Zootaxa 2496: 38–48 (2010)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

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

We describe here a new species of *Pseudopaludicola* (*P. murundu* sp. nov.) that is found in the central portion of the state of São Paulo, Brazil. This new species is related to *P. saltica*, from which it distinguishes by the presence of a single, dark, and subgular vocal sac with dark longitudinal folds in males, immaculate to light brown nuptial pads in males that cover the external part of the finger I, from the wrist to the second phalanx, and 11 pairs of chromosomes without heteromorphic sex chromosomes. From all other species of the genus it distinguishes by presenting very long hindlimbs, with tibio-tarsal articulation reaching beyond the end of the snout, and absence of T-shaped terminal phalanges. Tadpoles, advertisement call, and the karyotype are described as well.

Key words: Amphibia, *Pseudopaludicola*, new species, tadpole, advertisement call, karyotype

Resumo

No presente estudo é descrita uma nova species de *Pseudopaludicola* (*P. murundu*), encontrada no interior do estado de São Paulo, Brasil. Esta nova espécie é relacionada com *P. saltica*, da qual se distingue por possuir saco vocal subgular simples, escuro e com pregas longitudinais nos machos, calos sexuais de imaculados a marrom claro nos machos, os quais cobrem a porção externa do dedo I, do pulso até a segunda falange, e pela presença de 11 pares de cromossomos sem cromossomos sexuais heteromórficos. Das demais espécies do gênero se distingue por possuir pernas proporcionalmente mais longas, com a articulação tíbio-tarsial ultrapassando o focinho e pela ausência de falanges terminais em forma de T. Os girinos, canto de anúncio e cariótipo também são descritos.

Palavras chave: Amphibia, *Pseudopaludicola*, descrição de espécies, girino, canto de anúncio, cariótipo

Introduction

In the genus *Pseudopaludicola* Miranda-Ribeiro, 1926 twelve valid species are currently recognised (Frost 2009). These species are always small, with snout-vent lengths generally not exceeding 20 mm, and, with few exceptions, reproducing in slow-flowing clean water in open areas, where the species lay clutches containing

 Accepted by M. Vences: 16 Apr. 2010; published: 7 Jun. 2010

several eggs that are distributed isolated on the bottom of ponds (see further details in Giaretta & Facure 2009).

Lynch (1989) proposed two sub-groups for the genus: *P. falcipes* (Hensel, 1867) and *P. pusilla* (Ruthven, 1916) groups. In Brazil there are three species of the *P. pusilla* group (*P. boliviana* Parker, 1927, *P. canga* Giaretta and Kokubum, 2003 and *P. ceratophryes* Rivero and Serna, 1985), four species of the *P. falcipes* group (*P. falcipes*, *P. mystacalis* (Cope, 1887), *P. saltica* (Cope, 1887), and *P. ternetzi* Miranda-Ribeiro, 1937), and two species that were not assigned to any group (*P. mineira* Lobo, 1994 and *P. riopiedadensis* Mercadal de Barrio and Barrio, 1994). Among the populations assigned as *P. saltica* there may be other new species. Herein we describe a new species closely related to *Pseudopaludicola saltica* from the state of São Paulo, Southeastern Brazil.

Material and methods

Voucher specimens and description

Adults and tadpoles are deposited in the Célio F. B. Haddad amphibian collection (CFBH) at the Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil and 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) for 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) and Duellman (2001). Webbing formula follows Myers and Duellman (1982). For the acquisition of the morphometric data we used a digital measurement procedure using the software Image Pro-plus 4.5.1. Line drawings of the holotype and of a tadpole were made in a Zeiss stereomicroscope SV11 with a drawing tube.

Male vocalizations were recorded with a Marantz cassette tape recorder (PMD222), equipped with an external directional microphone (Audiotecnica AT835b) positioned ca. 50 cm from the calling male. We used chrome cassette tapes at 4.75 cm/s. We analyzed the calls using Raven 1.2 software (16 bits of resolution, 44 kHz of frequency sampling, and FFT and frame length of 256 samples). The terminology for the bioacoustic analyses follows that of Toledo & Haddad (2005).

The terminology for the morphological descriptions of the tadpoles follows Altig & McDiarmid (1999) and Lannoo (1999). The following measurements of tadpoles were taken according to Altig & McDiarmid (1999): total and body length. The following additional measurements were taken according to Eterovick and Brandão (2001): maximum body height, maximum body width, internarial distance, interorbital distance, eye–nostril distance, and eye diameter. Measurements were taken in millimeters using a digital caliper to the nearest 0.05 mm under a Zeiss stereomicroscope SV11 for total length and body length; other measurements were taken under a Zeiss stereomicroscope SV11 with an ocular micrometer. Larvae were obtained with a sieve. Developmental stages of tadpoles were determined according to Gosner (1960). Tooth row formula notation of tadpoles follows Altig (1970).

Chromosomal analysis

The analyzed specimens were obtained with permission of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA – permit number 02001.002003/2005-16). Four males and one female from Rio Claro, São Paulo, Brazil were analyzed. The voucher specimens were deposited in the ZUEC under the numbers ZUEC 14284–88.

Mitotic and meiotic metaphases were obtained from cell suspensions of intestinal epithelium and testes respectively of animals previously treated with 2 % colchicine for 5 h, according to King & Rofe (1976) and Schmid (1978) with few modifications. Chromosomes were stained with 10% Giemsa or submitted to the (Ag-NOR) silver staining (Howell & Black 1980) and C-banding (Sumner 1972) techniques, with a modification in the pre-treatment in which the samples were pre-treated with 50% acetic acid, according to Siqueira *et al.* (2008). The samples were examined under a photomicroscope Olympus BX60, and images

were captured using QCapture 2.81.0 and Image Pro-plus 4.5. The chromosomes were measured and classified according to Green & Sessions (1991).

Results

Pseudopaludicola murundu sp. nov.

Holotype. CFBH 8241, an adult male (Fig. 1) collected in a temporary pond in the "Sítio do Carlão", district of Itapé, Municipality of Rio Claro, state of São Paulo, southeastern Brazil (22°20'41.57"S; 47°40'1.52"W; approximately 600 m above sea level), by L. F. Toledo, J. G. R. Giovanelli, and F. B. Britto, on 09 January 2005.

Paratopotypes. Eleven males: CFBH 8235-40; 8242, collected on 09 January 2005 by L. F. Toledo, J. G. R. Giovanelli, and F. B. Britto; ZUEC 14286-87; 14289-90, collected on 31 January 2007 by L. F. Toledo, S. Siqueira, and E. F. Favero; and four females: CFBH 8243, collected on 09 January 2005 by L. F. Toledo, J. G. R. Giovanelli, and F. B. Britto; ZUEC 14284-85, 14288, collected on 31 January 2007 by L. F. Toledo, S. Siqueira, and E. F. Favero.

Diagnosis. *Pseudopaludicola murundu* is assigned to the genus *Pseudopaludicola* due to the presence of hypertrophied antebrachial tubercle (see Lynch, 1989). *Pseudopaludicola murundu* is a medium-sized species for the genus and is characterized by the following combination of characters: (1) single, dark, and subgular vocal sac with dark longitudinal folds in males; (2) immaculate to light brown, nuptial pads in males that cover the external part of finger I, from the wrist to the second phalanx; and (3) presence of 11 pairs of chromosomes without heteromorphic sex chromosomes (XX/XY).

Comparison with other species. *Pseudopaludicola murundu* is distinguished from all other species of the genus (except from *P. saltica*) by having very long hindlimbs, with tibio-tarsal articulation reaching beyond the end of the snout (in other species, except *P. saltica*, the tibio-tarsal articulation reach, at most, the nostrils). From species of the *P. pusilla* species group it is also distinguished by the absence of T-shaped terminal phalanges. From *P. saltica* it is distinguished by having the nostrils closer to the apical snout region (in *P. saltica* this distance is larger: mean snout-nostril distance = 1.1 ± 0.25 in males of *P. murundu* and 1.35 ± 0.21 in males of *P. saltica*), larger and clearer nuptial pads in adult males collected during peak of the breeding season (smaller and darker in adult males *P. saltica* collected during peak of the breeding season), longitudinal folds in the vocal sac (absent in *P. saltica*), and a darker vocal sac. Furthermore, it can be distinguished from *P. saltica* by having the chromosome pair 8 homomorphic between females and males, whereas in *P. saltica* this chromosome pair is heteromorphic in males, which can be considered a XX/XY sex chromosome system (Duarte *et al.*, 2010). In addition, the sequence divergence computed with MEGA Ver. 4.0 (Tamura et al. 2007) based on mitochondrial DNA (a fragment of Cyt-b) showed a higher degree of nucleotide divergence between *P. murundu* and *P. saltica* (8%) when compared to other close related species, P. ameghini – sensu Cope, 1887 and P. ternetzi (5%) (Veiga-Menoncello et al. unpublished data).

FIGURE 1. Adult male holotype of *Pseudopaludicola murundu* sp. nov. (CFBH 8241) in life.

Description of the holotype. Body elliptic and broad. Head triangular, longer than wide. 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 larger than 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<II<IV<III. Toe length I<II<V<III<IV. Finger and toe tips without disks (Figure 2C, 2D). Thumb with keratinized 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 very robust and long. Thigh shorter than tibia; foot slightly 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 with scattered tubercles. Cloacal region granular. Measurements of the holotype are presented in table 1.

TABLE 1. Measurements of the type series of *Pseudopaludicola murundu* sp. nov. Values presented in millimeters as mean ± standard deviation (range).

	Holotype	Paratypes	
Measurement	Male	Males (N = 11)	Females (N = 4)
snout-vent length	13.7	14.7 ± 0.4 (14.0–15.4)	15.0 ± 1.0 (14.1–15.9)
head length	5.5	5.7 ± 0.3 (5.1–6.1)	6.0 ± 0.4 (5.6–6.5)
head width	5.2	5.5 ± 0.3 (5.1–5.8)	5.3 ± 0.2 (5.1–5.6)
eye diameter	1.6	1.8 ± 0.3 (1.5–2.2)	1.9 ± 0.1 (1.8–2.1)
interorbital distance	1.3	1.4 ± 0.2 (1.0–1.7)	1.2 ± 0.2 (1.0–1.4)
internarial distance	1.4	1.5 ± 0.2 (1.4–1.9)	1.5 ± 0.1 (1.3–1.6)
thigh length	8.1	8.6 ± 0.4 (7.9–9.2)	8.5 ± 0.4 (8.0–8.9)
tibia length	9.5	9.9 ± 0.6 (8.8–10.6)	10.2 ± 0.6 (9.7–11.0)
foot length	8.9	9.2 ± 0.6 (7.4–9.8)	9.0 ± 0.7 (8.2–9.6)

In life, dorsum and limbs are brown with dark brown spots or stripes; vertebral line yellowish brown from the snout to vent; belly white; ventral view of legs yellowish white and vocal sac beige with dark dots; iris golden superiorly and brownish inferiorly (see Figure 1). In preservative, dorsum and dorsal part of limbs brown with dark brown spots or stripes; vertebral line beige from the snout to vent; belly light beige; legs yellowish white; vocal sac beige with dark dots and with dark longitudinal folds.

Variation. Females slightly larger than males, lacking vocal sac and nuptial pads (present in males). The nuptial pad can be brownish to light cream color in males. The snout shape in lateral view varies from rounded to slightly truncate. In 75% of the individuals (12 out of 16 examined) the vertebral line is absent. In life this line can be white, yellow, or red. In life, the two arc shaped granular folds on the scapula region are generally

darker than the general coloration of dorsum (see also Figure 1). Dorsal pigmentation varies among individuals, from brown to grayish. The number and form of the dorsal spots also varies, which can be small scattered brown dots up to large brown stripes over a lighter general dorsal coloration.

Natural history notes. Males were observed calling in open areas of a private farm. The region was previously a mosaic composed of Cerrado (Brazilian savannah) and semidesciduous Atlantic forest. Males called from the ground near shallow (about 2–3 cm of depth) slow-flowing water bodies, on the "murundus", which are small elevations on the ground (about 10 cm height), covered by grassy and/or arboreal vegetation. Calling males were spaced for at least 5 cm in distance, and it was possible to observe silent males even closer to the calling ones (however, we could not confirm if these males were performing satellite behavior). Males were observed calling at dawn and during the first hours of the night. One amplectant pair was observed and collected in the same microhabitat of the calling site. Egg laying was not observed, so we could not attribute any reproductive mode to *P. murundu*. Tadpoles were active at night in the shallow and slow-flowing water bodies, where males and the amplectant pair were observed. No other species was observed in syntopy.

Geographic distribution. The new species is known only for the type locality, which is an open grassland field of anthropogene origin used for cattle ranching in a particular farm.

Etymology. The specific name is a Tupi word that means small elevations on the ground, covered by grassy and/or arboreal vegetation. This is the most common calling site of this species.

Advertisement call. The advertisement call of *Pseudopaludicola murundu* is composed of groups of notes (each with three to six pulses) that are emitted in mean intervals of 92 ms (Figure 3A and 3B). The calls are generally long, with more than 10 seconds of duration (Figure 3A) and high pitched, with most of the frequencies higher than 5 kHz. The mean rate of notes emitted per minute is 225. Males can call in antiphony as the interval between notes are similar to the duration of notes. The first pulse of the note generally occupies lower frequencies than the last one. The intermediary pulses occupy the intermediary frequencies between the first and the last pulses. Further information of the numerical characteristics of the advertisement call is provided in Table 2.

TABLE 2. Physical characteristics of the advertisement calls of two males of *Pseudopaludicola murundu* sp. nov. recorded at the district of Itapé, municipality of Rio Claro, state of São Paulo, Brazil. Air temperature 25.5°C. Values presented as mean ± standard deviation (range).

Parameter	Values
Duration of the call (s) (n = 10)	12.79 ± 5.71 (4.55 – 24.85)
Duration of the note (s) (n = 14)	0.10 ± 0.01 (0.09 – 0.11)
Duration of the pulses (ms) (n = 14)	11.21 ± 2.29 (6.00 – 14.00)
Dominant frequency (kHz) (n = 10)	5.72 ± 0.32 (5.17 – 6.37)
Minimum frequency (kHz) (n = 10)	5.03 ± 0.20 (4.71 – 5.33)
Maximum frequency(kHz) (n = 10)	6.82 ± 0.26 (6.44 – 7.25)
Notes per call (n = 14)	45.60 ± 27.39 (15 – 82)
Pulses per note (n = 16)	5.07 ± 0.49 (3 – 6)
Interval between notes of the same call (s) (n = 14)	0.09 ± 0.03 (0.03 – 0.13)
Interval between pulses of the same note (ms) (n = 14)	3.36 ± 4.41 (00 – 12)

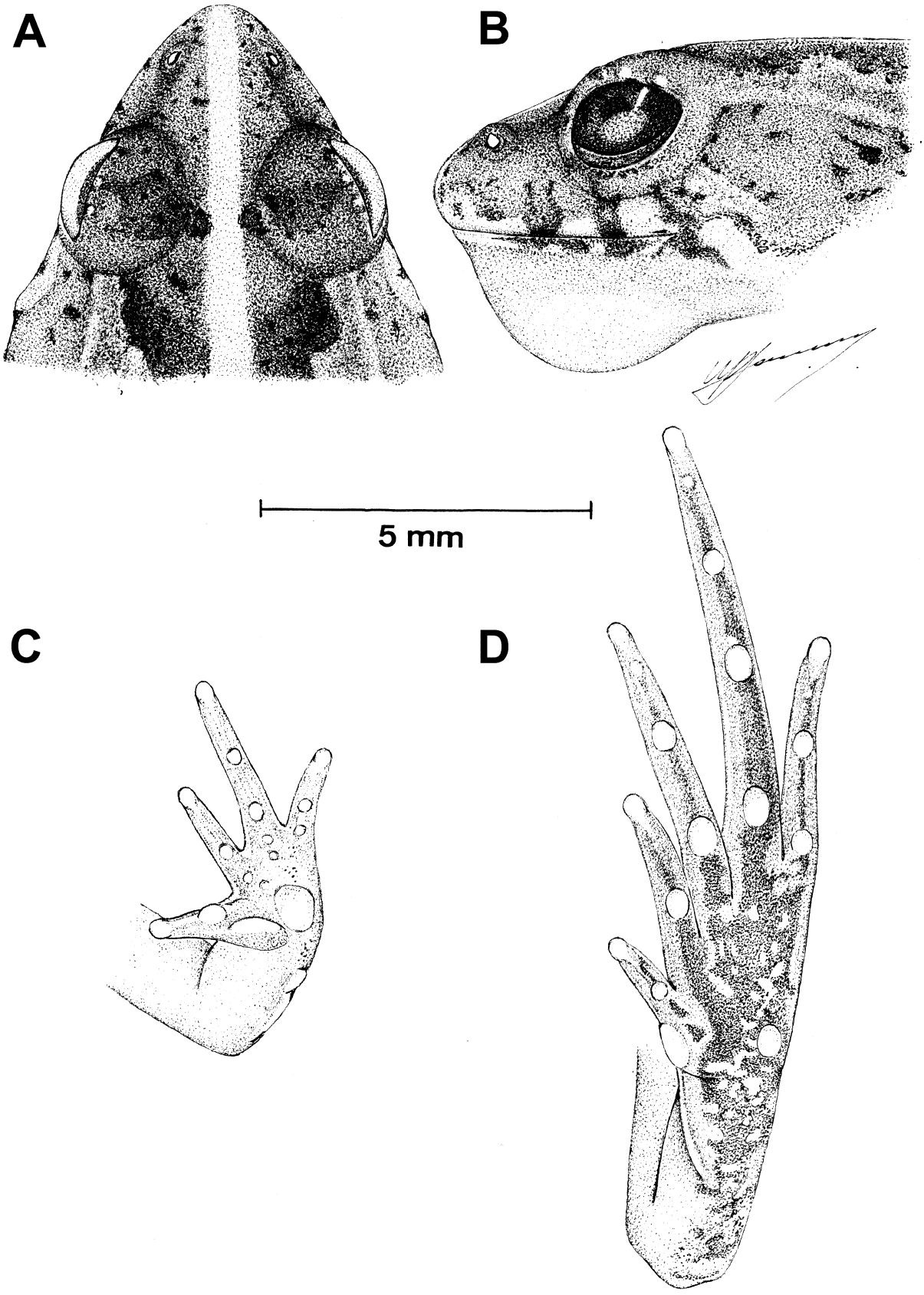

FIGURE 2. Drawings of the holotype of *Pseudopaludicola murundu* sp. nov. (CFBH 8241). Dorsal (A) and lateral (B) views of the head; ventral views of hand (C) and foot (D).

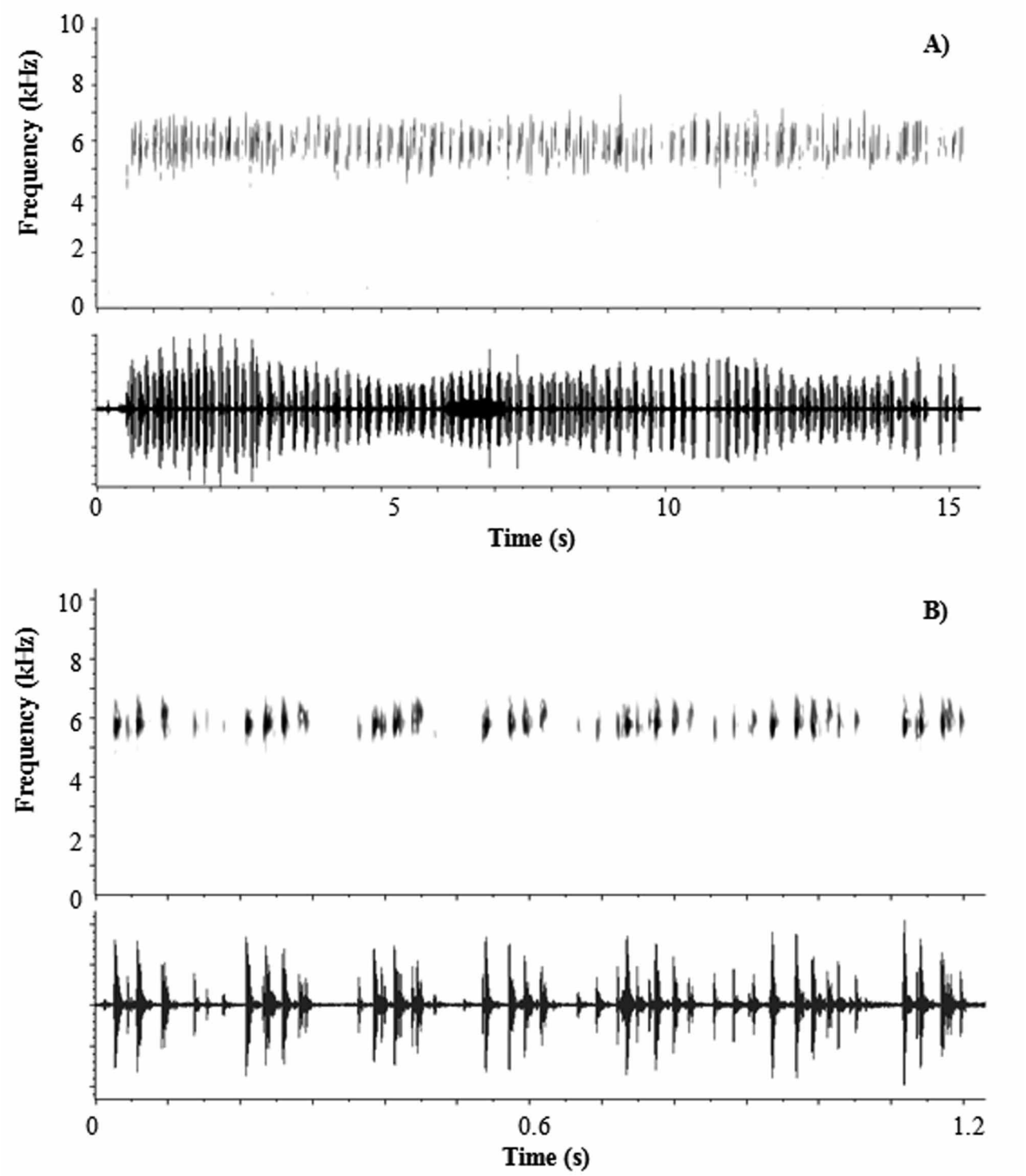

FIGURE 3. Waveform (below) and spectrogram (above) of one advertisement call (A) and detail of seven notes (B) of the advertisement call of *Pseudopaludicola murundu* sp. nov., recorded at the type locality. Air temperature 25.5°C.

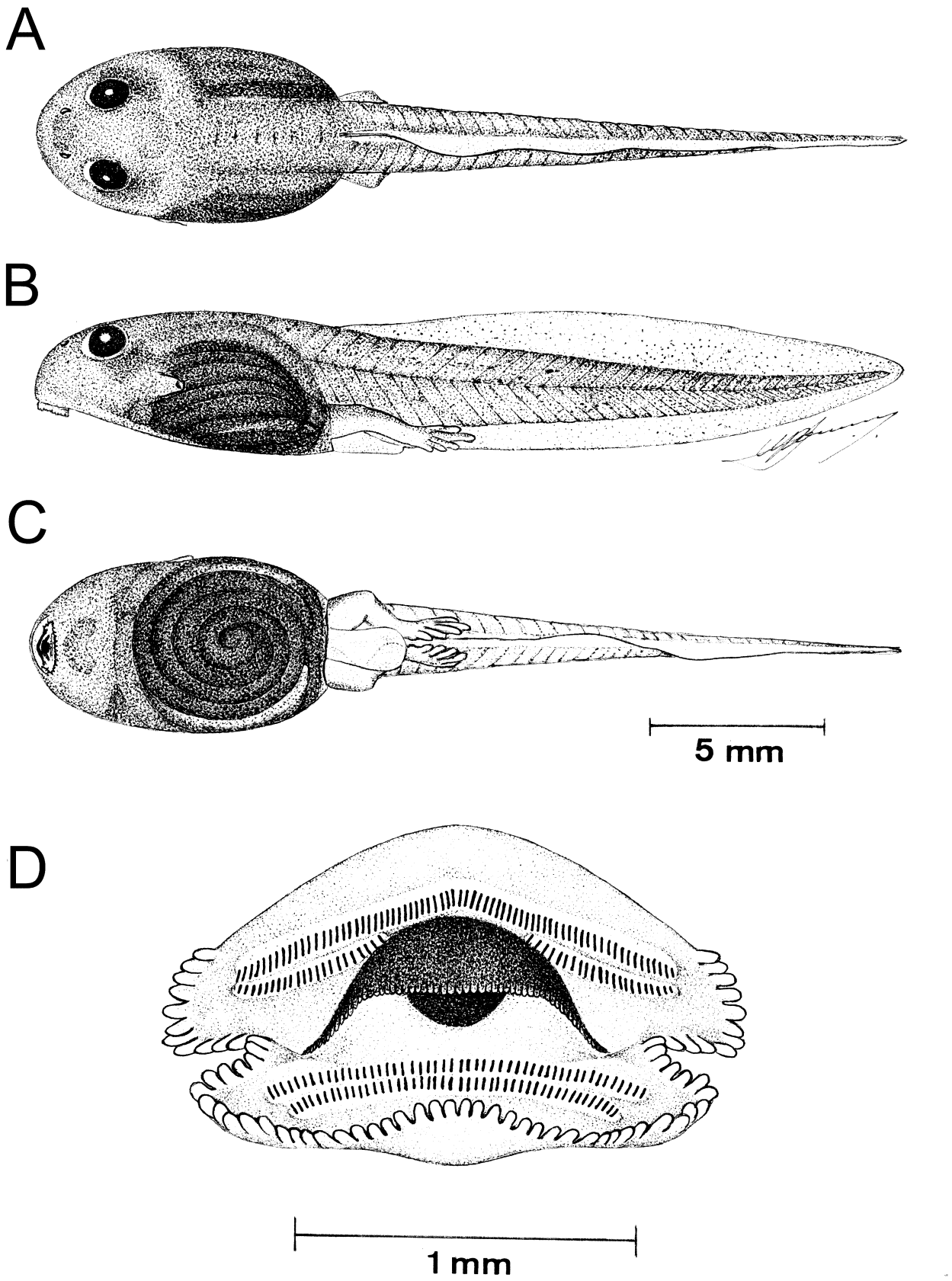

FIGURE 4. Drawings of the tadpole of *Pseudopaludicola murundu* sp. nov. (CFBH 22670). Dorsal view (A), lateral view (B), ventral view (C), and mouth parts (D).

Tadpoles. Larvae were obtained in shallow ponds at the type locality. As there was no other amphibian species in syntopy with *P. murundu* we assigned these tadpoles to this species (this tadpole confirm with general morphology of *Pseudopaludicola* tadpoles), however this identification is preliminary and not verified. The following description is based on seven tadpoles (CFBH 22670) in developmental stages 37-39 (Gosner 1960). Body elliptical in dorsal and ventral views (Figure 4A,C), depressed/globular with flattened venter (Figure 4B); body wider than high; snout rounded; eyes medium-sized, dorsolateral; nostrils dorsal, small, and reniform; 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. Oral disc directed ventrally, emarginated, and bordered by one row of well developed marginal papillae, interrupted along a large area on the anterior labium; tooth row formula 2(2)/2 or 2(2)/2(1); upper and lower jaw sheathes strongly developed and serrate (Figure 4D). In preservative, dorsum pale brown; throat and belly transparent; caudal musculature with very scattered pale brown pigmentation; fins transparent with scattered pale brown pigmentation.

Three tadpoles in developmental stage 37 measured (range, average, SD) total length 24.79–27.10 (25.65, 1.26); body length 8.05–8.90 (8.41, 0.44); maximum body height 3.67–4.36 (3.97, 0.36); maximum body width 5.02–5.68 (5.27, 0.36); internarial distance 1.24–1.27 (1.26, 0.02); interorbital distance 1.05–1.06 (1.06, 0.01); eye-nostril distance 0.39–0.49 (0.45, 0.05); eye diameter 1.26–1.33 (1.28, 0.04).

Karyotype description. The karyotype of the *P. murundu* has 2n = 22 chromosomes. Pairs 1, 2, 5, 7, 9, 10, and 11 are metacentrics, pairs 3, 4, and 6 submetacentrics, and pair 8 telocentric (Figure 5A–C). Besides the centromeric heterochromatic blocks, positive C-bands were detected in the interstitial regions on the long arms of the pairs 1, 2, 3, 4, and 6, and on the short arms of the pairs 1 and 2 (Figure 5B). The nucleolar organizing region (NOR) was detected by silver staining (Ag-NOR) in the telomeric region on the long arm of the chromosome 8 (Figure 5C; Table 3. The homologous of the pair 8, telocentric in both sexes, showed a notable size heteromorphism, and the Ag-NOR site was observed only in the larger homologous of this pair (Figure 5C) which were identified as 8 and 8’ (Table 3).

TABLE 3. Morphometric analysis of the chromosomes in the studied *Pseudopaludicola murundu* sp. nov. Classification according to Green & Sessions (1991). M = metacentric; SM = submetacentric; T = telocentric.

	Number of chromosomes											
	1	2	3	4	5	6	7	8	8’	9	10	11
Relative Size	17.23	14.08	12.40	11.19	8.60	7.62	7.24	4.59	3.01	4.99	4.61	4.39
Arm ratio	1.12	1.44	2.57	2.34	1.19	1.80	1.11	9.09	8.42	1.19	1.16	1.13
Classification	M	M	SM	SM	M	SM	M	T	T	M	M	M

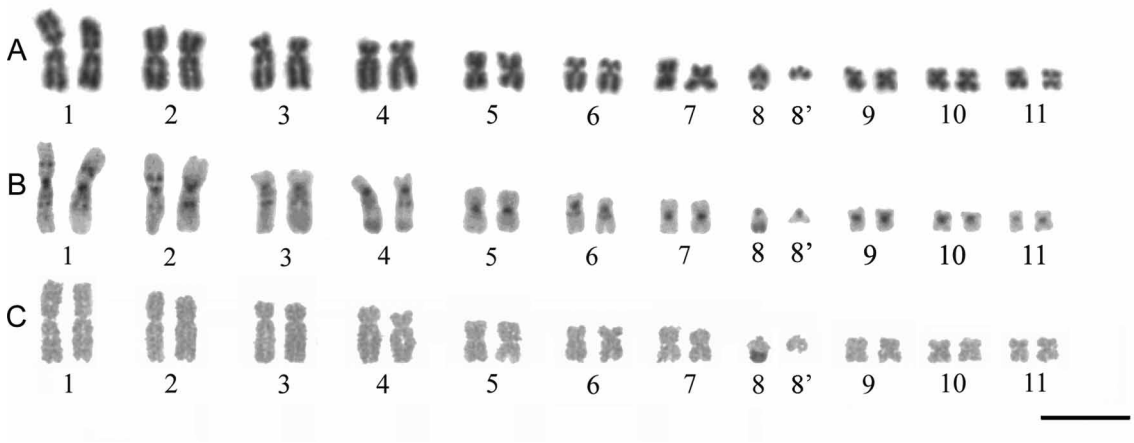

FIGURE 5. Karyotype of *Pseudopaludicola murundu* sp. nov. stained with Giemsa (A) and submitted to C-banding (B) and Ag-NOR (C) techniques. Bar = 5 µm.

Discussion

Except for the chromosomal pair 8, which consisted of telocentric homologous in both male and female specimens, the karyotype of *Pseudopaludicola murundu* was quite similar to that of *P. saltica* from the type locality (Chapada dos Guimarães, Mato Grosso state, Brazil) and from Uberlândia, Minas Gerais state, Brazil (Duarte *et al.*, 2010). In *P. saltica*, the morphological differentiation of the pair 8 between males and females evidenced a XX/XY sex-determination system (Table 4).

TABLE 4. Comparative summary of the karyotypes of *Pseudopaludicola murundu* sp. nov. and *P. saltica* (topotype). M = metacentric; SM = submetacentric; T = telocentric.

Species		Chromosome pairs				NOR in pair 8	C-band
		6	7	8	11		
P. murundu		SM/SM	M/ M	T/ T	M/ M	Telomeric in only one homologous	1, 2, 3, 4, 6, 8
P. saltica	♂	SM/SM	M/ M	T/SM	M/ M	Telomeric	1, 2, 3, 4, 6, 8
	♀	SM/SM	M/M	T/T	M/M	Telomeric	1, 2, 3, 4, 6, 8

Although the karyotypes of *Pseudopaludicola saltica* and *P. murundu* are highly similar regarding the chromosomal number, morphology, NOR position, and heterochromatin distribution pattern, the chromosome differentiation in the pair 8 suggested that *Pseudopaludicola murundu* is a distinct species, yet closely related to *P. saltica*, since the chromosome X of *P. saltica* has similar morphology to the pair 8 of *P. murundu*. The differentiation of the pair 8 might be one of the characters directly involved in speciation within *Pseudopaludicola*, since the NOR is also localized in this same chromosome pair in other species with 2n=22 in this genus, as for instance *P. mineira* and *Pseudopaludicola* sp. 1 (Duarte *et al.*, 2010), and *P. falcipes* and *Pseudopaludicola* sp. (Fávero, 2008).

The advertisement call of *P. murundu* is similar to that presented for the *P. saltica* population from municipality of Campinas, state of São Paulo, studied by Haddad & Cardoso (1987). Frequencies, number of pulses per note, interval between notes, and the increase of frequencies among the pulses of the same note are similar. There are differences in the interval between pulses (0 to 12 ms in *P. murundu* and 100 to 200 ms in *P. saltica* from Campinas), and in the number of notes per minute (mean of 225 in *P. murundu* and 400 in *P. saltica*) (see Haddad & Cardoso 1987). These differences are not explained by differences in the air temperature during the recordings, which were 24° C in Haddad & Cardoso (1987) and 25.5° C in the present study. The advertisement call of *P. saltica* from the type locality remains unknown, and we are not confident that the population of the municipality of Campinas is *P. saltica*.

It is possible that there are many undescribed species in the genus *Pseudopaludicola* in Brazil and it clearly needs taxonomic review, since several populations in the field and in the museum collections can not be assigned to any available name or are cryptic species on the basis of general external morphology (L. F. Toledo & C. F. B Haddad personal observation).

Acknowledgments

The authors are thankful to Jaime Somera for the line drawings, Fabio B. Britto, João G. R. Giovanelli, and Eduardo R. Fávero for helping during the field activities; Carlos A. T. De Lucca allowed the access to the private area where the species was found; Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, processes no. 2006/60055-0; 2008/50325-5; 2008/52847-9; 2008/50928-1), Fundação de Ensino de São Paulo (FUNDESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico for financial support.

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