Amphibia-Reptilia 28 (2007): 457-465

A new species of the genus *Physalaemus* Fitzinger, 1826 (Anura, Leiuperidae) from Southeastern Brazil

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Abstract. A new species of the genus *Physalaemus* from the Atlantic Rain Forest of the state of Minas Gerais is described. It belongs to *P. signifer* species group and is characterized by well-developed vocal sac, second finger with the same size of the fourth, granulate dorsal skin, large inguinal gland with black ocelli, presence of dorso-lateral folds, and advertisement call composed by harmonic notes, generally four, being the last shorter than the others. Information on tadpole external morphology are also presented.

Introduction

The neotropical genus *Physalaemus* Fitzinger currently contains 39 valid species (Nascimento et al., 2005; Pimenta et al., 2005) distributed from northern to southern South America, east of Andes. Among the seven species groups currently recognized (see Nascimento et al., 2005), the conspicuous *P. signifer* group is characterized by its small to moderate size (14.9-28.5 mm snout-vent length); slender body, smooth or rugose dorsal skin; absence of tarsal tubercle; metatarsal tubercles conical, without horned margins; presence of tarsal fold; small to large inguinal glands, associated to dark ocellus; and vocal sac well developed, except in *P. bokermanni*. This group is distributed along the Atlantic Rain Forest Domain (*sensu* Ab'Saber, 1977), from the State of Alagoas, in northeastern Brazil, to Rio Grande do Sul, in southern Brazil, and is currently composed of 11 species: *Physalaemus atlanticus* Haddad and Sazima, *P. bokermanni* Cardoso and Haddad, *P. caete* Pombal and Madureira, *P. camacan* Pimenta, Cruz, and Silvano, *P. crombiei* Heyer and Wolf, *P. maculiventris* (A. Lutz), *P. moreirae* (Miranda-Ribeiro), *P. nanus* (Boulenger), *P. obtectus* Bokermann, *P. signifer* (Girard), and *P. spiniger* (Miranda-Ribeiro) (Nascimento et al., 2005; Pimenta et al., 2005).

Herein, we describe a new species of the *P. signifer* group from the Atlantic Rain Forest of the State of Minas Gerais, southeastern Brazil. Advertisement call, tadpole morphology and some information on habitat are also provided.

Materials and methods

Comparisons of specimens of the new species with those of known species were based on observations of museum specimens and on literature information from Bokermann (1966), Caramaschi and Caramaschi (1991), Cardoso and Haddad (1985), Feio et al. (1999), Haddad and Pombal (1998), Haddad and Sazima (2004), Heyer and Wolf (1989), Heyer et al. (1990), and Pombal and Madureira (1997). Specimens used in the description or examined for comparisons are deposited in EI (Eugenio Izecksohn collection, housed in Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil), MCN (Museu de Ciências Naturais da Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, MG, Brazil), MNRJ (Museu Nacional, Rio de Janeiro, RJ, Brazil), MZUSP (Museu de Zoologia, Universidade de São Paulo, SP, Brazil), and ZUEC (Museu de História Natural, Universidade Estadual de Campinas, Brazil). Additional specimens examined are listed in the Appendix.

Abbreviations used for measurements of adult specimens are SVL (snout-vent length), HL (head length), HW (head

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width), ED (eye diameter), TD (tympanum diameter), END (eye-nostril distance), IND (internarial distance), IOD (interorbital distance), UEW (upper eyelid width), THL (thigh length), TBL (tibia length), and FL (foot length, including tarsus). All measurements are in millimetres and, except for FL, follow Duellman (1970). SVL, HL, HW, THL, TBL, and FL were measured with callipers, whereas other measurements were made with an ocular grid on a stereo dissecting microscope.

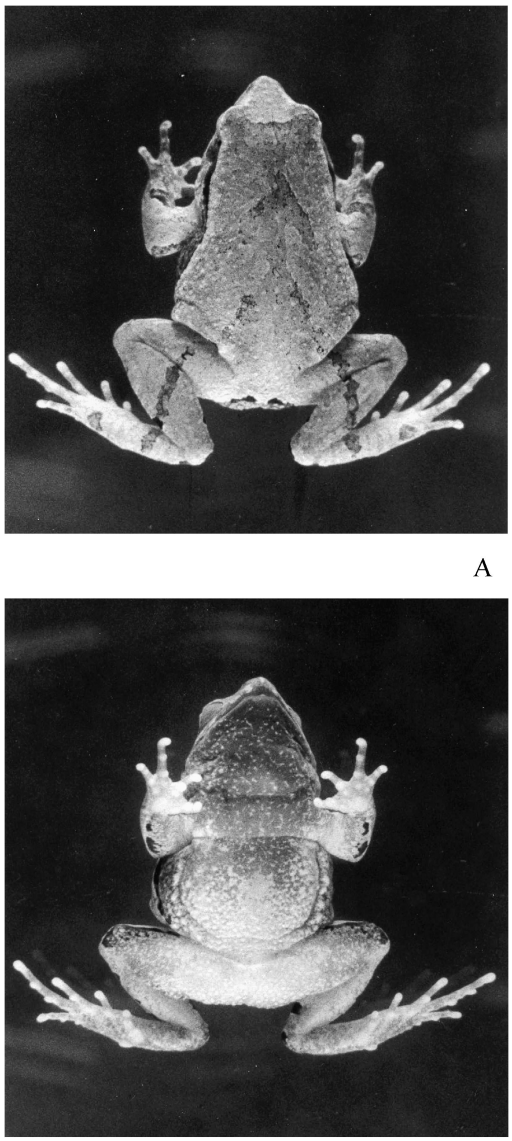
Tadpole description and measurements are based on 10 specimens in stage 36 (Gosner, 1960). Comparisons of the tadpole of the new species with those of known species were based on observations of museum specimens and on literature information from Bokermann (1963), Cardoso and Haddad (1985), Haddad and Sazima (2004), Haddad and Pombal (1998), Heyer and Wolf (1989), Heyer et al. (1990), Pombal and Madureira (1997), and Weber and Carvalho-e-Silva (2001). Nomenclature and measurements of tadpoles follow Altig and McDiarmid (1999), except for the interorbital and internarial distances, which were taken between the inner margins of eyes and nostrils, respectively. Measurements (in millimetres) were made using an ocular grid on a stereo dissecting microscope.

Vocalizations were recorded with a Panasonic RQ-L31 mini cassete recorder. Sonograms were produced with the software Avisoft-SASLab Light for Windows, version 3.74, using 16bit resolution, 16 kHz sampling frequency, and FFT with 256 points, and the waveform was produced with the software Sound Ruler, Acoustic Analysis, version 0.9.4.1. Terminology follows Duellman (1970). For comparison purposes, vocalizations of *Physalaemus obtectus* were obtained on 19 January 2004 (20.4°C air temperature), from the type locality, Reserva Biológica de Sooretama (18°33′ to 19°05′S, 39°55′ to 40°15′W), Municipalities of Sooretama, Jaguaré, and Vila Valério, State of Espírito Santo, Brazil.

Physalaemus irroratus sp. nov.
(figs 1-2)

Holotype: MNRJ 35124, adult male, collected at Fazenda Duas Barras (16°25′S, 40°03′W; 800 m above sea level), Municipality of Santa Maria do Salto, State of Minas Gerais, Brazil, by L.B. Nascimento, C.A.G. Cruz, R.N. Feio, P.L. Ferreira, M.G. Soares and D.P.R. Cabral, on 14-16 October 2003.

Paratypes: MNRJ 35125, adult male, collected with the holotype; MNRJ 35126, adult female and MNRJ 35127, adult male, collected at the type locality by L.B. Nascimento, D. Fernandes, M.G. Soares and D.P.R. Cabral, on 05-09 January 2004; MNRJ 40001, adult male, and MNRJ 40002, adult female, collected at the type locality, by L.B. Nascimento, C.A.G. Cruz, R.N. Feio, P.L. Ferreira, F. Leite and D.L. Pantoja, on 15 January 2005.



A

B

Figure 1. *Physalaemus irroratus* sp. nov., holotype (MNRJ 35124; SVL 21.6 mm), (A) dorsal and (B) ventral views.

Diagnosis: A species belonging to the *Physalaemus signifer* group, characterized by (1) small size (SVL males 20.5-22.5 mm, fe-

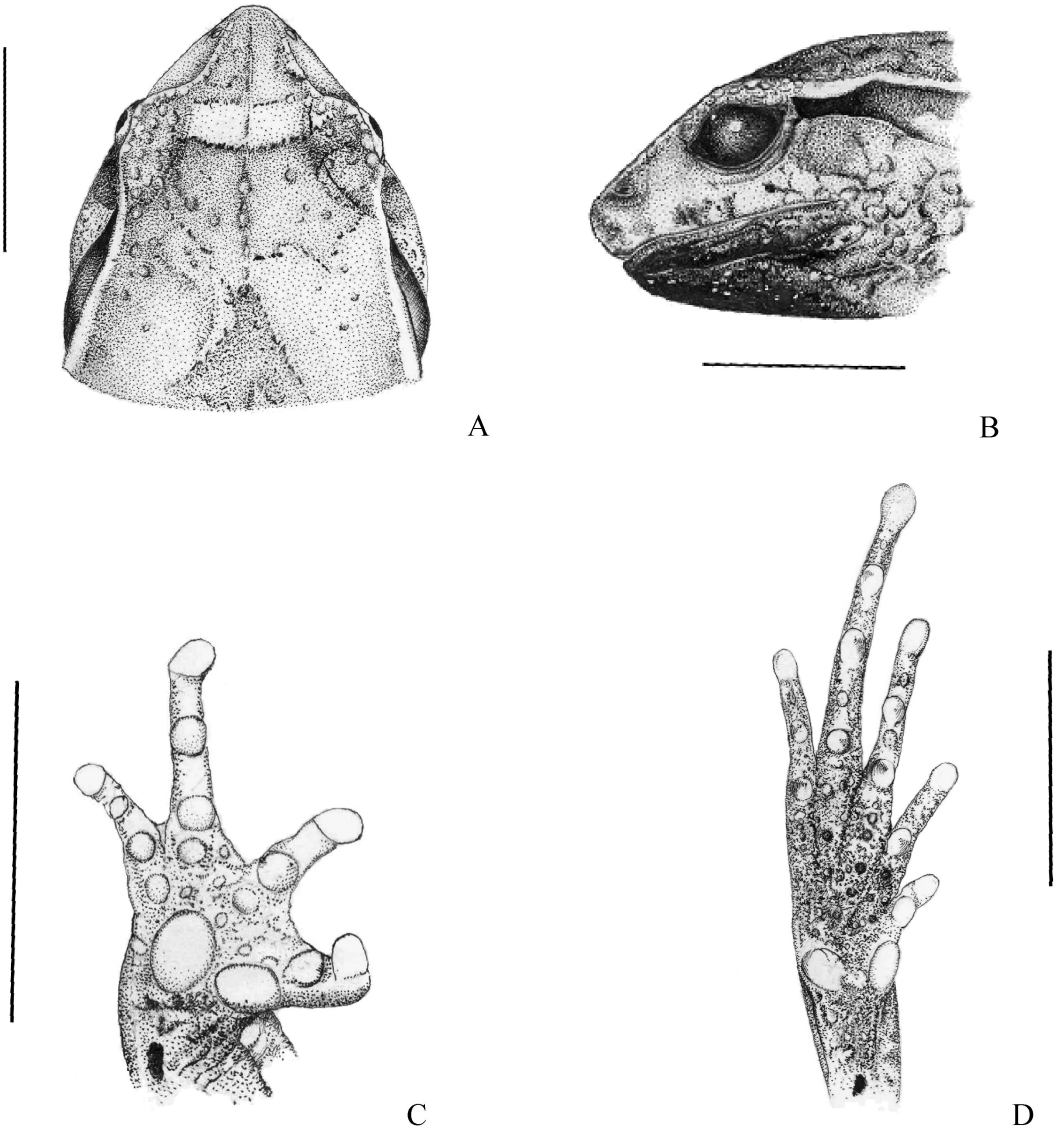


Figure 2. *Physalaemus irroratus* sp. nov., holotype (MNRJ 35124). (A) Dorsal and (B) lateral views of head; ventral views of (C) hand and (D) foot (scales = 5 mm).

male 22.5-22.9 mm); (2) robust body; (3) nostrils closer to the tip of snout than to eye; (4) tympanum distinct, large, close to the eye; (5) inguinal glands large with black ocelli; (6) vocal sac well-developed; (7) second finger with the same size of the fourth; (8) large, protruding tubercles on hands and feet; (9) dorsal skin granulated; (10) dorsolateral fold beginning at the posterior corner of eye and ending at the inguinal region; and (11) advertisement call with harmonic notes, generally four, being the last shorter than the others.

Comparison with other species: The new species is distinguished from all species of the *P. signifer* group by the granulated texture of dorsal skin (smooth to rugose in others species). The new species is distinguished from *P. atlanticus* by the distinct tympanum (indistinct in *P. atlanticus*), well developed dorsolateral fold

(weak in *P. atlanticus*), and by the granulated texture of posterior region of the belly and ventral surface of thighs (smooth in *P. atlanticus*). *Physalaemus irroratus* differs from *P. bokermanni* by the larger size (SVL for latter species, 14.9-20.4 mm) and of the inguinal glands (less than 10% of the body size in *P. bokermanni*, more than 15% in *P. irroratus*), and by the well developed vocal sac (poor developed in *P. bokermanni*). From *P. caete* the new species differs by its smaller size (SVL of *P. caete*, 22.2-25.3 mm), distinct tympanum (indistinct in *P. caete*) and prominent plantar supernumerary tubercles (not prominent in *P. caete*). *Physalaemus irroratus* is distinguished from *P. camacan* in having rounded snout in lateral view (protruding in *P. camacan*), the absence of a line of small white granules covering the dorsolateral fold (present in *P. camacan*), and prominent plantar supernumerary tubercles (not prominent in *P. camacan*). From *P. crombiei*, *P. irroratus* is distinguished by the presence of dorsolateral fold (absent in *P. crombiei*), the distinct supratympanic fold (extremely weak in *P. crombiei*), and the presence of plantar supernumerary tubercles (absent in *P. crombiei*). From *P. maculiventris* the new species is distinguished by a relatively light posterior belly (boldly dark and light mottled in *P. maculiventris*), rounded snout in lateral view (protruding in *P. maculiventris*), and the presence of plantar supernumerary tubercles (absent in *P. maculiventris*). From *P. moreirae*, *P. irroratus* is distinguished by rounded snout in lateral view (protuding in *P. moreirae*), the close proximity between tympanum and eye (not close in *P. moreirae*), and the presence of plantar supernumerary tubercles (absent in *P. moreirae*). *Physalaemus irroratus* differs from *P. nanus* by the distinct tympanum (indistinct in *P. nanus*) and presence of premaxillary and maxillary teeth (both absent in *P. nanus*). The new species is distinguished from *P. obtectus* by the rounded snout in lateral view (protruding in *P. obtectus*), the granulated texture of posterior region of the belly (smooth in *P. obtectus*), and the close proximity between tympanum and eye (not close in *P. obtectus*). From *P. signifer* the new species is distinguished by the rounded snout in lateral view (protruding in *P. signifer*), the granulated texture of posterior region of the belly (smooth in *P. signifer*), and the close proximity between tympanum and eye (not close in *P. signifer*). From *P. spiniger*, *P. irroratus* differs by the distinct tympanum (indistinct in *P. spiniger*), granulated texture of posterior region of the belly (smooth in *P. spiniger*), and presence of plantar supernumerary tubercles (absent in *P. spiniger*).

Description of holotype: Body robust (fig. 1); head wider than long; snout subelliptical in dorsal view, rounded in lateral view (fig. 2A, B); nostrils elliptical, slightly protuberant, located and oriented laterally, closer to tip of snout than to eye; canthus rostralis marked; loreal region almost vertical, nearly concave; eyes slightly protuberant; tympanum large, distinct, its diameter approximately 75% of eye diameter; anterior margin of *annulus tympanicus* close to posterior corner of eye; distinct supratympanic fold, straight from the posterior corner of the eye to shoulder; presence of a dorsolateral fold starting on the posterior corner of eye, immediately above the supratympanic fold, delimiting the dorsal region from the flank, and ending at the inguinal region; vocal sac subgular, well-developed, extending to the border of chest with belly; choanae small, round; tongue narrow, long, free posteriorly on approximately one third of length; maxillary and premaxillary teeth discernible by probe; vomerine teeth absent. Arms short, robust, upper arms as short and robust as forearms; fingers slender and short, not fringed, except for finger II; finger lengths I $<$ II $=$ IV $<$ III (fig. 2C); extensive nuptial asperities practically covering the thumbs, also covering half of the inner metacarpal tubercle; subarticular tubercles single, large, protruding, rounded; first subarticular tubercle absent in all fingers; outer metacarpal tubercle very large, elliptical, covering almost the entire carpal region; inner metacarpal tu-

bercle large, elliptical, approximately half the size of the outer metacarpal tubercle; presence of small supernumerary tubercles; finger tips slightly expanded. Legs moderately robust; tibia slightly longer than thigh, the sum of their lengths slightly shorter than SVL; a poorly developed inner tarsal fold on distal half of tarsus; toes slender, long, weakly fringed; toe lengths I < II < V < III < IV (fig. 2D); subarticular tubercles large, single, protruding, rounded; first subarticular tubercle absent in all toes; inner metatarsal tubercle medium-sized, ovoid; outer metatarsal tubercle medium-sized, elliptical, approximately the same size of the inner metatarsal tubercle; supernumerary tubercles small, but prominent; toe tips slightly expanded. Dorsal and ventral surfaces granulated; dorsolateral fold covered by a conspicuous line of small granules starting on *canthus rostralis*, also present as an arch above the vent, and on sides of the tibia. A distinct ventral disc is observed.

Color in preservative of the holotype: Dorsum pattern brown; a faint interocular transverse dark brown bar and a faint, dark brown double arrow shaped mark, both emarginated by a narrow white line; the posterior arrow extending to thigh, tibia and tarsus as a transverse bar. Loreal region brown, with four vertical cream bars; dorsolateral fold covered by a conspicuous line of small white granules; dark brown stripe from the corner of eye to near the inguinal region, immediately below the dorsolateral fold. A small black spot, emarginated by white line, covering the inguinal gland. Gular and chest regions dark brown, with scattered cream dots, resulting in a marbled aspect. Belly cream with scattered dark brown dots. Cloacal region with a triangular dark brown blotch, emarginated dorsally by a line of white granules. Dorsal surfaces of arms and legs brown; posterior surfaces of upper arms dark brown and ventral surfaces with two dark brown blotches; ventral surfaces of hands cream; nuptial asperities on thumbs brown. Anterior surfaces of thighs with a narrow dark brown stripe; anterior and posterior surfaces of tibias with scattered dark brown blotches or spots; heel with a dark brown blotch; posterior surfaces of tarsus dark brown; ventral surfaces of feet brown.

Measurements of the holotype (mm): SVL 21.6; HL 6.6; HW 7.3; ED 2.1; TD 1.6; UEW 1.8; IOD 2.6; IND 1.7; END 1.7; THL 10.0; TBL 10.2; FL 14.7.

Variation: Arms of females are slender than in males. General colour pattern varies from light brown to dark brown and from well-defined arrow pattern, stripes, and blotches to fading arrow pattern. One male (MNRJ 35127) and one female (MNRJ 40002) present scattered blotches with variable sizes and shapes on dorsum. Table 1 shows variations in measurements of four males and two females.

Tadpole: MNRJ 41460, Stage 36 (Gosner, 1960). Mean total length 16.6 ± 1.24 mm (n = 10). Body robust, oval in dorsal, ventral and lateral views; round-shaped snout in dorsal and lateral views (fig. 3A); body width approximately 63% of body length; body length about 38% of total length. Nostrils large, rounded, located and oriented dorsally, situated on midway between the eye and the tip of snout; eyes dor-

Table 1. Mean ($\bar{x}$), standard deviation (SD), and range of measurements (mm) of four males, and range of measurements of two females of *Physalaemus irroratus* sp. nov.

	Male (n = 4)			Female (n = 2)
	$\bar{x}$	SD	Range	
SVL	21.7	0.92	20.5-22.5	22.5-22.9
HL	6.7	0.10	6.5-6.8	7.5-7.7
HW	7.3	0.25	7.0-7.6	7.0-7.6
ED	2.0	0.10	1.9-2.1	2.1-2.2
TD	1.5	0.05	1.5-1.6	1.4-1.6
END	1.7	0.08	1.6-1.8	1.7-1.8
IND	1.6	0.09	1.5-1.7	1.8
IOD	2.6	0.17	2.4-2.8	2.6-2.8
UEW	1.8	0.17	1.6-2.0	1.7-1.9
THL	9.8	0.46	9.2-10.3	10.0-10.1
TBL	10.2	0.56	9.4-10.6	10.0-10.2
FL	14.1	0.73	13.1-14.7	14.4-14.8

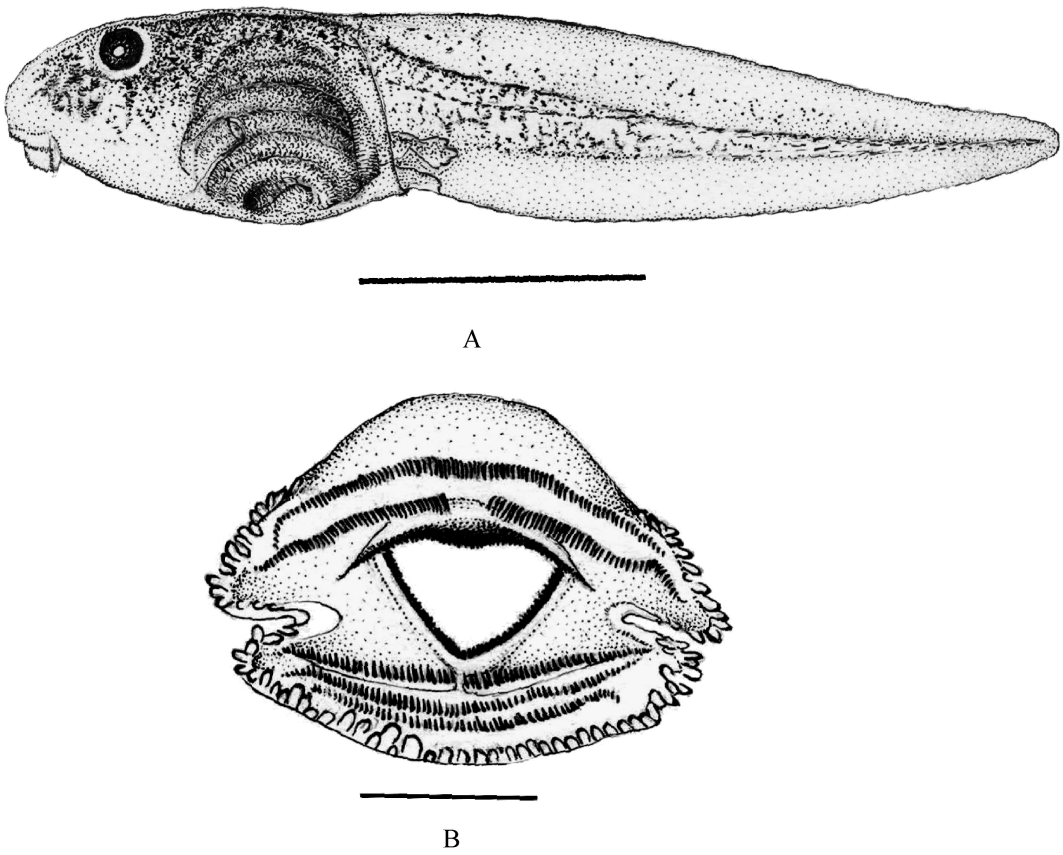


Figure 3. Tadpole of *Physalaemus irroratus* sp. nov. (MNRJ 41460, stage 36). (A) lateral view (scale = 5 mm); (B) oral disc (scale = 1 mm).

solateral; spiracle single, sinistral, oriented posterodorsally and attached to the body wall; its opening situated on the end of the second third of body length; cloacal tube medium sized, medial, attached to ventral fin, its opening oriented dextrally. Tail approximately 62% of total length; tail musculature slender; dorsal fin originating on posterior third of body and ventral fin originating on the beginning of tail; both fins arched, tail tip slightly directed upwards. Oral disc ventral, its width approximately 50% of body width, laterally emarginated (fig. 3B); a single marginal row of papillae on lips, interrupted by a wide medial gap on upper lip; labial tooth row formula 2(2)/3(1). The two anterior teeth rows with approximately the same length, longer than posterior rows; posterior teeth rows approximately the same size; upper jaw sheath arch-shaped and lower sheath "V"-shaped. Table 2 shows variations in measurements of fourteen tadpoles.

Color of tadpole in 5% formalin: General color pattern brown over a cream background, except for the ventral region, which is transparent. Tail musculature presents a cream background covered by many brown dots. Tail fins translucent with scattered brown dots. Legs cream with brown dots on dorsal surfaces.

The known tadpoles (*P. atlanticus*, *P. bokermanni*, *P. caete*, *P. camacan*, *P. maculiventris*, and *P. signifier*) of the *P. signifer* species group possess external morphology quite homogeneous, making hard to point diagnostic characters for them (Pimenta et al., 2005). *Physalaemus irroratus* differs from *P.caete* and *P. ca-*

Table 2. Mean ($\bar{x}$), standard deviation (SD), and range of measurements (mm) of 10 – tadpoles of *Physalaemus irroratus* sp. nov. in stage 36.

	$\bar{x}$	SD	Range
Total length	16.6	1.24	14.4-18.8
Body length	6.3	0.28	6.0-6.7
Body width	4.0	0.32	3.7-4.7
Body height	3.0	0.23	2.7-3.5
Tail height	3.1	0.30	2.6-3.7
Distance nostril-snout	0.6	0.08	0.6-0.7
Distance eye-nostril	0.6	0.07	0.5-0.7
Interorbital distance	1.2	0.09	1.1-1.3
Internarial distance	0.7	0.06	0.6-0.8
Eye diameter	0.7	0.06	0.6-0.8
Oral disc width	2.0	0.09	1.9 - 2.2

macan by the presence of a single row of marginal papillae (two rows in *P. caete* and *P. camacan*); the new species differs from *P. signifer* and *P. maculiventris* by its V-shaped lower jaw sheath (U-shaped in *P. signifer* and *P. maculiventris*); *P. irroratus* differs from *P. bokermanni* by the arched fins and rounded tail tip (fins straight and pointed tail tip in *P. bokermanni*); and from *P. atlanticus* the new species differs by the weakly developed jaw sheaths (jaw sheath strongly developed in *P. atlanticus*).

Vocalization: On 15 January 2005, specimen MNRJ 40001 was recorded emitting advertisement calls. Analysis of 25 advertisement calls demonstrated that they present harmonic structure and are generally composed by four notes (n = 23). One advertisemnt call shows five notes and another two notes, therefore the mean is 3.96 (SD = 0.45). The mean call duration is 0.73 s (SD = 0.11, range = 0.25-0.96); the mean intercall interval (defined in Pimenta et al., 2005) is 0.57 s (SD = 0.13, range = 0.45-0.92). In general, the three first notes are longer (first, second and third notes: $\bar{x}$ = 0.04 s, SD = 0.01) than the fourth ($\bar{x}$ = 0.02 s, SD = 0.001). When the advertsiment call shows five notes, the last one is shorter, but when there are two notes, both are long. Dominant frequency situated between 1.38 and 1.81 kHz ($\bar{x}$ = 1.77; SD = 0.31) (fig. 4).

Comparative data on advertisement calls of the *P. signifer* group are summarized in Pimenta et al. (2005). The advertisement calls of *P. irroratus*, *P. crombiei*, *P. obtectus*, and *P. signifier* present harmonic structure. However, the dominant frequency of *P. irroratus* (1.38-1.81 kHz) is higher than in *P. crombiei* (1.06 kHz, Heyer and Wolf, 1989) and *P. obtectus* ($\bar{x}$ = 1.18; SD = 0.36, present study). Additionally, despite of the similar number of notes per call ($\bar{x}$ = 3.96; SD = 0.45 for *P. irroratus*; $\bar{x}$ = 4.2; SD = 0.41 for *P. obtectus*), *P. irroratus* differs from *P. obtectus* by the shorter note at the end of the call. The advertisement call of *P. irroratus* differs from *P. signifer* by the number of notes (one for *P. signifier*, Wogel et al., 2002; generally four for *P. irroratus*).

Habitat: Fazenda Duas Barras is an unprotected area at Atlantic Rain Forest, characterized by the existence of large, well-preserved or secondary forest patches, associated with cattle breeding activities. Adults of *Physalaemus irroratus* sp. nov. were found during the wet season, in October 2003, January 2004 and 2005. Males call floating on small temporary ponds on the edge of trail of forested areas. The foam nests were found on October 2003, on a small temporary pond on a trail at forest fragment.

Distribution: The new species is known only from the type locality, at Fazenda Duas Barras, Municipality of Santa Maria do Salto, State of Minas Gerais, southeastern Brazil. This locality is in the mountain range called Serra do Cariri, which separates the States of Minas Gerais and Bahia, as well as the basins of Jequitinhonha and Buranhém rivers.

Etymology: The specific epithet "irroratus" is a latin vernacular name meaning "covered with granules". It refers to the granulated texture of the dorsal skin.

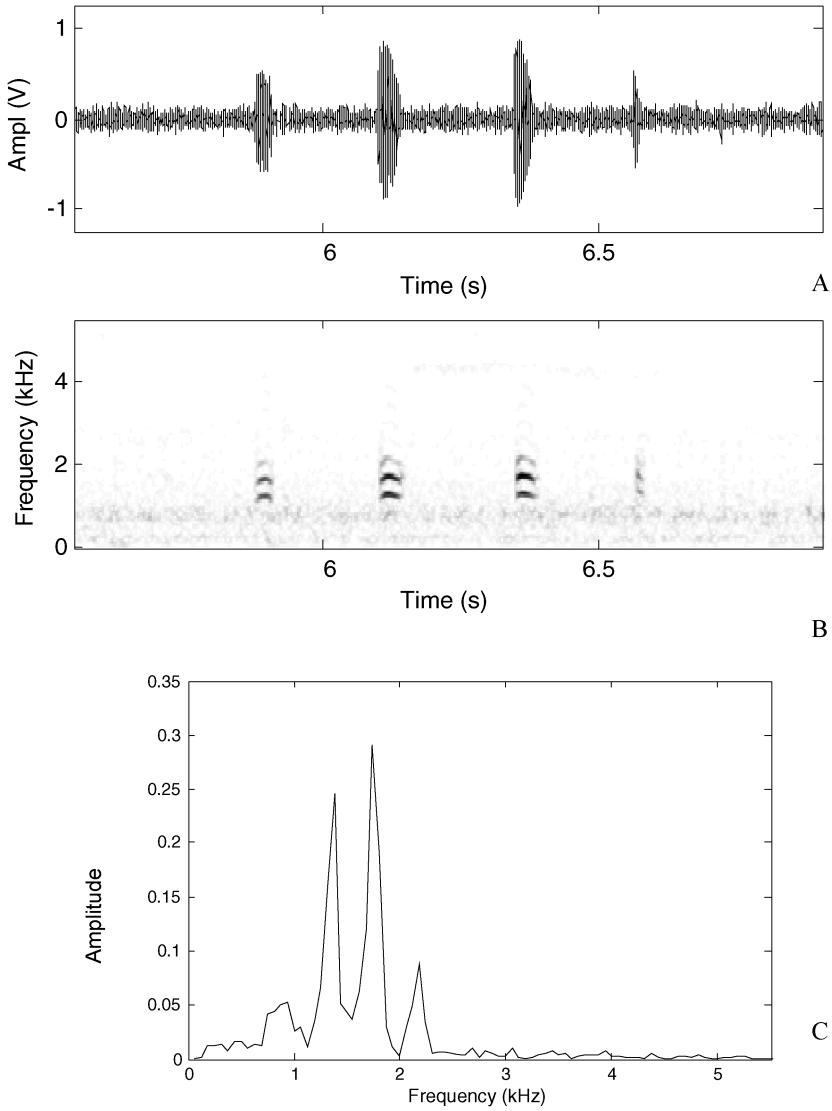


Figure 4. (A) Sonogram; (B) waveform; and (C) power spectrum of the second note of the advertisement call of *Physalaemus irroratus* sp. nov., recorded on 15 January 2005 at Fazenda Duas Barras, Municipality of Santa Maria do Salto, State of Minas Gerais. Air temperature 22°C. Voucher specimen MNRJ 40001.

Acknowledgements. We thank B.V.S. Pimenta (MN/UFRJ) for commenting on the manuscript; L. Aleixo, the owner of Fazenda Duas Barras, for logistical support; P.R. Nascimento for the drawings; Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA) provided licenses to collect the specimens (199/2002/Fauna MINAS GERAIS, 02015.018836/02 and 231/03 NUFAS/MINAS GERAIS, 02015.018836/02-16); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and CAGC fellowship. This work is part of the Project "Inventário de áreas prioritárias para conservação da biodiversidade nos vales dos rios Jequitinhonha e Mucuri", supported by Project on the Conservation and Sustainable Use of Brazilian Biological Diversity/Brazilian Ministry of the Environment (PROBIO/MMA) and coordinated by Conservação Internacional do Brasil (CI-Brasil).

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Appendix - Material Examined:

Physalaemus atlanticus: MNRJ 35116-18 (paratypes).
Physalaemus bokermanni: MZUSP 59551 (holotype), MZUSP 59552 (paratype), ZUEC 6845; MZUSP 125992-126002 (São Bernardo do Campo, SP).
Physalaemus caete: MNRJ 9803 (holotype), MNRJ 9850 (paratype).
Physalaemus camacan: MNRJ 33337 (holotype), MNRJ 33339, MNRJ 33338, MNRJ 33340-41, and MCN 2510 (paratype).
Physalaemus crombiei: MZUSP 66253-81 (paratypes).
Physalaemus maculiventris: MZUSP 67383-90 (Teresópolis – RJ).
Physalaemus moreirae: MZUSP 25867-70 (paratypes of *P. franciscae*), MZUSP 59935 (holotype of *P. franciscae*), MZUSP 77060-61 (Caraguatatuba – SP).
Physalaemus nanus: MNRJ 12827-12832, CFBH 3205-3206 (Florianópolis – SC), EI 2963(Rio Vermelho – SC), EI 2964-2967 (Blumenau – SC).
Physalaemus obtectus: EI 9466-67 (paratypes).
Physalaemus signifer: MNRJ 30861 (Bom Jesus do Itabapoana – RJ), MNRJ 32994-97 (Tinguá, Nova Iguaçu – RJ).
Physalaemus spiniger: MNRJ 28249-67 (Ribeirão Grande – SP), MZUSP 76561 (Estação Ecológica da Juréia – SP), MZUSP 83470-71 (Ilha do Cardoso, Cananéia – SP).

Received: August 31, 2006. Accepted: January 22, 2007.