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A NEW SPECIES OF ALLOPHRYNE (ANURA: ALLOPHRYNIDAE) FROM THE ATLANTIC RAIN FOREST BIOME OF EASTERN BRAZIL

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ABSTRACT: A new species of the genus *Allophryne* is described and, in contrast to its congeners that occur in the Amazon Basin, is based on specimens obtained in Uruçuca, State of Bahia, in the Atlantic Rain Forest of eastern Brazil. *Allophryne relicta* sp. nov. is characterized by a medium body size for the genus (snout–vent length range 19.9–21.9 mm in males); large head (head width about 35% of SVL); large, red-orange eyes, with a large black transversal stripe on iris; dorsum covered by few tubercles and many scattered black dots; dorsolateral surfaces cream with dark brown, elongate, anastomosed spots, and venter clear gray with scattered small white tubercles; presence of a line of tubercles on the ventrolateral surface of forearm; advertisement call formed by a multi-pulsed note of 0.509 ± 0.029 s in duration and emitted at intervals of 1.287 ± 0.500 s, with notes composed of 28.58 ± 1.84 pulses at a dominant frequency of 3828 ± 82.28 Hz; call-note emission rate of 34.46 notes/min and pulse emission rate of 56.13 ± 1.25 s. The molecular phylogeny supports the placement of *A. relicta* sp. nov. as sister taxon of a clade formed by *A. resplendens* + *A. ruthveni*.

Key words: *Allophryne relicta* sp. nov.; Amphibia; Taxonomy

THE FAMILIAL assignment of the genus *Allophryne* (Gaige, 1926) has been one of the most controversial among anurans (see summary in Frost, 2013), but it is currently recognized as an exclusive family, Allophrynidae, being the sister clade of the Centrolenidae (Pyron and Wiens, 2011; Frost, 2013). Representatives of the genus are known from the lowland rainforest of the Guiana Shield and Amazon. *Allophryne ruthveni* Gaige, 1926, a nonmontane forest dweller, is distributed from southern Venezuela, through Guyana, Surinam, and French Guiana, to north and central Brazil, in the states of Roraima, Amapá, Amazonas, Acre, Pará, Rondônia, and northern Mato Grosso. Additionally, it is expected to occur in adjacent Bolivia and eastern Colombia (Bernarde et al., 2006; Frost, 2013). *Allophryne resplendens* (Castroviejo-Fisher et al., 2012), is known from two

localities in Loreto, northeastern Peru (the Yavarí River drainage, and Quebrada Hungurahui, both in the Amazonian biome). This species is likely to occur in adjacent Brazil (Castruviejo-Fisher et al., 2012; Frost, 2013).

In this paper, we describe a new species of the genus *Allophryne* occurring outside of the Amazon region, viz., in the Atlantic Rain Forest in southern State of Bahia, eastern Brazil.

MATERIALS AND METHODS

Specimens and morphological analysis.— Specimens used in the description or examined for comparisons are deposited in the collections of the Museu Nacional, Rio de Janeiro, RJ, Brazil (MNRJ), the Museu de Zoologia, Universidade de São Paulo, SP, Brazil (MZUSP), the Museu de Zoologia, Universidade de Santa Cruz, Ilhéus, BA, Brazil (MZUESC), and the Célio F. B. Haddad Collection, Universidade Estadual Paulista, Rio Claro, SP, Brazil (CFBH). Comparative specimens examined are listed

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in the Appendix. The geographic coordinates of the type locality derive from Google Earth (accessed on 1 March 2013) based on the WGS84 datum.

Abbreviations used for measurements of adult specimens are SVL (snout–vent length), HL (head length), HW (head width), IND (internarial distance), END (eye–nostril distance), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance), TD (tympanum diameter), THL (thigh length), TBL (tibia length), tarsus length (TAL), FL (foot length), hand length (HAL), 4FD (4th finger disc diameter), and 4TD (4th toe disc diameter). All measurements were made with digital callipers (± 0.1 mm) using a stereo dissecting microscope; measurements followed Duellman (1970) and Napoli (2005). Snout profile terminology followed Heyer et al. (1990). Nomenclature of the fingers followed Fabrezi and Alberch (1996). Webbing formula notation followed the method of Savage and Heyer (1967), as modified by Guayasamin et al. (2006). All values are reported as means ± 1 standard deviation.

Bioacoustics.—The advertisement calls of two males of the new species (MZUESC 9226, SVL = 21.3 mm; MZUESC 9227, SVL = 20.7 mm) were recorded with a Sennheiser ME45 unidirectional microphone attached to a Marantz PMD 660 digital recorder. Recordings of a third individual (MZUESC 9225, SVL = 21.4 mm) were obtained using a Tascam DR1 digital recorder with internal microphone. In both cases, the microphone was placed at a distance of ≈ 20 cm from the recorded specimen.

The only species of the genus with a described advertisement call is *Allophryne ruthveni*. Caldwell and Hoogmoed (1998) described it based on the call of one male from Apiaú, State of Roraima, Brazil. In order to increase the number of analyzed calls to allow a better understanding of the variability of acoustic parameters within this species, we analyzed 28 advertisement calls from one male *A. ruthveni* recorded by W.E. Duellman in Puente Cuyuni, Venezuela, on 26 July 1974, at 2255 h, at an air temperature of 23°C. This sound file was provided by the Fonoteca Zoológica of the Museo Nacional de Ciencias

Naturales de Venezuela (Fonozoo number 8105), with the collector's permission.

Calls were analyzed using Raven Pro v1.4 (Ithaca, New York) and digitized at 44.1 kHz with 16-bit resolution. Waveforms and audio-spectrograms were made with fast Fourier transform using 256 points with a Hamming window function and 50% overlap. Terminology followed Duellman and Trueb (1986).

Molecular procedures.—We used the matrix of Castroviejo-Fisher et al. (2012)—12S and 16S trimmed for the tRNA's phenylalanine and valine (available from the Dryad Digital Repository; doi:10.5061/dryad.2kp7q7vb)—as a starting point, adding only sequences of our new species (GenBank accession number KF582053). To simplify future analyses, we deposited into GenBank the whole 12S + tRNA Valine + 16S (partial), the same fragments of Faivovich et al. (2005; for GenBank accession numbers, see Castroviejo-Fisher et al., 2012). Sequences of each fragment were independently realigned using MAFFT software (Katoh et al., 2002). Although Castroviejo-Fisher et al. (2012) used the E-INS-i alignment strategy, we retrieved good results for 12S using L-INS-I, and for 16S using Q-INS-I, strategies (i.e., less obvious misalignments than when using E-INS-i). The resulting multiple sequence alignments (available from the Dryad Digital Repository; doi:10.5061/dryad.9fq7q) were concatenated and analyzed under the maximum parsimony criterion using Sequence Matrix (Vaidya et al., 2010).

Given that we were only interested in assessing the position of the new species within Allocentroleniae (*sensu* Guayasamin et al., 2009) and confirm it as a member of *Allophryne*, we performed a parsimony search with the same data set used by Castroviejo-Fisher et al. (2012). The parsimony analysis was performed using TNT v1.1, Willi Hennig Society Edition (Goloboff et al., 2008) with gaps as a 5th character and using the “New Technology Search” option at level 50, including sectorial searches, ratchet (see Nixon, 1999), drift, and tree fusing (Goloboff, 1999), and requesting that the directed search hit 200 times the best length. The optimal trees were submitted to a final round of tree bisection and reconnection (TBR) branch-



FIG. 1.—*Allophryne relicta* sp. nov., holotype (MNRJ 75468; SVL 20.6 mm), dorsal and ventral views.

swapping. Jackknife support was evaluated through 1000 pseudo-replicates, with 10 RAS using TBR and 10 trees saved per replication. Uncorrected genetic distances were calculated from 761 bp of the 16S, and 923 bp of the 12S, sequences (maximum length overlapping for all the *Allophryne* sequences, Dryad Digital Repository: Found at <http://doi.org/10.5061/dryad.9fq7q>) using Molecular Evolutionary Genetics Analysis software (MEGA v5; Tamura et al., 2011).

RESULTS

Allophryne relicta sp. nov. (Figs. 1, 3)

Holotype.—MNRJ 75468, adult male (Fig. 1), collected at Uruçuca ($14^{\circ}35' S$, $39^{\circ}17' W$, 90 m altitude; datum = WGS84), State of Bahia, eastern Brazil, on 10 October 2009, by MS and IRD.

Paratypes.—MNRJ 75469–75470, MNRJ 84047–84048, CFBH 29207, CFBH 29208 (cleared and stained), CFBH 29209 (in ethanol), adult males, collected with the holotype; MNRJ 75471, MZUESC 9225–9227, adult males, collected at the type locality, on 28 January 2011, by MS and IRD.

Diagnosis.—The new species is assigned to the genus *Allophryne* because of the molecular evidence and the following combination of morphological characters: absence of metacarpal III process (M. Rada, personal observation); T-shaped terminal phalanges; tibiale and fibulare not fused (M. Rada, personal observation); absence of neopalatines and quadratojugals; protruding snout in lateral view. Note that, although these morphological character states have been used and accepted as diagnostic characters by Castroviejo-Fisher et al. (2012), none of them is a putative synapomorphy of *Allophryne*.

The new species is diagnosed by the following combination of traits: (1) medium size for the genus (range of SVL = 19.9–21.9 mm in males); (2) head large (HW about 35% of SVL); (3) eyes large and prominent, red-orange in color, with a large black transversal stripe on iris; (4) dorsum covered by few tubercles and many scattered black dots; (5) presence of a line of tubercles on the ventrolateral surface of forearm (ulnar tubercles of Castroviejo-Fisher et al., 2012); (6) webbing formula on feet, I 0^{+} – $2\frac{1}{2}$ II 1–2 III $1\frac{1}{2}$ – $2\frac{1}{2}$ IV $3\frac{1}{2}$ –2 V; (7) dorsolateral surfaces cream with dark brown, elongate, anastomosed stripes, and venter clear gray with

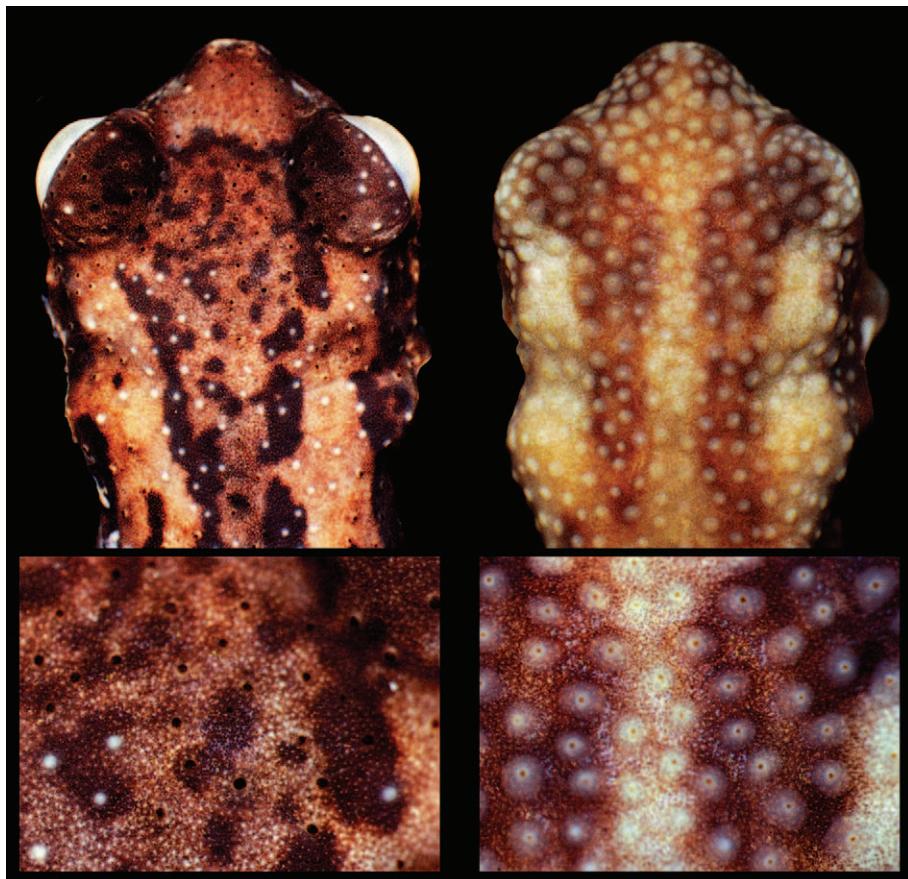


FIG. 2.—Dorsal view of the head and detail of the dorsal tubercles distribution in *Allophryne relictus* sp. nov. (left; holotype, MNRJ 75468, SVL 20.6 mm) and *A. ruthveni* (right; MZUSP 63850; SVL 22.4 mm).

scattered small white tubercles; (8) advertisement call formed by a multi-pulsed note of 0.029 ± 0.0509 s (range = 0.448–0.565 s) in duration, emitted at intervals of 1.287 ± 0.500 s (0.745–4.213 s), with notes composed of 25–32 pulses ($\bar{X} = 28.58 \pm 1.84$) and having a dominant frequency of 3828 ± 82.28 Hz (range = 3750–3962 Hz). The note emission rate was 34.46 notes/min and the pulse emission rate averaged 56.13 ± 1.25 s (50.51–59.39 s).

Allophryne relictus sp. nov. is distinguished from *A. ruthveni* by the larger head (HW about 35% of SVL in *A. relictus* sp. nov., compared with ≈30% of SVL in *A. ruthveni*), larger eyes, and a dorsum covered by few tubercles and many scattered black dots (dorsum covered by many tubercles with

keratinized spines, without black dots, in *A. ruthveni*; Fig. 2). *Allophryne relictus* sp. nov. presents a line of ulnar tubercles (absent in *A. ruthveni*) and longer legs (TBL about 45% of SVL in *A. relictus* sp. nov., compared with ≈42% of SVL in *A. ruthveni*). The labial and gular regions are immaculate in *A. relictus* sp. nov., whereas they contain distinctive white ocelli in *A. ruthveni*. The new species presents longer calls, more pulses per note, and a lower dominant frequency and pulse repetition rate than *A. ruthveni*.

Allophryne relictus sp. nov. is distinguished from *A. resplendens* by the former having larger eyes, a smaller tympanum, ulnar tubercles present (absent in *A. resplendens*; Castroviejo-Fisher et al., 2012), webbing less developed on feet (I $0^+ - 2\frac{1}{2}$ II 1–2 III $1\frac{1}{2} - 2\frac{1}{2}$

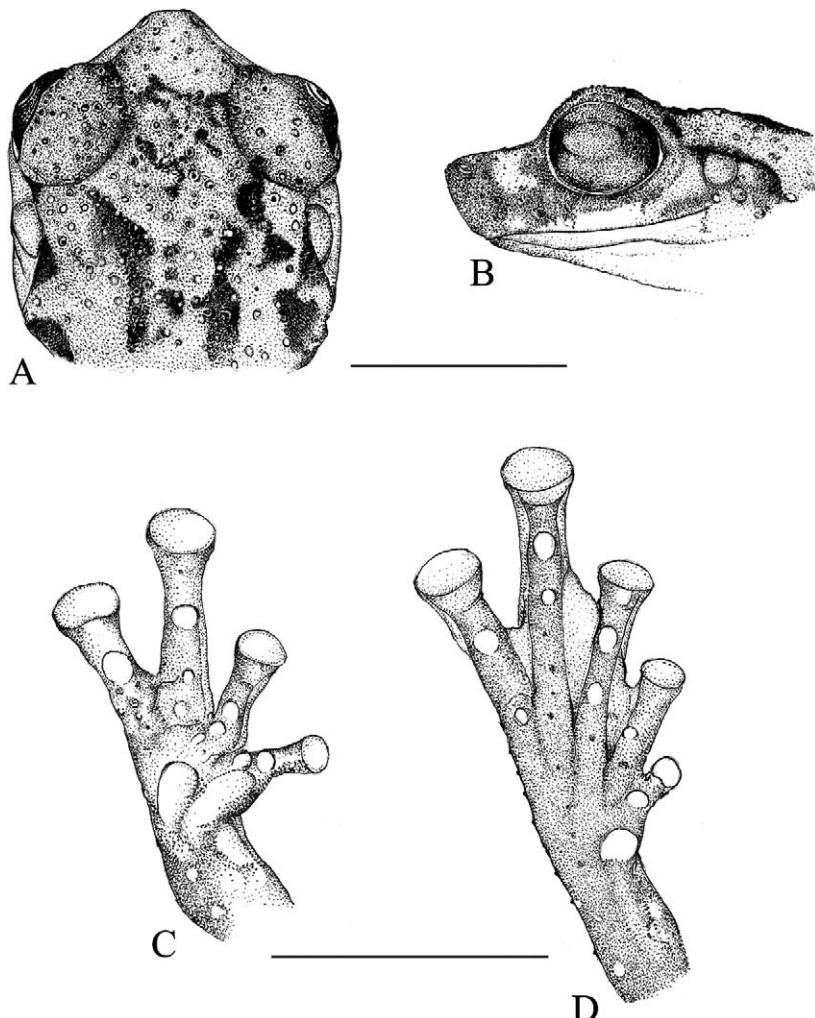


FIG. 3.—*Allophryne relicta* sp. nov., holotype (MNRJ 75468). (A) Dorsal view of head; (B) Lateral view of head; (C) Hand; (D) Foot. Bars = 5 mm.

IV $3\frac{1}{2}$ –2 V in *A. relicta* sp. nov.; I 0^+ –2 II 1 $^-$ –2 III 1–2 IV 3 $^-$ –2 $^-$ V in *A. resplendens*; Castroviejo-Fisher et al., 2012), and a distinct color pattern (dorsolateral surfaces cream to light brown with dark brown stripes and venter clear gray with scattered small white tubercles in *A. relicta* sp. nov.; dorsolateral and ventral surfaces dark brown to black with large and bright glossy yellow spots in *A. resplendens*; Castroviejo-Fisher et al., 2012). Additionally, *A. relicta* sp. nov. is diagnosed from *A. ruthveni* and *A. resplendens* by the color of the iris (eyes red-orange, with a large black transversal stripe on iris in *A. relicta* sp.

nov.; iris dark bronze with black reticulations in *A. ruthveni* and *A. resplendens*; see Bernarde et al., 2006; Castroviejo-Fisher et al., 2012).

Description of holotype.—Slender body; head width larger than head length (HL 84.1% of HW; HW 36% of SVL; HL 30.3% of SVL); snout truncate in dorsal view, protruding in profile (Fig. 3A–B); nostrils not protuberant, situated and directed laterally, nearer to the tip of snout than to eye; internarial distance smaller than eye–nostril distance (IND 59.8% of END), eye diameter (IND 47.3% of ED), upper eyelid width (IND

TABLE 1.—Range and mean values (± 1 standard deviation) of the measurements (mm) of the type specimens of *Allophryne relictica* sp. nov. ($n = 11$ adult males).

Characters	Range	Mean
SVL	19.9–21.9	20.9 \pm 0.57
HL	6.0–6.8	6.2 \pm 0.24
HW	7.0–7.5	7.2 \pm 0.20
IND	1.3–1.4	1.3 \pm 0.02
END	2.1–2.3	2.2 \pm 0.08
ED	2.4–2.9	2.7 \pm 0.17
UEW	1.9–2.6	2.2 \pm 0.19
IOD	2.3–2.8	2.6 \pm 0.18
TD	0.9–1.4	1.1 \pm 0.17
THL	8.5–9.3	9.0 \pm 0.25
TBL	8.7–10.1	9.4 \pm 0.34
TAL	4.4–5.3	4.9 \pm 0.26
FL	7.7–8.9	8.3 \pm 0.37
HAL	6.0–6.6	6.1 \pm 0.27
4FD	1.2–1.5	1.3 \pm 0.09
4TD	1.1–1.4	1.3 \pm 0.09

62% of UEW), and interorbital distance (IND 47.7% of IOD); eye–nostril distance smaller than eye diameter and interorbital distance (END 79.1% of ED; END 79.7% of IOD), and larger than upper eyelid width (UEW 96.4% of END); upper eyelid width smaller than eye diameter and interorbital distance (UEW 76.3% of ED; UEW 76.9% of IOD); eyes large, prominent, situated laterally, directed forward; canthus rostralis distinct, rounded; loreal region oblique, slightly concave; tympanum evident, small, circular, separated from posterior border of eye by approximately one and a half diameter of tympanum; dorsal margin of tympanum covered by the supratympanic fold; tympanum diameter smaller than internarial distance (TD 69.4% of IND), eye to nostril distance (TD 41.5% of END, eye diameter (TD 32.9% of ED), upper eyelid width (TD 43% of

UEW), and interorbital distance (TD 33.1% of IOD); tympanic annulus evident; supratympanic fold developed, covering dorsal edge of tympanum, curved downward and extending to insertion of arm; vocal sac poorly developed, subgular, single; vomerine, premaxillary, and maxillary teeth absent; choanae large; tongue small, rounded, slightly free and not notched posteriorly.

Forelimbs robust, with forearm diameter slightly larger than upper arms; a line of tubercles occurs along the ventrolateral aspect of forearm. Hands large (Fig. 3C), HL 29.1% of SVL; outer and inner carpal tubercles developed, elliptical; fingers, fringed, not webbed; fingers in crescent order of size, II < III < V < IV; adhesive discs developed, transversally elliptical; discs on Fingers II and III smaller than on Fingers IV and V; 4th finger disc diameter larger than tympanum diameter (TD 64.6% of 4FD); 4FD equals to 4TD; prepollex indistinct; nuptial pad on the internal base of the Finger II; subarticular tubercles large, rounded, single; few supernumerary tubercles.

Hind limbs short, slender; thigh length smaller than tibia length (THL 93.8% of TBL; THL 42.8% of SVL; TBL 45.7% of SVL); sum of thigh and tibia lengths smaller than SVL (THL + TBL 88.5% of SVL); a weak fold on posteroventral border of tarsus. Tarsal length smaller than foot length (TAL 58.5% of FL). Foot length smaller than thigh and tibia lengths (FL 93.2% of THL; FL 87.4% of TBL). Plantar surface with small, few, nearly aligned supernumerary tubercles (Fig. 3D); inner metatarsal tubercle large, rounded; outer metatarsal tubercle absent; toes short, fringed; discs on Toes I, II, and III smaller

TABLE 2.—Call parameters of *Allophryne relictica* sp. nov. and *A. ruthveni*. All means are reported ± 1 standard deviation.

Call parameters	<i>Allophryne relictica</i> sp. nov.	<i>Allophryne ruthveni</i> (this study)	<i>Allophryne ruthveni</i> (Caldwell and Hoogmoed, 1998)
Call duration (s)	0.509 \pm 0.029 (0.448–0.565)	0.364 \pm 0.017 (0.337–0.398)	0.352 (0.338–0.367)
Interval between call (s)	1.287 \pm 0.500 (0.745–4.213)	0.786–0.168 (0.480–1.043)	—
Calls / min	34.46	53.48	18
Number of pulses	28.58 \pm 1.84 (25–32)	24.75 \pm 1.109 (23–27)	24
Pulse rate (pulses / s)	56.13 \pm 1.25 (50.51–59.39)	68.06 \pm 0.656 (67.02–70.03)	69.6
Dominant frequency of call (Hz)	3828 \pm 82.28 (3750–3962)	5089 \pm 188.98 (4875–5250)	4710 (4120–5510)
Air temperature (°C)	23.1	23	23.8
Calls analyzed	90	28	2



FIG. 4.—Different color patterns of *Allophryne relicta* sp. nov. (specimens not identified).

than those on Toes IV and V; toes discs slightly smaller than fingers discs (4TD 94.4% of 4FD); subarticular tubercles rounded; plantar formula, I $0^+ - 2\frac{1}{2}$ II 1–2 III $1\frac{1}{2} - 2\frac{1}{2}$ IV $3\frac{1}{2} - 2$ V.

Dorsal surfaces smooth, with small rounded white tubercles and many small black dots scattered without forming definite pattern; calcar appendage and supracloacal crest absent; ventral surfaces areolate.

Measurements of holotype (mm).—SVL 20.6; HL 6.2; HW 7.4; IND 1.3; END 2.2; ED 2.8; UEW 2.2; IOD 2.8; TD 0.9; THL 8.8; TBL 9.4; TAL 4.8; FL 8.2; HAL 6.0, 4FD 1.4; 4TD 1.4.

Color of holotype.—In preservative, dorsum cream with a darker (brown) area extending middorsally from the interorbital region to urostyle region on each side, resembling an elongate hour-glass; laterally and delimiting the darker area, there are elongate, dark brown, irregular stripes and anastomosing spots; in the center of the area are irregular dark brown spots, smaller on the head and increasing in size toward the posterior. Flanks dark brown with scattered, irregular silvery spots. Arms dark brown anteriorly and white posteriorly; forearms cream with irregular dark brown spots. Legs bronze with irregular dark brown spots on dorsum and white spots on posterior surfaces of thighs and on knee. Venter clear gray; gular region densely white pigmented; chest with small irregular white spots; belly with white tubercles, giving a coarsely areolate appearance; ventral surfaces of thighs cream with small scattered white

dots. Eyes silver, with a transversal black stripe on iris.

Color in life.—In life, dorsum cream to yellow with variable amount of dark brown spots, from few, small, scattered spots (Fig. 4, left) to densely distributed, anastomosed, dark brown spots, forming an elongate hour-glass shape (Fig. 4, right); flanks dark brown to gray, with variable amount of irregular yellow spots; venter uniformly dirty white; eyes red-orange, with a black transversal stripe on iris.

Variation.—In addition to the variation in dorsal color pattern, the type specimens are similar in morphological traits (Table 1).

Etymology.—The specific epithet, a Latin adjective (“*relicta*”), meaning abandoned, forsaken, is an allusion to the occurrence of the new species associated to the “Hileia Bahiana,” a portion of the Atlantic Rain Forest that holds many biological components similar to, or directly related to, ones found in the Amazon; a relic of a past connection between these two biomes.

Advertisement call.—Males of *Allophryne relicta* were calling from herbaceous and shrubby vegetation (Fig. 5) between 20 cm and 120 cm height on the banks of an ephemeral stream inside a cacao plantation at 0130 h on 28 January 2011. We found about 10 vocalizing males, of which 3 were recorded. We analyzed 30 advertisement calls recorded from each individual, totaling 90 analyzed calls. The air temperature at the time of recording was 23.1°C.

The advertisement call of *A. relicta* (Table 2; Fig. 6) is formed by a multi-pulsed note of 0.509 ± 0.029 s (range = 0.448–0.565 s) in



FIG. 5.—Male *Allophryne relictica* sp. nov. in characteristic calling posture (specimen not identified).

duration, emitted at intervals of 1.287 ± 0.500 s (0.745 – 4.213 s). The notes consist of 25–32 pulses ($\bar{X} = 28.58 \pm 1.84$) with a dominant frequency of 3828 ± 82.28 Hz (range = 3750 – 3962 Hz). The note emission rate is 34.46 notes/min and pulse emission rate is 56.13 ± 1.25 s (50.51 – 59.39 s).

The advertisement of *A. relictica* differs from the call of *A. ruthveni* by a longer call duration and higher number of pulses per note, and by a lower dominant frequency and pulse repetition rate (Table 2; Caldwell and Hoogmoed, 1998; Chek et al., 2003).

Molecular analysis.—Tree search retrieved 25 equally most parsimonious trees with 12,141 steps; the strict consensus of which (Fig. 7) is similar to the one of Castroviejo-Fisher et al. (2012). *Allophryne relictica* is recovered as a sister taxon to the clade formed

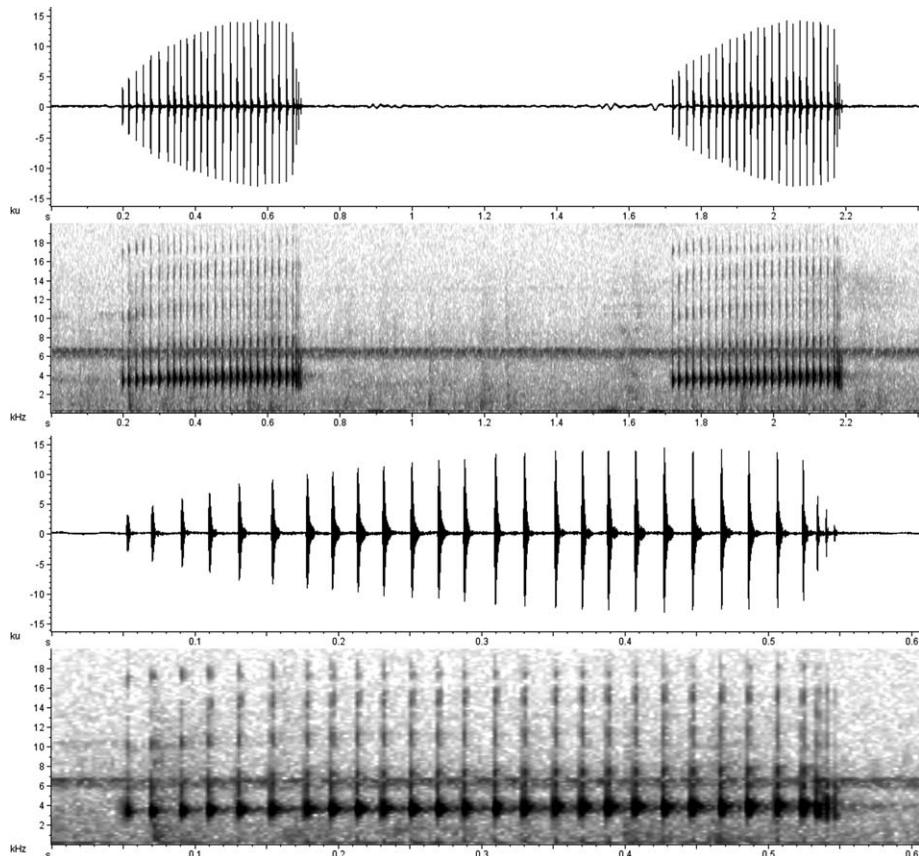


FIG. 6.—Waveform and audiospectrogram of the advertisement call of *Allophryne relictica* sp. nov., showing two calls (above) and one call (below).

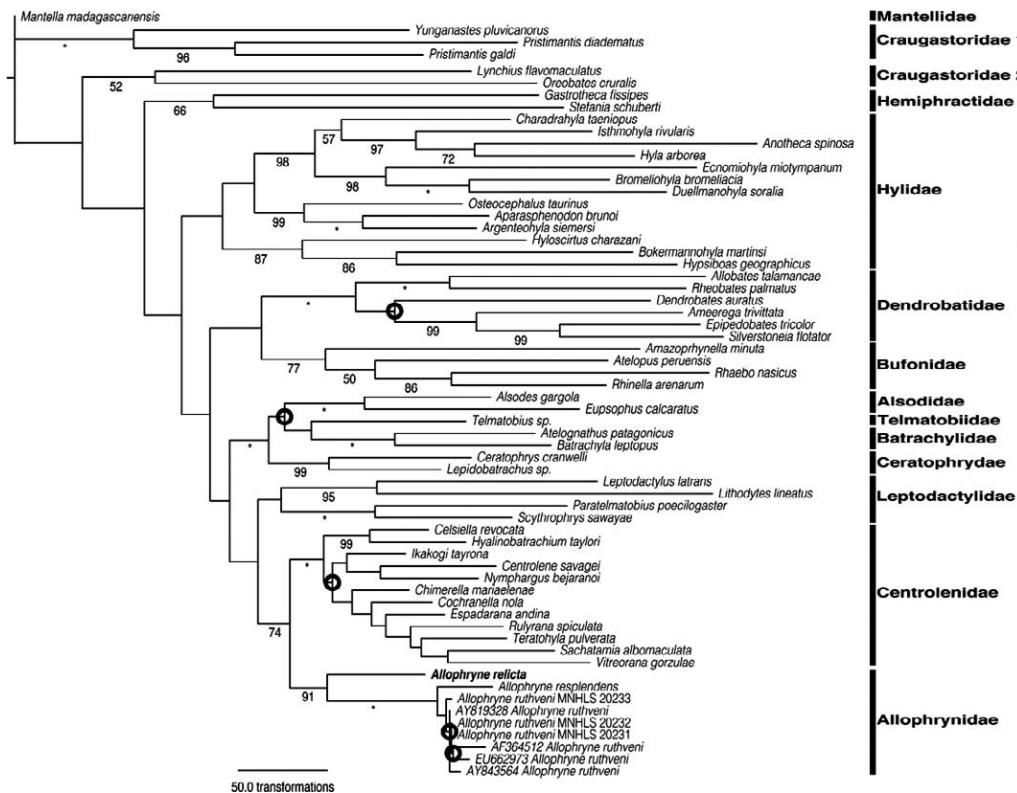


FIG. 7.—One of the 25 equally most parsimonious trees with 12,141 steps recovered by parsimony analysis. The new species is in bold. Numbers below nodes are Jackknife support values > 50%. Nodes marked with a black circle collapse in the strict consensus. Numbers before species names are GenBank accession numbers. Numbers after terminals of *A. ruthveni* are voucher numbers. MNHLS stands for “Museo de Historia Natural La Salle.”

by *A. resplendens* + *A. ruthveni*. Values of genetic divergence are presented in Table 3.

Geographic distribution.—*Allophryne relicta* is known only from the type locality, in Uruçuca, State of Bahia, eastern Brazil (Fig. 8).

Natural history.—Specimens of *Allophryne relicta* were found after heavy rainfall in small aggregations of 10–20 individuals on the leaves of shrubs on the banks of an approximately 2-m-wide temporary stream. The frogs remained at the stream for a short time, active only during the night of heavy rainfall that resulted in the filling of the stream. During the following night, only a few males were at the site calling sporadically, making their location difficult to determine. The stream that had originated the night before was dry again, with only some smaller puddles remaining. This pattern of explosive repro-

duction might explain why this species has not been discovered in other previous studies conducted in the region.

DISCUSSION

Our molecular analysis was aimed solely to confirm the new species as a member of the genus *Allophryne*, and to assess its position within the Allocentroleniae, as proposed by Guayasamin et al. (2009). In this respect, a well-supported monophyletic group was obtained, showing *A. relicta* as sister species of a group formed by the other two species of the genus, *A. resplendens* and *A. ruthveni*. This group was previously recovered by Castroviejo-Fisher et al. (2012). The Allocentroleniae were recovered as a monophyletic group involving the Allophrynidae and the Centrolenidae, as originally proposed by Guayasamin

TABLE 3.—Uncorrected genetic distances (%) between specimens of *Allophryne* inferred from 923 bp of the mitochondrial genes 12S (above the diagonal) and 761 bp of the 16S (below the diagonal). Available from the Dryad Digital Repository; doi: 10.5061/dryad.9fq7q.

	1	2	3	4	5	6	7	8	9
1. <i>Allophryne relicta</i> sp. nov.	°	6.7	6.1	6.3	6.1	6.0	7.2	—	—
2. <i>A. ruthveni</i> AY843564	9.5	°	0.8	0.9	0.8	1.0	2.8	—	—
3. <i>A. ruthveni</i> AY819328	—	—	°	0.1	—	0.2	2.0	—	—
4. <i>A. ruthveni</i> MHNLS 20231	9.3	0.6	—	°	0.1	0.3	1.9	—	—
5. <i>A. ruthveni</i> MHNLS 20232	9.3	0.6	—	—	°	0.2	2.0	—	—
6. <i>A. ruthveni</i> MHNLS 20233	9.5	1.0	—	0.7	0.7	°	1.8	—	—
7. <i>A. resplendens</i> MZUNAP 01–605	10.7	2.8	—	2.5	2.5	2.9	°	—	—
8. <i>A. ruthveni</i> EU662973	10.3	1.4	—	1.4	1.4	2.1	3.6	°	—
9. <i>A. ruthveni</i> AF364512	11.0	3.2	—	2.6	2.6	3.1	4.9	3.8	°

et al. (2009). The close relationship between these families has been recovered in previous studies (e.g., Frost et al., 2006), and so is not unexpected. Although we use the unranked taxon Allocentroleniae, this fact does not mean we support the maintenance of a monotypic taxon, or favor the alternative

suggestion to recognize Allophryninae within Centrolenidae; the use of Allocentroleniae is for convenience only, given that we do not aim to resolve high-level relationships.

The occurrence of *A. relicta* in the Atlantic Forest of Eastern Brazil was unexpected, but is not inexplicable. Besides *Allophryne*, sever-

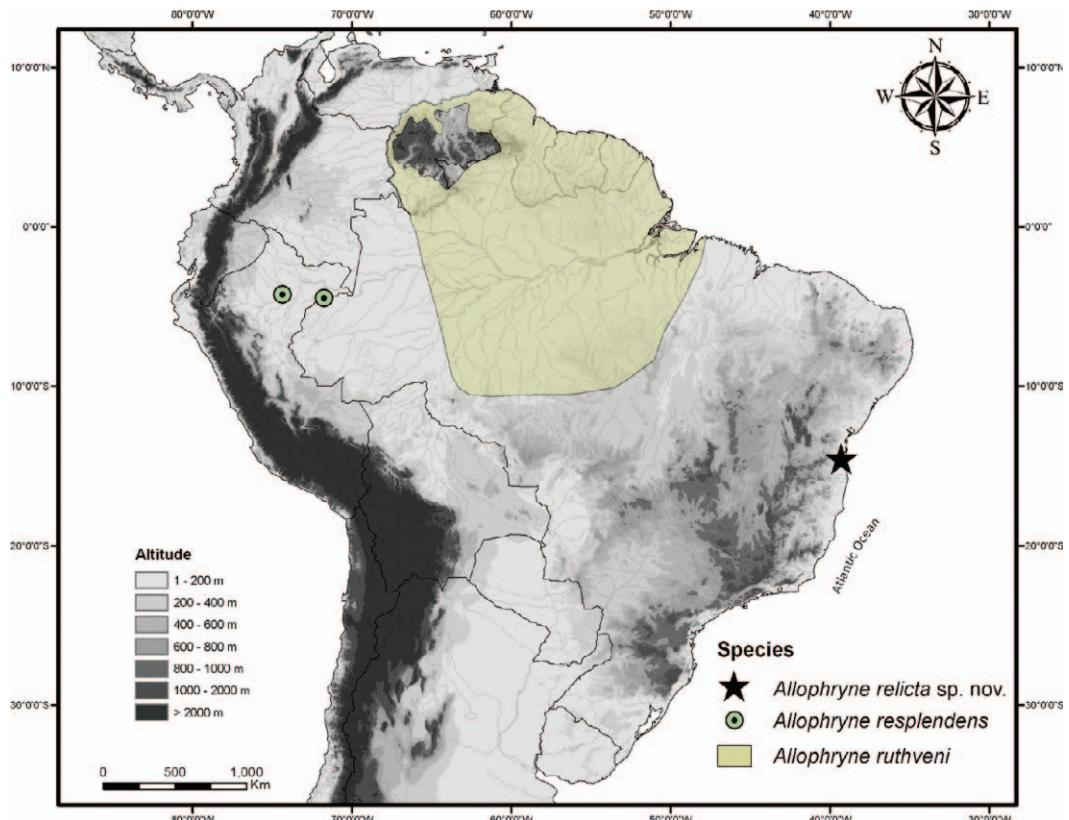


FIG. 8.—Geographic distribution of *Allophryne relicta* sp. nov. (star), *A. resplendens* (circles), and *A. ruthveni* (shaded distribution area follows IUCN, 2013).

al primarily Amazonian genera also have species distributed in Atlantic Forest, such as *Adelophryne* (Brachycephalidae; Hoogmoed et al., 1994; Fouquet et al., 2012), *Gastrotheca* (Hemiphractidae; Caramaschi and Rodrigues, 2007), and *Vitreorana* (Centrolenidae; Guayasamin et al., 2009). There is strong evidence that the Amazon Forest and the Atlantic Forest were united in the past, when wet and warm climate allowed the expansion of forested areas (see Carnaval and Bates, 2007; Fouquet et al., 2012). In the subsequent cold and dry period, the forests withdrew and these two biomes become isolated by open vegetation areas, such as the Caatinga and Cerrado biomes of northeastern and central Brazil. The putative ancestor of *A. relicta* may have originated in the Amazonian region and undergone a speciation process after the isolation of the Atlantic Forest.

The type locality in the region of Uruçuca commonly has cacao (*Theobroma cacao*) plantations that are shaded by large native or exotic species of trees. These plantations are locally known as “cabrucas,” and are characterized by a thick leaf-litter mantle and a large number of bromeliads that grow on the trees that shade the plantation, but also are less numerous on the cacao trees. Although these environments are quite different from a native forest, the cabrucas provide shelter for many forest species and maintain a higher humidity than open habitats. A large percentage of species in the landscape (e.g., 81% of litter frogs) can be found in cabrucas (Faria et al., 2007). However, cabrucas embedded within a landscape containing forest remnants have higher species richness than those found in landscapes where fragments of original forest are absent (Faria et al., 2007).

In the immediate surroundings of the site where the specimens were found, no large forest fragment remains, and the landscape is quite modified with some pastures among large areas of cabrucas. The largest forest fragment near the collection site of the specimens is located 35 km to the east: Parque Estadual da Serra do Conduru (14°28'S, 39°04'W; datum WGS84) with 9275 ha of protected area.

Major habitat modification is planned for this region of southern Bahia. A port complex called “Porto Sul,” in the city of Ilhéus, and a railroad passing through several municipalities in the state will be constructed. The first examples of this new species were found during field surveys for environmental licensing of the railway in the area of direct influence (OIKOS, 2010), and it is possible that the type locality will be affected during this construction project. The peculiarities of this new species—biogeographical significance (representing the only known member of the genus occurring in the Atlantic Rain Forest), rarity, and little knowledge of its life-history—make it pivotal that its type locality be kept intact. Although it is likely that this species has a wider distribution in the region, we should not take the risk that the only place known for the species suffers further habitat degradation or loss.

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LITERATURE CITED

- Bernarde, P.S., R.A. Machado, L.C. Macedo-Bernarde, G.R. Monção, W.V. Santos, and M.O. Silva. 2006. Amphibia, Anura, Centrolenidae, *Allophryne ruthveni* Gaige, 1926: Distribution extension for Rondônia, Southeastern Amazonia, Brazil. Check List 2:5–6.
- Caldwell, J.P., and M.S. Hoogmoed. 1998. Allophryniidae, *Allophryne*, A. *ruthveni*. Catalogue of American Amphibians and Reptiles 666:1–3.
- Caramaschi, U., and M.T. Rodrigues. 2007. Taxonomic status of the species of *Gastrotheca* Fitzinger, 1843 (Amphibia, Anura, Amphiognathodontidae) of the Atlantic Rain Forest of eastern Brazil, with description of a new species. Boletim do Museu Nacional (Nova Série) Zoologia 525:1–20.
- Carnaval, A.C., and J.M. Bates. 2007. Amphibian DNA shows marked genetic structure and tracks Pleistocene climate change in Northeastern Brazil. Evolution 61:2942–2957.
- Castroviejo-Fisher, S., P.E. Pérez-Peña, J.M. Padial, and J.M. Guayasamin. 2012. A second species of the family

- Allophrynididae (Amphibia: Anura). American Museum Novitates (3739):1–17.
- Chek, A.A., J.P. Bogart, and S.C. Lougheed. 2003. Mating signal partitioning in multi-species assemblages: A null model test using frogs. *Ecology Letters* 6:235–247.
- Duellman, W.E. 1970. The Hylid Frogs of Middle America. Monograph of the Museum of Natural History, The University of Kansas, USA.
- Duellman, W.E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill, USA.
- Fabrezi, M., and P. Alberch. 1996. The carpal elements of anurans. *Herpetologica* 52:188–204.
- Faivovich, J., C.F.B. Haddad, P.C.A. Garcia, D.R. Frost, J.A. Campbell, and W.C. Wheeler. 2005. Systematic review of the frog family Hylidae, with special reference to the Hylinae: Phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History* 294:1–240.
- Faria, D., M.L.B. Patience, M. Dixo, R.R. Laps, and J. Baumgarten. 2007. Ferns, frogs, lizards, birds and bats in forest fragments and shade cacao plantations in two contrasting landscapes in the Atlantic Forest, Brazil. *Biodiversity Conservation* 16:2335–2357.
- Fouquet, A., D. Loebmann, S. Castroviejo-Fisher, J.M. Padial, V.G.D. Orrico, M.L. Lyra, I.J. Roberto, P.J.R. Kok, C.F.B. Haddad, and M.T. Rodrigues. 2012. From Amazonia to the Atlantic forest: Molecular phylogeny of Phyzelaphryninae frogs reveals unexpected diversity and a striking biogeographic pattern emphasizing conservation challenges. *Molecular Phylogenetics and Evolution* 65:547–561.
- Frost, D.R. 2013. Amphibian Species of the World: An Online Reference. Version 5.6 (09 January 2013). Electronic Database. <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, USA. (Accessed 1 March 2013).
- Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C.F.B. Haddad, R.O. de Sá, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, B.L. Blotto, P.E. Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D.M. Green, and W.C. Wheeler. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297:1–370.
- Gaige, H.T. 1926. A new frog from British Guiana. *Occasional Papers of the Museum of Zoology, University of Michigan* 176:1–3.
- Goloboff, P.A. 1999. Analyzing large data sets in reasonable times: Solutions for composite optima. *Cladistics* 15:415–428.
- Goloboff, P.A., J.S. Farris, and K.C. Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24:774–786.
- Guayasamin, J.M., M.R. Bustamante, D. Almeida-Reinoso, and W.C. Funk. 2006. Glass frogs (Centrolenidae) of Yanayacu Biological Station, Ecuador, with the description of a new species and comments on centrolenid systematics. *Zoological Journal of the Linnaean Society* 147:489–513.
- Guayasamin, J.M., S. Castroviejo-Fisher, L. Trueb, J. Ayarzagüena, M. Rada, and C. Vilà. 2009. Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100:1–97.
- Heyer, W.R., A.S. Rand, C.A.G. Cruz, O.L. Peixoto, and C.E. Nelson. 1990. Frogs of Boracéia. *Arquivos de Zoologia* 31:231–410.
- Hoogmoed, M.S., D.M. Borges, and P. Cascon. 1994. Three new species of the genus *Adelophryne* (Amphibia: Anura: Leptodactylidae) from northeastern Brazil, with remarks on the other species of the genus. *Zoologische Mededelingen* 68:271–300.
- International Union for Conservation of Nature [IUCN]. 2013. The IUCN Red List of Threatened Species. Version 2013.1. Electronic Database. <http://www.iucnredlist.org> (Accessed 10 August 2013).
- Katoh, K., K. Misawa, K.-I. Kuma, and T. Miyata. 2002. MAFFT—a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30:3059–3066.
- Napoli, M.F. 2005. A new species allied to *Hyla circumdata* (Anura: Hylidae) from Serra da Mantiqueira, Southeastern Brazil. *Herpetologica* 61:63–69.
- Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15:407–414.
- OIKOS. 2010. Estudo de Impacto Ambiental (EIA) das obras de implantação da Ferrovia Oeste Leste (EF 334), entre Figueirópolis (TO) e Ilhéus (BA). Volume 2. I – Meio Biótico – Fauna. Ilhéus: Oikos [In Portuguese].
- Pyron, R.A., and J.J. Wiens. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* 61:543–583.
- Savage, J.M., and W.R. Heyer. 1967. Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. *Beiträge zur Neotropischen Fauna* 5:111–131.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28:2731–2739.
- Vaidya, G., D.J. Lohman, and R. Meier. 2010. Sequence-Matrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27:171–180.

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APPENDIX

Additional specimens examined

Allophryne ruthveni: BRAZIL: PARÁ: Serra dos Carajás (MNRJ 36529); Juruá, Rio Xingu (MZUSP 63843–63852). AMAPÁ: Serra do Navio (MZUSP 74444, holotype of *Sphaenorhylia seabrai*).