (4.7–5.6)	6.7
Head length	5.5	5.13 ± 0.57 (4.5–5.8)	6.7
Head width	5.4	5.30 ± 0.54 (4.5–6.0)	7.3
Eye diameter	1.3	1.34 ± 0.18 (1.2–1.6)	1.9
Upper eyelid width	1.2	1.13 ± 0.11 (1.0–1.2)	1.5
Interorbital distance	1.2	1.43 ± 0.19 (1.2–1.7)	1.7
Internarial distance	1.5	1.55 ± 0.12 (1.4–1.7)	2.1
Eye-nostril distance	1.3	1.42 ± 0.11 (1.3–1.6)	1.7
Snout-eye distance	3.0	2.88 ± 0.11 (2.8–3.0)	3.4
Humerus length	2.3	2.47 ± 0.27 (2.1–2.8)	2.3
Radio-ulna length	2.3	2.37 ± 0.22 (2.1–2.6)	3.2
Hand length	4.6	4.44 ± 0.20 (4.2–4.7)	4.6
Thigh length	8.5	8.85 ± 0.42 (8.4–9.4)	10.9
Tibia length	10.3	10.25 ± 0.39 (9.9–10.9)	12.9
Foot length	9.6	9.73 ± 0.23 (9.6–10.1)	12.5

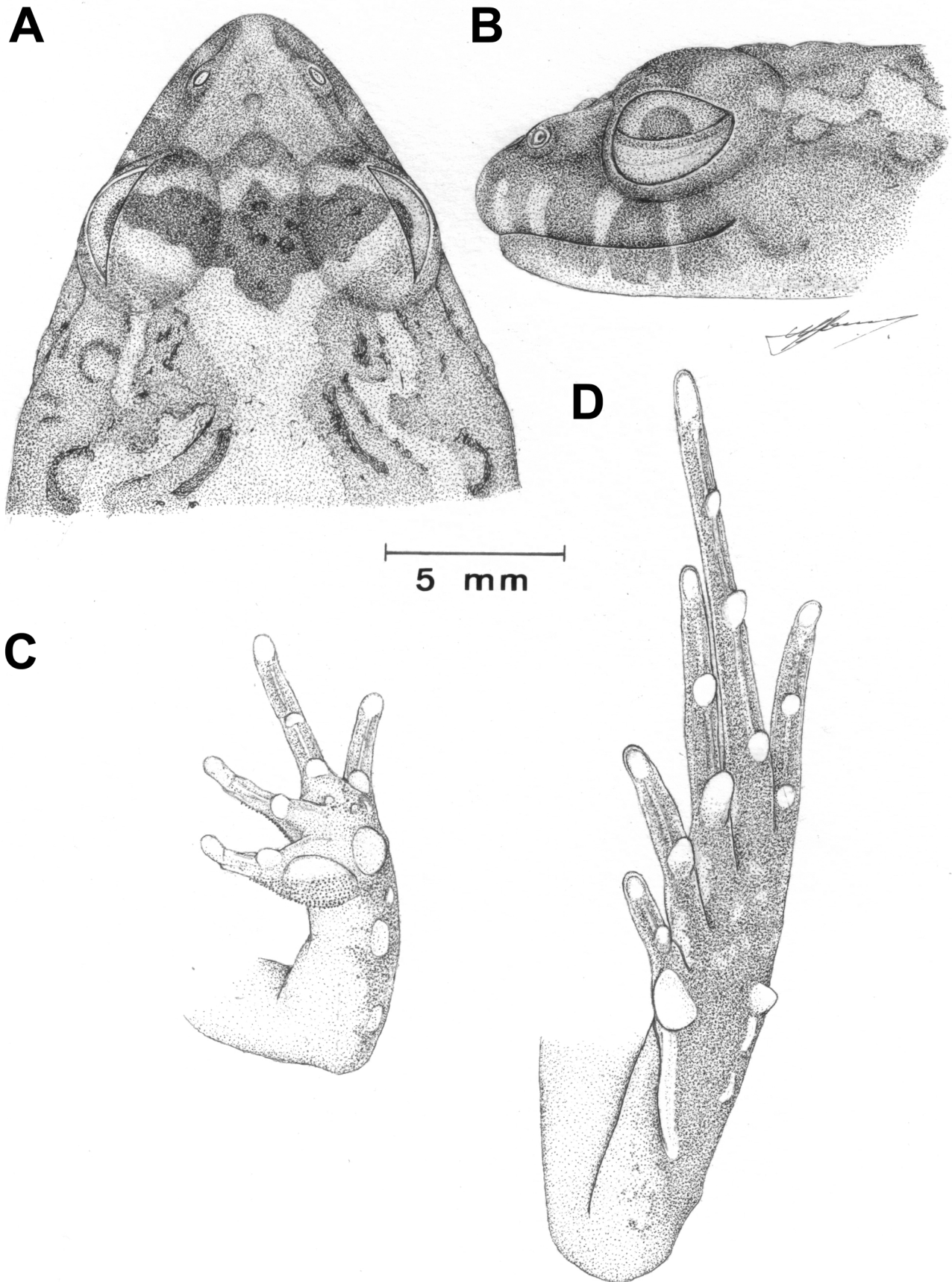


FIGURE 2. Holotype of *Pseudopaludicola serrana* sp. nov. Dorsal (A) and lateral (B) views of the head; ventral views of hand (C) and foot (D).

Color of the holotype. In life, dorsum and limbs are brown with green and brown spots or stripes; vertebral line from the snout to vent absent; belly white; ventral view of legs yellowish white and vocal sac beige with dark dots; iris reddish-brown superiorly and grayish-brown inferiorly; superior labium with vertical wide green stripes (see Figure 1A). In preservative, dorsum and dorsal part of limbs grayish brown with dark brown spots or stripes; belly and ventral surface of legs light beige; vocal sac grayish.

Variation. Females larger than males, lacking vocal sac and nuptial pads (present in males). Males present arms proportionally thicker than females. Nuptial pad can be brownish to light cream color. In 76.5 % of the individuals (13 out of 17 examined, including material not included in type series) the vertebral line is absent. In life this line can be yellow or red (see Figure 1B and C). Dorsal pigmentation varies considerably among individuals in the number and form of the dorsal spots, and in the general pattern, from brown to grayish, but always with some green blotches (Figure 1A, B, and C).

Natural history notes. Males were observed calling in open fields in the domain of Cerrado (Brazilian savannah). The species was observed calling in riparian fields (Campo Rupestre). Males called from the sandy, muddy or rocky ground, near shallow (from 2 to 5 cm of depth) slow-flow or still water bodies. Males were observed calling in the sundown and during the first hours of the night. Two defensive behaviors were observed: ceasing the calling activity and flee against human approach. Gravid females present ovules with black animal pole and yellowish-beige vegetative pole. A couple entered in axillary amplexus inside a plastic bag where they laid 32 eggs, which developed into tadpoles in laboratory until Gosner's stage 25.

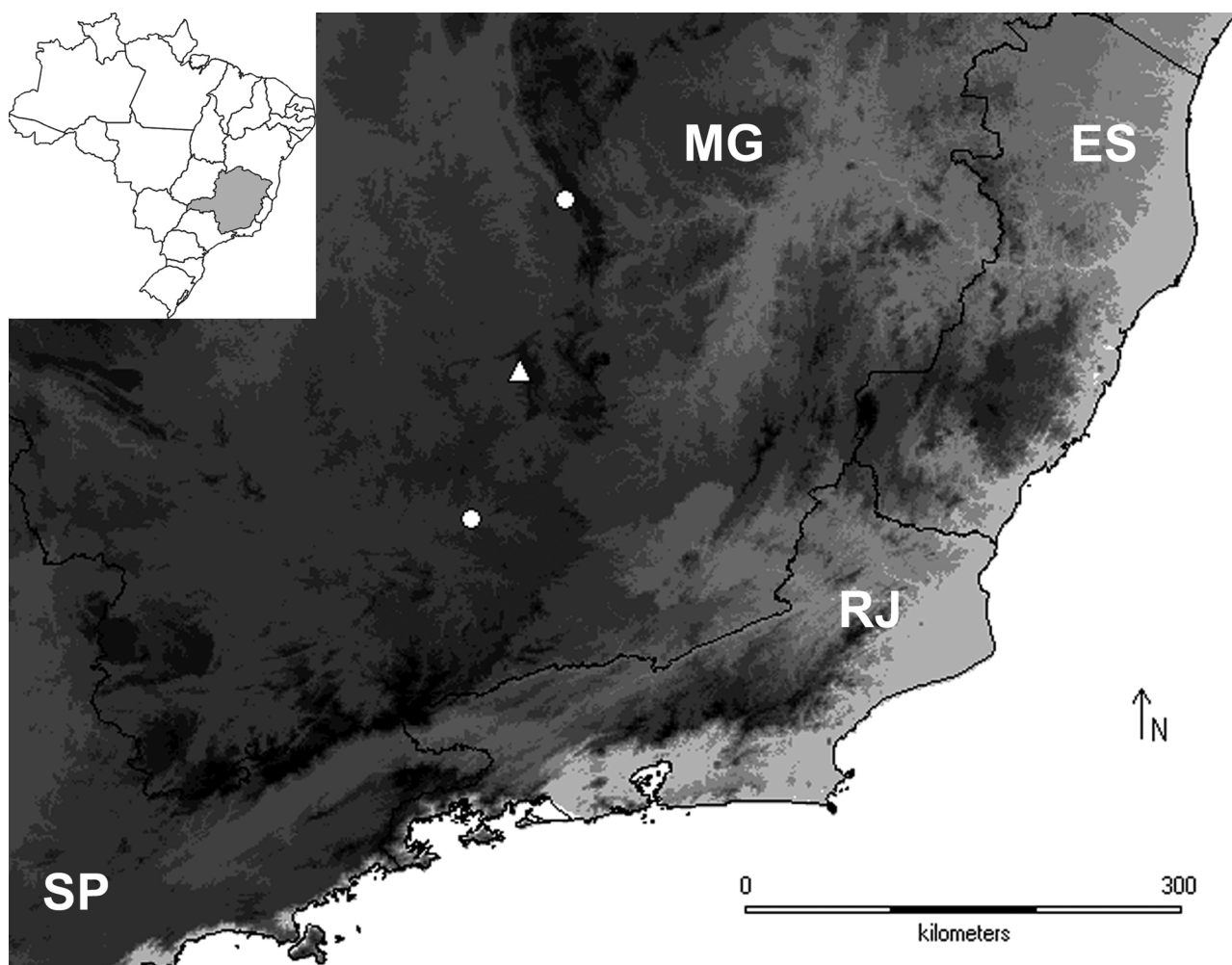


FIGURE 3. Geographic distribution of *Pseudopaludicola serrana* sp. nov. The triangle indicates type locality (Serra da Moeda) and the circles represent the other known localities of occurrence: Serra do Cipó (to the North) and Serra do Lenheiro (to the South). In the up left, map of Brazil indicating the locality of the state of Minas Gerais (in grey). SP: São Paulo; MG: Minas Gerais; RJ: Rio de Janeiro; ES: Espírito Santo.

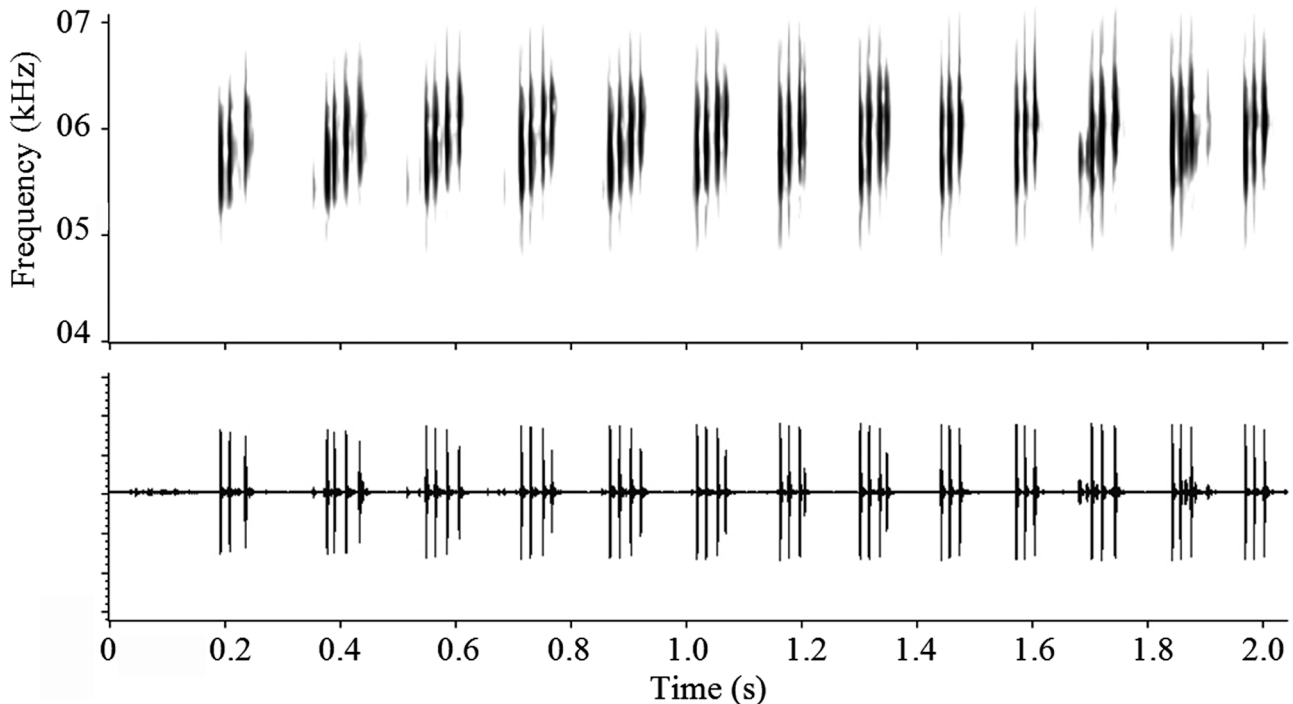


FIGURE 4. Waveform (below) and spectrogram (above) of the first two seconds of the advertisement call of *Pseudopaludicola serrana* sp. nov. recorded at type locality: Serra da Moeda, Minas Gerais.

TABLE 2. Physical characteristics of the advertisement calls of *Pseudopaludicola saltica* (n = 12 notes/ 1 call/ 1 male; air temperature 24°C), *P. murundu* (n = 14 notes/ 2 calls/ 2 males; air temperature = 25.5°C), and *P. serrana* sp. nov. (n = 15 notes/ 3 calls/ 3 males; air temperature = 25°C); all recorded at their type localities and analyzed for the present study. Values presented as mean ± standard deviation (range).

Species	Frequency (kHz)			Duration (ms)	
	Dominant	Minimum	Maximum	Note	Pulse
<i>P. saltica</i>	4.60 ± 0.18 (4.39–4.91)	3.05 ± 0.24 (2.52–3.34)	6.35 ± 0.35 (5.92–6.93)	76.33 ± 18.59 (31.0–97.0)	33.0 ± 4.43 (24.0–38.0)
<i>P. murundu</i>	5.72 ± 0.32 (5.17–6.37)	5.03 ± 0.20 (4.71–5.33)	6.82 ± 0.26 (6.44–7.25)	98.86 ± 8.57 (87.0–112.0)	11.21 ± 2.29 (6.0–14.0)
<i>P. serrana</i>	5.75 ± 0.11 (5.51–6.03)	3.56 ± 0.44 (3.12–4.50)	7.98 ± 0.41 (7.01–8.49)	85.33 ± 22.00 (25.0–115.0)	18.87 ± 3.04 (13.0–23.0)

continued.

Species	Interval between (ms)		Pulses / note (n)
	Notes	Pulses	
<i>P. saltica</i>	99.33 ± 15.99 (80.0–140.0)	19.25 ± 10.48 (0.0–32.0)	2.08 ± 0.51 (1–3)
<i>P. murundu</i>	92.50 ± 26.0 (31.0–132.0)	3.36 ± 4.41 (0.0–12.0)	4.93 ± 0.70 (3–6)
<i>P. serrana</i>	177.13 ± 64.00 (96.0–283.0)	11.53 ± 7.33 (0.0–26.0)	3.07 ± 0.80 (1–4)

Geographic distribution. The new species is known from three localities at the Espinhaço mountain range: Serra do Cipó, municipality of Santana do Riacho (approximately 19°08' - 43°40'; 1071 m; ZUEC 2323), Serra da Moeda, municipality of Brumadinho (type locality), and Serra do Lenheiro, municipality of São João del Rei (approximately 21°08' - 44°17'; 1081 m; ZUEC 16445–53; 16455–56), all higher than 1,000 m above sea level and in the state of Minas Gerais, Southeastern Brazil (Figure 3).

Etymology. The specific epithet is the feminine form from the Portuguese adjective “serrano” that means “the one who lives in the mountains”.

Description of the advertisement call. The advertisement call of *Pseudopaludicola serrana* is composed of groups of pulsed notes (with one to four pulses) that are emitted in mean intervals of 0.18 s. Calls are generally long, varying from about 14 to 45 seconds in the analyzed series. Calls are high pitched, with frequencies varying between about 3.0 and 8.5 kHz. The mean rate of notes emitted per minute is 227. The pulses within a note generally present an ascending modulation, i.e., the first pulse presents lower frequencies than the second, which, in turn, presents lower frequencies than the third, and so on (Figure 4). Further information of the physical characteristics of the advertisement call is in Table 2. Comparisons with other species are presented in table 3.

TABLE 3. Statistical outputs of the comparisons between physical characteristics of advertisement call of the species of *Pseudopaludicola* of the group of *P. saltica*, made by means of Kruskal-Wallis tests, followed by Mann-Whitney comparisons (Bonferroni corrected \ uncorrected). Bold values indicate significant differences ($P < 0.05$).

Pulse duration			
	<i>P. murundu</i>	<i>P. saltica</i>	<i>P. serrana</i>
<i>P. murundu</i>			0.00008
<i>P. saltica</i>	0.00005		0.00005
<i>P. serrana</i>	0.00023	0.00015	
Interval between notes			
	<i>P. murundu</i>	<i>P. saltica</i>	<i>P. serrana</i>
<i>P. murundu</i>		0.66200	0.00023
<i>P. saltica</i>	1.00000		0.00040
<i>P. serrana</i>	0.00068	0.00121	
Dominant frequency			
	<i>P. murundu</i>	<i>P. saltica</i>	<i>P. serrana</i>
<i>P. murundu</i>			0.21200
<i>P. saltica</i>	0.00026		0.00001
<i>P. serrana</i>	0.63600	0.00004	
Interval between pulses			
	<i>P. murundu</i>	<i>P. saltica</i>	<i>P. serrana</i>
<i>P. murundu</i>			0.00209
<i>P. saltica</i>	0.00327		0.03379
<i>P. serrana</i>	0.00628	0.10140	

Tadpoles. Larvae were obtained from eggs laid in plastic bags after collection of adult specimens in type locality. The following description is based on 16 tadpoles (ZUEC 16457) in developmental stages 25 (Gosner, 1960). Body elliptic in dorsal and ventral views (Figure 5), depressed/globular with flattened venter; body wider than high; snout rounded; eyes medium-sized, dorsolateral; nostrils dorsal, small, and oval; nostrils closer to the eyes than to the tip of snout; spiracle sinistral, its opening in the middle of body; cloacal tube large, medial; caudal musculature robust; dorsal fin originating on the anterior part of the tail; dorsal fin wider than ventral fin (Figure 5B). Oral disc directed ventrally, emarginated, and bordered by one row of marginal papillae, interrupted along a large area on the anterior labium; tooth row formula 2(2)/2(1); upper and lower jaw sheathes strongly developed and serrate. Some of the tadpoles present unkeratinized

mouthparts, probably due to *Batrachochytrium dendrobatidis* infection, acquired in the laboratory, as individuals collected in the same locality are apparently uninfected. In preservative and in life, dorsum pale brown; throat and belly transparent; caudal musculature with very scattered pale brown pigmentation; fins transparent with few scattered pale brown pigmentation (Figure 5). Lateral lines were not observed.

Three tadpoles at Gosner's stage 25 measured [mean \pm SD (range); size in mm] total length 9.34 ± 1.12 (8.05–10.05); body length 4.38 ± 0.56 (3.73–4.71); tail length 4.96 ± 0.56 (4.32–5.35); maximum body height 1.83 ± 0.31 (1.50–2.10); maximum body width 2.53 ± 0.39 (2.11–2.89); tail maximum width 1.61 ± 0.67 (1.00–2.32); internarial distance 0.48 ± 0.13 (0.34–0.60); interorbital distance 0.57 ± 0.07 (0.49–0.63); eye-nostril distance 0.31 ± 0.08 (0.26–0.40); eye diameter 0.46 ± 0.06 (0.40–0.56).

TABLE 4. Selected diagnostic characters of the species of the *Pseudopaludicola saltica* species group. See text for exact values and complementary descriptions.

Species	Size Male SVL	Morphology		Coloration Vocal sac	Vocalization	
		Nuptial pads	Head length proportion		Dominant frequency	Pulse duration
<i>P. murundu</i>	14–16 mm	Clearer	Long head	Dark	Above 5 kHz	06–14 ms
<i>P. saltica</i>	15–18 mm	Darker	Long head	Light	Below 5 kHz	24–38 ms
<i>P. serrana</i>	14–16 mm	Clearer	Short head	Dark	Above 5 kHz	13–23 ms

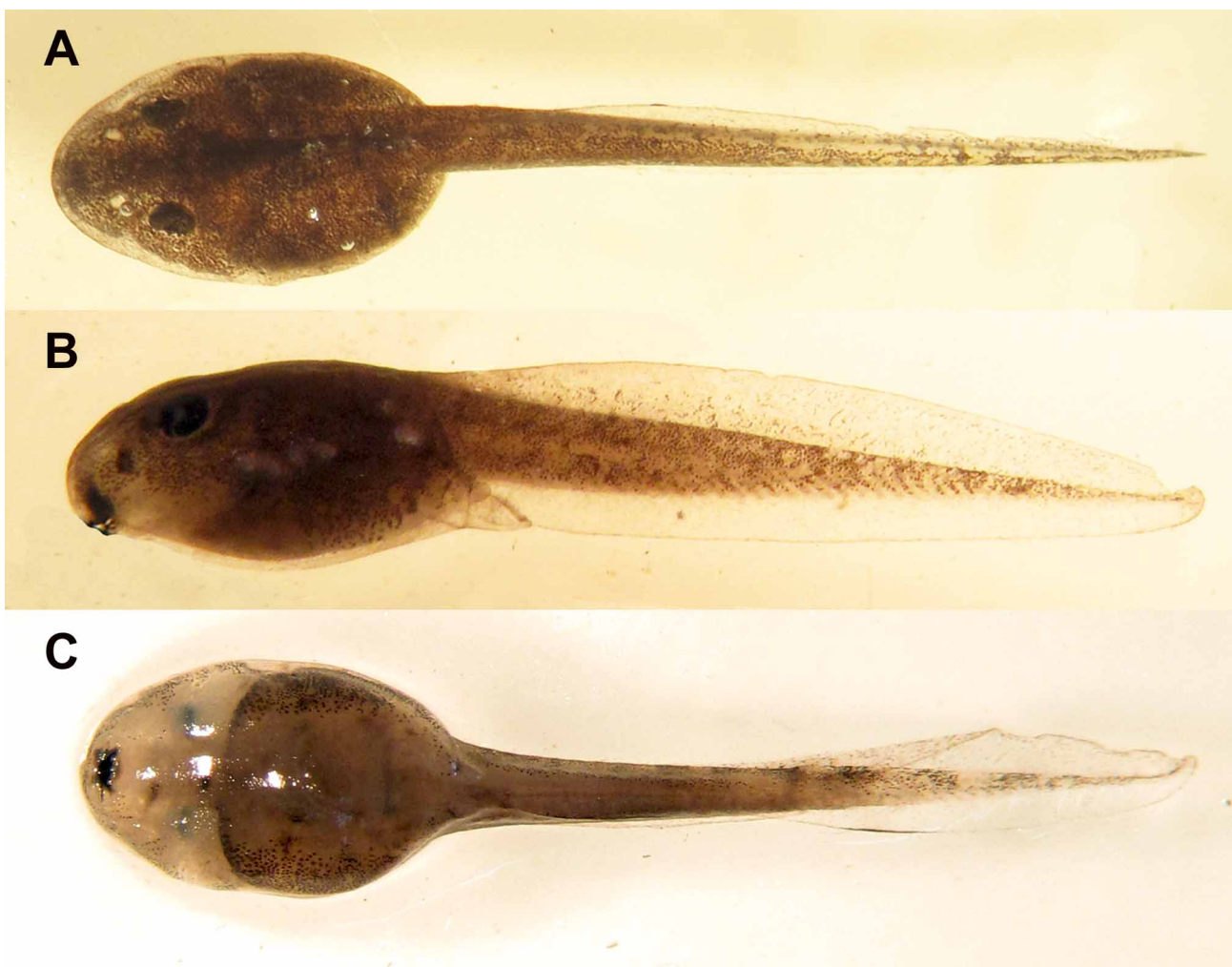


FIGURE 5. Tadpole of *Pseudopaludicola serrana* sp. nov. at Gosner's stage 25 (ZUEC 16457) in dorsal (A), lateral (B), and ventral (C) views.

Discussion

As commented in a previous work (Toledo et al., 2010), and based on my personal observations of material deposited in museums I assume that there are many species in the genus *Pseudopaludicola* in Brazil still waiting to be described. Furthermore, the genus clearly needs a striking taxonomic review. The use of bioacoustical, cytogenetic, and molecular analyses will facilitate the identification of several still unnamed species.

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