

A Second Species of the Family Allophrynidae (Amphibia: Anura)

SANTIAGO CASTROVIEJO-FISHER,¹ PEDRO E. PÉREZ-PEÑA,² JOSE M. PADIAL,¹
AND JUAN M. GUAYASAMIN³

ABSTRACT

We describe *Allophryne resplendens*, a new species from two localities in the Amazon rainforest of Loreto, Peru, of the family Allophrynidae, which was monotypic until this discovery. The new species can be readily differentiated from *Allophryne ruthveni* on the basis of dorsal and ventral coloration both in life and in preservative, transverse processes of presacral II oriented anterolaterally (oriented laterally in *A. ruthveni*), 19 nucleotide autapomorphies for 761 base pairs (bp) of the mitochondrial gene 16S, and 16 for 923 bp of 12S. Maximum parsimony analysis of the mitochondrial gene 12S and a fragment of up to 1060 bp of 16S supports the new species as sister to *A. ruthveni*.

INTRODUCTION

Discoveries and new descriptions of amphibian species have increased in recent years, with the number of nominal species growing of about 48.2% since 1985 (Frost et al., 2006) and 19.4% during the last decade (Köhler et al., 2005). The bulk of these new discoveries arise from applying recently developed advances in fields such as molecular biology, bioacoustics, and geography to clades from tropical regions (e.g., Meegaskumbura et al., 2002; Stuart et al., 2006; Fouquet et al., 2007; Vieites et al., 2009). Discovery of new species is, however, not randomly

1 Division of Vertebrate Zoology (Department of Herpetology), American Museum of Natural History.

2 Wildlife Conservation Society, Programa Loreto, Perú.

3 Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica, Av. Machala y Sabanilla, Quito, Ecuador.

distributed across clades of amphibians. Some groups account for most of the newly described diversity (e.g., Ranidae alone includes 25% of the new species of amphibians described since 2004: AmphibiaWeb, 2011) whereas for other groups it may be decades before a second species within a genus is discovered. For example, *Rhinatrema bivittatum* (Guérin-Méneville, 1838) remained as the only known species of *Rhinatrema* Duméril and Bribon, 1841, for 169 years until Gower et al. (2010) described a new species, although that discovery was quickly followed by another (Wilkinson and Gower, 2010).

Allophryne ruthveni Gaige, 1926, has stood as the only member of this monotypic genus and the family Allophrynidae for 85 years. It is known to occur in the lowland rainforest of the Amazon and Orinoco basins, from Venezuela and the Guianas to southwestern Brazil and probably Bolivia (Frost, 2011). Despite its ample distribution, records are rare and sparse. Phylogenetically, the relationship Centrolenidae + *Allophryne ruthveni* is the most accepted hypothesis, supported by molecular phylogenetics (Austin et al., 2002; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Guayasamin et al., 2008) and morphological studies (Duellman, 2001; Burton, 2004; Wiens et al., 2005; but see Fabrezi, 2006). In this work, we describe the second species in the family Allophrynidae, found in the Amazon rainforest of Loreto, Peru.

MATERIALS AND METHODS

NOMENCLATURE

Frost et al. (2006) placed *Allophryne ruthveni* in the monotypic subfamily Allophryninae, within Centrolenidae, whereas Guayasamin et al. (2009) argued to maintain the species in the monotypic family Allophrynidae. We followed the later arrangement (see Guayasamin et al., 2009, for argumentation).

TAXON SAMPLING AND MORPHOLOGY

Specimens were collected in the field according to approved animal use and care protocols (Heyer et al., 1994), and euthanized in Xylocaïne solution prior to fixation and preservation in ethanol 70%. Specimens studied are listed in appendix 1. Color characteristics are described according to digital photographs taken from life specimens in the field. External morphological characters were examined under a dissecting microscope. Measurements were taken with digital calipers on preserved specimens to the nearest 0.01 mm and rounded to 0.1 mm. Variables measured, as described in Duellman (2001), are as follows: snout-vent length (SVL); head length; head width; interorbital distance; eye diameter; upper eyelid; tympanum diameter; eye-nostril distance; distance from anterior margin of eye to tip of snout; internarial distance; eye-tympanum distance; length of forearm; hand length; finger I length; finger II length; width of disc of finger III; width of finger III; thigh length; shank length; foot length. Ontogenetic status was determined by examination of the development of gonads and presence of secondary sexual characteristics (e.g., vocal slits and sacs). Terminology for webbing is that described by Savage and Heyer (1967), as modified by Guayasamin et al. (2006). The osteological description



FIG. 1. Photographs of preserved specimens of *Allophryne*. **A–C**, *Allophryne resplendens*, new species, adult female holotype (SVL = 28.4 mm); **D–E**, *Allophryne ruthveni*, adult female, Surinam, AMNH 87687 (SVL = 27.0 mm). Photographs by JMP (A–C) and SCF (D–E).

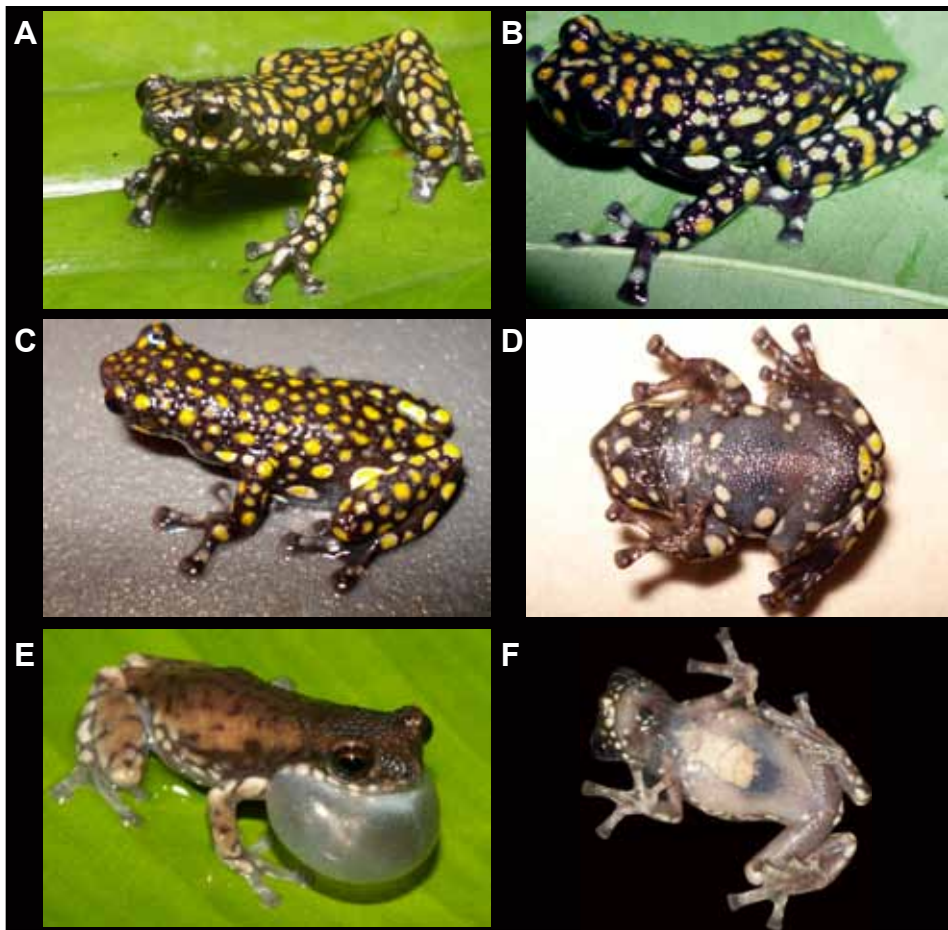


FIG. 2. Photographs of live specimens of *Allophryne*. **A**, *Allophryne resplendens*, n. sp., adult female holotype (SVL = 28.4 mm); **B**, *A. resplendens*, n. sp., specimen not collected, sex and size unknown, topotype; **C–D**, *A. resplendens*, new species, adult male, Quebrada Ungurahue (SVL = 25.0 mm); **E–F**, *A. ruthveni*, adult male, Venezuela, MHNLS 20231 (SVL = 23.5 mm). Photographs by Mark Bowler (A), WCS–Perú (B), William Lamar (C–D), and SCF (E–F).

was based on X-rays of the holotype; osteological terminology is that of Duellman and Trueb (1986), Fabrezi (1992, 1993), and Trueb (1973, 1993). Museum acronyms are: American Museum of Natural History (AMNH), Museo Historia Natural La Salle (MHNLS), and Museo de Zoología de la Universidad Nacional de la Amazonía Peruana (MZUNAP).

MOLECULAR ANALYSES

We sequenced the complete mitochondrial gene 12S (962 bp) and a fragment of the gene 16S (1060 bp) from the specimen of *Allophryne* collected in Loreto, Peru, and three specimens of *A. ruthveni* from Delta Amacuro, Venezuela. Genomic DNA was isolated using a standard phenol-chloroform extraction protocol (Sambrook et al., 1989). The selected markers were

amplified and sequenced using the same primers and PCR conditions as in Guayasamin et al. (2008) with the addition of primers 16L19 and 16H24 (Heinicke et al., 2007). Sequences from heavy and light strands were compared to generate a consensus sequence for each specimen using Sequencher 4.6 (Gene Codes Corp., 2006). We compared these sequences with those from GenBank for *Allophryne ruthveni*, as well as representative species of all other Neotropical families within Hyloidea, according to the taxonomy used in Frost (2011). We used sequences of *Mantella madagascariensis* to root phylogenetic trees. GenBank accession codes of all the sequences studied are provided in appendix 2.

Sequences of each marker were independently aligned in MAFFT (Kato et al., 2005) under the E-INS-i option. The resulting multiple sequence alignments (Dryad Repository: doi:10.5061/dryad.2kp7q7vb) were concatenated and analyzed under the maximum parsimony criterion (MP) using TNT 1.1, Willi Hennig Society Edition (Goloboff et al., 2008) with gaps as a fifth character and using the “New Technology search” option at level 100, including sectorial searches, ratchet (see Nixon, 1999), drift, and tree fusing (see Goloboff, 1999). Bootstrap support (BS, Felsenstein, 1985) was evaluated through 1000 pseudoreplicates, with 10 RAS using TBR and 10 trees saved per replication. Uncorrected genetic distances were calculated from 761 bp of the 16S and 923 of the 12S (maximum length overlapping for all the *Allophryne* sequences, Dryad Repository: doi:10.5061/dryad.2kp7q7vb) in PAUP* 4.0b10 (Swofford, 1998).

Allophryne resplendens, new species

Figures 1A–C, 2A–D

Allophryne, sp. nov., Rodríguez and Knell, 2003: 244.

HOLOTYPE: MZUNAP-01-605 (field number PEPP 500), adult female from Lago Preto (4°27'35.0"S, 71°45' 3.5"W; 120 m), Río Yavarí, Provincia Ramon Castilla, Departamento Loreto, Peru, collected by PEPP in November 2009. When collected, the distal section of the right hind limb of the specimen was missing; we cut a piece of tissue from the stump for molecular studies purposes.

REMARKS: We are aware of three other records of the new species. One is a noncollected but photographed adult (fig. 2B) found in Lago Preto, Yavarí, Loreto, Peru, in July 2008 (photographs by anonymous volunteers of Earthwatch and deposited at Wildlife Conservation Society, WCS–Perú). The second is reported by Rodríguez and Knell (2003) also from Lago Preto (4°28'S, 71°46'W; 90 m), Yavarí, Loreto, Peru. Rodríguez and Knell (2003) cite this specimen as deposited at the MUSM. However, in a visit to the MUSM in March 2008, we were unable to locate the specimen or to get a voucher code for it. The third specimen, an adult male (fig. 2C, D), was collected on the south bank of Quebrada Hungurahui (4°14'42.97"S, 74°21'36.02"W; 114 m), an affluent of Río Tigre, approximately 2 km north of Comunidad Monteverde, Loreto, Peru, by William Lamar on 1 August 2006. This specimen was collected and preserved in ethanol; unfortunately, the jar did not seal properly and the specimen totally decomposed and could not be used as a type.

GENERIC PLACEMENT: The new species is assigned to the genus *Allophryne* because of the following combination of morphological characters: absence of metacarpal III process (its pres-

TABLE 1. 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). Dryad Repository: doi:10.5061/dryad.2kp7q7vb.

	1	2	3	4	5	6	7	8
1 <i>A. resplendens</i>	*	2.0%	2.1%	1.7%	2.1%	–	–	2.1%
2 <i>A. ruthveni</i> 3301	2.6%	*	0.1%	0.3%	0.1%	–	–	0.1%
3 <i>A. ruthveni</i> 3302	2.6%	0.0%	*	0.2%	0.0%	–	–	0.0%
4 <i>A. ruthveni</i> 3303	3.0%	0.7%	0.7%	*	0.2%	–	–	0.2%
5 <i>A. ruthveni</i> AY843564	2.9%	0.5%	0.5%	0.9%	*	–	–	0.0%
6 <i>A. ruthveni</i> EU662973	2.9%	0.4%	0.4%	1.1%	0.4%	*	–	–
7 <i>A. ruthveni</i> AF364512	3.4%	0.9%	0.9%	1.4%	1.5%	1.4%	*	–
8 <i>A. ruthveni</i> AY819328	–	–	–	–	–	–	–	*

ence is a synapomorphy of centrolenid frogs), T-shaped terminal phalanges, tibiale and fibulare not fused, absence of neopalatines and quadratojugals, and protruding snout in lateral view; no phenotypic autoapomorphy is known for the genus. The molecular phylogeny also supports the placement of the new species in the genus *Allophryne*. The 10 most parsimonious trees (tree cost = 11,381 steps) resulting from 100 replicates (each including RAS + TBR + drift and tree fusing with the best tree hit 100 times) placed *Allophryne resplendens*, n. sp., as the sister species of *Allophryne ruthveni* (BS = 100; fig. 3).

DIAGNOSIS: *Allophryne resplendens*, n. sp., is distinguished from *A. ruthveni*, the only other known species in the genus and family (character states of the latter are in parentheses), by (1) color pattern (cf. photos in figs. 1 and 2): dorsolateral surfaces in life black (cream to light brown) with large and bright glossy spots due to accumulation of iridophores (absent), most of which containing irregular yellow blotches (absent), dorsolateral surfaces in preservative dark brown (cream to light brown with dark brown stripes) with large and bright glossy spots due to accumulation of iridophores (absent), ventral surfaces in life black and opaque (not pigmented, translucent) with large and bright glossy spots due to accumulation of iridophores except in palms and plantar surfaces (absent or just a few off-white spots on the throat/chest and distal parts of legs), same character states for both species for ventral surfaces in preservative than in life, but coloration dark brown instead of black for *A. resplendens*; (2) osteology: transverse processes of presacral II oriented anterolaterally (oriented laterally; observed in two cleared and stained specimens; see appendix 1, and fig. 2C in Fabrezi and Langone, 2000); and (3) DNA sequences: uncorrected genetic distance > 2.5 % for 761 bp of mitochondrial gene 16S, and \geq 1.7 % for 923 bp of 12S (table 1), 19 nucleotide autapomorphies for 761 bp of 16S, and 16 for 923 bp of 12S (table 2).

DESCRIPTION OF THE HOLOTYPE: Adult female, SVL 28.4 mm. Head wider than long (HL = 85.7% of HW); snout protruding in lateral profile and broadly rounded in dorsal view (fig. 1A, C); canthus rostralis indistinct, straight; loreal region flat; lips not flared; nostril small, not protuberant, closer to tip of snout than to eye. Eye of moderate size (ED = 10.6% of SVL). Tympanic membrane and tympanic annulus differentiated; tympanum relatively small (TD =

TABLE 2. Autapomorphies for *Allophryne resplendens* new species with respect to *A. ruthveni* inferred from a fragment of 761 bp and 923 bp of the mitochondrial genes 16S and 12S respectively. Dryad Repository: doi:10.5061/dryad.2kp7q7vb.

Marker	Position	<i>A. ruthveni</i>	<i>A. resplendens</i>
16S	91	G	A
	92	T	C
	101	G	A
	153	T	C
	174	C	T
	240	T	C
	280	A	-
	282	A	T
	333	-/T	C
	342	T	G
	464	A	G
	542	C	T
	544	T	C
	558	A	G
	584	T	C
	604	A	C
	624	T	C
	628	T	C
	638	A	T
12S	59	T	C
	91	A	G
	299	G	A
	341	T	C
	370	T	C
	375	T	C
	390	T	C
	459	T/-	C
	583	A	G
	625	A	T
	724	G	A
	748	C	T
	851	T	C
	852	T	C
	861	T	C
922	A	G	

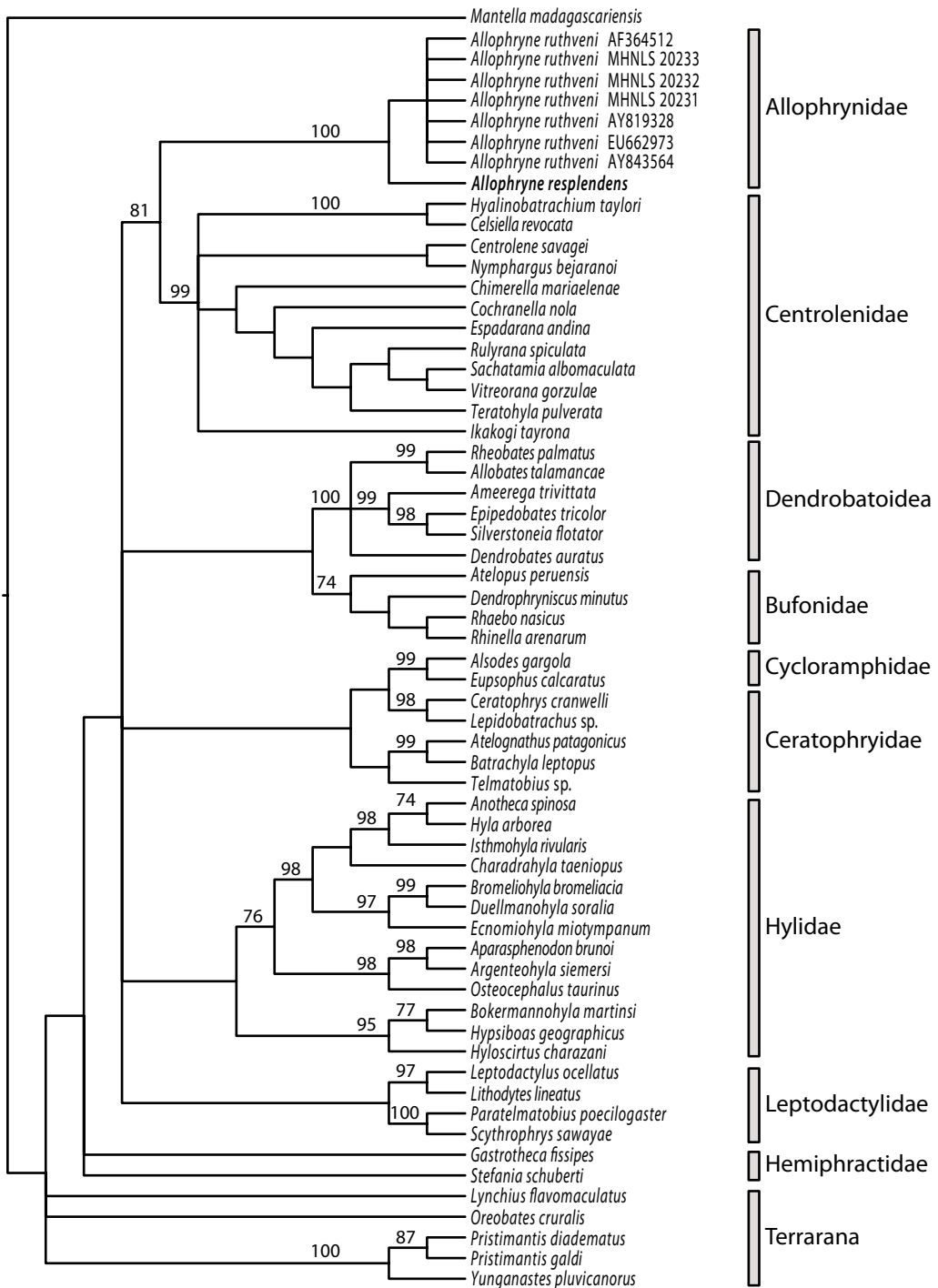


FIG. 3. Strict consensus of the 10 most parsimonious trees inferred from 12S and 16S sequences (tree cost = 11,381 steps). Bootstrap support values are indicated above branches when ≥ 70 .

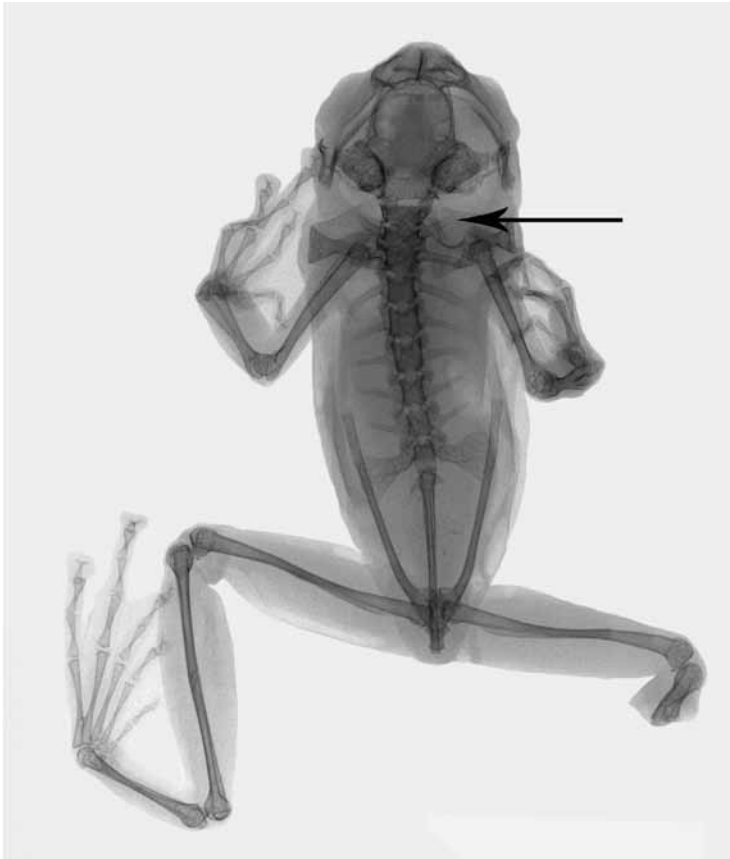


FIG. 4. X-ray of the holotype of *Allophryne resplendens*, n. sp. Arrow points to the anterolaterally oriented transverse processes of presacral II.

3.9% of SVL). Dentigerous process of vomer indistinguishable. Ulnar tubercles absent; relative lengths of fingers: III > IV > II > I webbing on hands basal. Discs expanded, truncate; subarticular tubercles round, conspicuous; few supernumerary tubercles; palmar tubercle oval, simple. Posterior limbs relatively long (FL = 40.8% of SVL; TL = 43.3% of SVL); tarsal tubercles absent; feet about 3/4 webbed, formula: I 0⁺-2 II 1⁻-2 III 1-2 IV 3⁻-2⁻ V; discs on toes elliptical; inner metatarsal tubercle ovoid; outer metatarsal tubercle small, barely evident; subarticular tubercles round, conspicuous; supernumerary tubercles absent. Skin on dorsal surfaces of head and body shagreen with hemispherical pustules having a central spicules; venter areolate. Cloacal opening directed posteriorly at midlevel of thighs, covered by a slightly developed cloacal sheath; cloacal tubercles absent.

COLOR IN LIFE: This description is based on photographs of the holotype and the other three mentioned specimens (fig. 2; Rodríguez and Knell, 2003: fig. 5C). Dorsolateral surfaces with large and bright glossy spots due to accumulation of iridophores, most of which contain yellow irregular blotches, set in a washed black reticulum; reticulum carries smaller and less bright oval to pentagon-shaped accumulations of iridophores from which brown spicules arise.

Ventral surfaces black and opaque with large and bright glossy spots due to accumulation of iridophores except in palms and plantar surfaces. Iris dark bronze with dark reticulations, pupillary ring absent, pupil black. There seems to be sexual dichromatism; the female has fewer spicules and more ventral glossy spots than the male, which is also the case in *Allophryne ruthveni*.

COLOR IN PRESERVATIVE:

This description is based exclusively on the holotype (fig. 1A–C). Dorsolateral surfaces with white large and bright irregular spots due to accumulation of iridophores, yellow irregular blotches absent, set in a dark brown reticulum that carries smaller and less bright oval to pentagon-shaped accumulations of iridophores from which brown spicules arise. Ventral surfaces dark brown with large and bright glossy spots due to accumulation of iridophores except in palms and plantar surfaces. Iris brown and pupil white.

OSTEOLOGY (fig. 4): The skull is widest posterior to the orbit and at the level of the articulation of the maxilla with the squamosal. The neopalatines and quadratojugals are absent. The anterior ramus of the pterygoid articulates with the posterior end of the maxilla. The vertebral column has eight presacral vertebrae that have transverse processes except presacral I. The vertebral profile in decreasing order of overall width of bony parts is: sacrum > III > IV > V ≈ VI ≈ VII > II > I. The orientations of the transverse processes of presacral VI are lateral, whereas those of presacrals II, VII, and VIII are anterolateral, and those of presacral III–V are posterolateral. The bony sacral diapophysis is broadly expanded distally, and has a slender base that is approximately a third the width of the distal margin. The leading edge of each diapophysis is slightly concave with an anterolateral orientation, whereas the posterior margin is nearly straight and oriented posterolaterally. The urostyle is slender and shorter than the length of the presacral portion of the vertebral column; the urostyle bears a dorsal crest on its anterior half.

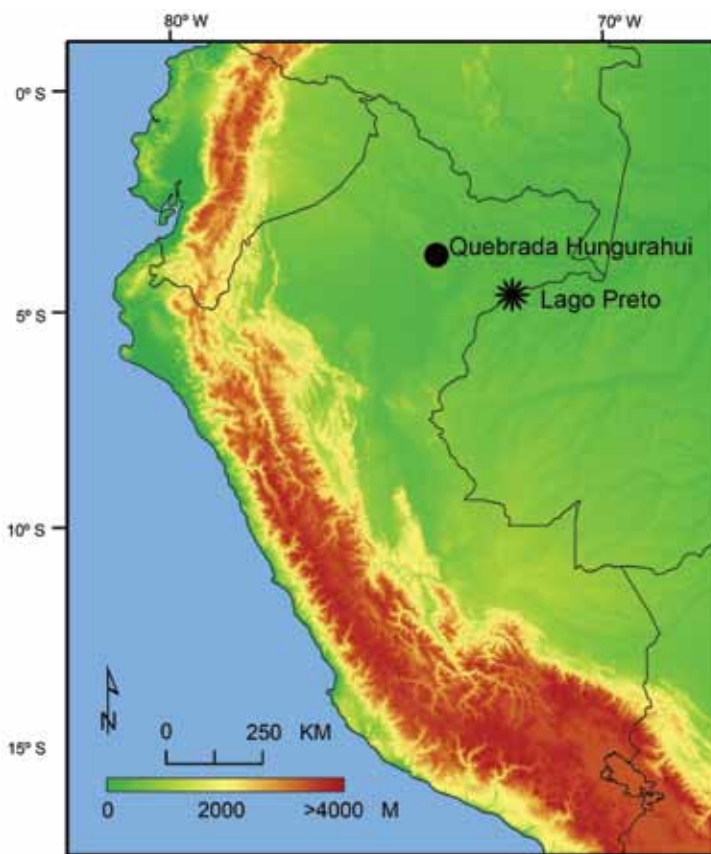


FIG. 5. Known distribution of *Allophryne resplendens*, n. sp. Star represents type locality.

TABLE 3. Search effort distributed by forest type and season in the Yavarí river.

	Flooding season (November)			Dry season (June–July)		
	Palm swamps	Varzea	Terra firma	Palm swamps	Varzea	Terra firma
Search hours	2.5	7.42	12.28	8.75	10.50	8.00
Number of people	2	2	1	2	2	2
Specimens found	1	0	0	0	0	0

The tibiale and fibulare are not fused medially. The phalangeal formulae for the hand and foot are standard, 2-2-3-3 and 2-2-3-4-3, respectively. In increasing order of length, the order of the digits on the hand is I < II < IV < III and that of the foot is: I < II < III < V < IV. The terminal phalanges of hands and feet are T-shaped.

MEASUREMENTS: The morphometric data for the female holotype are (in mm): SVL = 28.4; head length = 7.8; head width = 9.1; thigh length = 11.6; shank length = 12.3; foot length = 11.5; interorbital distance = 3.6; upper eyelid width = 2.0; internarial distance = 1.7; eye-to-nostril distance = 2.5; snout-eye distance = 3.3; eye diameter = 3.0; tympanum diameter = 1.1; eye-tympanum distance = 1.1; forearm length = 5.7; hand length = 8.7; finger I length = 6.1; finger II length = 6.5; and width of disc of finger III = 1.6.

DISTRIBUTION: *Allophryne resplendens* new species is currently known only from two localities in Peru (fig. 5). The type locality is in the Yavarí River drainage. This drainage includes other rivers, such as the Yavarí Mirín and Quebrada Esperanza. The region is dominated by the geological formation that covers much of northeastern Peru: the Pebas formation, a thick slab of clays and sands deposited in ancient lakes and rivers (Räsänen et al., 1998; Sánchez et al., 1999; de la Cruz et al., 1999). The southern sector, close to Angamos, is associated with an uplifted geological structure known as the Iquitos Arch, which stretches hundreds of kilometers across Loreto and into Colombia. The vegetation of the area includes upland (terra firma) forest concentrated along the Iquitos Arch, flooded (*varzea*) forest and palm swamps. The second known locality is the western side of the Amazon River, Quebrada Hungurahui (an affluent of Río Tigre), approximately 2 km north of Comunidad Monteverde, Loreto, Peru. *Allophryne resplendens*, n. sp., is likely present in other varzea forests at least around and between the two known localities. Thus, it is possible that this species also occurs in Brazil.

NATURAL HISTORY: This is an arboreal species that has been found perched on leaves and branches below 2 m. The holotype was found on a *Lepidocaryum tenue* (Arecaceae). These medium-size palm trees form particular forests known locally as *irapayales*. The specimen from Quebrada Hungurahui was also found in an irapayal forest. *Allophryne resplendens*, n. sp., is very difficult to find, which could reflect very low densities, at least in the lower strata of the forest outside the breeding season (the sister species is a explosive breeder). After 49.45 hours of search only one specimen was collected (table 3), and Rodríguez and Knell (2003) found only a single specimen in 200 hours of search. William Lamar (personal commun.) has visited the locality in Quebrada Hungurahui during the last 10 years in different months and has not

been able to locate by sight or sound any other specimens. At least 45 species of amphibians have been reported from the type locality and surroundings, of which 23 have been found in swamp palm forest.

ETYMOLOGY: The specific name *resplendens* is derived from the Latin verb *resplendo* meaning “to glitter,” which we used in allusion to the bright and ornate coloration of the frog.

DISCUSSION

TAXONOMY

Monotypic supraspecific taxa can result problematic. For example, different Linnean ranks convey the same information (*Allophrynidae* = *Allophryne* = *Allophryne ruthveni*) and phylogenetic definitions are repetitive (see Guayasamin et al.’s [2009] attempt to define *Allophrynidae* and *Allophryne*). Our discovery of a new species of *Allophryne* partly solves this problem. *Allophryne* is no longer equivalent to the species *Allophryne ruthveni* and we provide a new phylogenetic definition of the family and genus: a clade stemming from the most recent common ancestor of *Allophryne ruthveni* Gaige, 1926, and *Allophryne resplendens* Castroviejo-Fisher et al., 2011.

PHYLOGENETIC RELATIONSHIPS

Our MP tree shows the sequences of *A. resplendens*, n. sp., as sister to those of *A. ruthveni*. However, the MP analysis does not support the relationship (*Leptodactylidae*, (*Allophrynidae*, *Centrolenidae*)), which has been suggested in other phylogenetic studies (e.g., Frost et al., 2006; Guayasamin et al., 2008; Heinicke et al., 2009; Pyron and Wiens, 2011) although with low support. We explain this lack of resolution by the fact that our dataset was primarily designed to evaluate whether *A. resplendens*, n. sp., was more closely related to *Allophrynidae* than to representatives of all other families of Neotropical Hyloidea. Our results are therefore not comparable to studies using much larger taxon and character sampling (e.g., Frost et al., 2006; Pyron and Wiens, 2011).

The unique combination of phenotypic characters exhibited by *Allophryne ruthveni* has long puzzled herpetologists, resulting in different and conflicting phylogenetic hypotheses placing *Allophrynidae* as the sister taxon of the families *Bufo*nidae (Gaige, 1926; Laurent, 1980, 1986), *Hylidae* (Lynch and Freeman, 1966; Hoogmoed, 1969; Duellman, 1975; Duellman and Trueb, 1986), or *Centrolenidae* (Noble, 1931; Lutz, 1968; Duellman, 2001; Burton, 2004; Wiens et al., 2005). Although no unique autapomorphy has been described for *A. ruthveni*, the following combination of characters has been proposed as diagnostic: intercalary elements absent, biaxial articulation between last two phalanges, T-shaped terminal phalanges, tibiale and fibulare not fused, absence of neopalatines and quadratojugals, prepollex and prehallux formed by one proximal element each (Fabrezi and Langone, 2000, 2001; Fabrezi, 2006). Unfortunately, we have been able to secure only one specimen of *A. resplendens*, n. sp., so we could investigate only three of the characters mentioned above (i.e., T-shaped terminal phalanges, tibiale and fibulare not fused, absence of neopalatines and quadratojugals). The next logical step in the study of *Allophryne* would be to gather more information on these and other phenotypic characters and study them in combination with other relevant information and taxa in a cladistic framework.

ACKNOWLEDGMENTS

We thank Mark Bowler, Miguel Antúnez, Maria Soledad Riveros, Bray Torres, Franco Santana, Claudia Rios, Ciro Pinedo, Richard Bodmer, Pablo Puertas, and the volunteers of Earthwatch Institute for supporting the study in Lago Preto; Juan Carlos Chaparro for his support and help in the description of this species; and William Lamar for photographs and data on the new locality. The Wildlife Conservation Society–Perú (WCS–Perú) and AmazonEco provided financial support for fieldwork. Collection permits in Peru (authorization N° 320-2009) were issued by AG-DGFFS-DGEFFS. This study is included in the “Contrato Marco de Acceso a Recursos Genéticos N° 0001, 11 Enero 2007” subscribed between Fundacion La Salle de Ciencias Naturales and the Ministerio del Ambiente, Venezuela. The work of SCF was financed by a Fulbright/Ministry of Education postdoctoral research contract. The Universidad Tecnológica Indoamérica supports J.M.G.’s research. J.M.P.’s research is funded by a Gerstner Postdoctoral Fellowship at the American Museum of Natural History.

REFERENCES

- AmphibiaWeb. 2011. Information on amphibian biology and conservation. Berkeley, CA. Internet resource (<http://amphibiaweb.org/>), accessed June 14, 2011.
- Austin, J.D., et al. 2002. A molecular perspective on the evolutionary affinities of an enigmatic neotropical frog, *Allophryne ruthveni*. *Zoological Journal of the Linnean Society* 134: 335–346.
- Burton, T.C. 2004. Muscles of the pes of hylid frogs. *Journal of Morphology* 260: 209–233.
- de la Cruz, B., et al. 1999. Geología de los cuadrángulos de: Lagunas, Río Cauchío, Santa Cruz, Río Sacarita, Río Samiria, Breñaña, Requena, Remoyacu, Angamos, Santa Isabel, Tamanco, Nueva Esperanza, Buenas Lomas, Laguna Portugal, Puerto Rico, Tabalosos, Curinga, Quebrada Capanahua, Quebrada Betilia y Río Yaquerana. *Boletín del Instituto Geológico, Minero y Metalúrgico (INGEMMET), Carta Geológica Nacional, Sector Energía y Minas* 132, Serie A: 1–372.
- Duellman, W.E. 1975. On the classification of frogs. *Occasional Papers of the Museum of Natural History, University of Kansas* 42: 1–14.
- Duellman, W.E. 2001. *Hylid frogs of Middle America*. Ithaca, NY: Society for the Study of Amphibians and Reptiles.
- Duellman, W.E., and L. Trueb. 1986. *Biology of amphibians*. New York: McGraw-Hill.
- Duméril, A.M.C., and G. Bibron. 1841. *Erpétologie générale ou histoire naturelle complète des reptiles*. Vol. 8. Paris: Librairie Encyclopédique de Roret.
- Fabrezi, M. 1992. El carpo de los anuros. *Alytes* 10: 1–29.
- Fabrezi, M. 1993. The anuran tarsus. *Alytes* 11: 47–63.
- Fabrezi, M. 2001. A survey of prepollex and prehallux variation in anuran limbs. *Zoological Journal of the Linnean Society* 131: 227–248.
- Fabrezi, M. 2006. Morphological evolution of Ceratophryinae (Anura, Neobatrachia). *Journal of Zoological Systematics and Evolutionary Research* 44: 153–166.
- Fabrezi, M., and J.A. Langone. 2000. Los caracteres morfológicos del controvertido Neobatrachia arbórea *Allophryne ruthveni* Gaige, 1926. *Cuadernos de Herpetología* 14: 47–59.
- Faivovich, J., et al. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History*

294: 1–240.

- Fouquet, A., et al. 2007. Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE* 2 (10): e1109. [doi: 10.1371/journal.pone.0001109]
- Frost, D.R. 2011. Amphibian species of the world: an online reference. Version 5.5 (31 January, 2011). New York: American Museum of Natural History, electronic database (accessible at <http://research.amnh.org/vz/herpetology/amphibia/>)
- Frost, D.R., et al. 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.
- Gene Codes Corporation. 2006. Sequencher Version 4.1. Ann Arbor, Michigan.
- Goloboff, P.A. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428.
- Goloboff, P.A., et al. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.
- Gower, D.J., M. Wilkinson, E. Sherratt, and P.J.R. Kok. 2010. A new species of *Rhinatrema* Duméril & Bibron (Amphibia: Gymnophiona: Rhinatrematidae) from Guyana. *Zootaxa* 2391: 47–60.
- Grant, T., et al. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* 299: 1–262.
- Guayasamin, J.M., M.R. Bustamante, D. Almeida-Reinoso, and C.W. 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 Linnean Society of London* 147: 489–513.
- Guayasamin, J.M., S. Castroviejo-Fisher, J. Ayarzagüena, L. Trueb, and C. Vilà. 2008. Phylogenetic relationships of glassfrogs (Centrolenidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 48: 574–595.
- Guayasamin, J.M., et al. 2009. Phylogenetic systematics of Glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100: 1–97.
- Guérin-Ménéville, F.-É. 1838. *Iconographie du règne animal de G. Cuvier où représentation d'après nature de l'une des espèces les plus remarquables et souvent non encore figurées, de chaque genre d'animaux, avec un texte descriptif mis au courant de la science. Vol. 3 (Part Reptiles)*. Paris: J.B. Ballière.
- Heinicke, M.P., W.E. Duellman, and S.B. Hedges. 2007. Major Caribbean and Central American frog faunas originated by oceanic dispersal. *Proceedings of the National Academy of Sciences of the United States of America* 104: 10092–10097.
- Heinicke, M.P., et al. 2009. A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. *Zootaxa* 2211: 1–35.
- Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.-A.C. Hayek, and M.S. Foster (editors). 1994. *Measuring and monitoring biological diversity standard methods for amphibians*. Biological Diversity Handbook Series. Washington, DC: Smithsonian Institution Press.
- Hoogmoed, M.S. 1969. Notes on the herpetofauna of Surinam II. On the occurrence of *Allophryne ruthveni* Gaige (Amphibia, Salientia, Hylidae) in Surinam. *Zoologische Mededelingen* 44: 76–81.
- Katoh, K., K. Kuma, H. Toh, and T. Miyata. 2005. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.
- Köhler, J., et al. 2005. New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *Bioscience* 55: 693–696.
- Laurent, R.F. 1980 “1979.” *Esquisse d'une phylogénèse des anoures*. *Bulletin de la Société Zoologique de France* 104: 397–422.

- Laurent, R.F. 1986. Sous classe des lissamphibiens: Lissamphibia systématique, *In* P.-P. Grassé and M. Delsol (editors), *Traité de zoologie: anatomie, systématique, biologie*: 14, fasc. 1B (batraciens): 594–798. Paris: Masson.
- Lutz, B. 1968. Taxonomy of the Neotropical Hylidae. Texas Memorial Museum, Pearce-Sellards Series 11: 3–26.
- Lynch, J.D., and H.L. Freeman. 1966. Systematic status of a South American frog, *Allophryne ruthveni* Gaige. University of Kansas Publications, Museum of Natural History 17: 493–502.
- Meegaskumbura, M., et al. 2002. Sri Lanka: an amphibian hot spot. *Science* 298: 379.
- Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.
- Noble, G.K. 1931. *The biology of the Amphibia*. New York: McGraw-Hill.
- 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 (2): 543–583. [doi:10.1016/j.ympev.2011.06.012]
- Räsänen, M., A. et al. 1998. Geología y geoformas de la zona de Iquitos. *In* S. Kalliola and R. Flores-Paitán (editors), *Geoecología y desarrollo amazónico: estudio integrado en la zona de Iquitos, Perú*. *Annales Universitatis Turkuensis (Series A2)* 144: 59–137.
- Rodríguez, L.O., and G. Knell. 2003. Amphibians and Reptiles. *In* N. Pitman, C. Vriesendorp, and D. Moskovits (editors), *Perú: Yavarí. Rapid Biological Inventories Report 11*: 147–150, 244. Chicago: Field Museum.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press.
- Sánchez F., et al. 1999. Geología de los cuadrángulos de Puerto Arturo, Flor de Agosto, San Antonio del Estrecho, Nuevo Perú, San Felipe, Río Algodón, Quebrada Airambo, Mazán, Francisco de Orellana, Huanta, Iquitos, Río Manítí, Yanashi, Tamshiyacu, Río Tamshiyacu, Buen Jardín, Ramón Castilla, Río Yavarí Mirin y Buena Vista. *Boletín Instituto Geológico, Minero y Metalúrgico (INGEMMET), Carta Geológica Nacional, Sector Energía y Minas 132, Serie A*: 1–372.
- Savage, J.M., and W.R. Heyer. 1967. Variation and distribution in the tree-frog genus *Phyllomedusa*. *Beiträge zur Neotropischen Fauna* 2: 111–131.
- Stuart, B.L., R.F. Inger, and H.K. Voris. 2006. High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. *Biology Letters* 2: 470–474.
- Swofford, D.L. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b1. Sunderland, MA: Sinauer Associates.
- Trueb, L. 1973. Bones, frogs, and evolution. *In* J.L. Vial (editor), *Evolutionary biology of the anurans: contemporary research on major problems*: 65–132. Columbia, MO: University of Missouri Press.
- Trueb, L. 1993. Patterns of cranial diversity among the Lissamphibia. *In* J. Hanken and B.K. Hall (editors), *The skull, vol. 2: Patterns of structural and systematic diversity*: 255–343. Chicago: University of Chicago Press.
- Vieites, D.R., et al. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America* 106: 8267–8272.
- Wiens, J.J., J.W. Fetzner, C.L. Parkinson, and T.W. Reeder. 2005. Hylid frog phylogeny and sampling strategies for speciose clades. *Systematic Biology* 54: 719–748.
- Wilkinson, M., and D.J. Gower. 2010. A new species of *Rhinatrema* Duméril & Bibron (Amphibia: Gymnophiona: Rhinatrematidae) from Amazonas, Brazil. *Zootaxa* 2650: 63–68.

APPENDIX 1

ADDITIONAL SPECIMENS STUDIED

Allophryne ruthveni: VENEZUELA: DELTA AMACURO: Reserva Forestal Rio Grande, Coderforce (08°16'00.8"N, 61°45'16.3"W; 192 m), MHNLS 20231–6 (adult males). AMAZONAS: Base camp of Tapirapeco expedition, upper rio Mavaca (150 m), AMNH 131351 (adult female, cleared and stained). SURINAM: SARAMACCA: Raleigh Cataracts, Coppename river (50 m), AMNH 87687 (adult female), 876888 (adult male). GUYANA: IWOKRAMA: Pakatau creek, AMNH 163990 (adult female). Marudi creek, AMNH 44749 (male). UNKNOWN LOCALITY: AMNH 70108 (adult female), AMNH 70110 (unknown sex, cleared and stained)

APPENDIX 2

SPECIES, WITH THEIR CORRESPONDING SEQUENCES AND GENBANK CODES, USED IN THE STUDY.
Sequences newly generated for this study are in **boldface**.

Family	Genus	Species	12S	16S
Allophrynidae	<i>Allophryne</i>	<i>resplendens</i>	JQ436697	JQ436698
	<i>Allophryne</i>	<i>ruthveni</i>	AY819328	—
	<i>Allophryne</i>	<i>ruthveni</i>	JQ436702	JQ436700
	<i>Allophryne</i>	<i>ruthveni</i>	JQ436703	JQ436699
	<i>Allophryne</i>	<i>ruthveni</i>	JQ436704	JQ436701
	<i>Allophryne</i>	<i>ruthveni</i>	AY843564	AY843564
	<i>Allophryne</i>	<i>ruthveni</i>	—	AF364512
	<i>Allophryne</i>	<i>ruthveni</i>	—	EU662973
Aromobatidae	<i>Allobates</i>	<i>talamancae</i>	170026605	170026605
	<i>Rheobates</i>	<i>palmatus</i>	170026602	170026602
Bufonidae	<i>Atelopus</i>	<i>peruensis</i>	77176491	77176491
	<i>Dendrophryniscus</i>	<i>minutus</i>	61697193	61697193
	<i>Rhaebo</i>	<i>nasicus</i>	77176549	77176549
	<i>Rhinella</i>	<i>arenarum</i>	61697184	61697184
Centrolenidae	<i>Celsiella</i>	<i>revocata</i>	EU663379	EU663019
	<i>Centrolene</i>	<i>savagei</i>	EU663380	187729151
	<i>Chimerella</i>	<i>mariaelenae</i>	EU663350	EU662991
	<i>Cochranella</i>	<i>nola</i>	EU663375	EU663015
	<i>Espadarana</i>	<i>andina</i>	EU663335	EU662976
	<i>Hyalinobatrachium</i>	<i>taylori</i>	EU663420	EU663056
	<i>Ikakogi</i>	<i>tayrona</i>	EU663358	EU662999
	<i>Nymphargus</i>	<i>bejaranoi</i>	EU663422	EU663059
	<i>Rulyrana</i>	<i>spiculata</i>	EU663382	EU663022
	<i>Sachatamia</i>	<i>albomaculata</i>	EU663362	EU663003
<i>Teratohyla</i>	<i>pulverata</i>	EU663416	EU663053	

Family	Genus	Species	12S	16S	
Ceratophryidae	<i>Vitreorana</i>	<i>gorzulae</i>	EU663348	EU662989	
	<i>Atelognathus</i>	<i>patagonicus</i>	61697182	61697182	
	<i>Batrachyla</i>	<i>leptopus</i>	61697183	61697183	
	<i>Ceratophrys</i>	<i>cranwelli</i>	61697186	61697186	
	<i>Lepidobatrachus</i>	sp	37699560	37699560	
	<i>Telmatobius</i>	sp	61697380	61697380	
Cycloramphidae	<i>Alsodes</i>	<i>gargola</i>	61697176	61697176	
	<i>Eupsophus</i>	<i>calcaratus</i>	61697198	61697198	
Dendrobatidae	<i>Ameerega</i>	<i>trivittata</i>	170026722	170026722	
	<i>Dendrobates</i>	<i>auratus</i>	61697192	61697192	
	<i>Epipedobates</i>	<i>tricolor</i>	37624303	37624303	
	<i>Silverstoneia</i>	<i>flotator</i>	170026684	170026684	
Hemiphractidae	<i>Gastrotheca</i>	<i>fissipes</i>	61697203	61697203	
	<i>Stefania</i>	<i>schuberti</i>	61697379	61697379	
Hylidae	<i>Anotheca</i>	<i>spinosa</i>	61697177	61697177	
	<i>Aparasphenodon</i>	<i>brunoi</i>	61697178	61697178	
	<i>Argentohyla</i>	<i>siemersi</i>	61697181	61697181	
	<i>Bokermannohyla</i>	<i>martinsi</i>	61697252	61697252	
	<i>Bromeliahyla</i>	<i>bromeliacia</i>	61697223	61697223	
	<i>Charadrahyla</i>	<i>taeniopus</i>	61697290	61697290	
	<i>Duellmanohyla</i>	<i>soralia</i>	61697195	61697195	
	<i>Ecnomihyla</i>	<i>miotympanum</i>	61697256	61697256	
	<i>Hyla</i>	<i>arborea</i>	61697212	61697212	
	<i>Hyloscirtus</i>	<i>charazani</i>	61697229	61697229	
	<i>Hypsiboas</i>	<i>geographicus</i>	61697239	61697239	
	<i>Isthmohyla</i>	<i>rivularis</i>	61697270	61697270	
	<i>Osteocephalus</i>	<i>taurinus</i>	61697320	61697320	
	Leptodactylidae	<i>Leptodactylus</i>	<i>ocellatus</i>	61697299	61697299
		<i>Lithodytes</i>	<i>lineatus</i>	37699553	37699553
		<i>Paratelmatobius</i>	<i>poecilogaster</i>	159139110	159139110
<i>Scythrophrys</i>		<i>sawayae</i>	159139126	159139126	
Mantellidae	<i>Mantella</i>	<i>madagascariensis</i>	89255435	89255435	
Strabomantidae	<i>Lynchius</i>	<i>flavomaculatus</i>	166155972	166155972	
	<i>Oreobates</i>	<i>cruralis</i>	166155971	166155971	
	<i>Pristimantis</i>	<i>diadematus</i>	166155973	166155973	
	<i>Pristimantis</i>	<i>galdi</i>	166155975	166155975	
	<i>Yunganastes</i>	<i>pluvicanorus</i>	61697197	61697197	

Complete lists of all issues of *Novitates* and *Bulletin* are available on the web (<http://digitallibrary.amnh.org/dspace>). Order printed copies on the web from <http://www.amnhshop.com> or via standard mail from:

American Museum of Natural History—Scientific Publications
Central Park West at 79th Street
New York, NY 10024

Ⓢ This paper meets the requirements of ANSI/NISO Z39.48-1992 (permanence of paper).