# An extraordinary new species of
# *Melanophryniscus* (Anura, Bufonidae)
# from southeastern Brazil

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## ABSTRACT

We describe a new species of bufonid from a lowland, sandy soil, *restinga* habitat in the state of Espírito Santo, southeastern Brazil. Based on the shared occurrence of putative morphological synapomorphies of *Melanophryniscus* and the results of a phylogenetic analysis of DNA sequences of a broad sample of bufonids, and other anurans, we assign the new species to *Melanophryniscus*. The new species possesses several peculiar character states that distinguish it from all other *Melanophryniscus* including, but not limited to: fingers II, III, and V much reduced; nuptial pad with few enlarged, brown-colored spines on medial margin of finger II; seven presacral vertebrae, the last fused with the sacrum; and ventral humeral crest prominent, forming a spinelike projection.

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INTRODUCTION

Toads of the family Bufonidae are distributed in temperate and tropical areas worldwide, except for the Australo-Papuan region, Madagascar, Seychelles, and New Zealand, but they have been widely introduced in some of those areas where they did not naturally occur (Frost et al., 2006; Frost, 2011). It is a diverse family with nearly 50 genera and over 550 species. Although Bufonidae is monophyletic (Haas, 2003; Frost et al., 2006; Van Bocxlaer et al., 2010), the relationships among extant taxa are still not fully understood. *Melanophryniscus* is consistently recovered as the sister taxon of all other bufonids (Haas, 2003; Darst and Cannatella, 2004; Frost et al., 2006; Pramuk et al., 2007; Van Bocxlaer et al., 2010; Pyron and Wiens, 2011). Several morphological synapomorphies of Bufonidae exist (Ford and Cannatella, 1993; Frost et al., 2006), while few others support the less-inclusive clade of all Bufonidae excluding *Melanophryniscus* (and presumably *Truebella,* fide Frost et al., 2006; Pramuk, 2006).

We collected an unnamed and very peculiar bufonid toadlet during a herpetological survey in a *restinga* (sand-dune habitats covered mainly with herbaceous and shrubby xerophilous vegetation) fragment in the state of Espírito Santo, southeastern Brazil; restingas belong to the Atlantic rainforest biome and are common to the Brazilian coast (Suguio and Tessler, 1984; Eiten, 1992). Based on the presence of putative morphological synapomorphies of *Melanophryniscus* and results of a phylogenetic analysis of DNA sequences we assign the new species to *Melanophryniscus*.

MATERIAL AND METHODS

Fieldwork was carried out at Parque Estadual Paulo Cesar Vinha (PEPCV), a protected area in the restinga of Setiba, municipality of Guarapari, state of Espírito Santo, Brazil. Frogs were collected in pitfall traps with drift fences or by hand during active searches. Pitfall sampling occurred from December 2005 to November 2006 for 2–4 consecutive days, once a month, during that period. Traps were installed in sets of four buckets in a radial disposition (see Cechin and Martins, 2000, for details on pitfall traps). Three satellite buckets were placed 10 m from a central bucket and connected to it by 0.5 m tall drift fences. Five trap sets were installed in the area. Two sets in the open *Clusia* formation (for detailed description of vegetation formations at PEPCV see Pereira, 1990), two inside the forested area (*mata seca* of Pereira, 1990) and one in an area of herbaceous vegetation near the shore. Pitfall traps were checked once a day, in the morning. Traps were opened at 18:00 h (GMT -03:00 hrs) in the first day of sampling and closed at 18:00 h of the last day. Active searches for specimens were conducted from December 2005 through February 2007 for 2–4 consecutive days once a month. Searches were random in duration and habitat sampled. From November 2006–February 2007 a directed effort was made in search of evidence for reproductive activity of the new species. During that period searches focused on areas where individuals of the new species were previously found. Specifically, we actively searched the leaf litter, small crevices, burrows, small ponds, and phytotelmata (mostly bromeliads) looking for eggs, tadpoles, and breeding adults (i.e., calling males and amplectant pairs). Searches were conducted early in the morning and late in the afternoon, commonly

extending over the first few hours of night. Although several adults (of both sexes) and a few smaller specimens (apparently juveniles) were found during this directed effort, much to our frustration, no data on the reproductive biology of the species could be collected.

Live individuals were anesthetized and killed with a lethal dose of lidocaine, fixed in 10% formalin and preserved in 70% ethanol. Specimens used in the description and analyzed for comparisons are deposited in the following collections: American Museum of Natural History, New York (AMNH); Coleção de Anfíbios Célio F.B. Haddad, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil (CFBH); Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"–CONICET, Buenos Aires, Argentina (MACN); Museu Nacional, Rio de Janeiro, Brazil (MNRJ); Coleção Herpetologica Oswaldo Rodrigues da Cunha, Museu Paraense Emílio Goeldi, Pará, Brazil (MPEG).

Morphology: The following nine measurements were taken: SVL (snout-vent length), HL (head length; measured from tip of snout to posterior corner of the left eye), HW (head width; greatest width of head located between angles of the jaw), ED (eye diameter), IOD (interorbital distance; between anterior corners of the eyes), END (eye-nostril distance; from posterior margin of the nostril to anterior corner of the eye), THL (thigh length), TBL (tibia length), and FL (foot length; distal margin of tarsus to tip of fourth toe). All measurements are in millimeters, taken to the nearest 0.1 mm, and all were taken with an ocular micrometer attached to a Leica stereomicroscope, except for SVL, which was measured with digital calipers under the stereomicroscope. Descriptions of color in life were based on one paratype (CFBH 15745). Sex was determined by the presence of nuptial pads, vocal slits, and hypertrophied arms in males. This method of sexual identification proved successful by the dissection of one putative male and one putative female and direct observation of gonads and developing eggs.

Two paratypes, one male (CFBH 15745) and one female (CFBH 15735), were cleared and double-stained for osteological study, following the protocol by Taylor and Van Dyke (1985). Terminology follows Trueb (1973, 1993) for cranial and postcranial osteology and Fabrezi (1992, 1993) for carpal and tarsal osteology. Following Fabrezi and Alberch (1996), fingers are numbered II–V.

Phylogenetic Analyses: To hypothesize the phylogenetic placement of the new species, we analyzed DNA sequences from a broad sample of bufonid and several nonbufonid clades. The analysis included sequences of the mitochondrial genes 12S, the intervening tRNA$^{Val}$, and a fragment of 16S, and portions of the nuclear genes exon 1 rhodopsin, exon 2 of chemokine receptor 4 (Cxcr4), and recombination activating gene 1 (RAG-1). Sequences for most terminals were obtained from Genbank (appendix 2), We generated sequences from the new species and from *Frostius erythrophthalmus*. Through the courtesy of Diego Baldo we included sequences of *Melanophryniscus devincenzii*. Laboratory protocols and primers employed followed Faivovich et al. (2010).

Phylogenetic analyses were performed under direct optimization in POY 5.0 (Varón et al., 2010, 2012) using equal weights for all transformations (substitutions and insertion/deletion events) and the parsimony optimality criterion, following the justification of Kluge and Grant (2006; see also Grant and Kluge, 2009; Wheeler, 2012). Contiguous sequences were preliminar-

ily delimited in fragments of putative homology to allow incorporation of partial sequences and accelerate dynamic homology cost calculations (Wheeler et al., 2006). Analyses were performed using the command "search," which implements a driven search composed of random addition sequence Wagner builds (RAS), subtree pruning and regrafting (SPR) and tree bisection and reconnection (TBR) branch swapping, parsimony ratcheting (Nixon, 1999), and tree fusing (Goloboff, 1999), storing the shortest trees of each independent run and performing a final round of tree fusing on the pooled trees. Five independent runs, each consisting of four 12-hour driven searches, were implemented in parallel on a dual hexacore server at the Museu de Zoologia da Universidade de São Paulo. A final 24-hour run composed of three 8-hour driven searches of the implied alignment (Wheeler, 2003) derived from the optimal tree found under direct optimization was performed to search for additional most-parsimonious trees. Goodman-Bremer support values (Goodman et al., 1982; Bremer, 1988; see Grant and Kluge, 2008) were calculated using inverse constraints to search for next-most-optimal trees with 5 RAS + TBR analyses of the implied alignment.

## RESULTS

At least two character states shared by the new species and all the remaining bufonids justify its inclusion within the family: the origin of the m. depressor mandibulae solely from the squamosal and the absence of teeth (Ford and Cannatella, 1993; Frost et al., 2006). The absence of the zygomatic rami, exostosed frontoparietals that diverge anteriorly, and fusion of the parasphenoid with the sphenethmoid and prootic are putative synapomorphies of *Melanophryniscus* (McDiarmid, 1971; Graybeal and Cannatella, 1995) that also occur in the new species. Additionally, our phylogenetic analysis of DNA sequence data resulted in six most-

parsimonious trees of 33,389 steps that recovered the new species as the well-supported (Goodman-Bremer value of 26) sister of all included species of *Melanophryniscus*, which collectively are recovered as the sister of all remaining bufonids (fig. 1; see appendix 3 for entire tree). Although our analysis aimed only to study the position of the new species and should not be construed as a test of the most current hypotheses of bufonid relationships (Van Bocxlaer et al., 2010; Pyron
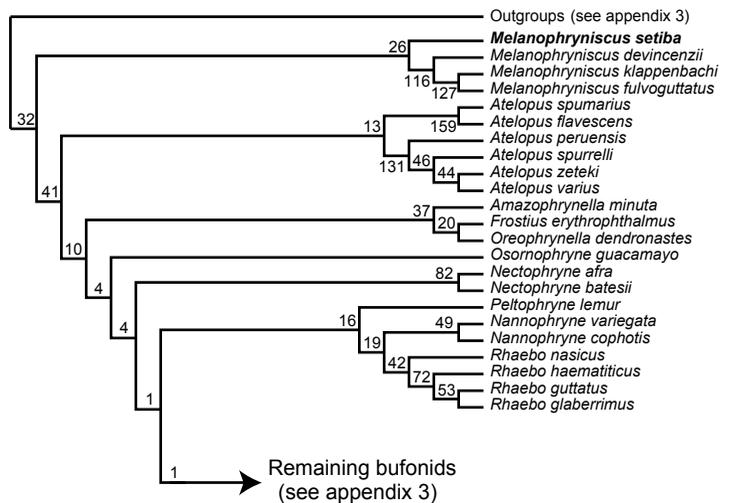


FIG. 1. Part of the strict consensus of 6 optimal trees of 33,389 equally weighted steps with Goodman-Bremer support values on each node. See appendix 3 for the full tree. The new species is in **boldface**.

and Wiens, 2011), we also note that *Frostius*, which has never been included in molecular phylogenetic analyses, was recovered as the sister taxon of our only exemplar of *Oreophrynella* (*O. dendronastes*) in a well-supported clade also containing *Amazophrynella minuta.*

## *Melanophryniscus setiba,* sp. nov.
### Figures 2–12, table 1

HOLOTYPE: CFBH 17036, male (figs. 3–4); from Parque Estadual Paulo César Vinha (20°36′25″S; 40°25′01″W, near sea level), approximately 1.5 km from the beach, restinga of Setiba, municipality of Guarapari, state of Espírito Santo, Brazil; collected on 10 December 2005 by P.L.V. Peloso, S.E. Pavan, N.B. Thomazini, and R. Montesinos.

PARATOPOTYPES: CFBH 15727–33, three adult females, one adult male, one adult female, and two adult males, respectively, collected on 26–29 December 2005 by P.L.V. Peloso, R. Montesinos, and R.V. Peloso; CFBH 15734–41, one adult male, one adult female (15735, adult female cleared and double-stained), three adult males, one adult female, one adult male, and one subadult, respectively, collected on 08–24 January 2006 collected by P.L.V. Peloso, S.E. Pavan, R. Montesinos, B. Becacici, and J. Albino; CFBH 15742, MPEG 21940–41 three adult females, collected on 17–18 March 2006 by P.L.V. Peloso and R. Montesinos; MPEG 21944, adult male, collected in May 2006 by R. Kawada; CFBH 15743–45, three adult males (15745 cleared and double-stained), collected on 17 December 2006 by P.L.V. Peloso and S.E. Pavan; MPEG 21942–43, one adult male, and one adult female, respectively, collected by P.L.V. Peloso, D. Barbosa, P. Rubens, R. Kawada, and S.E. Pavan on 15 December 2007.

DIAGNOSIS: A small *Melanophryniscus* (SVL 13.8–16.1; table 1) with a moderately robust body that can be diagnosed by the combination of the following character states: (1) head wider than long; (2) snout round in dorsal view and slightly protruding in lateral view (profiles follow Heyer et al., 1990); (3) frontal macrogland (see Naya et al., 2004) absent; (4) fingers II, III, and V much reduced; (5) subarticular tubercles distinguishable on finger IV; (6) nuptial pad, with few enlarged, brown-colored keratinized spines at medial margin of finger II present in males; (7) finger and toe tips rounded, unexpanded; (8) toes I–IV connected by barely noticeable webbing; (9) frontoparietals heavily exostosed, fused, not diverging anteriorly; (10) pectoral girdle completely ossified, triangular; (11) sternum heavily mineralized and greatly reduced; (12) seven presacral vertebrae, the last fused with the sacrum; (13) ventral humeral crest prominent, forming a spinelike projection, more evident in males than females; (14) surfaces of head and dorsum slightly granular without keratinous spines; throat and chest smooth; and (15) color pattern: dorsum reddish brown to dark brown in life; two dorsal marks present on dorsum—one in form of an "X" anteriorly, and one in form of a "Λ" posteriorly; venter light colored with variable amounts of brown markings and spots; a ventral dark brown stain usually present at midbody.

COMPARISONS WITH OTHER SPECIES: *Melanophryniscus setiba* differs from all remaining species of *Melanophryniscus* by the strong coossification of the dorsal skull elements. Males of the new species are readily distinguished from males of all remaining species of *Melanophryniscus* by the presence of a bifurcated humeral spine and by nuptial pads with a few enlarged,

TABLE 1 Measurements of the type series of *Melanophryniscus setiba*. The paratype CFBH 15741 is likely a juvenile (very small SVL) and was excluded from this analysis. SD = standard deviation.

| | Males (*n* = 14) | | | Females (*n* = 10) | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range |
| SVL | 15.2 | 0.6 | 14.4–16.1 | 15.2 | 0.8 | 13.8–16.0 |
| HL | 3.8 | 0.2 | 3.2–4.1 | 3.6 | 0.2 | 3.4–4.0 |
| HW | 5.7 | 0.2 | 5.3–6.1 | 5.7 | 0.2 | 5.3–6.1 |
| ED | 1.5 | 0.1 | 1.2–1.6 | 1.45 | 0.1 | 1.4–1.6 |
| IOD | 2.9 | 0.5 | 1.6–3.3 | 2.68 | 0.6 | 1.6–3.3 |
| END | 1.2 | 0.1 | 1.1–1.5 | 1.18 | 0.1 | 1.0–1.3 |
| THL | 6.7 | 0.1 | 6.4–7.0 | 6.26 | 0.4 | 5.6–6.7 |
| TBL | 6.2 | 0.2 | 5.8–6.7 | 5.73 | 0.5 | 5.2–6.9 |
| FL | 4.3 | 0.3 | 3.6–4.8 | 4.08 | 0.3 | 3.5–4.6 |

brown-colored spines on the base of finger II (taxonomic distribution of nuptial pads poorly known in *Melanophryniscus*, but, when present, composed of multiple minute, spines colored on its tips, on fingers II, III, and IV: see Discussion); presence of prominent ventral humeral crest, and reduced phalangeal formula of both hands and feet. The absence of a frontal macrogland readily separates the new species from all species in the *M. tumifrons* group (the macrogland is a putative synapomorphy of the *M. tumifrons* group; see Caramaschi and Cruz, 2002; Baldo and Basso, 2004; Naya et al., 2004). The new species further differs from all species in the *M. moreirae* and *M. stelzneri* groups by its almost smooth skin on dorsum and flanks (developed warts with an apical corneous spine in the *M. moreirae* group; and keratinized spines on skin in the *M. stelzneri* group). The diminutive size of *M. setiba* is rivaled only by *M. vilavelhensis* (12.8–17.2 mm; unassigned to any group; Steinbach-Padilha, 2008), with all other species >20 mm (summary in Steinbach-Padilha, 2008: 104).

Description of the Holotype (figs. 3–4): Body robust. Head large, wider than long; snout short, rounded in dorsal view and slightly protruding in lateral view (fig. 4A–B); nostrils small, not protuberant, directed anterolaterally, almost at tip of snout; frontal macrogland absent; canthus rostralis distinct, concave; loreal region nearly flat, vertical; eye diameter about half the interorbital distance, not protruding dorsally; postorbital crests absent; tympanum absent; supratympanic fold absent; vocal sac medial, not forming externally visible folds; vocal slits present, tongue long and narrow; premaxillary, maxillary, and vomerine teeth absent; choanae small, rounded. Strong coossification of the dorsal skull elements. Ventral humeral crest present, well developed, not projecting through the skin but externally visible as a protuberance. Arm and forearm hypertrophied (likely due to extensive development of musculature). Finger IV longest, followed by finger III; finger V reduced but distinguishable (the peculiar morphology of finger II and a much reduced finger V make judgments of relative finger lengths imprecise and unreliable); finger IV moderately robust; finger tips rounded; fingers connected by a thick web; nuptial pad with few enlarged, well-separated, brown-colored

keratinized spines along the proximal medial margin of finger II (fig. 4C); subarticular tubercles present and conspicuous on fingers III and IV; inner thenar tubercle elliptical, larger than outer metacarpal tubercle; palmar tubercle present, large, rounded; supernumerary tubercles on palm of hand present but barely distinguishable (fig. 4C). Legs short, slender; toes robust; toes I, II, and V very reduced; toe IV the longest, followed by toe III (others too small to make a confident statement of relative length; fig. 4D); toe tips rounded; barely noticeable webbing connecting toes II–V; subarticular tubercles present, large; inner metatarsal tubercles large, elliptical, larger than inner metatarsal tubercle (fig. 4D). Skin on head, dorsum, dorsal surfaces of arms, and legs very lightly granular. Gular region, chest, and ventral surfaces of arms and legs smooth.

Measurements of Holotype: SVL 15.1, HL 3.9, HW 5.5, ED 1.5, IOD 3.2, END 1.1, THL 6.4, TBL 5.9, FL 4.0.

Color of the Holotype in Preservative: Dorsum brown; two dorsal marks present, one in the form of an "X" anteriorly and one in the form of a "Λ" posteriorly; marks on the dorsum dark brown; dorsal arms and legs grayish brown. Throat brown (usually more pigmented in males than females); chest dark brown; venter light brown with a large dark brown stain at midbody and many dark spots and minor stains all over the ventral surface; ventral arms and legs cream color; tips of fingers and toes beige.

Color in Life (based on paratype CFBH 15475, male, fig. 2): Iris black; pupil ring golden. Dorsum reddish brown to dark brown; marks on the dorsum dark brown; a few white spots present dorsolaterally; arms and legs brown dorsally. Throat brown; chest dark brown; venter light orange with large dark brown blotch at midbody, ventral arms and legs light orange. Palm and sole brownish red; tips of fingers and toes reddish orange.

Variation and Sexual Dimorphism: Measurements of the type series are given in table 1. Males have robust arms and forearms (likely due to muscular hypertrophy) while they are slender in females (fig. 5). The humeral processes and bifurcated spine are variably visible externally among male specimens (fig. 5B–D), never visible externally in females (fig. 5A). Females lack nuptial spines and have slender arms and forearms. One cleared and double-stained female (CFBH 15735) possesses a distinct humeral spine, although it is not bifurcated and is much less developed than that of the male specimen (CFBH 15745). No sexual dimorphism in SVL was detected among 14 males and 10 females (Student's *t*-test; t = 0.139, df = 22, p = 0.891), although females have significantly shorter legs than males (t = -3.856, p < 0.05 for THL; t = -2.796, p < 0.05 for TBL; t = -2.021, p = 0.056 for FL). Bidder's organ is apparently absent (not detected from direct observation under a dissecting microscope or from histological sections).

The X- and Λ-shaped marks on the dorsum vary in intensity, but this variation does not seem to be ontogenetic or sexually dimorphic; some specimens have a light-brown dorsum while in others it is dark brown (colors in preservative, but such variations do occur in live specimens; reddish brown/dark brown). A few individuals have white spots on the dorsolateral surface near the thigh in life (fig. 2); in preservative these white spots tend to disappear. Ventral pattern of brown markings variable among individuals (fig. 5); throat uniformly dark colored

(brownish) or light (creamish) with brown spots and blotches; ventral midbody usually with a brown blotch, highly variable in shape and size, light or dark brown.

OSTEOLOGY: Description based on male CFBH 15745 and female CFBH 15735. Skull slightly longer than wide in dorsal view. Frontoparietals, prootics, exooccipitals, sphenethmoid complex, nasals, palatines, and, if actually present, vomers, forming a continuous, single, fused structure with unclear limits between individual endochondral bones (fig. 6A–D); dorsal surface of nasals and frontoparietals heavily exostosed with irregularly shaped outgrowths, allowing limits to be inferred (fig. 6B). Frontoparietals fused to each other and nasals, sphenethmoid, prootic, and ossified tectum synoticum. Frontoparietals with nearly triangular posterolateral processes covering posterior corner of orbit and anteromedial portion of otic capsule, extending laterally to anterolateral tip of otic capsule. Occipital groove covered along most of its length, exposed in some areas. Nasals fused medially, longer than wide, reaching anteriorly the tip of the snout; maxillary process short, not reaching pars facialis of the maxilla laterally. Premaxillae edentulous and in close contact with each other and with the maxillae (fig. 6C–D). Alary process of premaxilla laminar, expanded distally; directed slightly anteriorly in profile. Pars palatina well developed; pars dentalis nearly laminar. Palatine process pointed in ventral view, conspicuously thicker than pars palatina; maximum length about twice the width of the pars palatina. Maxilla edentulous; pars facialis of the maxilla with irregular free margin. Quadratojugal small and restricted to anteroventral part of quadrate (fig. 6E), contiguous with distal portion of ventral ramus of the squamosal; anterior process short and widely separated from the maxilla; quadrate unossified. Parasphenoid indistinguishably fused with sphenethmoid and prootic (fig. 6C). Vomers absent as discrete elements (i.e., either fused to underlying sphenethmoid complex or absent); dentigerous process absent; prechoanal process well developed; postchoanal process reduced. Palatines absent as discrete elements, likely fused to the sphenethmoid complex, as suggested by a distinct thickening of the bone in the corresponding area. Pterygoid triradiate; anterior ramus in contact with maxilla, reaching base of antorbital process, almost touching lateral tip of sphenethmoid complex; medial ramus in bony contact with otic capsule. Squamosal lacking zygomatic ramus; otic ramus with broad otic plate abutting crista parotica; ventral arm laterally flattened in its anterior section, resulting in bony sheath with pointed ventral process. Sphenethmoid extensively ossified, forming sphenethmoid complex due to fusion with several ossified nasal structures, including complete septum nasi. Orbitosphenoid cartilage completely ossified. Prootic fused with parasphenoid, exooccipital, and with the sphenethmoid complex anteriorly. Epiotic eminences low. Crista parotica broad, completely ossified. Occipital condyles broadly separated. Columellae and tympanic ring absent. Operculum cartilaginous, about two thirds of its volume ossified, with prominent point of attachment for the m. opercularis (fig. 6F). Fenestra ovalis with bony margins, except for the lateral wall in the male, which is cartilaginous.

Angulosplenial extended from the jaw articulation almost to mentomekelian bones. Dentary widest at anterior termination of angulosplenial, overlapping mentomeckelian. Mentomeckelian elements small and syndesmotically united. Distal portion of each half of the jaw, including mentomekelian, and distal portions of dentary and angulosplenial, curved ventrally, forming a wide V-shape in the area around the symphysis in frontal view.

FIG. 2. *Melanophryniscus setiba,* in life. Adult male, SVL 16.0 mm (CFBH 15745, paratype).
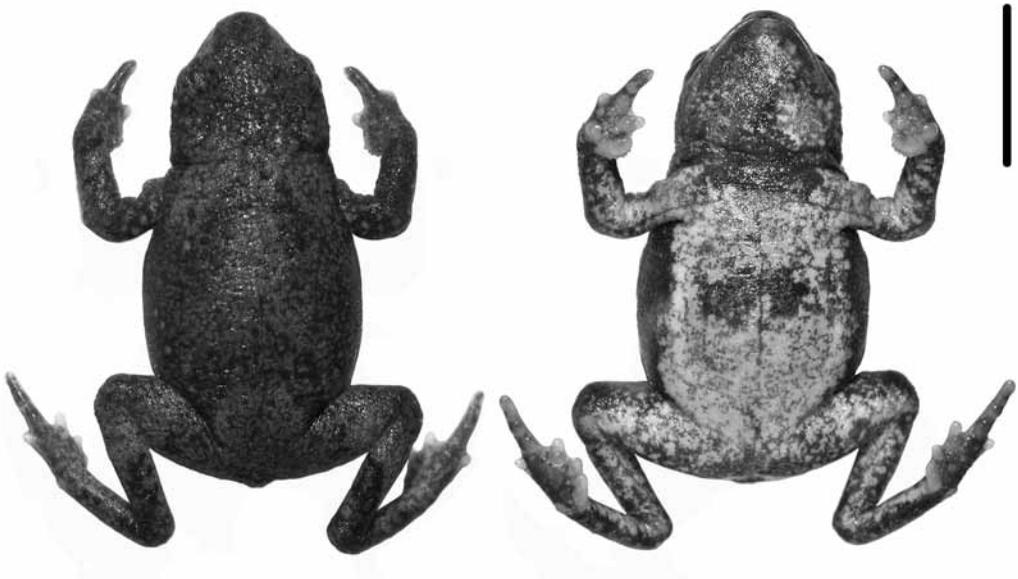


FIG. 3. *Melanophryniscus setiba.* Dorsal and ventral views of the holotype (CFBH 17036, male). Scale bar = 5.0 mm.
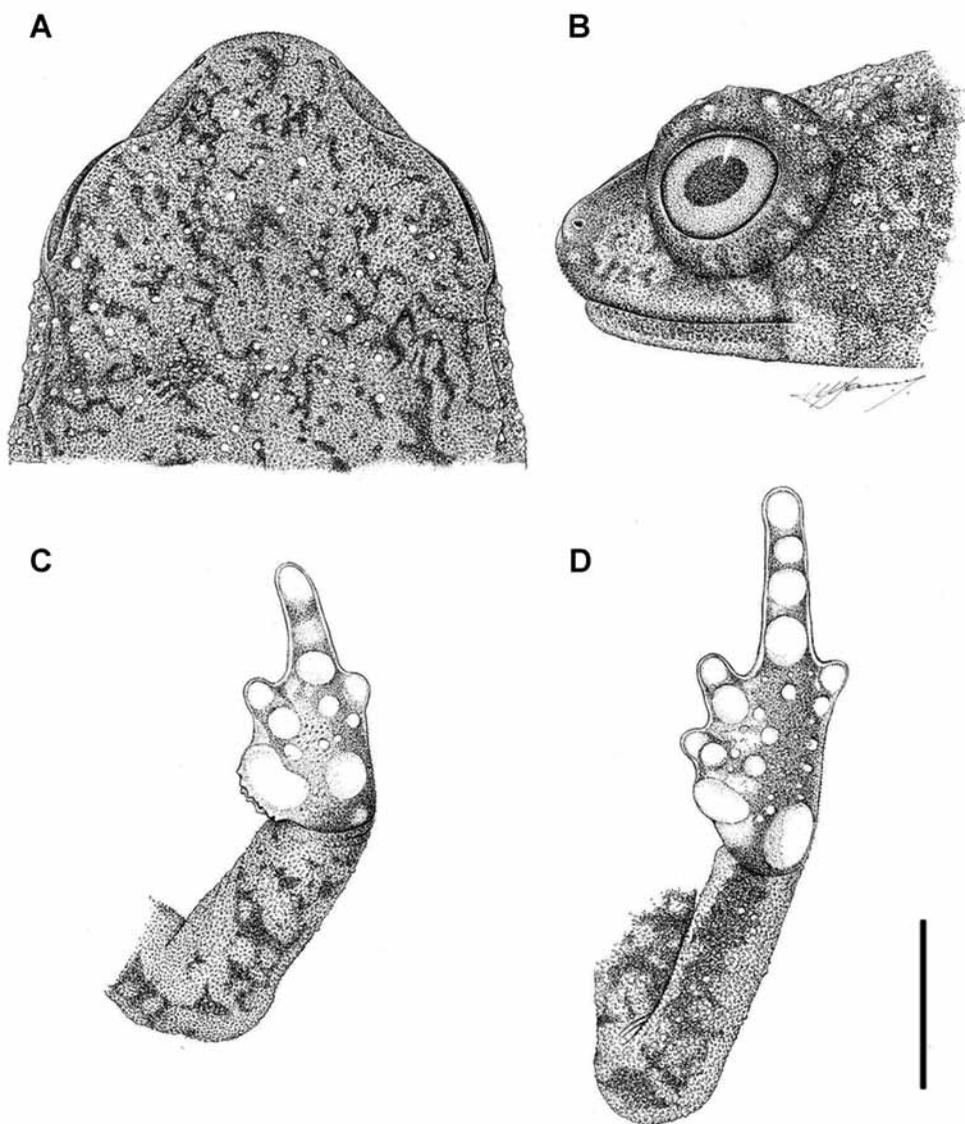
FIG. 4. *Melanophryniscus setiba.* Holotype (CFBH 17036, male). **A.** dorsal and **B.** lateral views of head; ventral views of **C.** hand and **D.** foot. Note the nuptial pad with keratinized spines at the medial margin of finger II. Scale bar = 2.0 mm.

Hyoid plate (fig. 7) about five times longer than wide (measured at narrowest point); not mineralized in female, slightly mineralized in male; hyalia moderately long, anterior processes oriented medially; hyalia inserted at limit between prootic and basal process. Anterolateral process present and broadly expanded, about 55% as long as hyoid plate; posterolateral processes absent. Posteromedial processes long, well ossified, noticeably curved dorsally. Larynx of male and female similar sized; female with complete cricoid; esophageal process small, triangular; bronchial processes long and slender (cricoid and bronchial processes of male damaged during preparation).
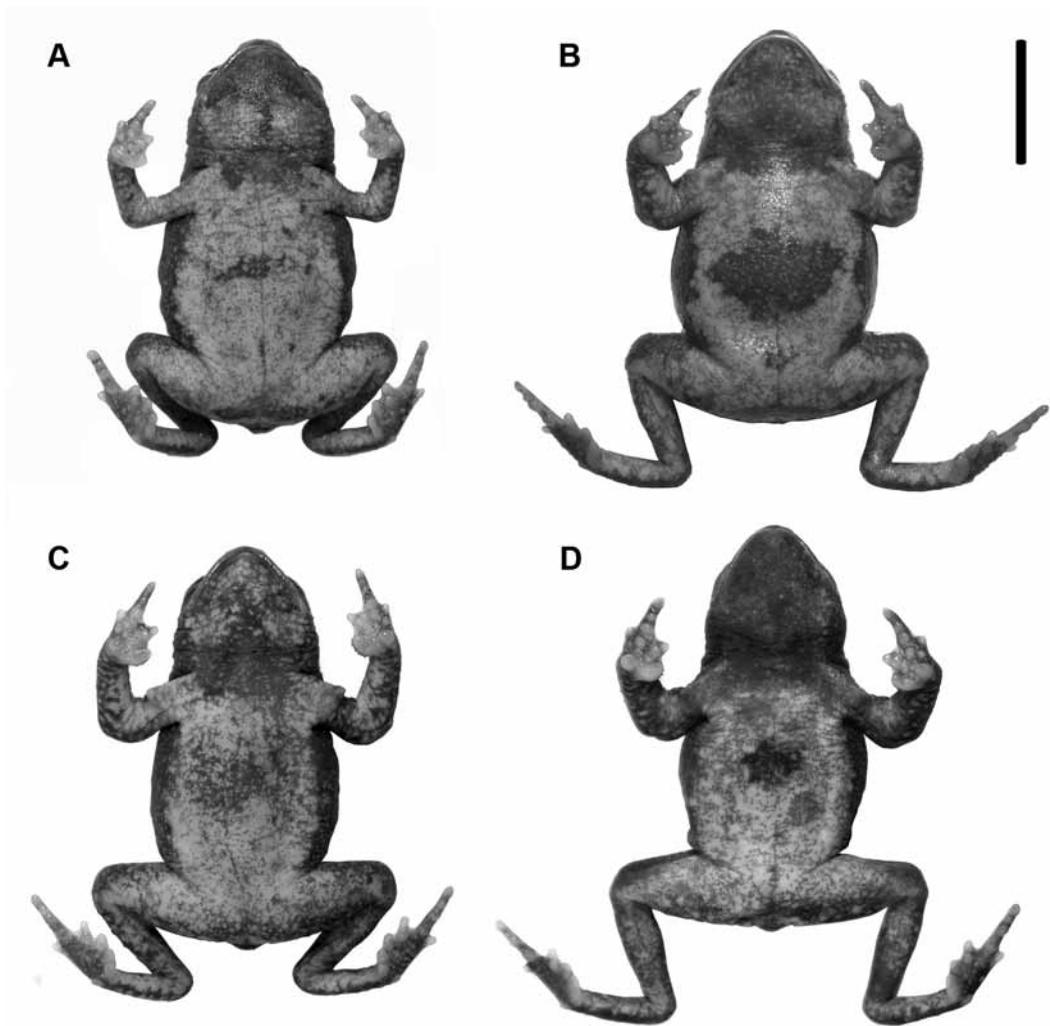
FIG. 5. Ventral pattern variation in *Melanophryniscus setiba*, **A.** CFBH 15739 female; **B.** CFBH 15745 male; **C.** CFBH 15733 male; and **D.** CFBH 15734 male. All paratypes. Scale bar = 5.0 mm.

Seven procoelous presacral vertebrae (fig. 8); first six vertebrae free, seventh fused with sacral vertebra, as evidenced by the intervertebral foramina between fused centra. Vertebrae II–VI with imbricated neural arches. Cotylar facets of atlas broadly separated. Transverse processes of presacral II directed anteriorly; transverse process of presacral III broader than those of presacrals II, IV–VI; processes with irregular flanges along its free margins. Neural arches of presacrals II–VI with irregular posterior margins; neural arches of presacrals III–V ornamented with elevated, anteriorly directed triangular shape with irregular base, those of presacrals VI and VII + sacrum ornamented with an irregular sagittal flange. Sacral diapophyses flattened, broadly expanded; irregular flanges along anterior and posterior margins. Iliosacral articulation with elongate sesamoid. Sacro-urostilar articulation bicondilar. Anterior half of
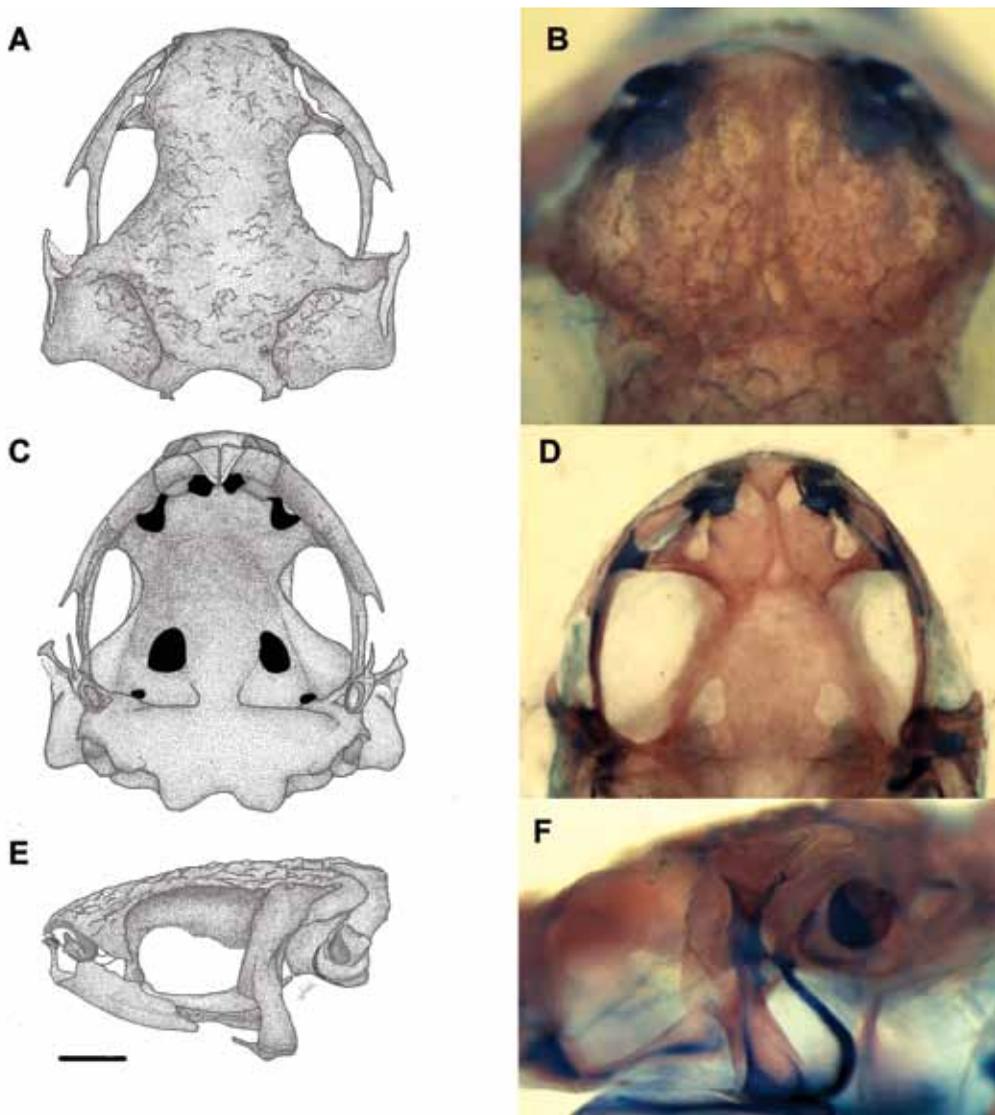
FIG. 6. Skull of *Melanophryniscus setiba.* Drawings (CFBH 15735, female) in **A.** dorsal, **B.** ventral, and **C.** lateral views. Photographs (CFBH 15745, male) showing details of **D.** nasal/frontoparietal region, **E.** ventral view of skull, and **F.** otic region.

urostyle bearing low, posteriorly tapered dorsal crest (fig. 8A); anterior portion of the crest with two parasagittal flanges; middle portion of urostyle with two lateral flanges, tapering and disappearing by the distal fifth of the bone.

Pectoral girdle (fig. 9) lacking omosternum. Clavicles straight and robust, fused to scapulae and coracoid. Partes acromialis larger than partes glenoidiales. Coracoids broadly dilated proximally. Epicoracoid cartilages heavily mineralized and reduced to a single, thin sliver of cartilage joining clavicles and coracoids. In the female the median section of the medial margin of the

coracoids is concave, and the epicoracoid discontinuous, establishing a fenestra between both coracoids. Sternum cartilaginous and heavily mineralized, round, greatly reduced. Cleithrum and ossified suprascapula indistinguishably fused.

Pelvic girdle (fig. 10) V-shaped. Ischia, pubis, and ilia fused. Ilial shaft cylindrical, elongate; dorsal crest developed along proximal 60% of ilium; dorsal prominence directed dorsolaterally. Preacetabular angle obtuse.

Humerus slender. In the male, it bears prominent ventral, medial, and lateral crests; ventral crest nearly 40% as long as humerus, bearing distinct, bicapitate, spinelike process near distal end of free margin (fig. 11); lateral and medial crests about 50% as long as humerus; medial crest higher than lateral crest. In the female, medial and lateral crests inconspicuous, forming low ridge; ventral crest present but less developed than in the male, lacking distinct spinelike process.

Carpus of right hand of female composed of radiale, ulnare, distal carpal 3–4–5, distal carpal 2, element Y, proximal element of prepollex, one sesamoid on the dorsal surface between radioulna and radiale, and one sesamoid on the ventral surface of distal carpal 3–4–5. Carpus of left hand differing in that proximal element of prepollex is absent as a discrete element, with equivalent space occupied by element Y; we consider it likely that in this hand the prepollex is fused with element Y. Terminal phalanges knoblike; reduced in



FIG. 7. Hyoid plate of *Melanophryniscus setiba* in ventral view (CFBH 15735, female). Scale bar = 1.0 mm.
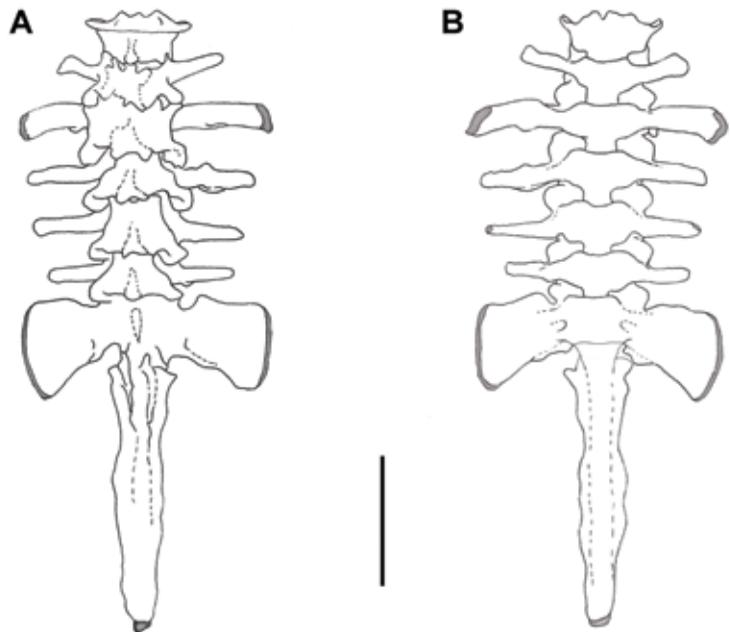


FIG. 8. Vertebral column of *Melanophryniscus setiba* (CFBH 15745, male) in **A.** dorsal, and **B.** ventral views. Scale bar = 2.0 mm.

digits II, III, V; elongate and slightly expanded in digit IV; penultimate phalanx of digit V reduced. Metacarpal II enlarged, bearing a peculiar, longitudinal bony outgrowth on medial margin, not ossified distally. Carpus of male (fig. 12A) with same morphology as female, with the addition that metacarpal II is even more



FIG. 9. Pectoral girdle of *Melanophryniscus setiba* (CFBH 15745, male) in ventral view. Scale bar = 2.0 mm.

enlarged medially due to hypertrophy of the bony outgrowth, which is fully ossified and has a distal process that covers the mediodistal section of metacarpal II. The relatively depressed longitudinal space delimited by this process and the element Y–prepollex coincident with space occupied by nuptial spines. Phalangeal formula of hand 1–2–3–2.

Tarsus (fig. 12B) composed of tibiale, fibulare, and three individual elements, including distal tarsal 2–3, an element that we interpret as distal tarsal 1 + element Y, and prehallux. Terminal phalanges knoblike, reduced on digits I–III and V, elongate on digit IV; penultimate phalanx on digit III reduced in the female. One sesamoid occurring in tarso-metatarsal joint at level of metatarsal IV, other between the proximal heads of tibiale and fibulare. Phalangeal formula of foot 1–2–2–4–2 (male), 1–2–3–4–2 (female).

Distribution and Natural History: *Melanophryniscus setiba* is known only from the type locality, where it was found in leaf litter of forested areas. Specimens were usually found in *mata seca* ("dry forest"). Several individuals were observed at the forest edge, less than 1 m away from an adjacent formation known as "open *Clusia* formation." The open *Clusia* formation occurs in sandy soil and is characterized by discontinuities in the vegetation with small and large shrubs forming a mosaic structure (fig. 13).

*Melanophryniscus setiba*, like most other *Melanophryniscus* (see Santos and Grant, 2011), is apparently a diurnal species with a peak of activity recorded in late afternoon. One specimen was collected at night while walking on the leaf litter, while 10 others were collected when daylight was available (all others collected in pitfall traps). Additional specimens were seen, but not collected, during the day (P.L.V.P., personal obs.). The species is a walking toad, with locomotion similar to that previously observed in other *Melanophryniscus* spp. and similar bufonids (McDiarmid, 1971; Baldo and Basso, 2004).

Despite a major, concentrated effort to obtain data on the reproductive biology of this species (see Material and Methods), no reproductive activity (e.g., calling males or amplectant pairs) or clues that could point to
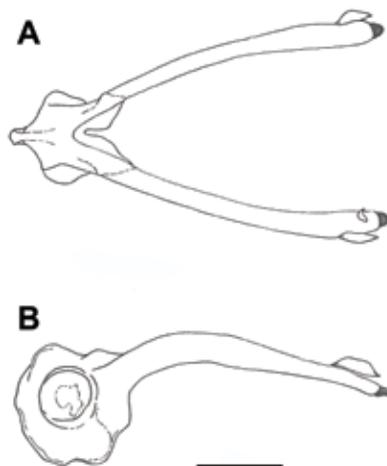


FIG. 10. Pelvic girdle of *Melanophryniscus setiba* (CFBH 15735, female) in **A.** dorsal, and **B.** lateral views. Scale bar = 1.0 mm.
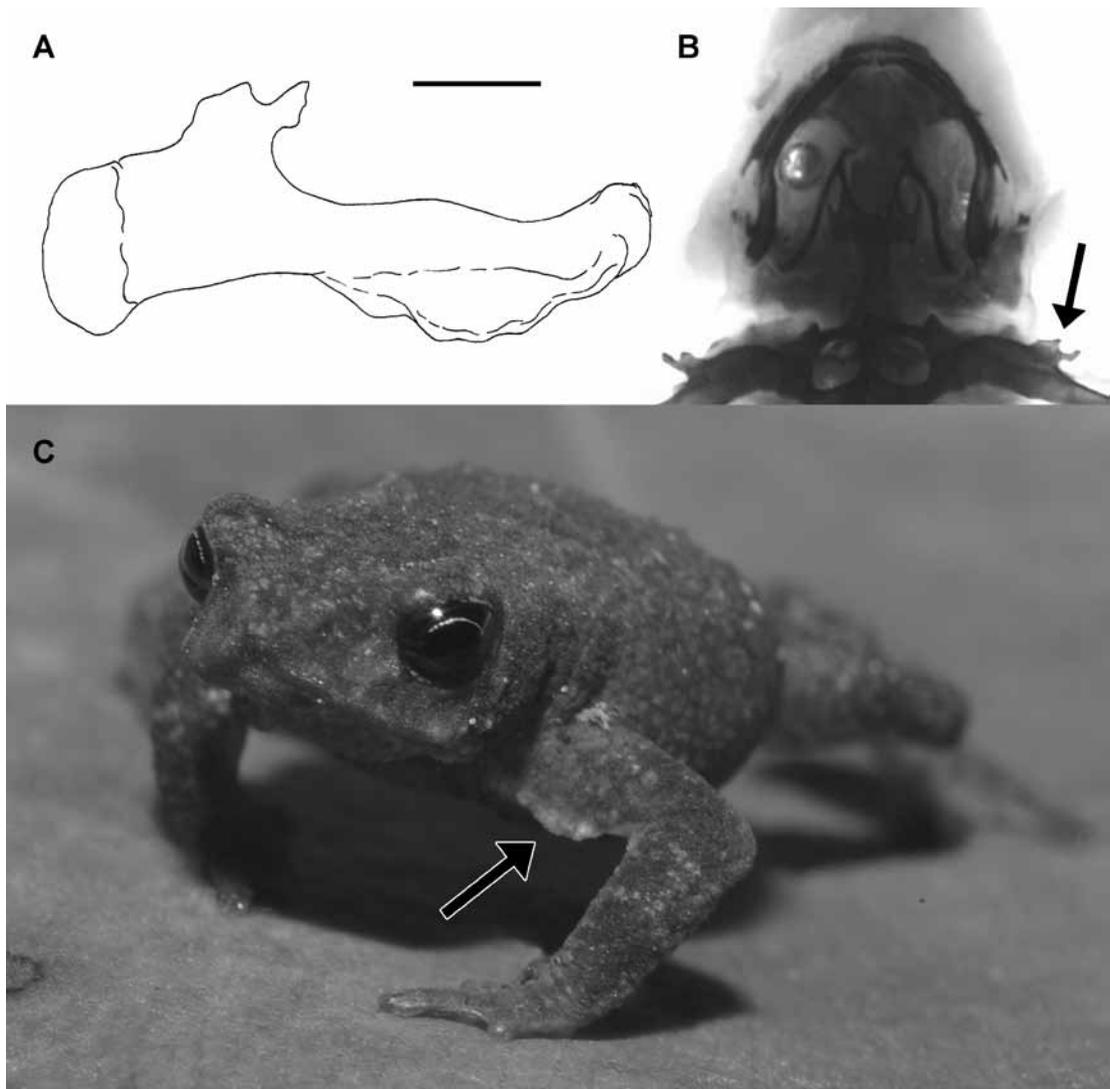
FIG. 11. Humerus and humeral crest/spine of a male *Melanophryniscus setiba* (CFBH 15745, paratype). **A.** Drawing of the humerus in dorsal view; scale bar = 2.0 mm. **B.** Photograph in ventral view; note slightly distinct morphology of spines in the right and left humerus. **C.** Same specimen, in life; note that humeral spine is externally visible in this sex but not protruding out of skin.

breeding site (e.g., eggs, tadpoles) were observed in the field. A dissected female (CFBH 15735) contained eight ova (ca. 2.0 mm each) with a pigmented animal pole and several other small immature eggs. The small ovarian complement and large size of ovarian eggs are suggestive of increased incubation time, and/or hatching in advanced developmental stages (Bradford, 1990; Summers et al., 2006, 2007). The type locality has a large quantity of bromeliad plants that could serve as a potential breeding site for *M. setiba* - phytotelm breeding is present at least in two *Melanophryniscus* species (*M. alipioi* Langone et al., 2008,

and *M vilavelhensis* Steinbach-Padilha, 2008). Nevertheless, we note that the diameter of oviposited ova in water-breeding *Melanophryniscus* are 1.7–2.0 mm in *M. krauczuki* (Baldo and Basso, 2004), 2.0 in *M. montevidensis* (Langone et al., 2008), 2.0–2.5 in *M. moreirae* (Bokermann, 1967; Starret, 1967), 1.4–2.0 in *M. stelzneri* (Echeverria, 1998; Bustos Singer and Gutiérrez, 1997), and egg diameter in the phytotelm-breeding *M. alipioi* is 2.5–3.0 mm (Langone et al., 2008). The presence of vocal slits and a vocal sac in males suggests that this species vocalizes. However, no calling males were detected, despite several targeted searches at the type locality by the senior author.

Stomachs of two specimens (CFBH 15735, female, and CFBH 15745, male) contained several ants and one mite. The diets of previously studied species



FIG. 12. Dorsal views of **A.** left hand and **B.** right foot of *Melanophryniscus setiba* (CFBH 15745, male). Scale bar = 2.0 mm.

of *Melanophryniscus* consist predominantly of ants and mites as well (reviewed by Daly et al., 2008; see also Bonansea and Vaira, 2007; Quiroga et al., 2011). Mites and ants are probably the dietary source of lipophilic alkaloids found in defensive skin secretions of *Melanophryniscus* (Saporito et al., 2011), which suggests that *M. setiba* might also secrete these alkaloids.

*Melanoprhyniscus setiba* seems to be locally abundant, although it is difficult to observe due to its small size and cryptic coloration. Most activity was recorded from December through March, which coincides with the rainy season. Other leaf-litter frogs captured in the same forest patch with the new species were *Chiasmocleis carvalhoi*, *Leptodactylus natalensis,* and *Physalaemus* cf. *crombiei*. Of these, *C. carvalhoi* and *P.* cf. *crombiei* were captured simultaneously with the new species in pitfall traps. Some hylids were also detected in the area where we found the new species, especially associated with bromeliads: *Aparasphenodon brunoi*, *Phyllodytes luteolus*, *Scinax agilis*, *S. alter* and *S. argyreornatus*. Among these, *S. argyreornatus* was the only one ever found in the leaf litter.

Etymology: The specific name, *setiba*, is derived from the Tupi Guarani language and means "seashells in abundance." Setiba is the popular name of the region where the type locality is located, likely due to the high number of mollusk shells found in the beaches of the region.

Suggested Common Names: Restinga toadlet (English); sapinho-da-restinga (Portuguese).
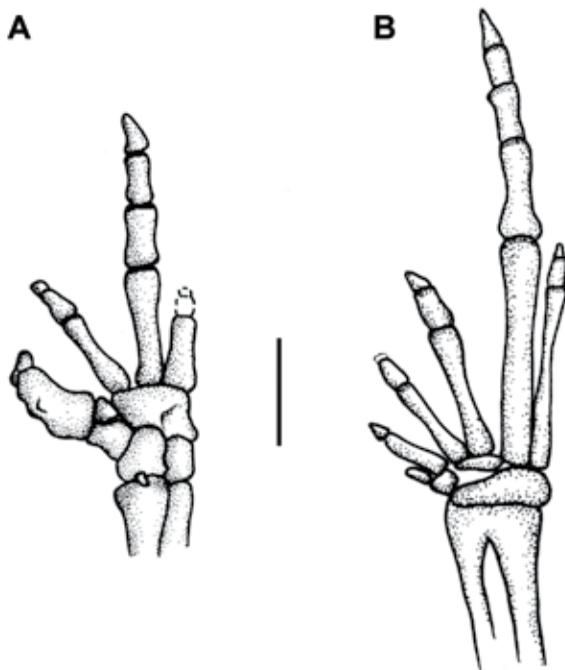
DISCUSSION

Consistent with previous findings (e.g., Graybeal, 1997; Darst and Cannatella, 2004; Frost et al., 2006; Pramuk, 2006; Van Bocxlaer et al., 2010; Pyron and Wiens, 2011), our phylogenetic analysis recovered *Melanophryniscus* as the sister taxon of all other bufonids. *Melanophryniscus setiba*, in turn, was placed as the sister taxon of the remaining exemplars of *Melanophryniscus*. Although our taxon sampling does provide a rigorous test of the placement of *M. setiba* among bufonids and other anurans,[7] it leaves the question of its placement among the species of *Melanophryniscus* largely unanswered. Of the 26 currently recognized species, only three were included in the present analysis. Following Cruz and Caramaschi (2003), *Melanophryniscus klappenbachi* and *M. fulvoguttatus* are exemplars of the *M. stelzneri* group, and *M. devincenzii* is an exemplar of the *M. tumifrons*



FIG. 13. Habitat of *Melanophryniscus setiba* at the type locality**. A.** General view of an open *Clusia* formation with a *mata seca* type of forest at background, and **B.** microhabitat of the species, sandy soil covered with leaf litter inside the *mata seca*.

groups. Unfortunately, we lack exemplars of the *M. moreirae* group and were also unable to include the recently named phytotelm breeders *M. alipioi* (Langone et al., 2008) and the diminutive *M. vilavelhensis* (unassigned to any group, Steinbach-Padilha, 2008). Importantly, we also failed to include *Truebella* (presumably related to *Melanophryniscus*; Pramuk, 2006). Sampling within *Melanophryniscus* must be greatly increased to test the placement of *M. setiba* relative to other species of this clade.

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[7] The number of recognized bufonid genera is a matter of ongoing discussion (see Frost et al., 2006, 2008, 2009; Pauly et al., 2009; Pyron and Wiens, 2011). We follow Frost (2011), who lists 48 genera, with the addition of *Amazophrynella* (Fouquet et al., 2012a, 2012b). We included 30 out of the 49 genera in our analysis. Most of the exclusions are unlikely to influence the position of the new taxa (e.g., the African genera *Altiphrynoides, Churamiti*), but some exclusions are, nonetheless, regrettable (see text). Our analysis was performed with the sole purpose of positioning the new species and we reserve ourselves to not comment any further on outgroup relationships and on the relationships within Bufonidae.

McDiarmid (1971) proposed several diagnostic features of *Melanophryniscus*, and Graybeal and Cannatella (1995) further listed as putative synapomorphies: the "absence of the zygomatic ramus of the squamosal, exostosed frontoparietals that diverge anteriorly, ossified orbitosphenoid cartilage, frontoparietals always fused posteriorly, and parasphenoid fused to the underlying chondrocranium…." Most of those synapomorphies were found in the specimens examined by us, including *M. setiba.* The only inconsistency we observed regards the "frontoparietals that diverge anteriorly." McDiarmid (1971) had already shown that the frontoparietals may not diverge anteriorly in some *Melanophryniscus* and this was confirmed by us when analyzing *M. moreirae.* The frontoparietals also do not diverge anteriorly in *M. setiba.* The apparent absence of a Bidder's organ in *M. setiba* is consistent with Echeverria's (1998) observations of *M. stelzneri.* The food items found on stomachs of two specimens of *M. setiba* (ants and mites) are the same food-item classes found on other studied species of *Melanophryniscus* (Filipello and Crespo, 1994; Daly et al., 2008, Quiroga et al., 2011).

In addition to putative synapomorphies *M. setiba* shares with other species of *Melanophryniscus*, it has a number of unusual character states:

(1) Nuptial pad structure. In addition to our report of nuptial pads in *M. setiba*, the taxonomic distribution of nuptial pads in *Melanophryniscus* is poorly known. Several papers dealing with the taxonomy of the genus have omitted any reference to nuptial pads (e.g., Klappenbach, 1968; Caramaschi and Cruz, 2002; Kwet et al., 2005; Di-Bernardo et al., 2006; Steinbach-Padilla, 2008), with the notable exceptions of Cei (1980), Baldo and Basso (2004), Langone et al. (2008), and Baldo et al. (2012), who mention its presence but do not discuss taxonomic distribution. Nuptial pads are known to be present in *M. alipioi* (Langone et al., 2008), *M. cambaraensis* (Santos et al., 2010), *M. krauczuki* (Baldo and Basso, 2004), *M. pachyrhynus* (Baldo et al., 2012), *M. rubriventris* (Vaira, 2005), and *M. stelzneri* (Cei, 1980), and we have observed them in adult males of *M. devincenzii*, *M. moreirae*, *M. simplex*, and *M. tumifrons*. In all these species, the pad occurs on finger II and most frequently on III or even IV (the case of *M. rubriventris*). Our observations indicate that the pad is composed of many minute, keratinized spines. In contrast, the nuptial pad of *M. setiba* has only a few, enlarged keratinized spines at medial margin of finger II (fig. 4C). The enlarged spines present in the pad of *M. setiba* are extremely infrequent in bufonids and are most reminiscent to those described and illustrated by Duellman and Ochoa (1991) in *Nannophryne corynetes* (as *Bufo corynetes*).

(2) Presence of anterior process of hyoid. The anterior process is absent in *M. moreirae, M. rubriventris, M. krauczuki,* and *M. pachyrhynus* (McDiarmid, 1971, 1972; Baldo and Basso, 2004; Baldo et al., 2012) but present and well developed in *M. setiba*. An anterior process on the hyale is also present in some species of *Atelopus*, (McDiarmid, 1971) and *Frostius* (Cannatella, 1986).

(3) Small ovarian complement. McDiarmid (1971) mentioned clutches with 78–237 eggs with black animal pole and light vegetal pole for *Melanophryniscus;* Baldo and Basso (2004) reported more than 109 eggs per clutch and up to 401 eggs per breeding event in *M. krauczuki.* We observed an ovarian complement of eight ova in *Melanophryniscus setiba,* the same number of eggs reported for a clutch in *M. vilavelhensis* (Steinbach-Padilha, 2008).

(4) Low phalangeal formula. Previously studied species of *Melanophryniscus* present phalangeal formulae of 2-2-3-3 and 2-2-3-4-3 for hands and feet, respectively (McDiarmid, 1971; Baldo and Basso, 2004). In contrast, the phalangeal formulae are 1-2-3-2 for hands and 1-2-2-4-2 or 1-2-3-4-2 in feet for *M. setiba*. Phalangeal loss, involving fingers II and V, and toes I, III, and V follows the general pattern described by Alberch and Gale (1985) in anurans (see also Yeh, 2002). Phalangeal losses occur also occur in other bufonids, such as some species of *Atelopus, Dendrophryniscus, Didynamipus, Incilius, Mertensophryne*, and *Osornophryne* (Savage and Kluge, 1961; McDiarmid, 1971; Ruiz-Carranza and Hernández-Camacho, 1976; Grandison, 1981).

(5) Spinelike humeral process. The highly developed ventral crest of the humerus is apparently a novelty among bufonids. While a notable ventral crest was reported by Perret (1972) and Boistel and Amiet (2001) for *Wolterstorffina mirei* and *W. chirioi* respectively, it is not elaborated into a spine. Similar processes, in shapes that vary from an elevated ridge to a sharply pointed, curved spine, are present at least in Centrolenidae (Cisneros-Heredia and McDiarmid, 2007), Hylidae (Bokermann, 1965), Microhylidae (Matsui, 2009), Telmatobiidae (Lavilla and Sandoval, 1991), and Rhacophoridae (Kuramoto and Joshy, 2003).

## CONSERVATION REMARKS

The coastal region of Brazil is one of the country's most disturbed and exploited areas (Morelato and Haddad, 2000). The demographic density of the coastal zone is much higher than the country's average (Ministério do Meio Ambiente, 2002) and, as a result, the remaining vegetated coastal strips have been intensely affected by human activity and a consequent degradation of the habitats (Morellato and Haddad, 2000).

Restingas are relatively fragile coastal environments and the clearing of vegetation results in further difficulties for habitat regeneration. Surprisingly, according to an extensive survey on the restingas of southeastern Brazil (Rocha et al., 2003), these coastal habitats, despite being in the highest human density zone, remain one of the least-known environments, not only in biodiversity, but also regarding conservation status of its remnants. The discovery of this species in Guarapari reinforces the notion that the Atlantic rainforest and its related habitats, such as restingas, are home to a great and still poorly known biodiversity. Several other vertebrate species have been recently described from restinga habitats across coastal Brazil (see examples in Rocha et al., 1997, 2000; Dias et al., 2002, Peixoto et al., 2003, Verrastro et al., 2003; Izecksohn et al., 2009). The high level of degradation of restinga habitats is a threat to survival of many species, especially to those endemic or rare.

Recognition of *Melanophryniscus setiba* as another case of endemism of restinga habitat seems tempting; however, it is possible that this species occurs in nearby forested areas outside the restinga domain. Some cases of amphibian species endemic to restinga habitats in southeastern Brazil were reported by Rocha et al. (2005)—*Scinax agilis, S. littoreus* and *Xenohyla truncata* (Hylidae), *Rhinella pygmaea* (Bufonidae), and *Leptodactylus marambaiae* (Leptodactylidae)—but at least *R. pygmaea* was shown not to be endemic to restingas (Silva et al., 2007).

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# APPENDIX 1

### Additional Specimens Examined

*Atelopus spumarius*: MPEG 2875 (Serra Norte, Carajás, Pará, Brasil). *Amazophrynella minuta*: MPEG 4260, 4560–61, 4553 (Faro, Pará, Brasil). *Frostius pernambucensis*: MNRJ 33431 *Melanophryniscus* sp.: MPEG 6076 (Itatiaia, São Paulo, Brasil). *Melanophryniscus moreirae*: AMNH 16997, 16999, CFBH 296–97; *Melanophryniscus krauczuki*: MACN 39957–58; *Melanophryniscus devincenzii*: MACN 36856; *Melanophryniscus montevidensis*: AMNH 71171–74; *Melanophryniscus rubriventris*: MACN 33218–19; *Melanophryniscus stelzneri*: AMNH 77710; *Melanophryniscus* **sp. (gr. *tumifrons*)** MACN 39959–61.

# APPENDIX 2

## SPECIES INCLUDED IN THE PHYLOGENETIC ANALYSIS

Genbank accession numbers for the sequences employed in the phylogenetic analysis. Sequences were produced by Darst and Cannatella (2004), Faivovich et al. (2005), Frost et al. (2006), Grant et al. (2006), Pramuk (2006), Pramuk et al. (2007), and Roelants et al. (2007). Species in bold are those for which we are providing original sequences.

|  | 12S + tRNAVal + 16S | RAG-1 | CXCR4 | Rhodopsin Exon 1 |
|---|---|---|---|---|
| *Acris crepitans* | AY843559 | — | EF107468 | AY844533 |
| *Adenomus kelaartii* | EF107161 | — | EF107447 | — |
| *Allobates trilineatus* | DQ501998 | — | — | DQ503184 |
| *Allophryne ruthveni* | AY843564 | — | — | AY844538 |
| *Alsodes gargola* | AY843565 | — | — | AY844539 |
| *Amazophrynella minuta* | AY843582 | DQ158346 | DQ306496 | AY844555 |
| *Ameerga trivittata* | DQ502201 | — | — | DQ503190 |
| *Amietophrynus kisoloensis* | DQ158464 | — | DQ306560 | — |
| *Amietophrynus maculatus* | DQ158469 | DQ158389 | DQ306533 | DQ284005 |
| *Amietophrynus poweri* | DQ158482 | DQ158401 | DQ306559 | — |
| *Amietophrynus regularis* | DQ158485 | DQ158404 | DQ306523 | DQ283858 |
| *Amietophrynus steindachneri* | DQ158488 | DQ158406 | DQ306546 | — |
| *Amietophrynus xeros* | DQ158499 | DQ158414 | DQ306561 | — |
| *Amietophrynus brauni* | DQ158437 | DQ158361 | DQ306514 | DQ284401 |
| *Amietophrynus camerunensis* | DQ158439 | DQ158363 | DQ306555 | DQ283979 |
| *Amietophrynus garmani* | DQ158453 | DQ158375 | DQ306547 | — |
| *Amietophrynus gracilipes* | DQ158456 | DQ158378 | DQ306522 | — |
| *Amietophrynus gutturalis* | DQ158460 | DQ158382 | DQ306556 | DQ284035 |
| *Amietophrynus latifrons* | DQ283343 | — | — | DQ283970 |
| *Amietophrynus tuberosus* | DQ283362 | — | — | DQ283984 |
| *Anaxyrus americanus* | DQ158426 | DQ158352 | DQ306520 | — |
| *Anaxyrus boreas* | DQ158498 | — | DQ306499 | DQ283871 |
| *Anaxyrus cognatus* | DQ158444 | DQ158367 | DQ306502 | — |
| *Anaxyrus debilis* | DQ158449 | DQ158371 | DQ306507 | — |
| *Anaxyrus exsul* | DQ158450 | DQ158372 | DQ306550 | — |
| *Anaxyrus fowleri* | DQ158451 | DQ158373 | DQ306505 | — |
| *Anaxyrus microscaphus* | DQ158476 | DQ158395 | DQ306563 | — |
| *Anaxyrus punctatus* | DQ283160 | — | — | DQ283855 |
| *Anaxyrus quercicus* | DQ158484 | DQ158403 | DQ306562 | — |
| *Anaxyrus terrestris* | DQ158489 | — | DQ306537 | DQ283854 |
| *Anaxyrus woodhousii* | DQ158498 | DQ158413 | DQ306551 | DQ283875 |
| *Ansonia longidigitata* | DQ283341 | — | — | DQ283968 |
| *Atelognathus patagonicus* | AY843571 | — | — | AY844545 |
| *Atelopus flavescens* | DQ283259 | — | — | DQ283928 |
| *Atelopus peruensis* | DQ158419 | DQ158345 | DQ306495 | — |
| *Atelopus spumarius* | DQ283260 | — | — | DQ283929 |
| *Atelopus spurrelli* | DQ502200 | — | — | — |
| *Atelopus varius* | AY325996 | — | — | — |

|  | 12S + tRNAVal + 16S | RAG-1 | CXCR4 | Rhodopsin Exon 1 |
|---|---|---|---|---|
| *Atelopus zeteki* | DQ283252 | — | — | — |
| *Batrachyla leptopus* | AY843572 | — | — | AY844546 |
| *Brachycephalus ephippium* | DQ283091 | — | — | — |
| *Bufo bufo* | DQ158438 | DQ158362 | DQ306504 | — |
| *Bufo gargarizans* | DQ158428 | DQ158353 | DQ306531 | DQ283905 |
| *Centrolene prosoblepon* | AY843574 | AY364223 | AY364193 | AY844548 |
| *Ceratophrys cornuta* | AY326014 | AY364218 | DQ306491 | — |
| *Ceratophrys cranwelli* | AY843575 | — | — | — |
| *Chacophrys pierottii* | DQ283328 | — | — | — |
| *Cochranella bejaranoi* | AY843576 | — | — | AY844372 |
| *Crossodactylus schmidti* | AY843579 | — | — | AY844552 |
| *Cruziohyla calcarifer* | AY843562 | — | — | AY844536 |
| *Cryptobatrachus sp.* | AY326050 | — | — | — |
| *Cycloramphus boraceiensis* | DQ283097 | — | — | DQ283813 |
| *Dendrobates auratus* | AY843581 | — | AY364184 | AY844554 |
| *Didynamipus sjostedti* | AY325991 | — | — | — |
| *Duttaphrynus melanostictus* | DQ158475 | DQ158394 | DQ306508 | DQ283967 |
| *Edalorhina perezi* | AY843585 | — | — | AY844558 |
| *Epipedobates boulengeri* | DQ283037 | — | — | — |
| *Eupsophus calcaratus* | AY843587 | — | — | AY844560 |
| *Fejervarya limnocharis* | AY843588 | — | — | AY844561 |
| **Frostius erythophthalmus[a]** | **JX961680** | — | — | — |
| *Gastrotheca cornuta* | AY843591 | — | — | — |
| *Gastrotheca fissipes* | AY843592 | — | — | AY844564 |
| *Gastrotheca pseustes* | AY326051 | — | — | — |
| *Heleophryne purcelli* | AY843593 | AY364221 | AY364191 | AY844565 |
| *Hemiphractus helioi* | AY843594 | AY844382 | — | AY844566 |
| *Hemisus marmoratus* | AY326070 | AY364216 | AY364186 | DQ284029 |
| *Hyalinobatrachium fleischmanni* | DQ283453 | — | — | DQ284043 |
| *Hyla cinerea* | AY549327 | AY844408 | DQ306493 | AY844597 |
| *Hylodes phyllodes* | DQ283096 | — | — | DQ283812 |
| *Hypodactylus sp.[b]* | AY326010 | — | — | — |
| *Incilius alvarius* | DQ158425 | DQ158351 | DQ306516 | DQ283933 |
| *Incilius coccifer* | DQ158443 | DQ158366 | DQ306526 | — |
| *Incilius valliceps* | DQ158493 | DQ158409 | DQ306545 | — |
| *Incilus coniferus* | DQ158445 | — | DQ306534 | DQ283860 |
| *Incilus luetkenii* | DQ158467 | DQ158387 | DQ306565 | — |
| *Ingerophrynus divergens* | DQ283149 | — | — | DQ283849 |
| *Ingerophrynus galeatus* | DQ158452 | DQ158374 | DQ306506 | DQ283995 |
| *Ingerophrynus macrotis* | DQ158468 | DQ158388 | DQ306525 | — |
| *Kaloula conjuncta* | AY326064 | — | — | — |
| *Lepidobatrachus laevis* | DQ283152 | — | EF107461 | DQ283851 |
| *Leptodactylus latrans* | DQ158417 | DQ158343 | DQ306492 | AY844681 |
| *Leptodactylus petersi* | DQ283063 | — | — | DQ283790 |
| *Leptophryne borbonica* | EF107164 | — | EF107450 | — |

|  | 12S + tRNAVal + 16S | RAG-1 | CXCR4 | Rhodopsin Exon 1 |
|---|---|---|---|---|
| *Limnodynastes salmini* | AY326071 | AY364219 | AY364189 | — |
| *Limnomedusa macroglossa* | AY843689 | — | — | AY844682 |
| *Lithodytes lineatus* | AY843690 | — | — | AY844683 |
| *Litoria aurea* | AY843691 | — | — | AY844684 |
| *Litoria freycineti* | AY843693 | — | — | — |
| *Litoria infrafrenata* | AY843694 | — | — | AY844687 |
| *Litoria meiriana* | AY843695 | — | — | AY844688 |
| *Lysapsus limellum* | AY843697 | — | — | AY844690 |
| *Mantidactylus femoralis* | AY843698 | — | — | — |
| *Megaelosia goeldii* | DQ283072 | — | — | DQ283797 |
| ***Melanophryniscus devincenzii**[c]* | **JX961678** | — | — | **JX961676** |
| *Melanophryniscus fulvoguttatus[d]* | DQ158421 | DQ158347 | DQ306494 | — |
| *Melanophryniscus klappenbachi* | AY843699 | — | — | DQ283765 |
| ***Melanophryniscus setiba**[e]* | **JX961679** | — | **JX961675** | **JX961677** |
| *Mertensophryne anotis* | AF220910 | — | — | — |
| *Mertensophryne micranotis* | EF107207 | — | EF107491 | — |
| *Nannophryne cophotis* | DQ158446 | DQ158369 | DQ306540 | — |
| *Nannophryne variegatus* | DQ158494 | DQ158410 | DQ306515 | — |
| *Nectophryne afra* | DQ283360 | — | — | DQ283981 |
| *Nectophryne batesii* | DQ283169 | — | | — |
| *Nectophrynoides tornieri* | DQ283413 | — | EF107490 | DQ284018 |
| *Neobatrachus sudelli* | AY843700 | — | — | AY844691 |
| *Odontophrynus americanus* | AY843704 | — | — | AY844695 |
| *Oreophrynella dendronastes* | DQ158422 | — | — | — |
| *Osornophryne guacamayo* | AY326036 | — | — | — |
| *Paratelmatobius sp.* | DQ283098 | — | — | DQ283814 |
| *Pedostibes hosii* | DQ283164 | — | EF107449 | DQ283859 |
| *Peltophryne lemur* | DQ158465 | DQ158386 | DQ306513 | — |
| *Phrynoidis asper* | DQ158431 | DQ158356 | — | DQ283848 |
| *Phrynoidis juxtasper* | DQ158463 | DQ158385 | DQ306542 | — |
| *Phyllobates bicolor* | DQ502181 | — | — | — |
| *Phyllobates terribilis* | DQ502157 | — | — | DQ503244 |
| *Phyllodytes luteolus* | AY843721 | — | — | AY844708 |
| *Physalaemus cuvieri* | AY843729 | — | — | AY844717 |
| *Platymantis sp.* | AY326061 | — | — | |
| *Pleurodema brachyops* | AY843733 | — | — | AY844721 |
| *Pristimantis rhabdolaemus[6]* | AY843586 | — | — | AY844559 |
| *Pristimantis thymelensis* | AY326009 | — | — | — |
| *Pristimantis w-nigrum* | AY326004 | DQ158344 | — | — |
| *Pseudepidalea viridis* | DQ283279 | — | — | DQ283940 |
| *Pseudopaludicola falcipes* | AY843741 | — | — | AY844728 |
| *Pseudophryne bibroni* | AY843988 | — | — | AY844729 |
| *Rana temporaria* | AY326063 | — | — | — |
| *Rhacophorus bipunctatus* | AY843750 | — | — | — |
| *Rhaebo glaberrimus* | DQ158454 | DQ158376 | DQ306510 | — |
| *Rhaebo guttatus* | DQ158459 | DQ158381 | DQ306497 | DQ283994 |

| | 12S + tRNAVal + 16S | RAG-1 | CXCR4 | Rhodopsin Exon 1 |
|---|---|---|---|---|
| *Rhaebo haematiticus* | DQ158461 | DQ158383 | DQ306501 | DQ283861 |
| *Rhaebo nasicus* | DQ158477 | DQ158396 | DQ306512 | — |
| *Rhinella amboroensis* | DQ283386 | — | — | DQ284003 |
| *Rhinella arenarum* | AY843573 | AY844370 | — | AY844547 |
| *Rhinella arenarum* | DQ158429 | DQ158354 | DQ306529 | AY844547 |
| *Rhinella arequipensis* | DQ158430 | DQ158355 | DQ306564 | — |
| *Rhinella arunco* | DQ158442 | DQ158365 | DQ306552 | DQ283857 |
| *Rhinella atacamensis* | DQ158433 | DQ158357 | DQ306541 | — |
| *Rhinella castaneoticus* | DQ158440 | DQ158364 | DQ306539 | — |
| *Rhinella cf. dapsilis* | DQ158448 | DQ158370 | DQ306532 | — |
| *Rhinella cf. margaritifer* | DQ158471 | DQ158390 | DQ306554 | — |
| *Rhinella cf. margaritifer* | DQ158491 | — | DQ306511 | — |
| *Rhinella festae* | DQ158423 | DQ158349 | DQ306521 | — |
| *Rhinella granulosa* | DQ158457 | DQ158379 | DQ306557 | DQ283966 |
| *Rhinella humboldti* | DQ158434 | DQ158358 | — | — |
| *Rhinella limensis* | DQ158466 | — | DQ306509 | — |
| *Rhinella marina* | DQ158473 | DQ158392 | DQ306530 | DQ283789 |
| *Rhinella nesiotes* | DQ158478 | DQ158397 | DQ306500 | — |
| *Rhinella ocellatus* | DQ158479 | DQ158398 | DQ306538 | — |
| *Rhinella poeppigii* | DQ158481 | DQ158400 | DQ306517 | — |
| *Rhinella schneideri* | DQ158480 | DQ158399 | DQ306528 | DQ283791 |
| *Rhinella spinulosa* | DQ158487 | DQ158405 | DQ306566 | DQ283775 |
| *Rhinella vellardi* | DQ158495 | DQ158411 | DQ306527 | — |
| *Rhinella veraguensis* | DQ158496 | DQ158412 | DQ306524 | — |
| *Rhinoderma darwinii* | DQ283324 | AY364222 | AY364192 | DQ283963 |
| *Scaphiophryne marmorata* | AY843751 | AY364205 | AY364175 | — |
| *Schismaderma carens* | DQ158424 | DQ158350 | DQ306519 | DQ284027 |
| *Scythrophrys sawayae* | DQ283099 | — | — | DQ283815 |
| *Sphaenorhynchus lacteus* | AY549367 | — | — | AY844754 |
| *Telmatobius bolivianus* | AY843769 | — | — | AY844757 |
| *Telmatobius verrucosus* | DQ283040 | — | — | DQ283770 |
| *Thoropa miliaris* | DQ283331 | — | — | — |
| *Trachycephalus venulosus* | AY549362 | AY844493 | — | AY844707 |
| *Trichobatrachus robustus* | AY843773 | AY364212 | AY364182 | AY844760 |
| *Vitreorana eurygnatha* | AY843595 | — | — | AY844567 |
| *Werneria mertensi* | DQ283348 | — | — | DQ283974 |
| *Wolterstorffina parvipalmata* | DQ283346 | — | — | DQ283972 |

[a] *Frostius erythophthalmus* MNRJ 32399, Brazil: Bahia: Uruçuca: Parque Estadual Serra do Canduru.

[b] These sequences were reported as *Phrynopus* sp. (KU 202652) by Darst and Cannatella (2004). However, they blast as *Hypodactylus brunneus* (KU 178258) with 99% of coverage and 99% of similarity; for that reason we label them as *Hypodactylus* sp.
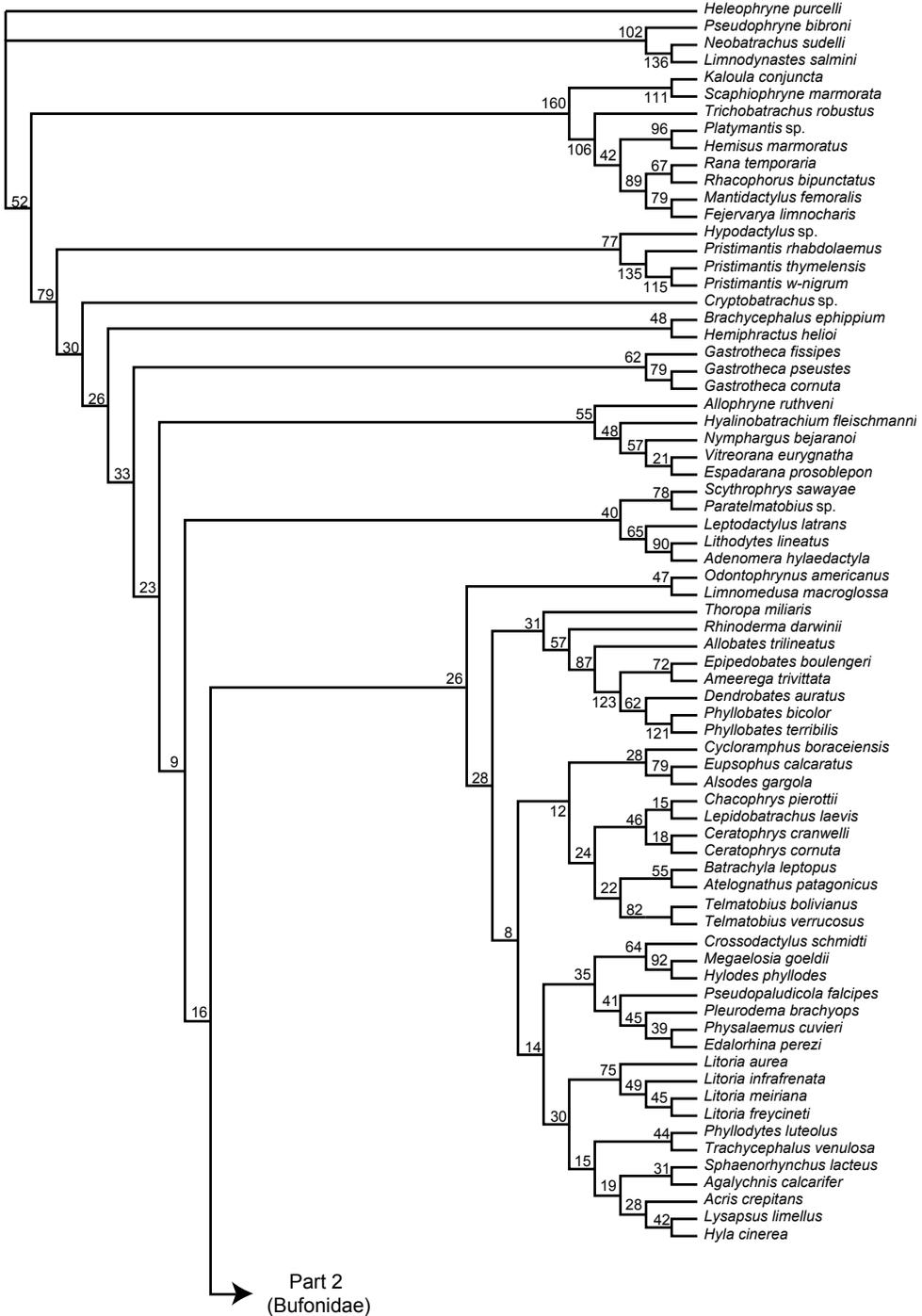
[c] *Melanophryniscus devincenzii* BKT 0074, Uruguay: Tacuarembó: Punta del Laureles.

[d] These sequences had been reported as *Melanophrynuscus stelzneri* (KU 289071) by Pramuk (2006). The specimen is actually *M. fulvoguttatus* (Diego Baldo, personal commun.).

[e] *Melanophrynuscus setiba* CFBH 15748 (tissue collection number, CFBH-T 5088), Brazil: Espírito Santo: Guarapari: Parque Estadual Paulo César Vinha (20°36′25″S; 40°25′01″W).

## APPENDIX 3: Phylogenetic Tree

Strict consensus of six optimal trees of 33,389 equally weighted steps with Goodman-Bremer support values on each node. Part of the tree is shown in figure 1. The new species, *Melanophryniscus setiba*, is shown in **boldface**.