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Systematics of the endangered toad genus *Andinophryne* (Anura: Bufonidae): phylogenetic position and synonymy under the genus *Rhaebo*

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Abstract

Bufonidae is one of the most diverse amphibian families. Its large-scale phylogenetic relationships are relatively well understood with the exception of few Neotropical genera that may have diverged early in the evolution of the family. One of those genera is *Andinophryne*, a poorly known group of three toad species distributed in the western slopes of the Andes of northern Ecuador and southern Colombia. Their phylogenetic position is unknown due to lack of genetic data. We estimated a new phylogeny (over 200 species) of the family Bufonidae based on DNA sequences of mitochondrial and nuclear genes to assess the phylogenetic position of *Andinophryne* based on recently collected specimens of *A. colomai* and *A. olallai* from Ecuador and Colombia. We also examined external and internal morphology of *Andinophryne* to explore its congruence with the new phylogeny. The mtDNA and nuclear phylogenies show that *Andinophryne* is embedded within *Rhaebo*, a genus that belongs to a large clade characterized by the presence parotoid glands. Morphological characters confirmed the affinity of *Andinophryne* to *Rhaebo* and a close relationship between *Andinophryne colomai* and *Andinophryne olallai*. *Rhaebo* was paraphyletic relative to *Andinophryne* and to solve this problem we synonymize *Andinophryne* under *Rhaebo*. We discuss putative morphological synapomorphies for *Rhaebo* including *Andinophryne*. We provide species accounts for *R. atelopoides* new comb., *R. colomai* new comb. and *R. olallai* new comb. including assessments of their conservation status. We suggest that the three species are Critically Endangered. Their altitudinal distribution and association with streams are characteristic of endangered Andean amphibians.

Key words: Bufonidae, Colombia, conservation status, Ecuador, *Rhaebo*, synonymy, systematics

Resumen

Bufonidae es una de las familias de anfibios más diversas. Sus relaciones filogenéticas a gran escala están relativamente bien entendidas, con la excepción de algunos géneros Neotropicales que pueden haber divergido temprano en la evolución de la familia. Uno de esos géneros es *Andinophryne*, un grupo pobremente conocido de tres especies de sapos distribuidos en la vertiente occidental de los Andes en el norte de Ecuador y el sur de Colombia. Su posición filogenética es desconocida por falta de datos genéticos. Estimamos una nueva filogenia (casi 200 especies) de la familia Bufonidae basada en secuencias de ADN de genes mitocondriales y nucleares para evaluar la posición filogenética de *Andinophryne* utilizando especímenes recientemente colectados de *A. colomai* y *A. olallai* en Ecuador y Colombia. También examinamos la morfología externa e interna de *Andinophryne* para explorar su congruencia con la nueva filogenia. Las filogenias con los ADN mitocondriales y nucleares mostraron que *Andinophryne* está anidado dentro de *Rhaebo*, un género que pertenece a un clado grande caracterizado por la presencia de glándulas parotoideas. Los caracteres morfológicos confirmaron la afini-

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dad de *Andinophryne* con *Rhaebo* y una estrecha relación entre *Andinophryne colomai* y *Andinophryne olallai*. *Rhaebo* resultó parafilético en relación a *Andinophryne* y solucionamos este problema sinonimizando *Andinophryne* bajo *Rhaebo*. Discutimos sinapomorfías morfológicas putativas para *Rhaebo* incluyendo *Andinophryne*. Proveemos descripciones de especies para *R. atelopoides* nueva comb., *R. colomai* nueva comb. y *R. olallai* nueva comb., incluyendo evaluaciones de su estatus de conservación. Sugerimos que las tres especies están Críticamente Amenazadas. Su distribución altitudinal y asociación con arroyos son características de los anfibios andinos amenazados.

Palabras clave: Bufonidae, Colombia, Ecuador, estatus de conservación, *Rhaebo*, sinonimia, sistemática

Introduction

Bufonidae is one of the largest families of anuran amphibians with a total of 590 formally described species distributed in all continents except Australia and Antarctica (AmphibiaWeb 2015). This frog family has been used as a model to study the influence of phenotype on geographic range (Van Bocxlaer *et al.* 2010), global patterns of diversification and biogeography (Pramuk *et al.* 2007) as well as the role of climate change and disease in population extinctions (Pounds *et al.* 2006; Lips *et al.* 2008). These studies and comprehensive reviews of their systematics have allowed reaching a reasonably good understanding of their large-scale phylogenetic relationships (e.g., Pauly *et al.* 2004; Pramuk 2006; Roelants *et al.* 2007; Pyron & Wiens 2011).

Despite this progress, the phylogenetic position of the Neotropical genera *Andinophryne*, *Metaphryniscus*, and *Truebella* remain uncertain because they have not been included in molecular-based phylogenetic analyses. Determining their phylogenetic position is crucial because they have been hypothesized to have diverged early in the evolution of Bufonidae (Hoogmoed 1985; Señaris *et al.* 1994) and, therefore, they could have a strong influence on the interpretation of character evolution within Bufonidae.

The genus *Andinophryne* Hoogmoed, 1985 contains three poorly known species distributed in the western slopes of the Andes in Northern Ecuador and southern Colombia. *Andinophryne* was characterized as having an omosternum, parotoid glands, a well-developed ear, extensive webbing between the toes and fingers and an anteriorly firmisternal and posteriorly arciferal pectoral girdle (Hoogmoed 1985). These and other characteristics lead to Hoogmoed (1985) to hypothesize that *Andinophryne* was "primitive" and closely resembling the ancestor of several Neotropical bufonid genera. Progress in the assessment of its evolutionary relationships has been hindered by the scarcity of collections, a consequence, at least in part, of their threatened conservation status.

Available evidence suggests that the genus is highly endangered (Lynch *et al.* 2014). *Andinophryne colomai* Hoogmoed 1985 is listed as Critically Endangered in the IUCN Red List and was recorded in nature for the last time in 1984. It is only known from two populations in northern Ecuador (Hoogmoed 1989). *Andinophryne atelopoides* (Lynch & Ruíz-Carranza 1981) is listed as Data Deficient with only two known specimens from the type locality in southwestern Colombia. It was recorded for the last time in 1980 when the type specimens were collected (Castro & Lynch 2004). The least known species is *Andinophryne olallai* Hoogmoed 1985. Until 2012, the only specimen known was the holotype, an adult female collected in Tandayapa, northern Andes of Ecuador, in 1970. The type locality is part of a reserve frequently visited by naturalists. The lack of records in over 40 years suggests that the population is extinct. Although there was a report of this species from Reserva Natural Río Ñambí (hereafter Río Ñambí), Colombia, by Murillo-Pacheco *et al.* (2005), examination of recently collected specimens from that population by PDG, MAR y SRR indicate that they actually are *A. colomai*. *Andinophryne olallai* is listed as Data Deficient (Coloma *et al.* 2010b) or Endangered (Ron *et al.* 2014).

Recent fieldwork in Ecuador resulted in the discovery of a population of *Andinophryne olallai* after 43 years since the last record of the species (Lynch *et al.* 2014). Those samples and recently collected material of *A. colomai* allow us to evaluate, for the first time, the phylogenetic position of the genus *Andinophryne*. In addition, we provide new species accounts and evaluate their conservation status. The molecular phylogenies show that *Andinophryne* is embedded within the bufonid genus *Rhaebo*. The morphological characters are consistent with these molecular phylogenetic results.

Material and methods

Ethics statement. Permits to carry out this study were obtained from Ministerio de Ambiente of Ecuador and

CORPONARIÑO of Colombia. Toads were painlessly euthanized with an overdose of Roxicaine (anesthetic spray). This study was evaluated and approved by Pontificia Universidad Católica del Ecuador DGA (Dirección General Académica) in accordance with the guidelines for environmental and social impacts for research projects.

Phylogeny. To determine the phylogenetic position of *A. colomai* and *A. olallai* we sequenced the mitochondrial genes 12S and 16S, the intervening tRNA-Val (up to 2393 bp) and the nuclear gene RAG-1 (up to 744 bp). The newly generated DNA sequences are available at GenBank under accession numbers KP892883 (PSO-CZ2119, RAG-1), KP892884 (QCAZ 55561, RAG-1), KP845427 (PSO-CZ2119, 12S and 16S), and KP845426 (QCAZ 55561, 12S and 16S).

We combined those sequences with 12S, 16S and RAG-1 sequences retrieved from the GenBank. To obtain largely independent evidence of *Andinophryne* phylogenetic affinities, we analyzed the mitochondrial and nuclear genes separately. Mitochondrial and nuclear genes provide independent evidence of phylogenetic relationships because their inheritance is unlinked. We did not carry out a combined analysis because both trees showed, with strong support, the same position of *Andinophryne* relative to other bufonid genera. We deleted from the matrix redundant samples (e.g., > 1 sample of a single species). We also included samples of Batrachylidae, Centrolenidae, Ceratophryidae, Cycloramphidae, Hylidae, Leptodactylidae, Odontophrynidae, Rhinodermatidae, and Telmatobiidae as outgroups (family content as in AmphibiaWeb 2015). Sequence alignment was done with Geneious 5.4.4 software (GeneMatters Corp.) using the Geneious alignment algorithm. The final mitochondrial matrix had 222 terminals while the RAG-1 matrix had 149. Because it is likely that each of our sampled genes evolved under different processes, we partitioned the matrix to allow the independent inference of models of evolution for each of three mitochondrial genes or codon positions in RAG-1. We used software PartitionFinder v. 1.1.1 (Lanfear *et al.* 2012) to simultaneously estimate both the best-fit model for each partition and the best partition strategy for our data.

Phylogenetic trees were obtained using maximum likelihood searches with the software GARLI 2.0 (Zwickl 2006). We carried out 10 replicates and increased the setting "genthreshfortopoterm" until all searches resulted in similar likelihood values, indicating an efficient search. The final setting of "genthreshfortopoterm" was 300.000 for the mitochondrial search and 500.000 for the RAG-1 search. Node support was assessed with non-parametric bootstrapping with 200 pseudoreplicates with the same settings of the full search but with a single replicate per search.

Morphology. Examined material is listed in Supplemental Information. Due to the threatened conservation status of *A. olallai*, we limited the number of preserved specimens to two adult males. However, our description of external morphology was also based on photographs of ~20 live adults. Sex and reproductive condition were determined by direct gonadal examination. Sex determination in live adults was difficult. However, we were able to confidently sex two females on which eggs were visible through the body wall. Morphometric measurements were made following the methodology of Mueses-Cisneros (2009). The conditions of the omosternum and *Musculus adductor longus* were determined by dissection in the chest and the thigh, respectively. Snout-vent length is abbreviated as SVL.

Examined specimens are deposited in the following collections: Amphibian Collection at Instituto de Ciencias Naturales, Universidad Nacional de Colombia (ICN, Bogotá, Colombia), Amphibian Collection at Instituto Alexander von Humboldt (IAvH, Villa de Leyva, Colombia), Herpetology Collection at Universidad de Nariño (PSO-CZ, Pasto, Colombia), Herpetology Division at Museo Ecuatoriano de Ciencias Naturales (DHMECN, Quito, Ecuador) and Amphibian Division at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ).

Climate and conservation status. We obtained climatic information and remaining habitat for known localities of *Andinophryne olallai* from digital climate maps published by Ministerio de Ambiente del Ecuador (2013). Assessment of conservation status was based on IUCN (2001) guidelines. Estimates of extent of occurrence were based on the minimum convex polygon (i.e., the smallest polygon in which no internal angle exceeds 180 degrees and which contains all the known localities for the species) with a 10 km buffer zone for each locality.

Results

Phylogeny. According to PartitionFinder, the strategy of two partitions (12S + rRNA-Val and 16S) was the best to analyze the mitochondrial data. The selected model of evolution for both partitions was GTR + G + I. For the RAG-1 phylogeny, the best strategy was a single partition under the GTR + G + I model of evolution.t

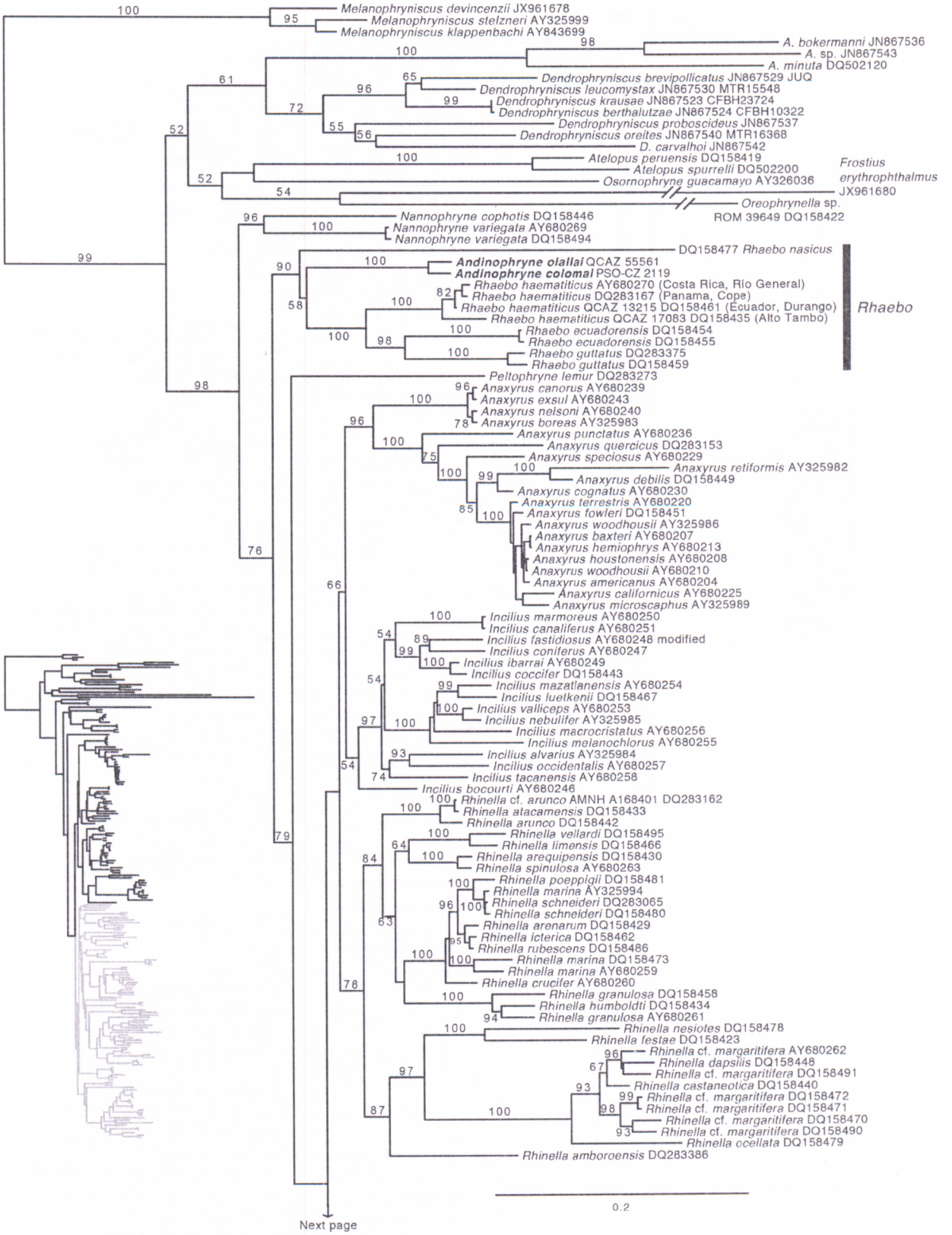


FIGURE 1. Phylogenetic relationships of Bufonidae based on mitochondrial DNA sequences. Maximum likelihood tree obtained under the GTR + Γ + I model of character evolution and two partitions of a matrix of 2393 bp of mitochondrial DNA genes 12S, tRNA-Val, and 16S using GARLI 2.0 (lnL = -91975.7). Numbers at branches are nonparametric bootstrap values (from 200 pseudoreplicates). Bold characters highlight samples of *Andinophryne*. Numbers to the right of the species name are GenBank accession numbers and Museum catalog numbers.

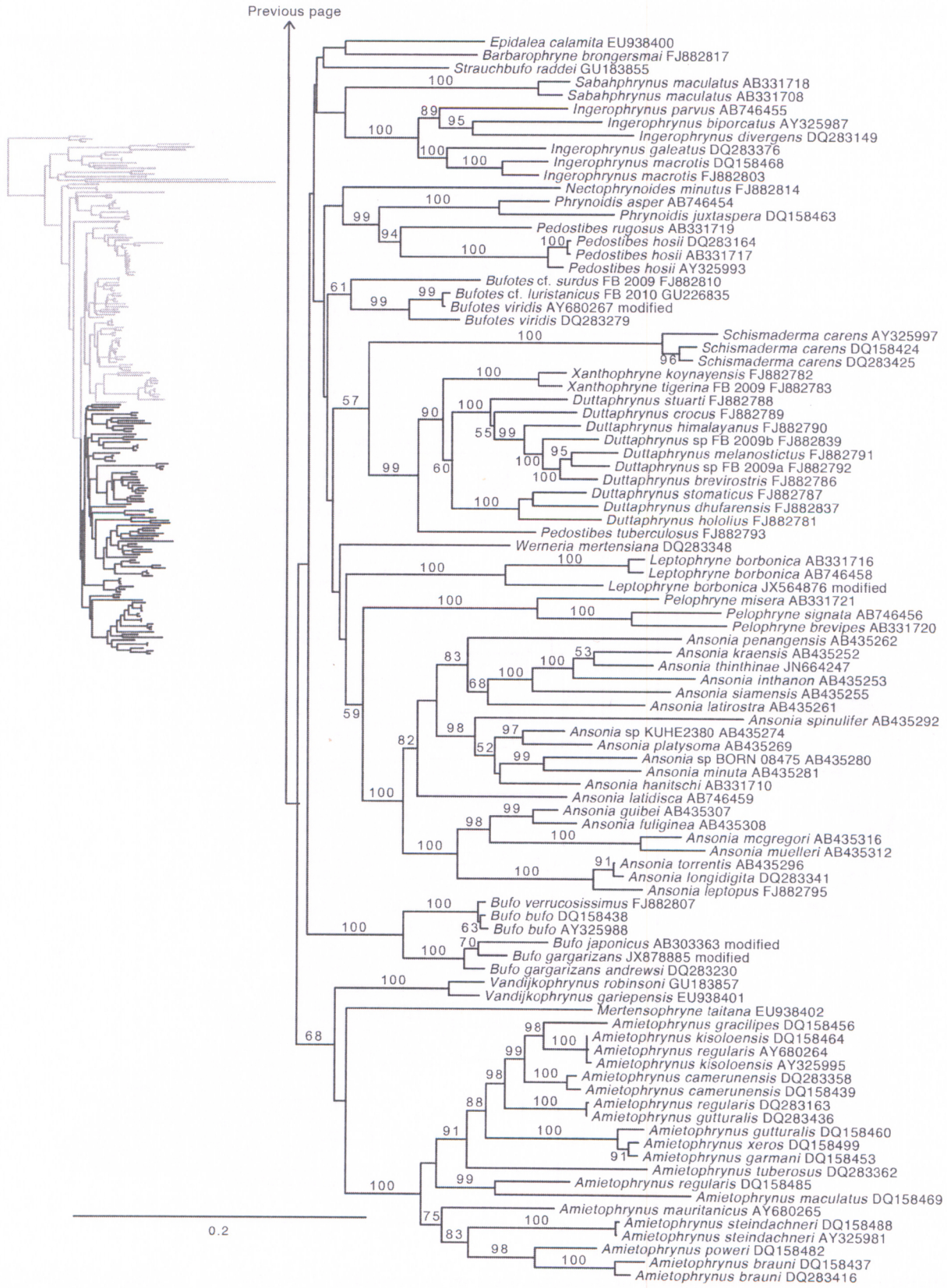


FIGURE 1. (Continued)

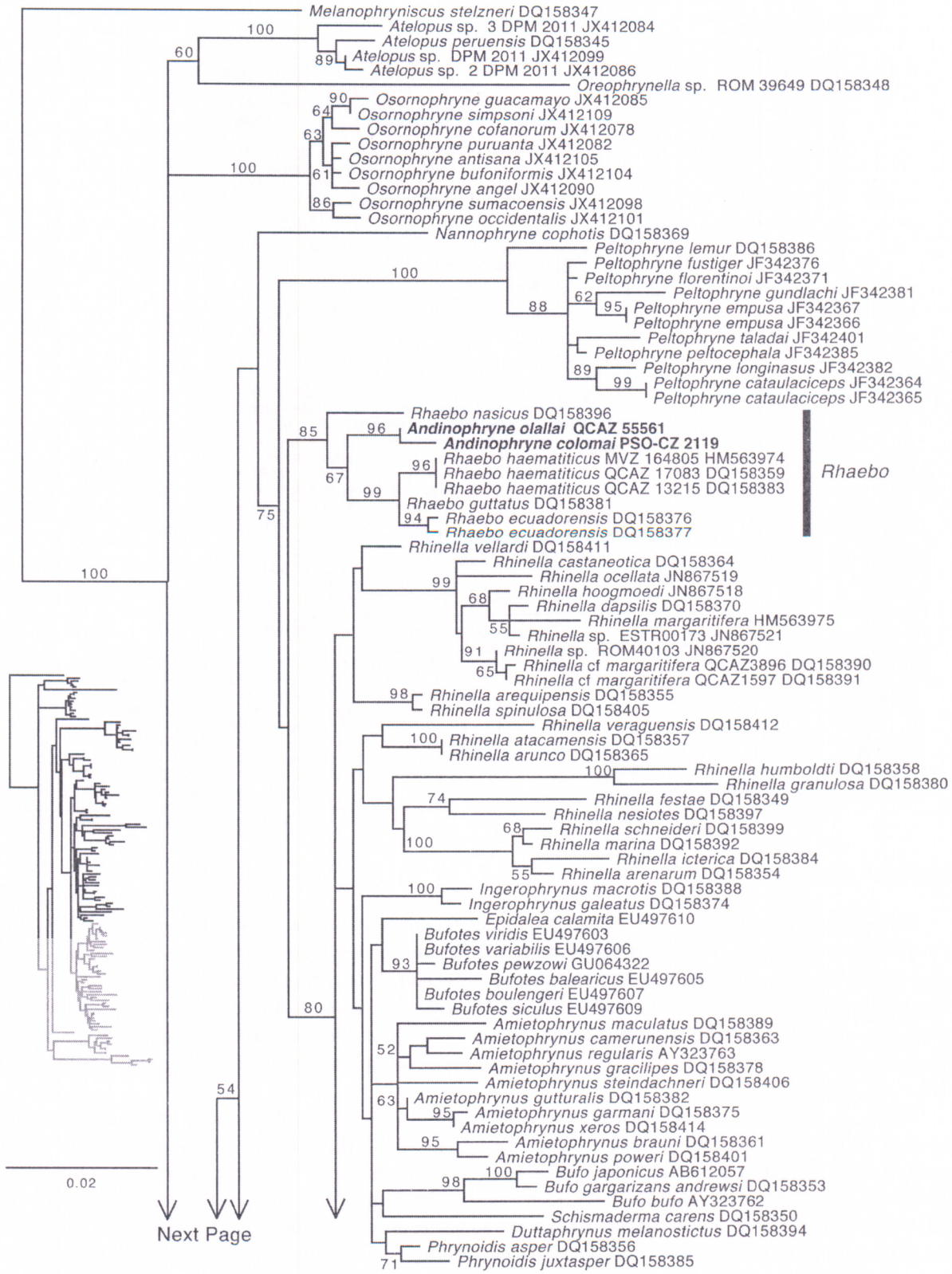


FIGURE 2. Phylogenetic relationships of Bufonidae based on RAG-1 DNA sequences. Maximum likelihood tree obtained under the GTR + Γ + I model of character evolution under a single partition of a matrix of 744 bp of nuclear DNA gene RAG-1 using GARLI 2.0 (lnL = -8827.6). Numbers at branches are nonparametric bootstrap values (from 200 pseudoreplicates). Bold characters highlight samples of *Andinophryne*. Numbers to the right of the species name are GenBank accession numbers and Museum catalog numbers.

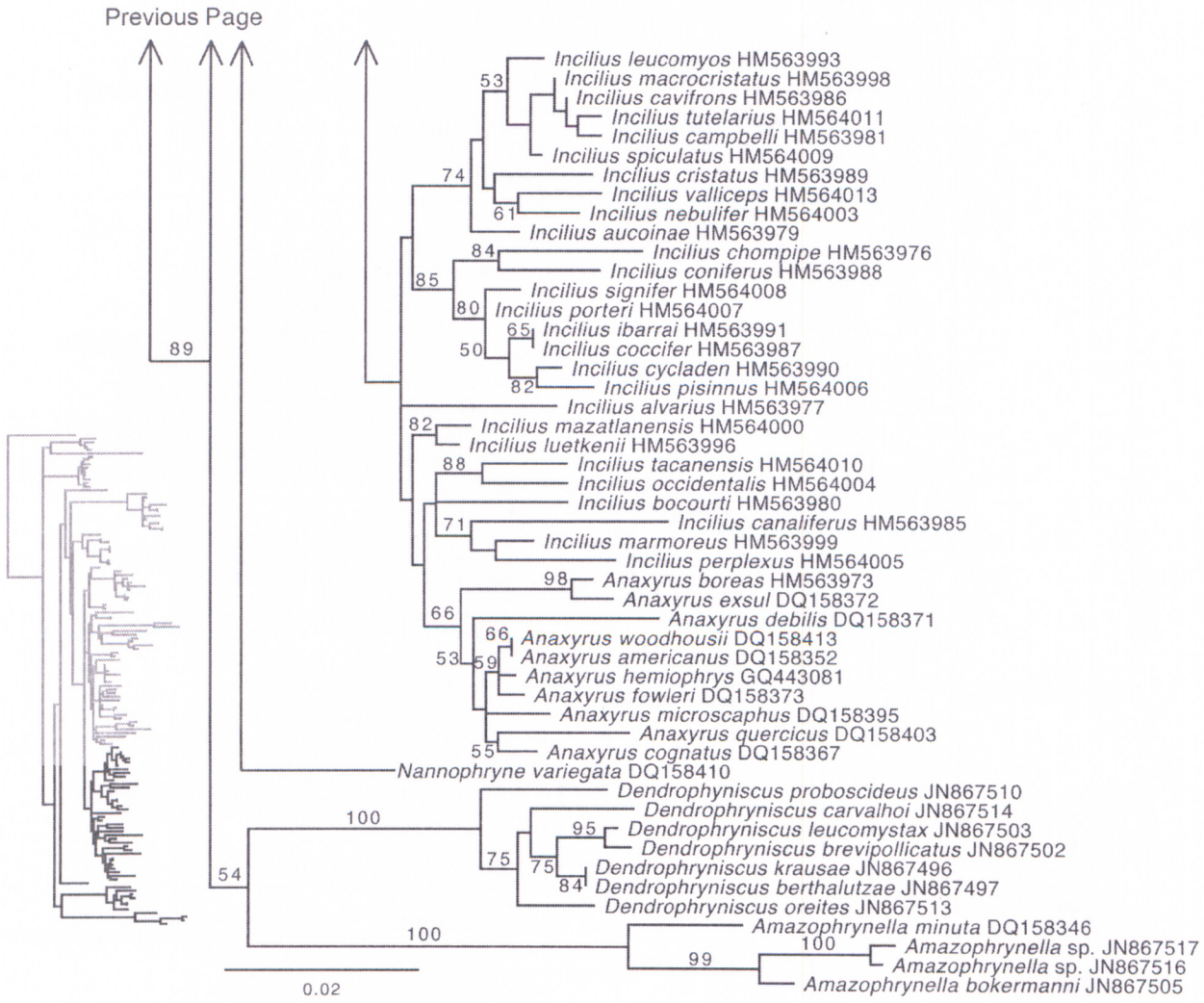


FIGURE 2. (Continued)

Both phylogenies show a strong support for a clade that unites *Rhaebo* + *Andinophryne* (mtBT = 90, nBT = 85). In both phylogenies, *Rhaebo* is paraphyletic relative to *Andinophryne* because *R. haematiticus* Cope, *R. ecuadorensis* Mueses-Cisneros, Cisneros-Heredia, and McDiarmid and *R. guttatus* (Schneider) are more closely related to *Andinophryne* than to *R. nasicus* (Werner). Within *Rhaebo*, we found a strong support for a basal divergence of *R. nasicus* and a close relationship between the Amazonian species *R. guttatus* and *R. ecuadorensis*. We also found strong support for the monophyly of *Andinophryne* (mitochondrial Bootstrap, mtBT, = 100; nuclear, nBT, = 96; Figs. 1–2).

Within *R. haematiticus* there is evidence of the existence of one cryptic species in the Chocó region of Ecuador. In the mitochondrial phylogeny, specimen QCAZ 13215 (Esmeraldas Province, Durango) was more closely related to specimens of *R. haematiticus* from Panamá and Costa Rica than to a sample collected at a distance of less than 20 km (QCAZ 17083, Esmeraldas, Alto Tambo; Fig. 1). Genetic distance between QCAZ 13215 and Central America samples had a range 0.6 to 0.8%; distance between QCAZ 13215 and QCAZ 17083 was 4.9%. Neither of the specimens corresponds to the recently described *R. andinophrynoides* Mueses-Cisneros.

The uncorrected genetic distance between *Andinophryne colomai* and *A. olallai* is 2.7% for the 16S gene. Although this value is rather low, the concordance between genetic distance and differences in external morphology between specimens from Manduriacu and Río Ñambí (see below) confirms that both are valid species.

Morphology. Similarly to the phylogeny, our examination of the external morphology of *A. colomai* and *A. olallai* revealed close affinities with species of the genus *Rhaebo* including the absence of prominent cranial crests

and the presence of orange parotoid secretions (Figs. 3–5). Available osteological data for *A. colomai* also suggest a close affinity with *Rhaebo*. Pramuk (2006) suggested two osteological synapomorphies for *Rhaebo*: (1) sphenethmoid distinctively wide and (2) anterior process of the prootic prominent and notched. We define the sphenethmoid condition as "wide" if its lateral edges are in contact with the frontoparietals (Mueses-Cisneros 2009; Fig. 6B). The wide condition is evident in *A. colomai* (Fig. 6C). The second character, the "anterior process of the prootic" (*sensu* Pramuk 2006) corresponds to the occipital condyles, which are part of the exoccipital instead of the prootic (Mueses-Cisneros 2009). The condition in *A. colomai* corresponds to the one observed in *Rhaebo*: condyles prominent and notched. Overall, the morphological characters indicate that *A. colomai* and *A. olallai* are closely related to the genus *Rhaebo*.

Taxonomic review. We solve the paraphyly of the genus *Rhaebo* Cope 1862 by proposing to treat *Andinophryne* as its junior synonym. We find this solution preferable to creating a new genus to accommodate *Rhaebo nasicus* because the phylogenetic relationships within *Rhaebo* are still unknown for a majority of the species. Our phylogeny only includes four out of ten species of *Rhaebo.* The assignment of the excluded species to either *Rhaebo* or the new genus would be highly speculative. The assignment of the *Andinophryne* species to *Rhaebo* generates the following name changes: *Rhaebo atelopoides* (Lynch & Ruíz-Carranza 1981) new combination, *Rhaebo colomai* (Hoogmoed 1985) new combination, and *Rhaebo olallai* (Hoogmoed 1985) new combination. Below we present species accounts for each of them. Under this new taxonomy, the genus *Rhaebo* contains 13 species distributed from Honduras to the Pacific lowlands of western Ecuador, northern Colombia, northwestern Venezuela, the Guiana region, and the Amazonian lowlands of Venezuela, Colombia, Ecuador, Peru, Bolivia, and Brazil (Frost 2014; Mueses-Cisneros 2008).

Species accounts

Rhaebo atelopoides (Lynch & Ruíz-Carranza 1981) new combination

(Fig. 4F)

Bufo atelopoides Lynch & Ruíz-Carranza 1981. Holotype: ICN 06373, by original designation. Type locality: Quebrada Sopladero, Parque Nacional Natural de Munchique, road from Uribe to La Gallera (ca. km. 54), western slope of Cordillera Occidental, Departamento del Cauca, Colombia; 2190 m.
Andinophryne atelopoides (Hoogmoed 1985)

Diagnosis. A medium sized bufonid (SVL 41.2–42.3 mm in males, females unknown) characterized by the absence of enlarged cephalic crests, short and rounded parotoids, extensive webbing on the toes and flanks with a row of medium sized tubercles connecting the parotoid gland with the groin. The color of its skin secretions and the size of testicles are unknown.

Comparisons with other species. *Rhaebo atelopoides* is most similar to *R. colomai* and *R. olallai*, species that also have extensive webbing in the toes. It differs from both species by the presence of light dorsal marks (yellowish green in life; Fig. 4F). Short and rounded parotoid glands are also distinctive (elongated in *R. colomai* and *R. olallai*). It further differs from *R. colomai* in having a weakly truncated snout in profile (slightly projected in *R. colomai*).

Description. Based on two adult males. A medium sized bufonid, SVL in males 41.2 and 42.3 mm (n = 2); females unknown. Head narrower than body, slightly wider than long, head width is 104.4% of head length and 47.4% of SVL; head length is 45.7% of SVL; head subacuminate in dorsal view, weakly truncated in profile; vertical fleshy fold at rostrum tip; distance between nostril and tip of the snout 73.5–75.7% of the distance from the nostril to the eye; nostril posterior to the anterior edge of the upper lip, below *canthus rostralis* on slightly protruding area; nostril oval to elongated, oblique, directed laterally; area between the nostrils concave; area from tip of the snout to anterior border of eyelid concave; interorbital and occipital region flat, with numerous high rounded tubercles extending to the interparotoideal region; interorbital area much wider than the upper eyelid; upper eyelid with numerous tubercles and few spinules, internal and external border not delineated with tubercles, external border fleshy and protruding; cephalic crests absent; *canthus rostralis* flat and fleshy, projected into the loreal region; loreal region concave with numerous rounded and low tubercles; lips not prominent; eyes with horizontally rounded pupil; tympanum oval, visible anteriorly over 3/5 of its area, 35.5 to 37.0% of eye diameter,

with a fleshy fold between the posterior corner of the eyelid and the parotoid gland; parotoid glands ovoid, pointed anteriorly, rounded posteriorly, longer than wide, elevated, without evident pores and with undulations in the outer edge; parotoid width 39.7 to 41.2% of the length of the gland and 31.0 to 32.0% of the distance between the two parotoid glands; two elevated crests are present between parotoids, from the anterior border or from mid-gland length to the posterior border; dorsal skin with abundant elevated subacuminate tubercles and spicules; oblique lateral row of 5 to 8 elevated tubercles extending from the parotoid gland to the groin, with a ventrolateral line of 6 to 13 isolated tubercles; ventral skin with abundant low tubercles, much more abundant at the tip of the gular region and the chest.

Forelimbs slender, long, with dorsal and ventral spicules, more abundant dorsally; length of hand is 29.9 to 34.3% of SVL; fingers basally webbed, webbing formula I1–2II1.5–4III3–2IV, fingers thin, with fleshy tip, not swollen; Finger I much shorter than II; smooth palms, supernumerary tubercles inconspicuous, subarticular tubercles low, slightly visible; palmar and thenar tubercles evident, palmar tubercle rounded, 1.1 times larger than the elongated thenar tubercle; ulnar tubercles and ulnar fold absent; metacarpal fold absent.

Hindlimbs thin, densely tuberculate, with abundant spicules; tibia and foot length equal 37.5–40.4% and 41.1–45.2% of SVL, respectively; toes with thin fleshy tip, not bulbous, with extensive membranes, all toes distinguishable from it; webbing formulae I0–1II1–1.5III1–4IV3.5–2.5V; supernumerary plantar tubercles inconspicuous; subarticular tubercles ill defined; conspicuous inner metatarsal tubercle, oval, 1.2–1.3 times the size of oval external metatarsal tubercle; inner tarsal fold absent.

Tongue oval, much longer than wide, attached to the mouth floor anteriorly along three fifths of its length, unnotched posteriorly; choanae round, small; males with subgular vocal sac and elongated vocal slits; nuptial pads on Finger I with minute low spicules; cloacal opening directed dorsolaterally.

Coloration in life. dorsum brown with green to yellowish green marks; venter cream with a greenish tone and brown reticulations; posterior areas of thighs brown with yellow dots; anal patch black; iris yellow above, gray below, with a mid-horizontal reddish brown band and black reticulations (Lynch & Ruíz-Carranza 1981).

Distribution and ecology. Only known from its type locality in Munchique Natural National Park, Departamento de Cauca, 2190 m above sea level. The area where the species was collected was very humid and covered by primary forest. Individuals were captured next to a highway and on vegetation 40 cm above ground.

Conservation status. The species has not been found after the collection of the type material (two individuals) in 1980. Searches at the type locality and nearby sites by F.J. López-López in 2003 were unsuccessful. Because of its restricted extent of occurrence, limited number of known localities and absence of records since 1980, we suggest that *R. atelopoides* is assigned to the Critically Endangered category (CR) under criteria B1a,b(v).

Remarks. Lynch and Ruíz-Carranza (1981) assigned this species to the "*Bufo margaritifer*" (= *Rhinella margaritifera*) group. Hoogmoed (1985) transferred it to the genus *Andinophryne*. Lynch and Ruíz-Carranza (1981) provide a description and drawings of holotype (dorsal view) and the paratype (pectoral girdle, hands and feet).

Rhaebo colomai (Hoogmoed, 1985) new combination
(Fig. 3)

Andinophryne colomai Hoogmoed, 1985: 264. Holotype: RMNH 21905, by original designation. Type locality "Cabeceras del Río Baboso, cerca de Lita", Provincia Carchi, Ecuador.
Andinophryne colomai Hoogmoed, 1989, Zoologische Verhandelingen (Leiden), 250: 1–32.
Andinophryne olallai —Murillo-Pacheco, *et al.* 2005. Herpetological Review 36 (3): 331.

Diagnosis. A medium sized bufonid (SVL 32.6–38.4 mm in males, 54.9–59.1 mm in females) characterized by the absence of enlarged cephalic crests, yellowish-orange skin secretions (Fig. 5), elongated parotoids, extensive webbing in the toes, and flanks with areolate skin and a row of medium sized tubercles connecting the parotoid gland with the groin (Fig. 3). Testes size unknown.

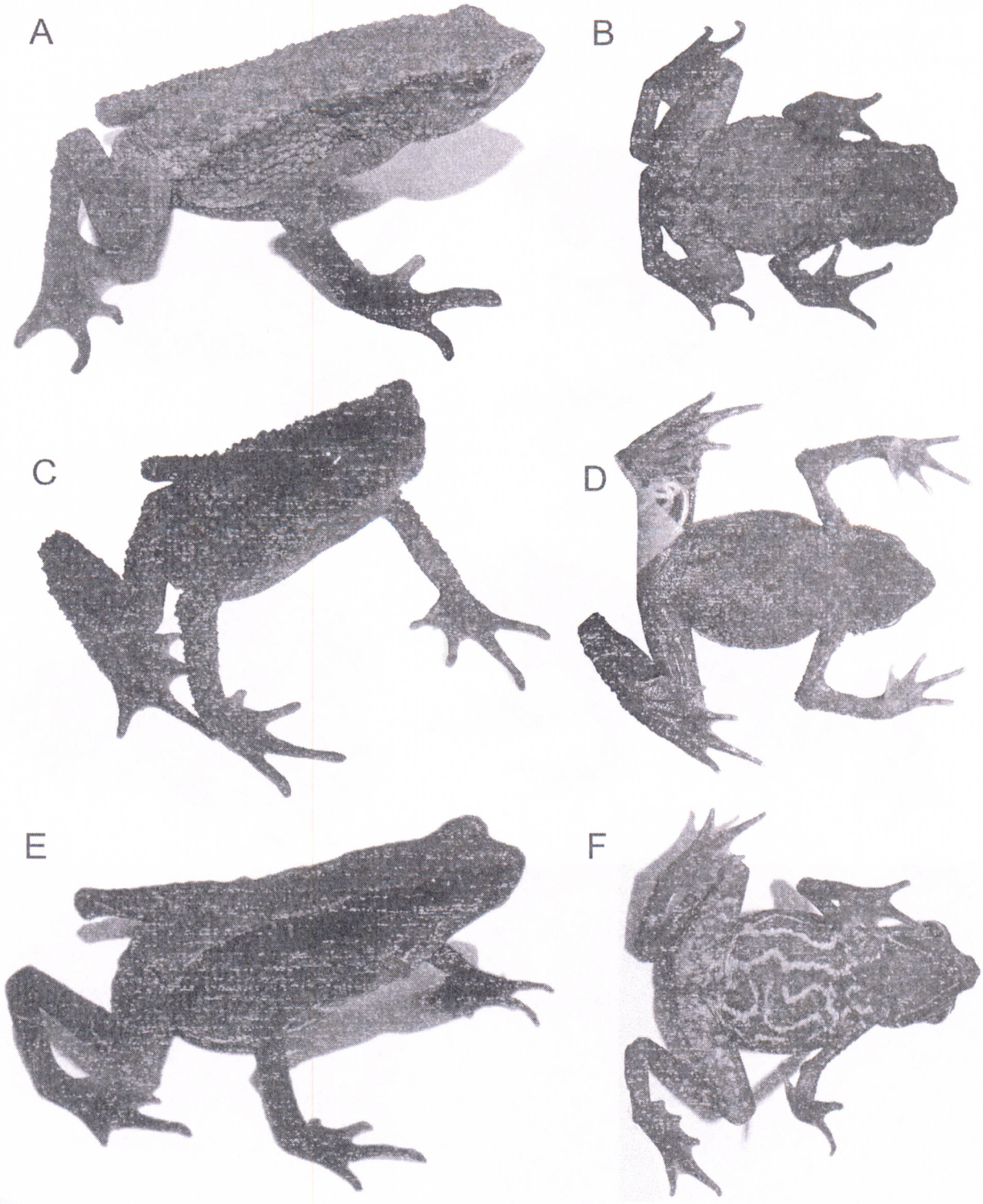


FIGURE 3. Dorsolateral and ventral views of *Rhaebo colomai*. Colombia, Departamento de Nariño, Reserva Natural Río Ñambí. A–B: adult female (PSO-CZ 2115), SVL = 34.29 mm. C–D: adult male (PSO-CZ 2117), SVL = 36.65 mm. E–F: juvenile (not collected), SVL = 23.48 mm, showing both the narrow light dorso- and oblique lateral lines, and the contrasting light lines in the venter. Photographs by PDAGC.

Comparisons with other species. The most similar species is *R. olallai*. Both species differ in the pattern of tuberculation in the flanks. In *R. colomai* the skin on the flanks is areolate (females) to strongly areolate (males) with medium-sized tubercles, most of them arranged along an oblique lateral row from the parotoid gland to the

groin (Fig. 3). In *R. olallai* the skin of the flanks is smooth to weakly areolate but with large, fleshy and prominent tubercles (Fig. 4). Because the amount of tuberculation is sexually dimorphic in both species, some female *R. colomai* may resemble the pattern seen in male *R. olallai*. *Rhaebo atelopoides* differs in having short and rounded parotoid glands (elongated and large in *R. colomai*).

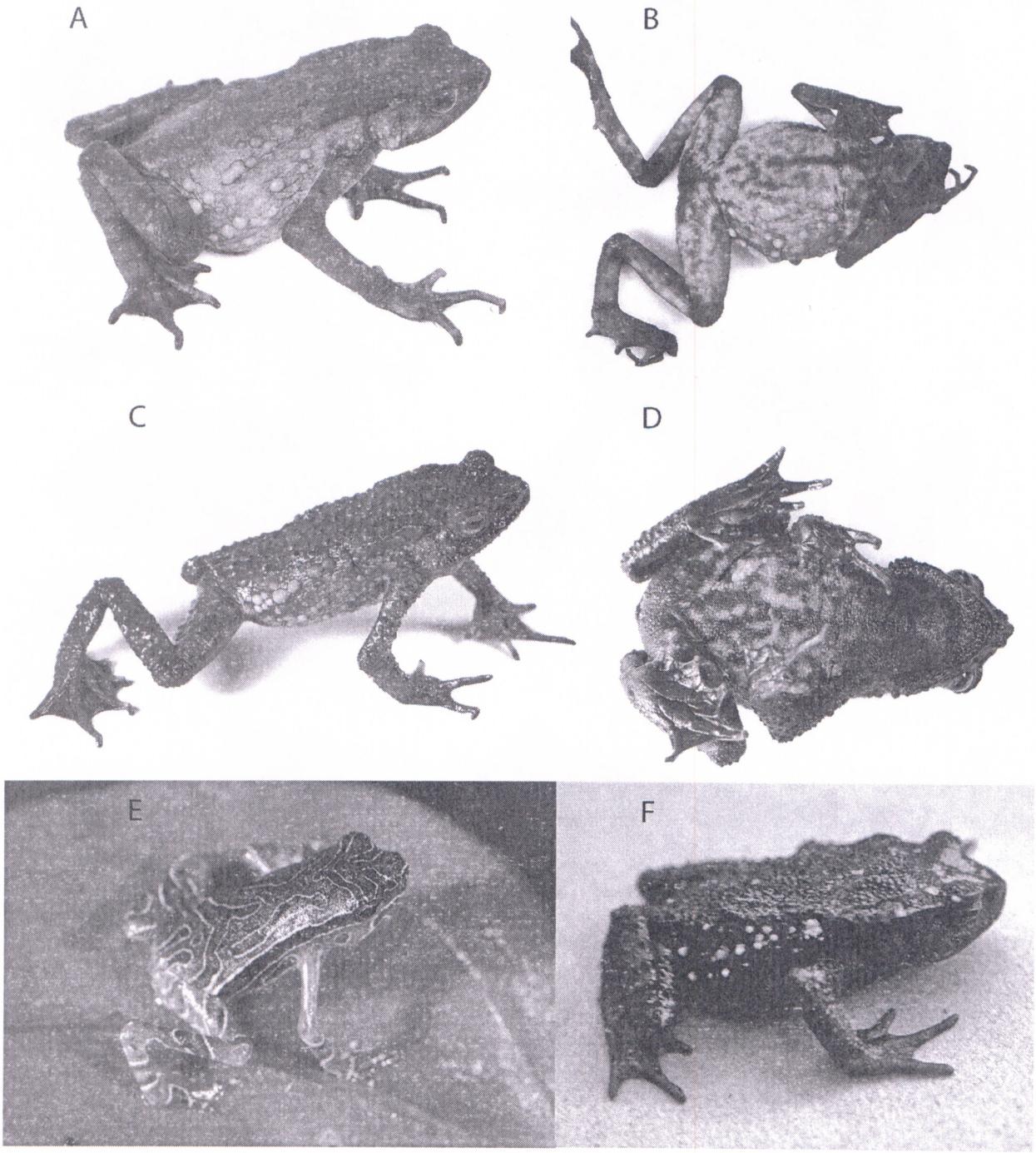


FIGURE 4. Dorsolateral and ventral views of *Rhaebo olallai* and *Rhaebo atelopoides*. A–E: *Rhaebo olallai*, from Ecuador, Provincia de Imbabura, Manduriacu. F: *Rhaebo atelopoides*, from Colombia, type locality. A–B: adult female, SVL = 60.0 mm. C–D: adult male (QCAZ 56576), SVL = 43.9 mm. E: metamorph showing pattern of contrasting clear lines in the dorsum. F: adult male (IAvH 278), SVL = 42.3 mm. Photographs by SRR (*R. olallai*) and J. D. Lynch (*R. atelopoides*).

Description. Based on three adult females and six adult males. A medium sized bufonid, SVL in males 32.6–38.4 mm (n = 6), females 54.9 to 59.1 mm (n = 3). Morphometric measurements are shown in Table 1. Head width

88.5–95.6% of head length and 33.4–34.6% of SVL in males, 97.5–100.0% of head length and 31.1–32.6% of SVL in females; head length is 35.2–38.9% of SVL in males and 31.1–33.5% in females; head subacuminate in dorsal view, projected in profile; vertical fleshy fold at rostrum tip; distance between nostril and tip of the snout 58.8–74.2% of the distance from the nostril to the eye in males, 23.3–31.8% in females; nostril posterior to the anterior edge of the upper lip, below *canthus rostralis* on slightly protruding area; nostril oval to rounded, oblique, directed laterally; area between the nostrils concave; area from tip of the snout to anterior border of eyelid flat; interorbital and occipital region flat; females with few low tubercles from the internarial region and eyelids to interglandular region; males with numerous dense tubercles and spicules extending along the entire dorsum; interorbital area much wider than the upper eyelid; internal border of eyelid delineated or not with low isolated tubercles, external border fleshy and protruding; cephalic crests absent; *canthus rostralis* fleshy, gently concave, not projected into the loreal region; loreal region strongly concave, in females with low tubercles extending to the area below the tympanum with four postrictal spicules and some larger tubercles and spicules reaching the junction of the forelimbs, in males numerous tubercles from the loreal region to the groin, with three to four postrictal spicules and some infraparotoidal spicules; lips not prominent, outlined dark brown in females; eyes with horizontally rounded pupil; tympanum not visible in males, completely or partially (1/2 to 3/5 of its area) visible in females, rounded to oval, 27.4 to 38.0% of eye diameter, with a fleshy and wavy fold between the posterior corner of the eyelid and the parotoid gland; parotoid glands elongated with anterior and posterior borders rounded or pointed, some pores present and undulations in the outer edge; parotoid width 25.6 to 34.6% of the length of the gland and 27.4 to 41.8% of the distance between the two parotoid glands in males, 27.5 to 62.8% and 31.3 to 61.2%, respectively, in females; dorsal skin with abundant tubercles and elevated spicules in males, smooth with small tubercles in females with micro granulations smooth in appearance, with some low tubercles on the head and dorsum; oblique lateral row of 6 to 9 tubercles isolated or fused, fleshy and prominent extending from the parotoid gland to the groin, with or without a ventrolateral line of low tubercles; ventral skin tuberculate to strongly areolate with some thin spicules at the tip of the gular region, much more evident in males.

TABLE 1. Morphometric measurements for adult specimens of *Rhaebo colomai*. Range, average and standard deviations are provided for each variable. All measurements are in mm. Measurement abbreviations: SVL = Snout-vent length; IOD = inter-orbital distance; IND = Inter-narial distance; IPD = Inter parotoid distance; PED = Parotoid-eye distance; TD = Tympanum diameter; ED = Eye diameter; END = Eye-nostril distance; NRD = nostril-rostrum distance; TL = Tibia length; FOOT = Foot length; HAND = hand length.

Measurements	Males (n = 6)	Females (n = 3)
SVL	32.6–38.4 (34.3±2.2)	54.9–59.1 (56.6±2.2)
Head length	11.6–13.6 (12.5±0.7)	18.3–18.7 (18.5±0.2)
Head width	10.9–13.0 (11.7±0.7)	17.9–18.4 (18.2±0.3)
IOD	4.3–5.6 (5.0±0.5)	7.5–8.6 (7.9±0.6)
Eyelid width	3.4–3.9 (3.6±0.2)	4.4–5.1 (4.6±0.4)
IND	3.5–4.0 (3.7±0.2)	5.2–5.6 (5.4±0.2)
Parotoid length	7.4–9.3 (8.1±0.6)	11.2–12.8 (11.7±0.9)
Parotoid width	2.0–2.9 (2.4±0.4)	3.5–7.1 (4.7±2.1)
IPD	6.7–8.8 (7.4±0.8)	9.7–11.6 (10.8±1.0)
PED	2.5–3.2 (2.8±0.3)	3.9–5.2 (4.6±0.7)
TD	--	1.5–1.9 (1.7±0.2)
ED	3.0–3.7 (3.4±0.2)	5.0–5.7 (5.3±0.4)
END	3.1–3.5 (3.4±0.1)	4.3–4.4 (4.3±0.1)
NRD	2.0–2.5 (2.2±0.2)	1.0–1.4 (1.1±0.1)
TL	15.0–17.5 (15.8±0.9)	24.7–27.0 (25.7±1.2)
FOOT	15.0–17.8 (16.0±1.0)	25.8–29.0 (27.1±1.7)
HAND	10.7–12.3 (11.5±0.6)	20.3–21.6 (20.9±0.7)

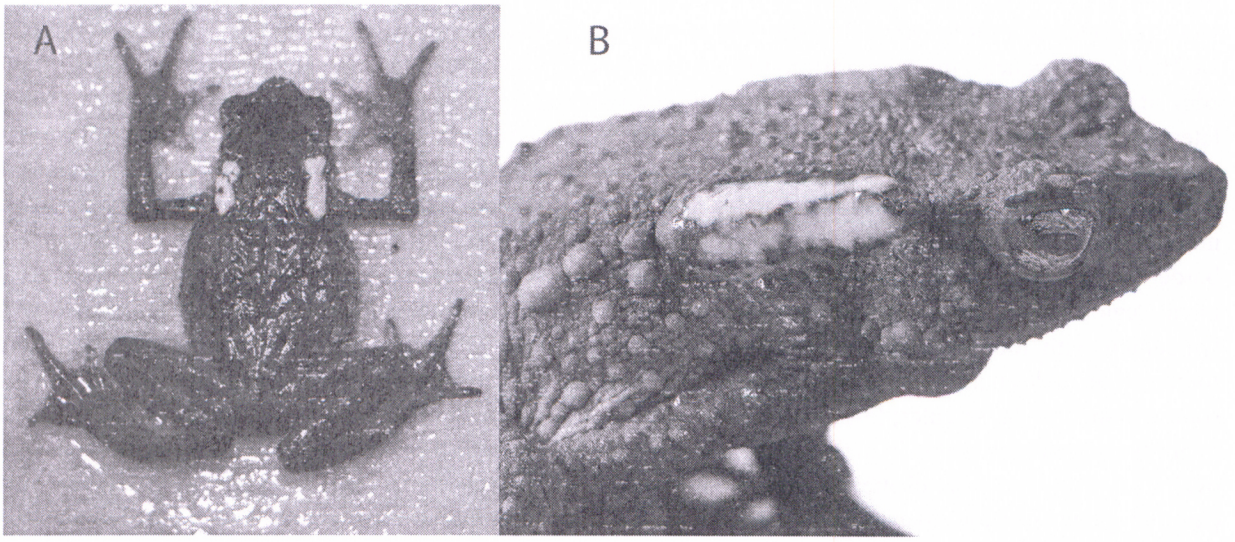


FIGURE 5. Skin secretions in *Rhaebo. Rhaebo colomai* (left) and *R. olallai* (right). Note yellow-orange color. Photos by J. H. Mueses-Cisneros (left) and SRR (right).

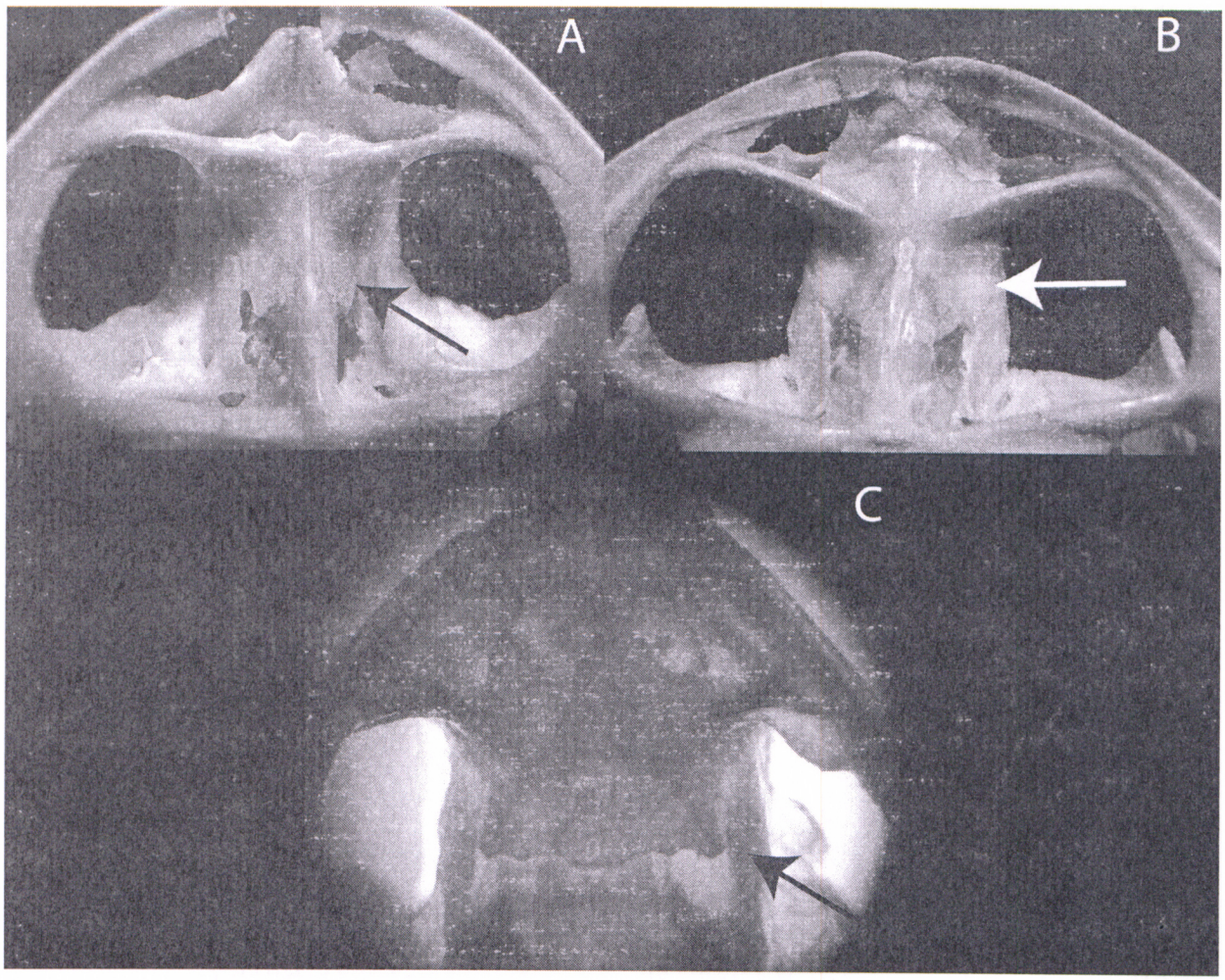


FIGURE 6. Shape of the sphenethmoides in ventral view. A: Narrow, *Rhinella marina*, ICN 54685. B: Wide, *Rhaebo blombergi*. ICN 54714. C: Wide, *Rhaebo colomai*. Photos J. J. Mueses-Cisneros.

Forelimbs slender, long, with numerous subconical tubercles and dorsal and ventral spicules (less abundant in females); length of hand is 31.8 to 35.8% of SVL in males and 36.5 to 37.1% in females; fingers basally webbed; fingers thin, long, with fleshy tip, not swollen; Finger I shorter than II; smooth palms, supernumerary tubercles inconspicuous, subarticular tubercles low, slightly visible, rounded; palmar and thenar tubercles evident, palmar tubercle rounded, 1.2 to 1.3 times larger than the elongated thenar tubercle; ulnar tubercles and ulnar fold absent; metacarpal fold absent.

Hindlimbs thin and long; in males densely tuberculate dorsally and ventrally, in females only few tubercles present dorsally; inner tarsal fold absent; outer edge of tarsus without tubercles; toes thin and long, with fleshy tip, not bulbous, with extensive membranes, all fingers distinguishable from it, webbing formula I(1.5–2)–(2–2.5)II(1.5–2⁻)–3III(2.5–3)–2.5IV in males, I1.5–2II(2–2⁻)–3III(2.5–3)–3IV in females; supernumerary plantar tubercles inconspicuous; subarticular tubercles ill defined; conspicuous inner metatarsal tubercle, oval, 1.5 times the size of the rounded external metatarsal tubercle.

Tongue oval, longer than wide, thin anteriorly, rounded posteriorly, attached to the mouth floor anteriorly along three fifths of its length, unnotched posteriorly; choanae round, small; males with subgular vocal sac and vocal slits; nuptial pads low, not swollen; cloacal opening directed dorsolaterally.

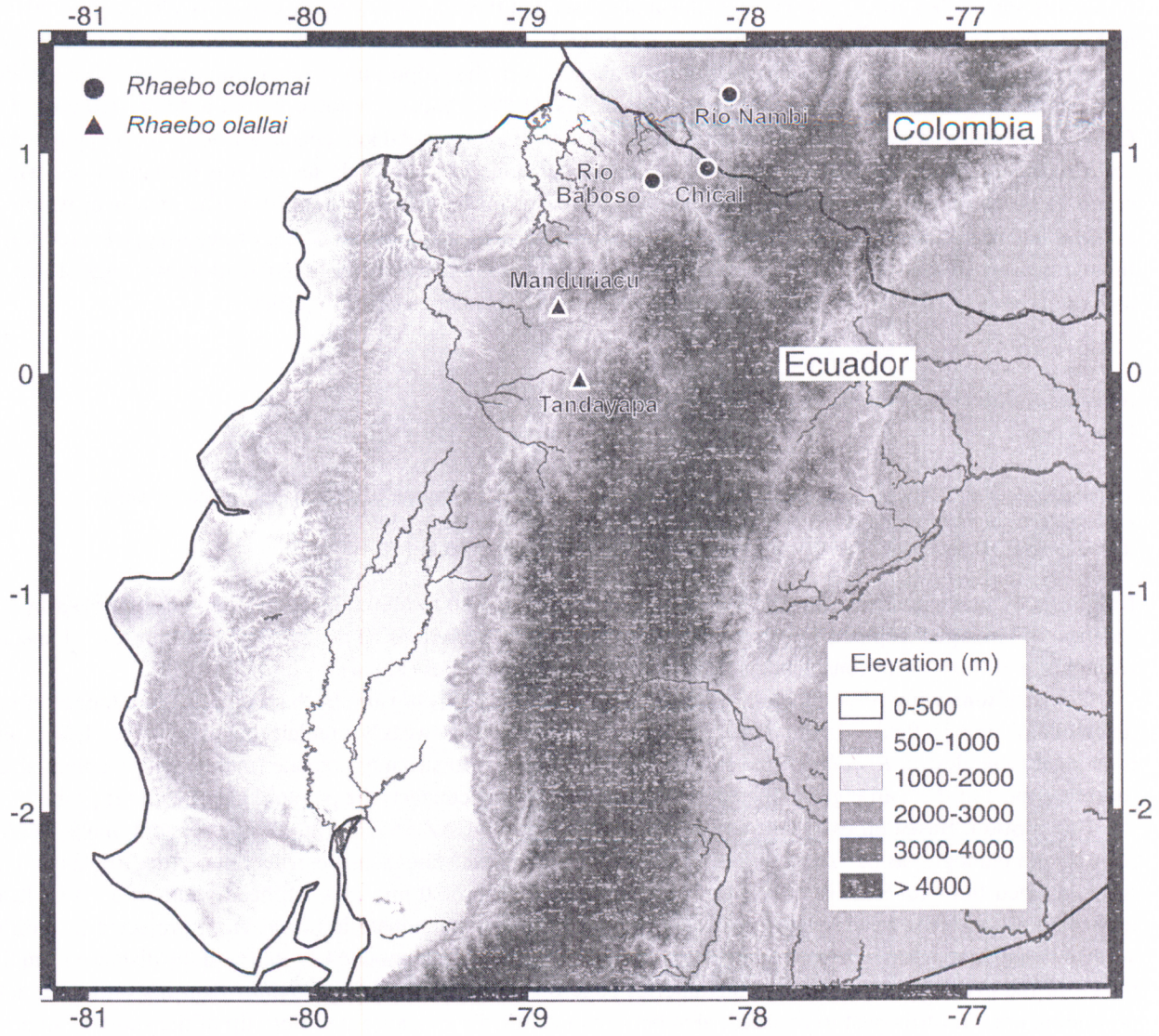


FIGURE 7. Known localities for *Rhaebo colomai* and *Rhaebo olallai*. The map is based on Hoogmoed (1985, 1989) and localities reported herein.

Distribution and ecology. *Rhaebo colomai* is known from three localities in the western slopes of the Andes in northern Ecuador and southern Colombia, between 1180 and 1500 m above sea level (Hoogmoed 1989; Murillo-Pacheco *et al.* 2005; Fig. 7). Vegetation types, according with the Ministerio de Ambiente del Ecuador (2013) classification system are Evergreen Foothill Forest of the Andean Western Cordillera (headwaters of the Baboso river and Chical); in Colombia, this species inhabits Premontane Rain Forests (*sensu* Holdridge 1987). In Ecuador, annual precipitation at headwaters of the Baboso river is 3394 mm and at Chical 2610 mm; mean annual temperature is 22.4 and 20.5 °C, respectively; in Colombia, annual precipitation at Río Ñambí is > 7100 mm; mean annual temperature is 19.3±1.59 °C (range 17.1–19.7 °C; Salaman 2001).

At Río Ñambí, *R. colomai* is restricted to old growth secondary forests. Individuals are uncommon, being observed at night perched on fallen logs, rock crevices, and on shrub or tree branches or leaves along the margins of small streams and inside the forest. Perch height of the active individuals was on average 0.87 m above ground (range 0.10–3.96 m; n = 42), with adults (mean = 1.28 m, range 0.35–3.96 m, n = 21) generally perching higher than younger individuals (mean = 0.48 m, range 0.10–1.34 m, n = 19). On individual (not collected) was observed perching on epiphytic Araceae *ca.* 4 m high. Amplexus and egg clutches remain unknown. One adult female (JJM 658) released a yellowish-orange secretion when it was captured.

Conservation status. Extent of occurrence is 575 km^2, including the Ecuadorian localities. However, the lack of records since 1984 at Río Baboso and Chical suggests that these populations are extinct (Coloma *et al.* 2010a) and therefore the current extent of occurrence might be less than 100 km^2. At both localities the forest has been logged and fragmented. At the headwaters of the Baboso River, the proportion of remaining forest, within a radius of 10 km of the collection site, is 65.3%; at Chical it is 71.8%. Chical is located within a deforested area. The Colombian locality is a natural reserve, with a forested area of about 14000 km^2 characterized by a canopy between 25 to 30 m high and a very dense understory that has a high concentration of shrubs and low diameter trees, which seems to be correlated with the selective removal of trees (Franco-Roselli *et al.* 1997). The area over which *R. colomai* has been recorded at Río Ñambí is 0.48 km^2. Because of its restricted extent of occurrence (< 100 km^2), limited number of known localities (≤ 5) and increasing habitat alteration and fragmentation, we suggest that *R. colomai* is assigned to the Critically Endangered category (CR) under criteria B1a,b(iii).

Rhaebo olallai (Hoogmoed, 1985) new combination

(Fig. 4 A–E)

Andinophryne olallai Hoogmoed, 1985: 264. Holotype: BM 1970.98, "Tandayapa", Provincia Pichincha, Ecuador.
Andinophryne olallai Hoogmoed, 1989, Zoologische Verhandelingen (Leiden), 250: 1–32.
Andinophryne olallai Lynch *et al.* 2014. Amphibian & Reptile Conservation 8: 1–7.

Diagnosis. A medium sized bufonid (SVL 43.8 mm in males, 57.0 to 60.0 mm in females) characterized by the absence of enlarged cephalic crests, yellowish-orange skin secretions (Fig. 5), elongated testes, elongated parotoids, and flanks with conspicuous, fleshy and prominent tubercles (Fig. 4).

Comparisons with other species. The most similar species is *R. colomai*. Both species differ in the pattern of tuberculation in the flanks. In *R. olallai* the skin on the flanks is weakly areolate but with large, fleshy and prominent tubercles; in *R. colomai* the skin is areolate (females) to strongly areolate (males) with medium-sized tubercles, most of them arranged along an oblique lateral row that connects the parotoid gland with the groin.

Description. Based on two adult females, one adult male (QCAZ 56576; Fig. 4), one subadult male (QCAZ 55561), and photographs of ~20 adult individuals of unknown sex. Male measurements refer to the single adult. A medium sized bufonid, SVL in males 43.9 mm, females 57.0 to 60.0 mm (n = 2); head width 105.3% of head length, 33.8% of SVL, head length is 32.2–32.8% of SVL; head acuminate in dorsal view, projected in profile; vertical fleshy fold at rostrum tip; distance between nostril and tip of the snout 64.1–70.3% of the distance from the nostril to the eye; nostril posterior to the anterior edge of the upper lip, below *canthus rostralis* on moderately protruding area; nostrils oval to rounded; area between the nostrils concave; area from tip of the snout to anterior border of eyelid flat to slightly convex; interorbital and occipital region flat; interorbital area much wider than the upper eyelid; internal border of eyelid not delineated with low isolated tubercles, external border fleshy and protruding; cephalic crests absent; *canthus rostralis* fleshy, gently concave to gently convex, not projected into the loreal region; loreal region strongly concave; lips not prominent to slightly prominent, outlined dark brown in some

individuals; eyes with horizontally round pupil; tympanum not visible to barely visible in life; preserved specimen QCAZ 56576 shows a conspicuous rounded tympanum (diameter = 1.5 mm), concealed on its upper half; parotoid glands elongated with anterior and posterior borders rounded or pointed, some pores present and undulations in the outer edge; parotoid secretion yellowish-orange (Fig. 5B); two adult females lack dorsal tubercles except for few indistinct flat tubercles in sacral region (Fig. 4A); male QCAZ 56576 has numerous tubercles and spicules over the entire dorsum (Fig. 4C); a majority of individuals of undetermined sex have scant, scattered dorsal tubercles; few individuals have abundant tubercles; if present, tubercles are more abundant between the sacrum and the vent; oblique lateral row of 6 to 9 large tubercles isolated or fused, fleshy and prominent, frequently extending from the parotoid gland to the groin; large to medium sized tubercles are usually present in the flank with irregular arrangements; medium sized tubercles can reach the distal margins of the venter; ventral skin tuberculate.

Forelimbs slender, long, with or without subconical tubercles; fingers basally webbed; fingers thin, long, with fleshy tip, not swollen; Finger I shorter than II; smooth palms, supernumerary tubercles absent or scant and inconspicuous, subarticular tubercles low, slightly visible, rounded; palmar tubercles evident, rounded; thenar tubercle inconspicuous; ulnar tubercle and ulnar fold absent; metacarpal fold absent.

Hindlimbs thin and long; hindlimb tuberculation varying from abundant in dorsal and ventral faces to scant and ill-defined in the dorsal face only. Inner tarsal fold absent; outer edge of tarsus with or without tubercles; toes thin and long, with fleshy tip, not bulbous, with extensive membranes; supernumerary plantar tubercles absent; subarticular tubercles ill-defined; nearly flat inner metatarsal tubercle, oval, 3 times the size of ill-defined rounded external metatarsal tubercle.

Tongue oval, longer than wide, thin anteriorly, rounded posteriorly, attached to the mouth floor anteriorly along one half of its length, unnotched posteriorly; choanae ovoid; males with subgular vocal sac and vocal slits; nuptial pads brown and keratinous, low, not swollen; cloacal opening transversal. Testis elongated and hypertrophied, its length is 23% of SVL (QCAZ 56576); male QCAZ 55561 (SVL = 36.9 mm) is a subadult with extremely small testicles.

Distribution and ecology. *Rhaebo olallai* is known from two localities in the western slopes of the Andes in Northern Ecuador (elevation 1300–1500 m above sea level; Fig. 7). Vegetation types, according with the Ministerio de Ambiente del Ecuador 2013 classification system, are Evergreen Foothill Forest of the Andean Western Cordillera (Manduriacu) and Evergreen Low Montane Forest of the Andean Western Cordillera (Tandayapa). Annual precipitation at Tandayapa is 2731 mm and at Manduriacu 2935 mm; mean annual temperature is 17.1 and 19.6 °C, respectively. At Manduriacu all field observations took place at night in *Terra Firme* forest. Most adults and juveniles were observed along streams perching on vegetation up to 2 m above ground. Some individuals were observed moving actively suggesting that activity is nocturnal. Adult individuals were also observed within the forest and away from streams (distance > 50 m).

Conservation status. Assuming that populations survive at the two known localities, extent of occurrence is 1079 km^2. However, Tandayapa, the type locality, is a reserve frequently visited by naturalists and the lack of records since 1970 suggests that the population is extinct. If the single surviving population is Manduriacu, current extent of occurrence is < 100 km^2. At Tandayapa, the proportion of remaining habitat, within a radius of 10 km is 74%; at Manduriacu it is 83.3%. At both localities the forest is fragmented within distances < 1 km of population site. Because of its restricted extent of occurrence, limited number of known localities and increasing habitat alteration and fragmentation, we suggest that *R. olallai* is assigned to the Critically Endangered category (CR) under criteria B1a,b(iii).

Discussion

Genetic and morphological evidence show unequivocally that *Andinophryne* is part of *Rhaebo*, a genus of toads distributed in tropical South and Central America. Before our work the phylogenetic position of *Andinophryne* was uncertain, a consequence of the scarcity of collections resulting from a restricted distribution and lack of field records for up to four decades.

The conservation status and distribution of species of the genus *Rhaebo*, including the three former *Andinophryne*, is consistent with the ecological and phylogenetic patterns associated with the precipitous population declines that many Neotropical amphibians have experienced (see Bolaños *et al.* 2008 for a review).

Amphibians that have declined rapidly in the Neotropics are stream-associated species in mid to high elevations (Lips *et al.* 2003; Ron *et al.* 2003; La Marca *et al.* 2005). Within *Rhaebo*, four species are threatened: *R. atelopoides*, *R. colomai*, *R. olallai* and *R. caeruleostictus* (based on our categorization and IUCN 2013). These species are associated with streams and distributed primarily above 1000 m of elevation. In contrast, four species are non-threatened: *R. guttatus*, *R. haematiticus*, *R. nasicus*, and *R. blombergi* (IUCN 2013). They are distributed below 1000 m and breed on temporary or permanent ponds or along lowland streams and rivers (Mueses-Cisneros *et al.* 2012; IUCN 2013). A similar pattern is evident in *Atelopus*, a diverse bufonid genus that is among the most affected by population declines (La Marca *et al.* 2005). *Atelopus* has been used as a model system to study the role of climate change and disease in population extinctions (Pounds *et al.* 2006; Lips *et al.* 2008). In *Atelopus*, 90% of its 93 species are Endangered or Critically Endangered IUCN 2013. Their conservation status has a strong elevational signal with a higher proportion of declining species above 1000 m (La Marca *et al.* 2005). In *Atelopus* and *Rhaebo*, Endangered and Critically Endangered species share similar ecological traits. Rigorous phylogeny-based comparative analyses could allow testing the generality of these observations and even predicting the conservation status of Data Deficient species based on their phylogenetic position and the ecological traits of closely related taxa.

Phylogenetic relationships. The affinity between *Andinophryne* and *Rhaebo* was hinted by Hoogmoed (1985) and Graybeal and Cannatella (1995) who noticed that the presence of an omosternum was shared between *Andinophryne*, *R. haematiticus* and *R. guttatus*. Hoogmoed (1985) states "it seems possible that this genus [*Andinophryne*] is the most primitive of the group and closely resembles the ancestral stock (most probably *Bufo haematiticus* like) that gave rise to this group of genera [*Atelopus*, *Osornophryne* or *Rhamphophryne*]". A close relationship with *Rhaebo* was also suggested by Mueses-Cisneros 2008 who reviewed the systematics of the genus *Rhaebo* based on morphological characters. He included in his analyses the genus *Andinophryne* and concluded that while *Andinophryne* lacked synapomorphies, putative synapomorphies for *Rhaebo* were also present in *Andinophryne*. He highlighted that some species of *Rhaebo* had a morphology intermediate between both genera as exemplified by *R. andinophrynoides*, a species described by Mueses-Cisneros (2009). Frost *et al.* (2006) suggested that *Andinophryne* might have a closer relationship with *Rhinella*, a hypothesis that is incompatible with our phylogeny.

In our phylogeny, *Rhaebo* and *Nannophryne* occupy a basal position within a clade of bufonids characterized by the presence of parotoid glands. This position is consistent with previous phylogenies (Pramuk *et al.* 2007; Van Bocxlaer *et al.* 2010) except for a weakly supported sister clade relationship between *Rhaebo* and *Peltophryne* in Van Bocxlaer *et al.* (2010). The divergence between *Rhaebo* and the other bufonids took place ~ 35 million years ago (Van Bocxlaer, *et al.* 2010). Species richness in both clades differs by over one order of magnitude (*Rhaebo* has 13 species vs. ~412 for its sister clade) suggesting markedly different diversification rates. Comparative analyses between both clades could help to understand the reasons for the high diversification rates and wide geographic range of the sister clade of *Rhaebo*.

The phylogeny based on mt-DNA shows support for the monophyly of all bufonid genera, as defined by Frost (2014), except for *Pedostibes*, a genus distributed in India and southeast Asia. *Pedostibes hosii* and *Pedostibes rugosus* are more closely related to *Phrynoidis* spp. than to *Pedostibes tuberculosus*. Because the later is the type species of *Pedostibes*, transferring *Pedostibes hosii* and *Pedostibes rugosus* to the genus *Phrynoidis* could solve the polyphyly of *Pedostibes*.

Morphological synapomorphies of *Rhaebo*. The genus *Rhaebo* has been characterized as lacking cephalic crests, having omosternum, an sphenethmoid distinctively wide, exoccipital condyles prominent and notched and yellowish-orange skin secretions (Frost *et al.* 2006; Pramuk 2006; Mueses-Cisneros 2009). The morphology of *R. atelopoides*, *R. colomai* and *R. olallai* is consistent with this characterization because they lack cephalic crests, have an omosternum (Hoogmoed 1985), and have yellowish orange skin secretions. The condition of the sphenethmoid and exoccipital condyles was evaluated only in *R. colomai* and is similar to other species of *Rhaebo*.

It is unclear, however, which diagnostic characters of *Rhaebo* are derived (i.e., synapomorphies) and which are ancestral. Yellowish parotoid secretions are a putative synapomorphy for *Rhaebo* because secretions from most toads are white (Frost *et al.* 2006). A few populations of *R. haematiticus* and *R. glaberrimus* have white secretions (J.J. Mueses-Cisneros and S.R. Ron, pers. obs.) making necessary a comprehensive evaluation of this character to confirm it as a synapomorphy. Parotoid glands are the only know synapomorphy for the most speciose clade within Bufonids. Characterization of the chemical composition of parotoid secretions could be helpful to understand

diversification patterns within Bufonidae because there is a marked difference in diversification rates between *Rhaebo* and the other bufonids with parotoid glands.

An additional synapomorphy for *Rhaebo* seem to be the coloration pattern of juveniles. With few exceptions (e.g. *R. haematiticus*), juveniles of *Rhaebo* have dorsal coloration consisting of a dark background with contrasting thin clear stripes or dots (Figs. 3–4). This coloration pattern, which disappears in the adults, is absent in other bufonids suggesting that it is derived in *Rhaebo* (see also Mueses-Cisneros 2008 and Mueses-Cisneros 2009: Fig 3).

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