

A NEW SPECIES OF ARBOREAL *RHINELLA* (ANURA: BUFONIDAE) FROM CLOUD FOREST OF SOUTHEASTERN PERU

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ABSTRACT: A new arboreal species of *Rhinella* is described from the humid montane forest of Manu National Park in the Cordillera Oriental of southern Peru. The new species can be distinguished from all known *Rhinella* by a unique combination of external and osteological characters as well as by molecular data. The new toad is compared to *R. arborescandens* and *R. veraguensis* with respect to external characters. On the basis of morphological and molecular data, the new taxon is closely related to *R. chavin*, *R. nesiotes*, and *R. festae*. Although DNA data indicate that a member of the *R. veraguensis* group (*R. nesiotes*) is its sister taxon, the new species is not closely related to other members of this species group (e.g., *R. veraguensis*). In addition, DNA data indicate that the *R. veraguensis* group as it currently is defined is paraphyletic. Until additional studies are completed on the phylogeny of these South American toads, we refrain from assigning the new taxon to a species group.

Key words: Anura; Bufonidae; DNA; Manu National Park; Morphology; New species; Peru; *Rhinella*

RESUMEN: Se describe una nueva especie arborícola del género *Rhinella* de los bosques montanos húmedos del Parque Nacional del Manu en la Cordillera Oriental del sur del Perú. La nueva especie se distingue del resto de las especies del género *Rhinella* por una combinación de características externas y osteológicas así como por datos moleculares. Esta nueva especie se compara con *R. arborescandens* y *R. veraguensis* respecto a las características morfológicas externas y osteológicas.

THE FAMILY Bufonidae contains 486 species (see Amphibiaweb.org, accessed 2006), with the number of species increasing annually. Several species of Andean toads have been described recently, suggesting that much anuran diversity within this region yet remains to be discovered (e.g., Duellman and Ochoa, 1991; Duellman and Schulte, 1992; Gluesenkamp, 1995; Harvey and Smith, 1993; Harvey and Smith, 1994; Lehr et al., 2001; Lehr et al., 2005; Lötters and Köhler, 2000; Padial et al., 2006). Nine new species have been assigned to the *Rhinella veraguensis* group, largely endemic to the Andes of South America (Lehr et al., 2005) within the past 18 yr. Herein, we describe a new species of Andean *Rhinella* and note that it is closely related to some members (e.g., *R. nesiotes*, and *R. chavin*) of the *R. veraguensis* group based on evidence from external and internal morphological characters and phylogenetic analysis of DNA data.

MATERIALS AND METHODS

Coordinates were recorded with a GPS device. Specimens were photographed, fixed in 10% formalin and preserved in 70% ethanol. Measurements were taken with calipers to the nearest 0.1 mm. Specimens examined were deposited in the Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco (MHNC; Cusco, Peru) and Museo de Historia Natural San Marcos (MHNSM; Lima, Peru). Osteological information for the new species was obtained by examination of radiographs and a single cleared-and-stained specimen prepared following the protocol of Dingerkus and Uhler (1977). Abbreviations used herein are: SVL (direct line distance from tip of snout to posterior margin of vent), TIB (length of flexed hind leg from knee to heel), FL (foot length, distance from base of inner metatarsal tubercle to tip of fourth toe), HL (distance from tip of snout to articulation of jaw), HW (greatest width of head measured between jaw articulations), EW (greatest width of eyelid

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measured perpendicular to medial axis of skull), IOD (shortest distance between medial margins of upper eyelids), ED (eye diameter measured along horizontal axis), EN (distance from anterior corner of eye to posterior border of nostril), NR (distance from anterior margin of nostril to tip of rostrum), IND (distance between nostrils), TY (tympanum diameter, horizontal), and GLAND (parotid gland diameter, vertical). Our terminology for webbing formula follows Savage and Heyer (1967) and Myers and Duellman (1982). Specimens examined are listed in Appendix 1.

Molecular data used in this study were collected for a prior study on relationships of South American bufonids (Pramuk, 2006). We added newly sequenced, complementary data for one specimen of the new *Rhinella* to a data set of 3987 bps of combined mitochondrial (12S) and nuclear (POMC and RAG-1) DNA data. Prior to extraction, tissues were fixed in 95% ethanol. The DNA was extracted from small amounts (~50 ng) of muscle or liver tissue with the Dneasy Tissue Kit® (Qiagen, Inc.) and visualized on 1% high-melt agarose gels in TAE buffer. Polymerase chain reactions were performed in 50 µl reactions containing 0.5 units of *Taq* polymerase (Fisher), ~1 µg of genomic DNA, 10 pmol of each primer, 15 nmol of each dNTP, 50 nmol of MgCl₂, and buffer. Amplification followed published PCR conditions (Palumbi, 1996). Cycle sequencing reactions were completed with Big Dye Sequencing kits (ABI, Inc.). Amplified DNA was purified with Sephadex columns and sequencing was performed directly using an ABI 3100 automated sequencer. The program Sequencher 3.1.1 (Gene Codes Corp.) was used to edit sequences. Clustal X (Thompson et al., 1997) was employed to perform preliminary alignment using default parameters, with adjustments by eye. Alignment of protein coding sequences was straightforward. These sequences were translated into amino acids to verify alignment. Additional information regarding data alignment protocols can be found in Pramuk (2006).

Data were analyzed with maximum likelihood (ML) and maximum parsimony (MP) using the program PAUP* (v4.0; Swofford, 2002), and Bayesian analysis was performed



FIG. 1.—Photograph of the holotype in life of *Rhinella manu* sp. nov. (MHNC 3005; female, SVL = 41.8 mm).

with the application MrBayes (ver. 3.0b4; Huelsenbeck & Ronquist, 2001; details of data analyses are identical to those discussed elsewhere [Pramuk, 2006]). The sole purpose of the phylogenetic analysis presented herein was to place the new species within its appropriate species group and not to conduct a detailed study of relationships among South American bufonids, as this is covered in detail elsewhere (Pramuk, 2006). In addition, all analysis methods converged on the same result with regards to the placement of the new species and the relationships within South American bufonids (i.e., the *Rhinella* clade). Therefore, we only present and discuss the results of the Bayesian analysis herein.

Rhinella manu sp. nov.

Holotype.—MHNC 3005 (Fig. 1), an adult female obtained near Tres Cruces, approximately 134 km northeast of Cusco (13°06'19.2"S, 71°17'00"W) at 2750 m elevation, Distrito de Paucartambo, Provincia de Paucartambo, Departamento de Cusco, Peru, by Juan C. Chaparro on 13 January 2000.

Paratypes.—All from Distrito de Paucartambo, Provincia de Paucartambo, Departamento de Cusco: an adult female MHNC 3007 collected on 13 January 2000, by John L. Achicahuala; three adult females, MHNC 3009 (MHNSM 24883) collected on 15 January 2000, MHNC 3003 collected on 22 August 2000, MHNC 3004 collected on 13 January 2000 by Juan C. Chaparro; two adult males MHNC 3006, 3008, collected on 13 January 2000 by José A. Ochoa; and two juveniles MHNC 3010 and MHNC 3011

collected on 21 August 2000, by Juan C. Chaparro. All specimens were collected at 13°06'19.2"S, 71°17'00"W between 2700–2800 m.

Diagnosis.—A medium-sized species of *Rhinella* attaining a maximum SVL of 41.8 mm in adult females (MHNC 4206; Fig. 1). The new species is distinguished from all congeners by the following combination of characters: (1) canthus rostralis raised with pronounced border distinct, orbitotypanic and postorbital crests weak; (2) tympanum distinct, oval; (3) parotoid glands large (about twice ED), oblong, taller than wide, exaggerated to the point of being nearly spherical, not contacting eye; (4) numerous small round glands dispersed on dorsal surfaces of body; (5) forearm moderately long and slim; (6) dorsolateral, round, enlarged, and elevated granules; (7) tarsal fold absent, entire surfaces of limbs granular; (8) webbing on hand and foot fleshy; (9) first finger shorter than second; (10) males without vocal sacs or vocal slits; (11) small keratinous spines on dorsal surfaces of thumb in breeding males; and (12) iris reddish orange in life. We note that *Rhinella manu* is closely related to *R. chavin* and *R. nesiotetes* of the *Rhinella veraguensis* group (sensu Duellman and Schulte, 1992) based on external characters and evidence from DNA analysis (see Remarks).

Rhinella manu can be distinguished from a similar arboreal species *R. arborescandens*, mainly by having a visible tympanic membrane (absent in *R. arborescandens*); a concave internarial area (flat); webbing on hand and foot well developed (not developed); lacking a cream dorsal line on midventral surface of tarsus (with cream stripe present); a black dorsum with red tint, and without middorsal stripe in males or females (reddish with brown and yellow middorsal stripe present in males). *Rhinella manu* can be distinguished from the recently described and similar arboreal species *R. tacana* by having a reddish orange iris (green in *R. tacana*). *Rhinella manu* females are larger than *R. arborescandens* or *R. tacana* females (*R. manu* female SVL = 41.8, *R. arborescandens* female SVL = 38.0, *R. tacana* female SVL = 34.2; Duellman and Schulte [1992] and Padial et al. [2006], respectively). *Rhinella manu* has a visible tympanic mem-

brane while *R. amboroensis*, *R. justinianoi*, *R. quechua*, and *R. veraguensis* lack a visible tympanic membrane. *Rhinella fissipes* has a barely visible tympanum. *Rhinella amboroensis*, *R. justinianoi*, *R. quechua* are larger (*R. manu* adult male SVL = 32.3; *R. amboroensis* adult male SVL = 38.5; Harvey and Smith, 1993); (*R. justinianoi* adult female SVL = 53.6; Harvey and Smith, 1994); adult *R. quechua* (putative female) SVL = 62.0; (Gallardo, 1961). *Rhinella fissipes* also is larger than *R. manu* (mean female SVL = 68.1; range = 65.8–71.9, $n = 8$; Köhler [2000]) and has two prominent dorsolateral rows of tubercles. Moreover, unlike *R. manu*, *R. fissipes* has the first finger longer than second and serrated foot webbing, with parotoid glands slightly elevated and not protruding laterally. The new species can be distinguished from *R. multiverrucosus* and *R. chavin* by having nuptial spines on the first finger in breeding males (Fig. 3) and by the lack of enlarged glands on dorsum and extremities; *R. multiverrucosus* and *R. chavin* are larger than *R. manu* (*R. multiverrucosus* mean adult female SVL = 61.45; range = 54.0–68.9, $n = 12$; Lehr et al. [2005]; *R. chavin* mean adult female SVL = 59.5; range = 54.8–64.9, $n = 4$; Lehr et al. [2001]). *Rhinella manu* differs from *R. inca* by having the first finger shorter than the second. The new species differs from *R. nesiotetes* by having pronounced parotoid glands (low and diffuse in *R. nesiotetes*); *R. manu* has a row of enlarged dorsolateral warts, (absent in *R. nesiotetes*); and *R. nesiotetes* is smaller (adult female SVL = 29.0; Duellman and Toft [1979]). *Rhinella manu* differs from *R. rumbolli* by having long and slender limbs, inconspicuous cranial crests, and first finger longer than second (first finger shorter than second in *R. rumbolli*).

Description of holotype: external morphology.—Body robust; head longer than wide; tip of snout pointed in dorsal and lateral views (Fig. 2), protruding past anterior margin of jaw in all views; head flat dorsally with cephalic crests extending from posterior margin of eyelid to anterior margin of parotoid gland. Skin of head co-ossified with underlying bones, with scattered, granular tubercles. Upper eyelid bulbous, with numerous granu-

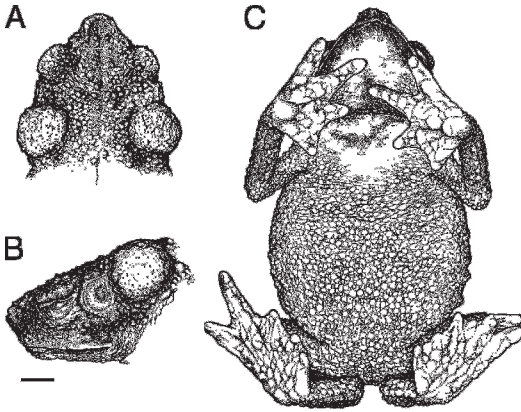


FIG. 2.—(A) Dorsal and (B) lateral views of the head and (C) ventral views of *Rhinella manu* (MHNC 4211; adult male, SVL = 32.3 mm). Scale bar = 1 cm. Drawings by T. Howard.

lar tubercles with external margin of eyelid flared; internarial area concave, forming a V-shaped trough; canthus rostralis raised with pronounced border, angled sharply antero-ventrally immediately anterior of eye; naris slightly protuberant, directed laterally; loreal region slightly concave and nearly vertical, with indistinct granular tubercles extending into infraorbital region and becoming pronounced in infratympanic region. Upper lip flared and smooth; cluster of tubercles at angle of jaw; tympanum distinct (Fig. 1), equal in size to eye, with distinct raised, granular margin; parotoids large (about twice ED), much larger than tympanum, oblong, taller than wide, exaggerated to the point of being nearly spherical; forelimbs moderately

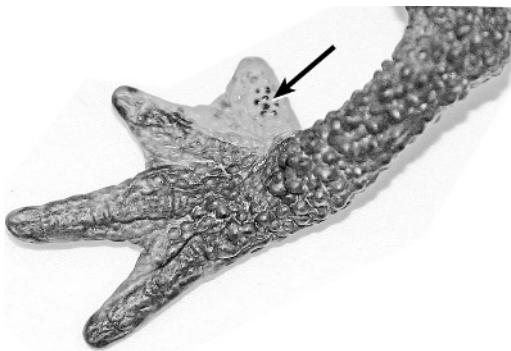


FIG. 3.—Arrow indicating keratinous spines on digit I of the hand of an adult male *Rhinella manu* (MHNC 4211).

long, robust; hand broad with relatively long fingers; relative length of fingers $I < II < I - V < III$, webbing fleshy; lateral fringes of fingers are present; tips of digits round; subarticular tubercles low, round to ovate; larger than supernumerary tubercles; supernumerary tubercles abundant, small, irregular or rounded; palmar tubercles round and low, accessory palmar tubercles subtriangular and avoid, flat in profile; thenar tubercle low and ovoid, and flat in profile; limbs long and thin, tibia length shorter than foot length; tarsal fold absent; inner metatarsal tubercle ovoid and flat; three times the size of outer metatarsal tubercle; outer metatarsal tubercle round, round in profile; toes moderately short; relative length of adpressed toes $I < II < III < V < IV$; foot webbing completely covering digits I–III and most of digit V; webbing fleshy; foot webbing formula $II-1III1-1III1-3IV2-1V$; lateral fringes present; subarticular tubercles small and round to ovoid; supernumerary tubercles smaller than subarticular tubercles; dorsal surface of forearm, tibia and femur with numerous scattered small warts; dorsolateral row of elevated warts. Snout protuberant, extending well past anterior margin of jaw in all views; tympanum oval, vertical dimension greater than horizontal, slightly larger than eye; dorsum with scattered small warts on a smooth background; dorsal surfaces of limbs covered with small rounded tubercles, skin of gular and pectoral regions smooth; that of inguinal region slightly rugose with numerous small warts becoming more pronounced laterally, skin of venter in coccygeal region with escutcheon of pronounced glandular pustules (Fig. 2); digits of hand and foot elongate and extensively webbed; relative lengths of digits of the hand: $I < II < IV < III$; webbing completely covering digits I and II of hand and up to base of second phalange on digits III and IV; ultimate and penultimate phalanges of digit IV extend beyond webbing; webbing fleshy; webbing formula of fingers $II-1III1-2III2-2IV$; cloaca projecting slightly downward, cloacal tube present; muscles and peritoneum suffused with coral tint in preservative. Choanae small, round; vomerine teeth absent; tongue elongate and ovoid, longer than wide. Measurements (in mm) of holotype: SVL = 41.8; TIB = 15.0; FL =

17.4; HWID = 13.1; HLEN = 12.2; IOD = 5.2; IND = 2.8; EN = 4.0; NR = 2.2; EW = 3.7; GLAND = 5.4; TY = 3.1.

Internal morphology.—Origin of tensor fasciae latae just posterior to midpoint of ilium (on ventrolateral surface) and inserts on lateral surface of distal half of cruralis, adductor longus absent; pectineus inserts proximally about one third length of femur; sartorius broad; mandibular branch of trigeminal nerve passes medial to m. adductor mandibulae externus (“S condition” sensu da Silva and Mendelson, 1999); m. depressor mandibulae exhibits the “SQ condition” (da Silva and Mendelson, 1999).

Osteology.—The cranium of *Rhinella manu* resembles that of other, recently illustrated members of the *R. veraguensis* group (e.g., *R. chavin*, *R. multiverrucosus*, and *R. veraguensis*) in overall shape and in having a lightly exostosed dorsal surface of the skull, an exposed sphenethmoid in dorsal view, and a truncated cultriform process of the parasphenoid in ventral view (Lehr et al., 2005). In dorsal view, the nasals are not in contact with each other medially (Fig. 4), slightly asymmetrical, with anteromedial margins crenulate; frontoparietals contacting medially; posteromedial margins of nasals not contacting anterior margins of frontoparietals making sphenethmoid visible in dorsal view. In ventral view, the vomers are well developed, with anterior margins not in contact with maxillae; optic foramen large and circular, posterior margin formed by anterior margin of orbitosphenoid, anterior margin formed by cartilage spanning from orbitosphenoid to sphenethmoid; quadratojugal well developed and in contact with maxilla and quadrate; columella robust; alary process of premaxilla well developed; discrete septomaxilla present with anteriorly-directed nasal process and medially directed U-shaped depression. A large sesamoid of the hand is present at the base of metacarpal 3; radiale, ulnare, and centrale (digits 2, 3, 4) present; centrale, carpal, and two prepollical elements present at base of prepollex (digit 1); tips of terminal phalanges moderately expanded (Fig. 5). The hyales of the hyoid apparatus are without anterior processes; posterior medial process well ossified, with spur on medial surface, approximately one fifth of the distance from anterior end,

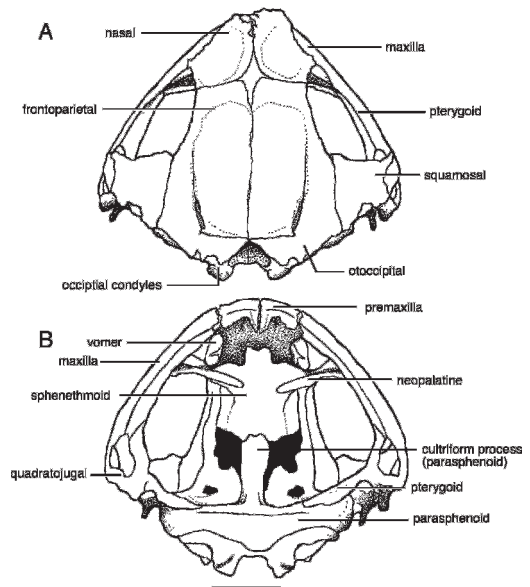


FIG. 4.—Dorsal and ventral views of the skull of *R. manu*, illustrating cranial structures described in the text (MHNC 4404; female, SVL = 41.0 mm). Scale bar = 5 mm.

posterolateral processes slightly expanded distally, anterior lateral processes very stout, broadly connected to hyoid (Fig. 6).

Seven discrete presacral vertebrae present; atlas and axis fused; width of transverse processes $1 < 6 < 5 < 7 < 4 < 2 < 3$; transverse processes of atlas/axis complex are laterally dilated, tapered on all other vertebrae; sacrum fused to coccyx, sacral diapophyses broadly expanded; lateral crests present on urostyle, two paired expansions creating a faintly “waisted” appearance (Fig. 7).

Color in preservative.—Dorsum brown-black in adult males and females, with juveniles having a light brown dorsal coloration. Venter pink orange to beige, with or without brown blotches on the chest and tiny cream blotches dispersed on venter.

Color in life.—Dorsum dark umber (black with red tint); venter red with darker blotch covering underside of throat, gular, and pectoral regions. Ventral margin of jaw darkly pigmented. Palmar and plantar surfaces of hand and foot with dark spots and the outer digits and distal margins darkly pigmented. Lateral regions of the venter light red. Iris reddish orange.

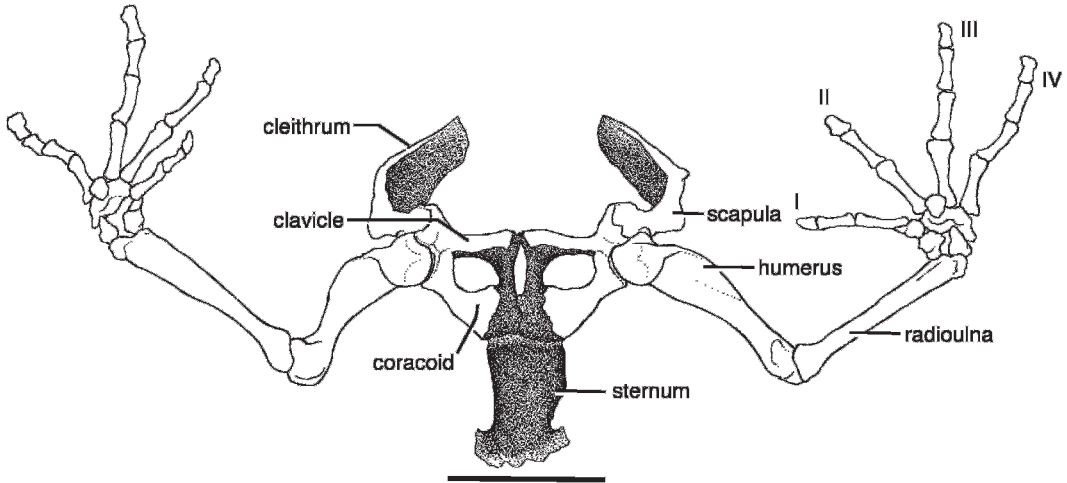


FIG. 5.—Ventral views of the forelimbs and pectoral girdle of *R. manu* (MHNC 4404). Scale bar = 5 mm.

Variation.—The type series includes three adult females, one adult male, and two juveniles. All adults are similar in coloration. Skin of juveniles rufous with scattered, irregular, creamy yellow patches and overlying bluish white blotches. One female specimen (MHNC 4404) had highly convoluted oviducts with 35–40 eggs per ovary (mean diameter of 2 mm). Adult males with pointed keratinous spikes on the first finger of the hand (Fig. 3).

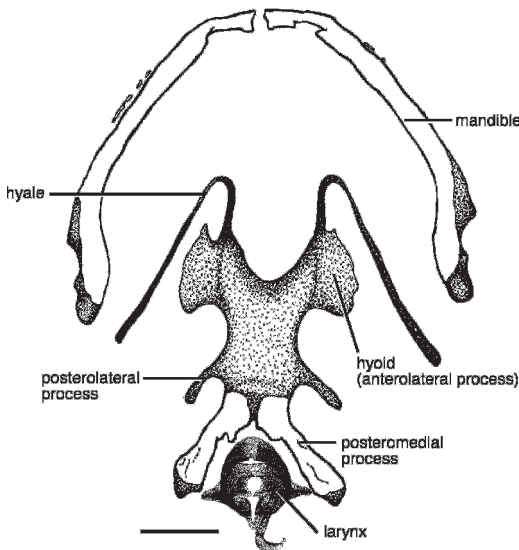


FIG. 6.—The hyoid apparatus of *R. manu* (MHNC 4404). Scale bar = 5 mm.

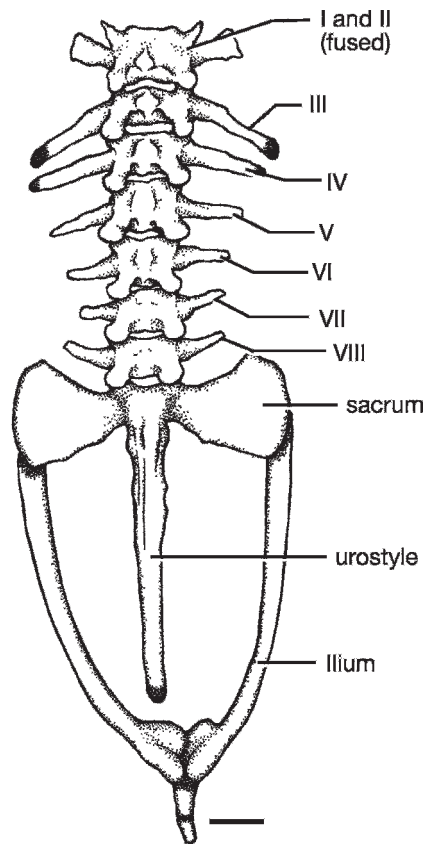


FIG. 7.—Dorsal view of the vertebral column of *R. manu* (MHNC 4404). Note that vertebrae I and II are fused and that the urostyle (coccyx) is fused to the sacrum. Scale bar = 5 mm.

TABLE 1.—Measurements taken from the type series (ranges in mm). See Materials and Methods for definitions of acronyms.

Specimen	SEX	SVL	TIB	FL	HWID	HLEN	IOD	IND	EN	ED	NR	EW	GLAND	TY
MHNC 4206	F	41.8	15.0	17.4	13.1	12.2	5.2	2.8	4.0	4.0	2.2	3.7	5.4	3.1
MHNC 4404	F	41.0	14.4	16.3	12.6	11.7	5.0	2.7	3.9	3.6	2.0	3.7	5.2	3.1
MHNC 4214	F	39.1	14.2	16.0	11.4	11.4	4.9	2.6	3.8	3.5	1.9	3.3	5.6	3.0
MHNC 4213	F	36.6	13.5	14.9	11.5	11.2	4.5	2.4	3.8	3.3	1.8	3.1	5.3	2.7
MHNC 4212	F	32.4	12.2	13.5	10.6	10.2	4.1	2.4	3.4	3.1	1.7	3.0	5.2	2.6
MHNC 4211	M	32.3	12.0	14.2	10.5	10.1	4.1	2.3	3.3	3.0	1.7	2.8	5.0	2.5
MHNC 4215	M	31.5	11.4	13.1	10.4	9.7	4.1	2.2	3.2	2.7	1.6	2.7	4.9	2.5
MHNC 4394	J	24.2	9.2	9.4	8.4	7.9	3.1	1.7	2.2	2.3	8.0	2.3	3.6	1.9
MHNC 4393	J	15.3	5.2	5.5	5.5	5.2	2.2	1.3	1.6	1.7	0.9	1.9	1.8	1.3

Morphological measurements are provided in Table 1.

Etymology.—The specific name is a noun in apposition in reference to the type locality, in Manu National Park. The meaning of the word Manu and its language of origin are unknown; however, it has been suggested that word is derived from the Machiguenga word, Mañu, which means mosquito (W. Maldonado, personal communication).

Ecology and behavior.—Specimens were found in cloud forest during and directly following the rainy season. *Rhinella manu* is likely nocturnal and arboreal as all specimens were encountered at night, on leaves and branches of arboreal ferns (*Cyathea*), 50 cm–2 m above the ground. Most specimens (7/9) were collected in areas without standing water. The habitat is composed of montane, humid primary forest. Floristic composition of the area includes the following genera: *Clusia*

(Gittiferae), *Miconia* (Melastomataceae), *Hedyosmum* (Chloranthaceae), *Schefflera* (Araliaceae), *Oreocallis* (Proteaceae), *Piper* (Piperaceae), *Chusquea* (Bambusoideae), and *Cyathea* (tree ferns). Other anuran species in the area of the type locality include *Eleutherodactylus rhabdolaemus*, *E. cf. cruralis*, *Phrynopis cf. peruvianus*, and *P. cophites*. Additional information on the general characteristics of the area of the Kosñipata Valley was provided by Duellman (1978).

Comments.—Species of the *Rhinella veraiguensis* group display varied behaviors. For example, some species such as *R. amboroensis* (Harvey and Smith, 1993) and *R. rumbolli*

TABLE 2.—Ranges (in mm) of adult males and females of *Rhinella manu*. For abbreviations, see Material and Methods.

Characters	Males (n = 2)	Females (n = 5)
SVL	31.5–32.3	32.4–41.8
TIB	11.4–12.0	12.2–15.0
FL	13.1–14.2	13.5–17.4
HWID	10.4–10.5	10.6–13.1
HLEN	9.7–10.1	10.2–12.2
IOD	4.1–4.1	4.1–5.2
IND	2.2–2.3	2.4–2.8
EN	3.2–3.3	3.4–4.0
ED	2.7–3.0	3.1–4.0
NR	1.6–1.7	1.7–2.2
EW	2.7–2.8	3.0–3.7
GLAND	4.9–5.0	5.2–5.6
TY	2.5–2.5	2.6–3.1

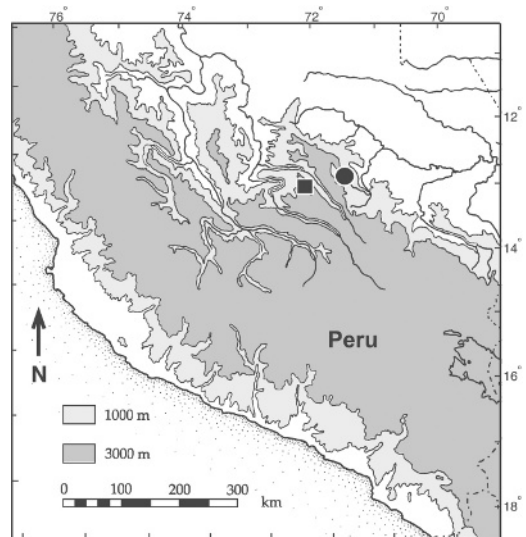


FIG. 8.—Map illustrating the distribution of *R. manu*, only known from the type locality, Valle de Kosñipata, within the Reserva Biósfera del Manu in southeastern Peru (indicated with a circle); the square represents the city of Cusco.

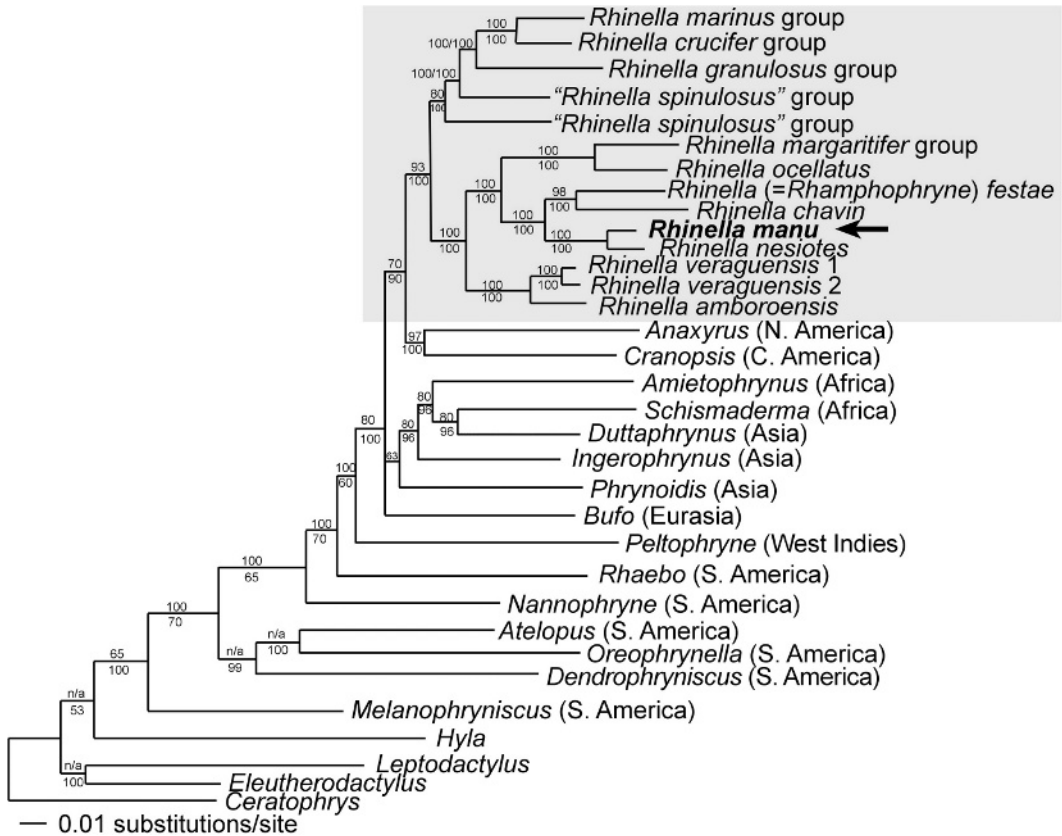


FIG. 9.—Bayesian consensus tree resulting from analysis of 3987 bps of combined 12S mtDNA data, and POMC and RAG-1 nuclear DNA data. Maximum parsimony bootstrap values and Bayesian posterior probabilities (the latter, multiplied by 100) are indicated above and below the nodes, respectively. The gray box highlights the South American *Rhinella* clade. This tree has been reduced to show placement of species groups within South American *Rhinella* and their relationship to *R. manu* (indicated with an arrow). These data indicate that taxa from the *R. veraguensis* group included in our analysis do not form a natural group. For this reason, and because our data set does not include all known species of the *veraguensis* group, we refrain from assigning *R. manu* to an existing species group of South American *Rhinella*.

(Carrizo, 1992) are largely aquatic, whereas others are at least partially arboreal (e.g., *Rhinella chavin*, *R. veraguensis* [Lehr et al., 2001], *R. multiverrucosus* [Lehr et al., 2005], and *R. nesiotis* [Duellman and Toft, 1979]). *Rhinella arborescendens* (Duellman and Schulte, 1992) and *R. manu* are arboreal, whereas *R. inca* and *R. justinianoi* are primarily terrestrial (J. C. Chaparro, personal observation; Harvey and Smith [1994], respectively). Field observations revealed that *manu* climbs rather than hops.

Distribution.—*Rhinella manu* is known only from the type locality, Trocha Union Km 6, situated in the Valle de Kosñipata, within the

Reserva Biósfera del Manu in southeastern Peru (13°06'19.2"S, 71°17'00"W), between 2700–2800 m in elevation (Fig. 8).

Remarks.—Our phylogenetic topology agrees largely with the topologies of Pauly et al., (2004) and Pramuk (2006) and for most part with that of Frost et al. (2006); however, it differs markedly from the latter study in its placement of the *Bufo margaritifera* group (*Rhinella*, *vide* Frost et al., 2006). We note that Frost et al. (2006) state “if ‘*Rhinella* Fitzinger, 1828 [is] found to be nested within *Chaunus* Wagler, 1828, the name *Rhinella* will take precedence for the inclusive group”. Therefore, we use the generic name *Rhinella*

proposed by Frost et al. (2006) to include most species groups of South American toads that were formerly assigned to the genus *Bufo* (except *Rhaebo* [formerly the *Bufo guttatus* group] and *Nannophryne* [formerly *Bufo variegatus*]).

Maximum parsimony (MP), maximum likelihood (ML), and Bayesian (Fig. 9) analyses of DNA data performed for this study converged on congruent topological relationships (ML bootstraps and Bayesian posterior probabilities [bpp] are very similar, therefore only MP bootstraps and bpp are illustrated on the branches). Genbank accession numbers for sequences produced for this study are: *R. manu* (MHNC 4215, male) collected from Trocha Union, Km 6, Kosñipata: 12S: DQ404394; POMC: DQ404396, and RAG-1: DQ404395. All other GenBank accession numbers are provided in Pramuk (2006). Topologies resulting from all analyses recover high support (e.g., bpp = 100) for a sister group relationship between *R. manu* and *R. nesiototes*. This analysis agrees with results of a prior analysis (Pramuk, 2006) indicating that the *R. veraguensis* group as it is currently defined (*R. amboensis*, *R. arborescandens*, *R. chavin*, *R. fissipes*, *R. inca*, *R. justinianoi*, *R. multiverrucosus*, *R. nesiototes*, *R. quechua*, *R. rumbolli*, and *R. veraguensis*) is not a monophyletic (i.e., “natural”) group. Our Bayesian tree indicates that taxa from the *R. veraguensis* group included in our analysis form two sister lineages: one clade comprising *Rhinella festae* (= *Rhamphophryne festae*), *R. manu*, *R. nesiototes*, and *R. chavin*, which is sister to the *R. margaritifera* group + *R. ocellatus*. The other clade assigned to the *R. veraguensis* group (*R. veraguensis* + *R. amboensis*) falls out as sister to the above-mentioned clades. It is apparent that additional work yet remains in resolving the relationships of the 12 currently described species of this group and their relationships to other species of South American *Rhinella*. For more complete discussions of South American bufonid phylogeny see Pauly et al. (2004), Frost et al., (2006), and Pramuk (2006). Because the *R. veraguensis* group is not monophyletic (Pramuk, 2006), we refrain from assigning *R. manu* to it. However, we note that on the basis of morphological characters, *R. manu* is most similar to species

currently assigned to this group and that based on molecular and morphological evidence it cannot be assigned with confidence to any other species group of South American *Rhinella*. Additionally, because our results are based on incomplete taxon sampling, at this time we refrain from assigning a new species group to the clade comprising *R. chavin*, *R. festae*, *R. manu*, and *R. nesiototes*.

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APPENDIX I

Material Examined

Rhinella arborescandens: Peru: Amazonas: pass 5 km NW Mendoza, 2400 m, KU 209394 (paratype). *Rhinella chavin*: Peru: Departamento de Huánuco: Provincia de Pachitea: Distrito Chaglla, along the road from Chaglla to Tomayrica, MHNSM 20028 (holotype), MHNSM 20025, 20026, 20029, 20030, 20031, 20032, 20033 (paratypes). *Rhinella multiverrucosus*: Peru: Departamento de Pasco: Provincia de Pasco: Distrito de Paucartambo, MHNSM 17820, (holotype); 17836, 20653–55, 20665, 22653 (paratypes), MHNSM 19035–36, MHNSM 18612–13. *Rhinella nesiotis*: Peru: Huanuco: NA: W slope Serranía de Sira, “Pato Rojo”, 1100 m, KU 209394. *Rhinella veraguensis*: Peru: Cusco: NA: 4 Km WSW Santa Isabel, 1700 m, KU 139115. Note: Unfortunately, while in route to Peru, specimen numbers MHNC 4404, MHNC 4214, MHNC 4215, and MHNC 4394 were lost by the international shipping company DHL Express in May 2005.