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Source: South American Journal of Herpetology, 18(1) : 13-23

Published By: Brazilian Society of Herpetology

URL: https://doi.org/10.2994/SAJH-D-18-00069.1

South American Journal of Herpetology, 18, 2020, 13–23

Description and Phylogeny of a New Species of Andean Lizard (Gymnophthalmidae: Cercosaurinae) from the Huancabamba Depression

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Abstract. We describe a new species of *Macropholidus* lizard from the Andean highlands of southern Ecuador and northern Peru based on recent collections. Among other characters, the new species differs from other species of *Macropholidus* in having a paired series of enlarged middorsal scale rows restricted to the nape, striated dorsal scales, as well as ocelli on tail, flanks, scapular region and neck. We also present a molecular phylogeny of Cercosaurinae and genetic distances as additional evidence supporting delimitation of the new species, which is sister to *M. annectens* from southern Ecuador. Data on Andean orogeny and age estimates of cercosaurine lizards suggest that the Huancabamba Depression, long recognized for its dual role as a biogeographic barrier and a migration corridor, has also served as a center of origin and diversification of *Macropholidus* lizards.

Keywords. Hemipenes; *Macropholidus;* South America; Systematics; Tropical Andes.

INTRODUCTION

Gymnophthalmid lizards in the clade Cercosaurinae Gray, 1838 have radiated along the Northern and Central Andes for the last 60 million years (Torres-Carvajal et al., 2016). More than one third of the known diversity within Cercosaurinae (i.e., 52 of 147 species) has only been discovered during this century, at a rate of ~2.5 species per year. In addition, recent DNA-based phylogenetic studies have provided major insights into the phylogeny of Cercosaurinae along with numerous taxonomic changes including new generic names and new combinations (e.g., Castoe et al., 2004; Doan and Castoe, 2005; Goicoechea et al., 2012; Sánchez-Pacheco et al., 2017; Moravec et al., 2018). In this context, the sister clades *Pholidobolus* Peters, 1863 and *Macropholidus* Noble, 1921, traditionally ranked as genera, were recently redefined. The former was defined as the largest crown clade containing *Pholidobolus montium* (Peters, 1863), but not *Macropholidus ruthveni* Noble, 1921; conversely, *Macropholidus* is the largest crown clade containing *Macropholidus ruthveni,* but not *Pholidobolus montium* (Torres-Carvajal and Mafla-Endara, 2013).

Macropholidus lizards are small (snout–vent length ≤ 60 mm, females larger than males), terrestrial and oviparous (Montanucci, 1973; Cadle and Chuna, 1995; Reeder, 1996). They are restricted to the Andes of southern Ecuador and northern Peru, where a region of relatively low-elevation mountains known as the Huancabamba Depression separates the northern and central Andean cordilleras. *Macropholidus* is currently known to include four species—*M. annectens* Parker, 1930, *M. ataktolepis* Cadle and Chuna, 1995, *M. huancabambae* (Reeder, 1996), *M. ruthveni*—occurring approximately between 470 and 3000 m (Torres-Carvajal et al., 2015). Based on recent collections in northern Peru and southern Ecuador, here we describe a new species of *Macropholidus* using data on morphology and color pattern. We also present molecular evidence by including DNA sequences of the new species in a phylogenetic analysis of Cercosaurinae.

MATERIALS AND METHODS

The taxonomic conclusions of this study are based on the study of external morphological features and color pattern, as well as inferred phylogenetic divergences from DNA sequence data. We interpret this information as species delimitation criteria following the evolutionary species concept (Simpson, 1951, 1961; de Queiroz, 1998, 2007).

How to cite this article: Torres-Carvajal O., Venegas P.J., Nunes P.M.S. 2020. Description and phylogeny of a new species of andean lizard (Gymnophthalmidae: Cercosaurinae) from the Huancabamba depression. *South American Journal of Herpetology* 18: 13–23. http://doi.org/10.2994/SAJH-D-18-00069.1

Submitted: 15 October 2018
Accepted: 10 May 2019
Available Online: 15 September 2020

Handling Editor: Juan Diego Daza
http://doi.org/10.2994/SAJH-D-18-00069.1
ZooBank: urn:lsid:zoobank.org:pub:21B92B67-A93F-4128-9F08-1FE2BE043C94

Morphology

All specimens examined in this paper were deposited in the herpetological collections of Museo de Zoología, Pontificia Universidad Católica de Ecuador, Quito (QCAZ), and Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru. Specimens used for comparisons are listed in Appendix 1. The following measurements were taken with digital calipers and recorded to the nearest 0.1 mm, except for tail length (TL), which was taken with a ruler and recorded to the nearest millimeter: head length (HL), head width (HW), shank length (ShL), axilla-groin distance (AGD), and snout–vent length (SVL). Measurements and counts were summarized as minimum–maximum, $\bar{x}$ ± SD. Sex was determined either by dissection or by noting the presence of everted hemipenes. We follow the terminology of Montanucci (1973) and Reeder (1996) for lepidosis. Morphological data from other species of *Macropholidus* were taken from the literature (Noble, 1921; Montanucci, 1973; Cadle and Chuna, 1995; Reeder, 1996) and by examination of the specimens listed in Appendix 1.

Hemipenes were prepared following the procedures described by Manzani and Abe (1988), modified by Pesantes (1994) and Zaher (1999). The retractor muscle was manually separated and the everted organ was filled with stained petroleum jelly. The hemipenes were immersed in an alcoholic solution of Alizarin Red for 24 h to stain calcified structures (e.g., spines or spicules), in an adaptation of the procedures described by Uzzell (1973) and Harvey and Embert (2008) proposed by Nunes et al. (2012). The terminology for hemipenial structures follows previous literature (Dowling and Savage, 1960; Savage, 1997; Nunes et al., 2012).

DNA sequences and phylogenetic inference

Total genomic DNA was digested and extracted from liver or muscle tissue using a guanidinium isothiocyanate extraction protocol. Using primers and amplification protocols from the literature (Torres-Carvajal et al., 2016), we obtained DNA sequences of mitochondrial genes *12S, 16S,* and *ND4* from 10 individuals of the new species described herein, as well as one individual of *Macropholidus annectens*. GenBank accession numbers are MK844642–MK844674.

We added the 11 sequences generated in this study to the Cercosaurinae dataset of Torres-Carvajal et al. (2016) as expanded by Betancourt et al. (2018). Thus, the data matrix used herein for phylogenetic analyses contained 187 taxa and 1,920 characters. DNA sequences were assembled and aligned in Geneious v9.1.8 (Kearse et al., 2012) under default settings for MAFFT (Katoh and Standley, 2013). Ribosomal (*12S* and *16S*) gene regions with multiple gaps were realigned to minimize indels and optimize nucleotide identities among different individuals. *ND4* and *c-mos* sequences were translated into amino acids for confirmation of alignment. After partitioning the concatenated data matrix by gene and codon position (i.e., eight partitions total), the best partitioning scheme was chosen using PartitionFinder v2.1.1 under the Bayesian Information Criterion (BIC), and the "greedy" algorithm with branch lengths of alternative partitions "linked" to search for the best-fit scheme (Guindon et al., 2010; Lanfear et al., 2012, 2017). A maximum likelihood analysis was run using the selected partitioning scheme in RAxML v8.2.10 (Stamatakis, 2014) under the GTRCAT approximation. Nodal support was assessed with the rapid bootstrapping (BB) algorithm (Stamatakis et al., 2008) on 1,000 replicates. These analyses were performed in the CIPRES Science Gateway (Miller et al., 2010). Phylogenetic trees were rooted with *Alopoglossus viridiceps* (Torres-Carvajal et al., 2016) and visualized and edited using FigTree v1.4.2 (Rambaut, 2014). In addition, we calculated pairwise genetic distances for all mitochondrial genes among species of *Macropholidus* using DIVEIN (Deng et al., 2010).

RESULTS

The new species described here is assigned to *Macropholidus* based on morphological and phylogenetic

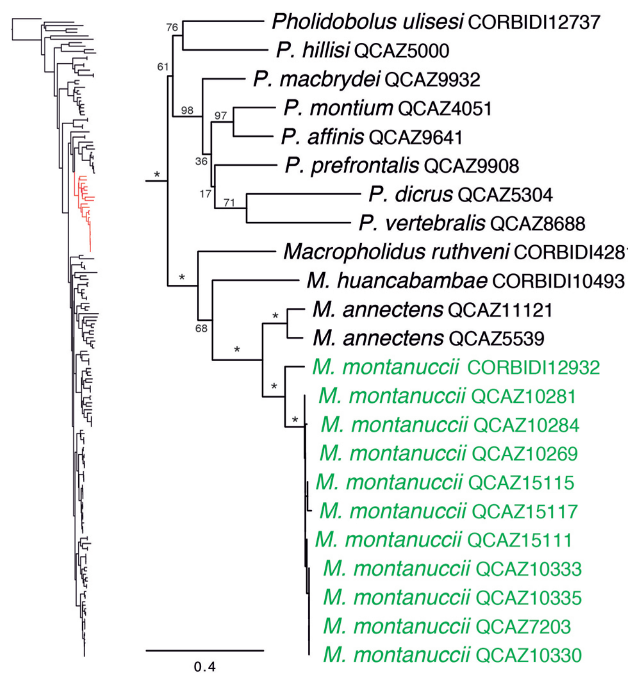

Figure 1. Phylogeny of Cercosaurinae, with a close-up of the clade (*Pholidobolus, Macropholidus*). Maximum likelihood tree obtained under a RAxML analysis of 187 taxa and 1,920 base pairs of mitochondrial and nuclear DNA. Bootstrap support values are indicated next to branches; asterisks represent values ≥ 99.

evidence. It possesses the diagnostic morphological apomorphies of *Macropholidus* (Torres-Carvajal and Mafla-Endara, 2013): presence of a single transparent palpebral disc in the lower eyelid, and absence of a lateral fold between fore and hind limbs. In addition, it is phylogenetically nested within *Macropholidus* with strong support (BB ≥ 99; Fig. 1).

Macropholidus montanuccii sp. nov.

Macropholidus sp. Torres-Carvajal et al., 2016: 70.
Suggested common name in English: Montanucci's Cuilanes.
Suggested common name in Spanish: Cuilanes de Montanucci.

Holotype

CORBIDI 12931 (Figs. 2–4), adult male from Peru, Departamento de Piura, Provincia Ayabaca, Bosque de Cuyas, 4°35′45.9″S, 79°42′46.6″W, WGS84, 2,526 m, collected on 20 May 2013 by P.J. Venegas, L. Echevarría and M. Gulman.

Paratypes (*n* = 67)

ECUADOR: **Provincia Loja:** Guachaurco, 4°1′59.81″S, 79°52′24.6″W, 3,078 m, 21 February 2010, collected by S. Aldás and F. Velásquez, QCAZ 10269 (female); Guachaurco, 4°1′56.96″S, 79°52′29.53″W, 3,001 m, 24 February 2010, collected by S. Aldás and F. Velásquez, QCAZ 10279 (juvenile); Guachaurco, 4°2′6.86″S, 79°52′17″W, 2,999 m, 24 February 2010, collected by S. Aldás and F. Velásquez, QCAZ 10280 (male), 10281–282 (females); Guachaurco, 4°2′14.78″S, 79°52′12.18″W, 2,958 m, 24 February 2010, collected by S. Aldás and F. Velásquez, QCAZ 10284 (male), QCAZ 10285 (female); Guachaurco, 4°2′33.07″S, 79°51′40.36″W, 2,824 m, 25 February 2010, collected by S. Aldás and F. Velásquez, QCAZ 10287, 10290, 10292, 10294–296 (females), 10289, 10291, 10293 (males); Guachaurco, 4°2′32.53″S, 79°51′46.04″W, 2,841 m, 25 February 2010, collected by S. Aldás and F. Velásquez, 10298–10301, 10303–304 (females), 10297, 10302, 10305 (males); Huajala, 4°5′41.28″S, 79°58′36.77″W, 2,116 m, 27 February 2010, collected by S. Aldás, F. Velásquez, and E. Tapia, QCAZ 10318, 10320, 10321, 10323, 10330, 10332, 10335, 10337 (females), 10316,

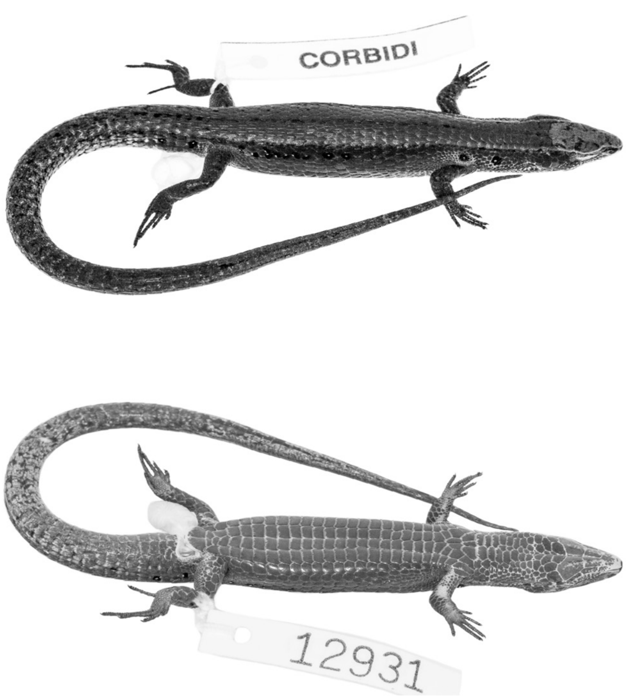

Figure 2. Holotype (CORBIDI 12931; SVL = 53.5 mm) of *Macropholidus montanuccii* **sp. nov.** in dorsal (**top**) and ventral (**bottom**) views. Photographs by D. Quirola.

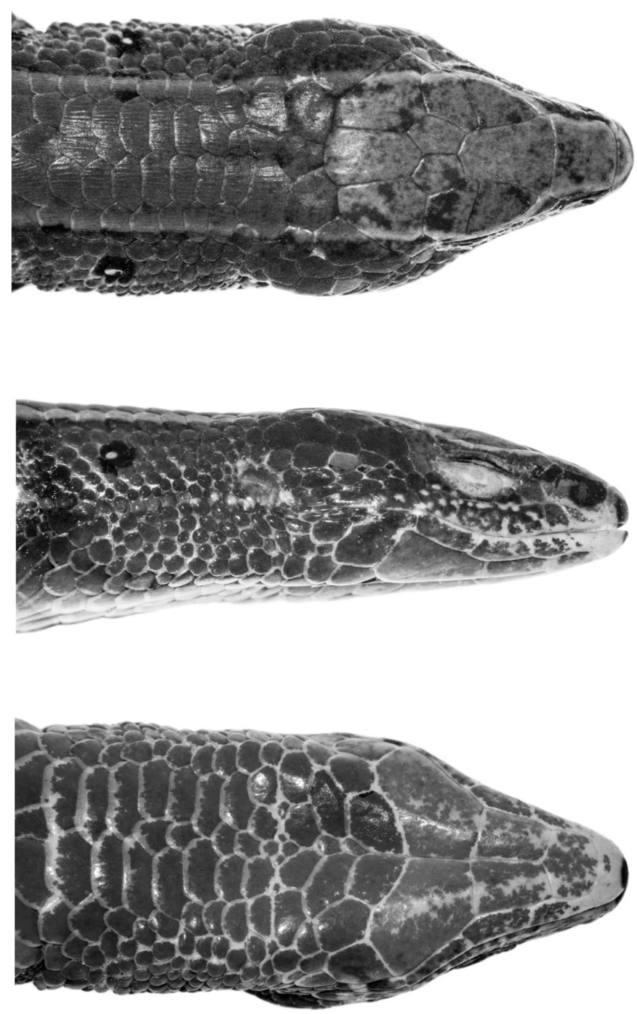

Figure 3. Head of the holotype (CORBIDI 12931) of *Macropholidus montanuccii* **sp. nov.** in dorsal (**top**), lateral (**middle**), and ventral (**bottom**) views. Photographs by D. Quirola. Scale bar = 5 mm.

10322, 10326–328, 10333 (males); Buenavista-Cosanga road, 4°11′27″S, 79°58′37.99″W, 1,947 m, 27 October 2000, collected by David A. Kizirian, QCAZ 10867 (female); Celica, 4°5′56.9″S, 79°57′21.74″W, 30 December 2004, collected by G. Onore, QCAZ 7203 (juvenile); PERU: **Departamento de Piura:** Provincia Ayabaca: Cerro Chacas, near Bosque de Cuyas, 4°36′8.6″S, 79°42′20.1″W, 2,768 m, 8 May 2006, collected by P.J. Venegas and D. Vasquez, CORBIDI 948–49 (adult females), 11164–65 (adult females); Cerro Yantuma, near Bosque de Cuyas, 4°36′10.7″S, 79°42′47.7″W, 2,462 m, 9 May 2006, collected by P.J. Venegas and D. Vasquez, CORBIDI 950 (adult female), 951 (adult male), 952 (adult female), 953–55 (juveniles); Bosque de Cuyas, collected with the holotype, CORBIDI 12932 (adult female), 12934–35 (adult males), 12938 (adult male), 12940 (adult female), 12941 (adult male), 12942–44 (adult females), 12947 (adult female); Cerro Yantuma 4°35′50.2″S, 79°42′14.6″W, 2,982 m, 18 May 2013, collected by P.J. Venegas, L. Echevarria and M. Gulman, CORBIDI 12933 (adult female), 12936–37 (adult males), 12939 (adult male), 12945–46 (adult males).

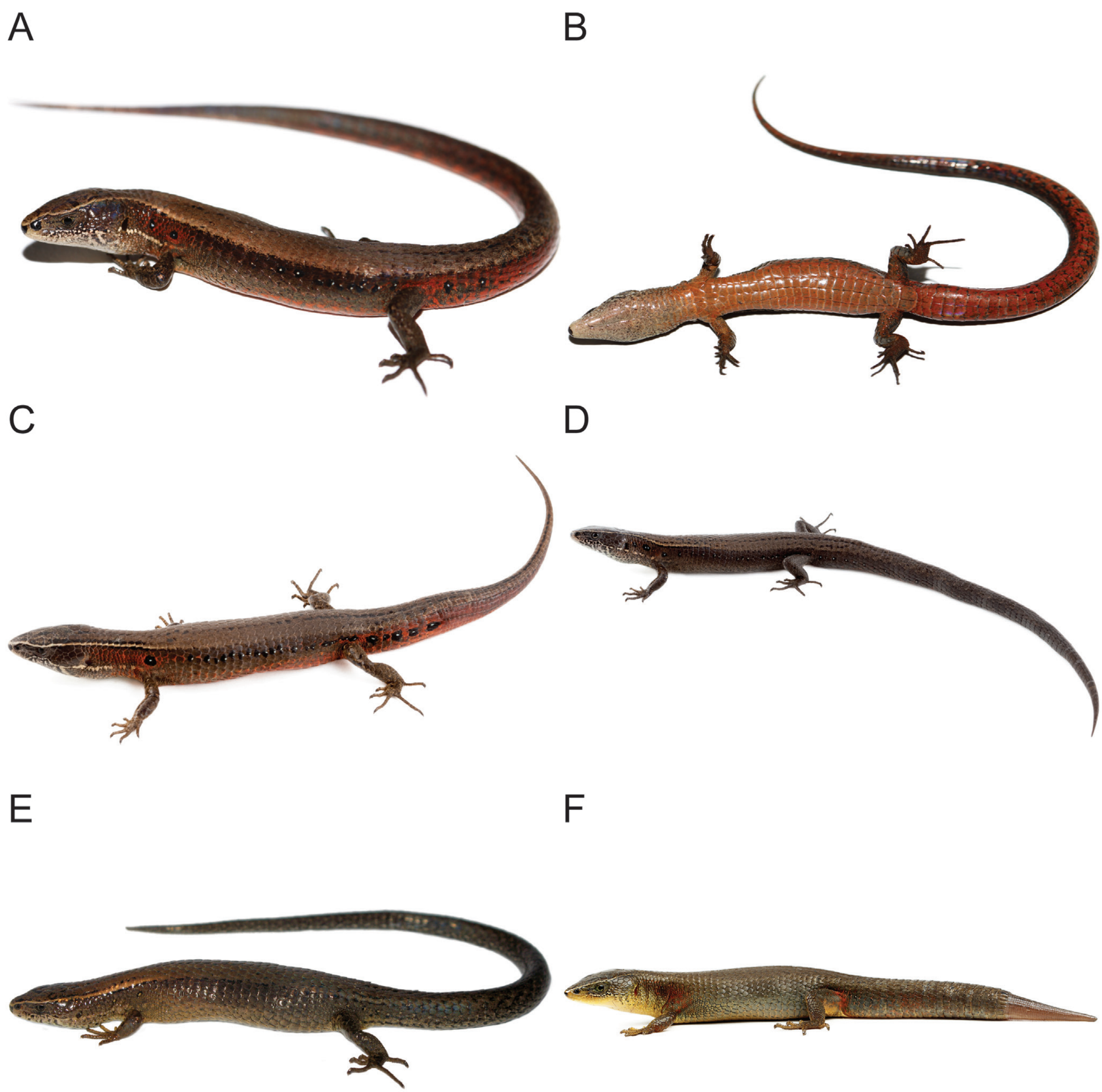

Figure 4. Specimens of *Macropholidus montanuccii* **sp. nov.** (**A–E**) and *M. annectens* (**F**) in life. **A, B:** holotype CORBIDI 12931, SVL = 53.5 mm; **C:** paratype QCAZ 10316, SVL = 52.18; **D:** paratype QCAZ 10326, SVL = 44.94; **E:** paratype QCAZ 10292, SVL = 49.33; **F:** QCAZ 13870, SVL = 48.86.

Diagnosis

Macropholidus montanuccii **sp. nov.** can be distinguished from both *M. ataktolepis* and *M. ruthveni* by having a paired series of enlarged middorsal scale rows restricted to the nape (Figs. 2, 3; series continuous to anterior half of body in *M. ataktolepis,* and continuous to sacral region in *M. ruthveni*). From *M. ataktolepis, M. montanuccii* **sp. nov.** further differs in lacking prefrontal scales and having more (33–42, 36.78 ± 1.80) transverse rows of dorsal scales from occipital scale to posterior margin of hind limbs (29–35, 32.8 ± 1.92 in *M. ataktolepis*). From *M. huancabambae, M. montanuccii* **sp. nov.** differs in having shorter and striated dorsal scales (elongate and strongly keeled in *M. huancabambae*), and more transverse rows of dorsal scales from occipital scale to posterior margin of hind limbs (32–35, 32.2 ± 0.2 in *M. huancabambae*). The new species is more similar in morphology to its sister species *M. annectens* (Fig. 4; character states in parentheses), from which it differs in having fewer transverse rows of dorsal scales from occipital scale to posterior margin of hind limbs (40–48, $\bar{x}$ = 42.6); fewer—21–28, 23.85 ± 1.77—transverse rows of ventral scales between collar fold and preanals (25–30, $\bar{x}$ = 27.3); usually a series of black speckles forming a more or less continuous line on middorsum and onto tail (dorsum usually uniform without speckles forming lines; Fig. 4); irregular dark marks on lower lips (lower lips uniform in color); conspicuous ocelli above fore limbs, on neck, and sometimes along flanks extending onto tail (ocelli absent); distinct white dorsolateral stripe from snout to scapular region (white stripe shorter, from snout to nape or to a level above tympanum); and five or more paired, enlarged middorsal scales behind occiput (1–2).

Characterization

(1) Two supraoculars, anteriormost larger than posterior one; (2) prefrontals absent; (3) femoral pores present in males and sometimes (16%) in females; (4) single transparent palpebral disc in lower eyelid; (5) nape with a paired series of five or more widened middorsals; (6) scales on dorsal surface of neck and dorsum striated (multiple longitudinal ridges on each scale); (7) dorsal scales more or less arranged in transverse rows; (8) 33–42 transverse rows of dorsal scales from occipital scale to posterior margin of hind limbs; (9) rows of lateral granules at midbody absent; (10) lateral body fold absent; (11) dorsum brown with medial black flecks sometimes forming continuous or fragmented vertebral stripe; (12) one ocellus above insertion of fore limb followed anteriorly by 1–2 ocelli on neck (more conspicuous in adult males); (13) 0–15 ocelli longitudinally arranged on flanks; (14) 0–12 ocelli longitudinally arranged on anterior half of tail; (15) lips with irregular black flecks and dots; (16) dark stripe (solid or irregular) extending from posteriormost infralabial onto lateralmost pregular usually present; (17) distinct white dorsolateral stripe from snout to scapular region; (18) hemipenis bilobate with two apical folds and a series of spinulate flounces along body; (19) maximum SVL 57.32 mm in males, 55.83 mm in females.

Description of holotype

Adult male (CORBIDI 12931; Figs. 2–4); SVL 53.5 mm; TL 110 mm; dorsal and lateral head scales juxtaposed, smooth; rostral hexagonal, 1.86 times as wide as high; frontonasal roughly quadrangular, slightly wider than long, smaller than frontal, laterally in contact with nasal, loreal and anteriormost superciliary; prefrontals absent; frontal pentagonal, longer than wide, wider anteriorly, in contact with the first (anteriormost) superciliary, and first and second supraoculars on each side; frontoparietals pentagonal, longer than wide, separated from each other by complete medial suture, each in contact with second supraocular and parietal laterally, frontal anteriorly and interparietal posteriorly; interparietal roughly hexagonal, lateral margins parallel to each other; parietals slightly larger than interparietal, irregularly hexagonal and positioned anterolaterally to interparietal, each in contact laterally with one postocular and two supratemporals; postparietals three, medial one smaller than lateral ones; supralabials seven, fourth one longest and below center of eye; infralabials five, suture between third and fourth below center of eye; temporals enlarged, irregularly pentagonal, juxtaposed, smooth; supratemporal scales two, large, smooth; nasal divided, irregularly quadrangular, longer than high, in contact with rostral anteriorly, first and second supralabials ventrally, frontonasal dorsally and loreal posteriorly; nostril on ventral aspect of nasal, directed laterally, piercing nasal suture; loreal irregularly quadrangular; frenocular pentagonal, similar in size to loreal and separating it from supralabials; supraoculars two, anteriormost the largest; superciliaries three, elongate, first one in contact with loreal and frenocular; palpebral disk single, unpigmented; suboculars four, the anteriormost shorter than the others; postoculars three, medial one smaller than the others; ear opening anteroventrally oval, without denticulate margins; tympanum slightly recessed into auditory meatus; mental semicircular, wider than long; postmental roughly pentagonal, slightly wider than long; genials in two pairs in contact medially, contacting infralabials, second pair separated from gulars by six juxtaposed pregulars; gulars imbricate, smooth, widened in two longitudinal rows; gular fold incomplete; posterior row of gulars (collar) with two widened scales.

Scales on nape wider than dorsals on body, with an irregular longitudinal series of two widened middorsals; scales on sides of neck small and granular; dorsal scales striated, imbricate, more or less arranged in transverse rows, similar in size to scales on flanks; scales on dorsal surface of neck and body striated; dorsal scales between occipital and posterior margin of hind limbs 36; dorsal scale rows in a transverse line at midbody 23; one row of smooth, enlarged ventrolateral scales on each side; lateral fold on body flanks absent; ventrals smooth, wider than long, arranged in 22 transverse rows between collar fold and preanals; ventral scales in a transverse row at midbody seven; subcaudals smooth; limbs overlapping when adpressed against body; axillary region with granular scales; scales on dorsal surface of fore limb smooth, imbricate; scales on ventral surface of forearm granular, those on arm imbricate; manual subdigital lamellae single or divided, 14 on Finger IV; groin region with small, imbricate scales; hind limbs with striated and imbricate scales dorsally and smooth scales ventrally; scales on posterior surface of thighs granular; femoral pores two on each leg; pedal subdigital lamellae divided, 20 pairs on Toe IV; preanal pores absent; cloacal plate paired, bordered by four scales anteriorly, of which the two medialmost are enlarged. Additional measurements (mm) and proportions: HL 11.48; HW 8.75; ShL 5.19; AGD 28.35; TL/SVL 2.05; HL/SVL 0.21; HW/SVL 0.16; ShL/SVL 0.09; AGD/SVL 0.52.

Hemipenial morphology of holotype (Fig. 5)

Fully everted and expanded left hemipenis: 8 mm long; bilobate; hemipenial body roughly conical in shape, thinner at base, ending distally in two small lobes (almost fully everted) with apical folds. *Sulcus spermaticus* narrow and shallow, broader proximally, originating medially at base of organ and extending in a straight line to lobular crotch, where it gets divided by a small fleshy fold into two branches that run medially on each lobe and end apically among folds; area on sulcate face of body between *sulcus spermaticus* and flounces nude.

Lateral and asulcate aspects of hemipenis ornamented with 25 approximately equidistant flounces (most extending around lateralmost aspect of sulcate side) bearing rows of calcareous spicules and extending along body; except for first proximal five, flounces separated medially by nude area on asulcate side; first and second proximal flounces restricted to asulcate side; first four proximal flounces nearly horizontal, fifth one chevron-shaped; except for first proximal seven, flounces continuous and chevron-shaped (apices directed towards base of organ) on lateral aspects of hemipenial body, progressively shorter towards lobes.

Color of holotype in life (Fig. 4)

Dorsal background from head to base of tail brown, with a darker tone along the rest of the tail; sides of head dark brown, nostril surrounded by a bold black ring; anterior margin of rostral and mental black, giving the appearance of black-painted lips; supralabials and infralabials dark brown with cream flecks; dark irregular stripe extending from posteriormost infralabial onto lateralmost pregular; pale dorsolateral stripe extending from tip of snout to scapular region; sides of neck, flanks and limbs dark brown; cream narrow stripe extending from tympanum to arm insertion; distinct ocelli (black with white center) present, one above insertion of fore limb, another one on neck, nine on posterior half of flanks, and two at base of tail; ventrolateral region of body orange; throat greyish brown with dark brown flecks; chest, belly and base of tail dark orange; iris pale brown.

Variation

Variation in measurements and scale counts of *Macropholidus montanuccii* **sp. nov.** is presented in Table 1. Generally three superciliaries on both sides (79%), sometimes four on one side (16%) and less frequently four on both sides (5%); usually three postoculars on both sides (96%), rarely two on one (2.9%) or both (1.5%) sides; postparietals three in most specimens (97%), rarely (1.5%) four or five; usually seven supralabials on both sides (69%), sometimes six on one side (18%), less frequently six on both sides (7.3%), or eight on one side (5.9%); generally five infralabials on both sides (66%), rarely six on one side (8.8%), six on both sides (10.3%), seven on one side (1.5%) or seven on both (2.9%); usually four anterior and four posterior (4/4) cloacal plates (46%), sometimes 4/2 (38%), and rarely 2/2 (8.8%), 2/3 (1.5%), 3/2 (2.9%), 4/3 (2.9%); loreal scale very rarely (1.5%) absent on one or both sides. Males are slightly larger than females (maximum SVL in mm 57.32 and 55.83, respectively), and always have femoral pores (only present in 16% of examined females). A distinct ocellus above fore limbs and 1–2 ocelli on neck are present in all specimens. Tail ocelli vary between 3–12, whereas flank ocelli vary from none to 13. Ocelli are generally more conspicuous in adult males than in females and juveniles.

The hemipenis of paratype QCAZ 10326 (Fig. 5) only differs from the hemipenis of the holotype in having the apical folds fully everted into pleats, and 22 spinulate flounces, of which the first proximal four are continuous medially and the first is restricted to the asulcate side of the organ.

Phylogenetic relationships and genetic variation

The maximum likelihood phylogram of Cercosaurinae strongly supports a clade containing *Pholidobolus* and *Macropholidus* as sister taxa (Fig. 1). The new species is monophyletic with maximum support and deeply nested within *Macropholidus* as sister to *M. annectens*. Pairwise

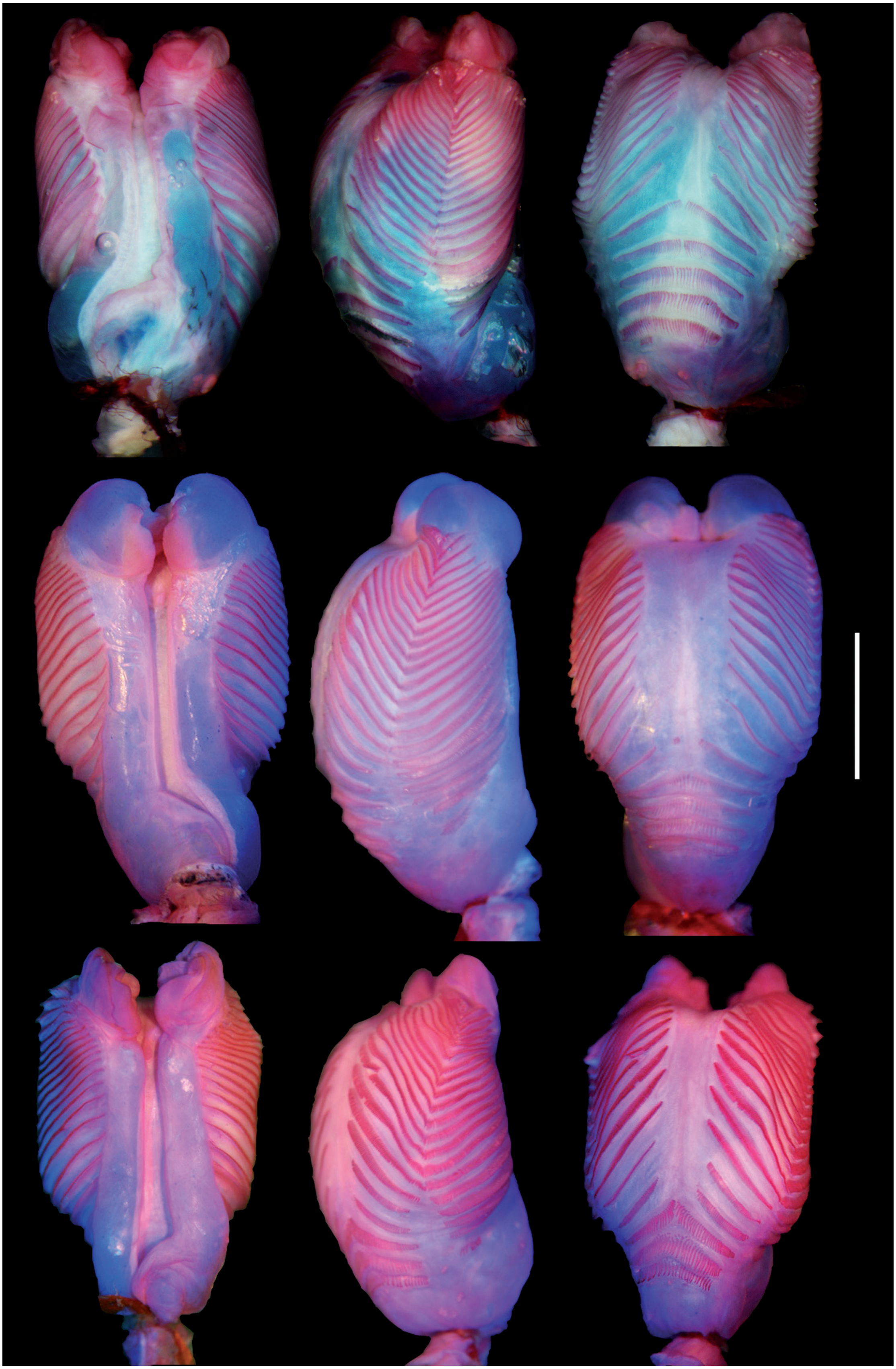

Figure 5. Hemipenes of *Macropholidus* in sulcate (**left**), lateral (**middle**), and asulcate (**right**) views. **Top:** *M. montanuccii*, holotype (CORBIDI 12931); center: *M. montanuccii* **sp. nov.,** paratype (QCAZ 10326); **bottom:** *M. annectens* (QCAZ 13870). Scale = 3 mm. Photographs by P. Nunes.

Table 1. Variation in lepidosis and measurements of *Macropholidus montanuccii* **sp. nov.** Range followed by $\bar{x}$ ± SD are given for adult males, adult females and all specimens including juveniles of undetermined sex. Femoral pores where counted on both legs (i.e., *n* corresponds to number of legs). Sample size is given in parentheses if different from headings.

Character	Males (*n* = 25)	Females (*n* = 37)	All (*n* = 68)
Dorsal scales between occipital and posterior margin of hind limb	33–39 36.04 ± 1.43	34–42 37.32 ± 1.99	33–42 36.78 ± 1.80
Dorsal scale rows in a transverse line at midbody	15–19 16.92 ± 0.97 (*n* = 24)	14–19 16.95 ± 1.25	14–19 16.84 ± 2.34 (*n* = 67)
Ventral scales between collar fold and preanals	21–27 23.56 ± 1.78	21–28 24.08 ± 1.72	21–28 23.85 ± 1.77
Ventral scale rows in a transverse line at midbody	6–7 6.21 ± 1.31 (*n* = 24)	6–8 6.11 ± 0.39	6–9 6.21 ± 0.92 (*n* = 67)
Subdigital lamellae on Finger IV	10–17 13.88 ± 1.56	12–15 13.89 ± 0.88	10–17 13.88 ± 1.18
Subdigital lamellae on Toe IV	17–22 18.80 ± 1.29	15–29 18.89 ± 2.33	15–29 18.85 ± 1.95
Femoral pores	1–5 3.44 ± 1.08 (*n* = 36)	0–4 0.52 ± 1.13 (*n* = 66)	—
Maximum SVL in mm	57.32	55.83	—
TL/SVL	1.19–2.45 1.70 ± 0.39 (*n* = 10)	1.04–2.35 1.80 ± 0.44 (*n* = 19)	—

genetic distances between *M. annectens* and *M. montanuccii* **sp. nov.** are 0.01–0.02 for *12S,* 0.02–0.04 for *16S,* and 0.10–0.12 for *ND4,* which correspond to the lowest distances among species of *Macropholidus* sampled to date (Fig. 6).

Distribution and natural history

Macropholidus montanuccii **sp. nov.** is known from the highlands and Pacific slopes of the Andes in southern Ecuador and northern Peru (Fig. 7). It occurs at elevations between 1,947 and 3,078 m in the province of Loja in Ecuador and the department of Piura in Peru. The type locality lies within Cordillera de Huancabamba in northwestern Peru and corresponds to the Eastern Cordillera Real Montane Forest (Olson et al., 2001); it forms part of a patch of cloud forest of approximately 1,200 ha on the western slope of Cerro Chacas. During our field surveys in the type locality and nearby areas, *M. montanuccii* **sp. nov.** was abundant between 8:00 h and 11:00 h, both under sunny conditions and partially clouded sky with sun intervals. All individuals of *M. montanuccii* **sp. nov.** were collected active by day, foraging on the leaf litter and between the herbaceous vegetation close to the edge of trails and along road cuts. When individuals of this species were disturbed, they took refuge in the herbaceous vegetation, shrubs, and under fallen trunks or rocks. Some individuals were found inactive under fallen trunks or rocks in cloudy or rainy days.

Four females collected on February 2010 in southern Ecuador laid two eggs each, which ranged between 10.59–12.41 mm in length and 6.02–6.66 mm in width. Two gravid females collected in May 2006 in Peru contained one egg on each oviduct ranging between 4.26–6.34 mm in length and 2.85–4.25 mm in width.

Other sympatric squamates collected with *Macropholidus montanuccii* **sp. nov.** were *Dipsas jamespetersi, D. oreas, Erythrolamprus albiventris, Atractus carrioni, Mastigodryas heathii, Andinosaura vespertina, Stenocercus carrioni, S. humeralis, S. limitaris,* and *S. ornatus.*

Etymology

The specific epithet is a noun in the genitive case and is a patronym for Richard R. Montanucci, who published a seminal work on the systematics of *Pholidobolus* lizards in the early 1970's (Montanucci, 1973) after intensive work along the Andes of Ecuador. Richard Montanucci has dedicated his life to the study of lizards. His work on *Pholidobolus* lizards is of great importance for anyone interested in gymnophthalmid lizards from the Tropical Andes.

DISCUSSION

The hemipenes of *Macropholidus* and *Pholidobolus*

The hemipenial morphology of species of *Macropholidus* and *Pholidobolus* has been described for most species (*M. annectens, M. ruthveni, M. montanuccii* **sp. nov.,** *P. affinis, P. hillisi, P. macbrideyi, P. prefrontalis* and *P. ulisesi*) and

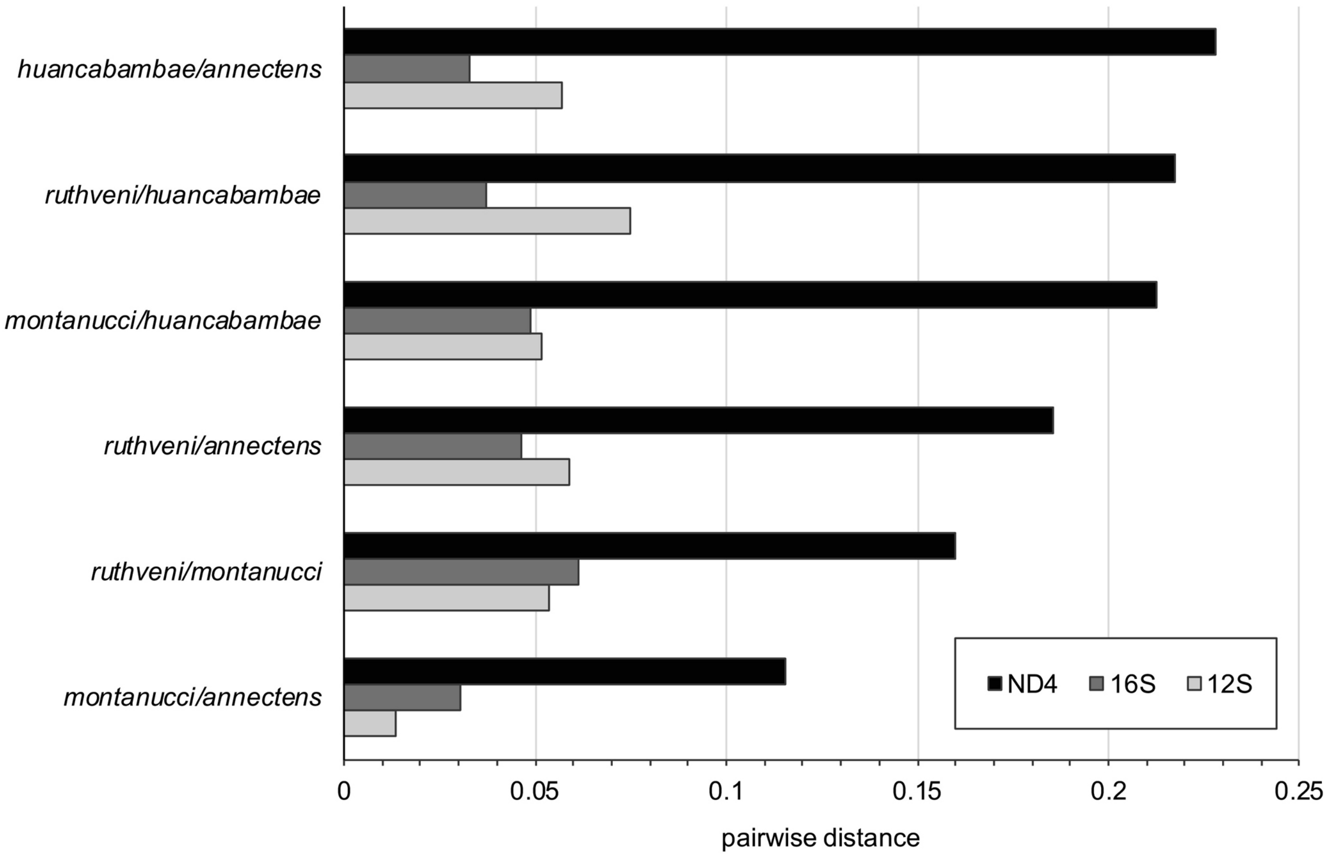

Figure 6. Histogram illustrating mean pairwise genetic distances among species of *Macropholidus* for three mitochondrial gene fragments.

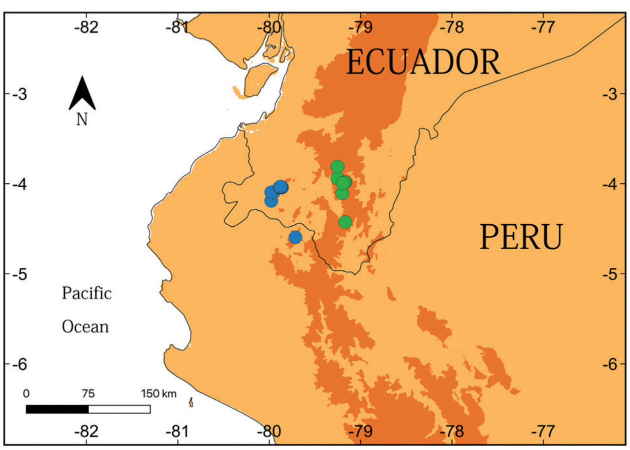

Figure 7. Distribution of *Macropholidus annectens* (green) and *M. montanuccii* **sp. nov.** (blue) in South America. Areas > 2000 m are in darker color.

is overall conservative. All species share a conical hemipenial body, laterally and basally (asulcate face) ornamented with flounces bearing calcified spicules. There are only slight differences among species. For example, the flounces on the asulcate side vary from nearly transverse (e.g., *P. hillisi;* Torres-Carvajal et al., 2014) to chevron-shaped (Fig. 5) with only the basalmost flounces being transverse (Nunes, 2011; Venegas et al., 2016). Other slight differences in body and lobular shape are usually difficult to attribute to specific or individual differences, because the sample available for each taxon is frequently small and subject to artefacts during preparation. Nonetheless, all species of *Macropholidus* and *Pholidobolus* examined to date share a bulge marking the margins of the asulcate face (Nunes, 2011; Torres-Carvajal et al., 2014; Venegas et al., 2016, Parra et al., 2020), which we propose as a putative synapomorphy of the clade *Macropholidus* + *Pholidobolus* as it has not been described in other species of Cercosaurine lizards. The conservative hemipenial morphology of this clade contrasts with the variation reported in some clades of gymnophthalmid lizards, in which hemipenes bear diagnostic characters at the species level (e.g., Nunes et al., 2012; Rodrigues et al., 2017; Kok et al., 2018). Only a more comprehensive examination of the hemipenes within the Cercosaurinae will shed some light on their evolution.

Biogeography of *Macropholidus*

The Huancabamba Depression or Huancabamba Deflection has long been recognized as a major biogeographic barrier for some Andean organisms (Vuilleumier, 1969; Duellman, 1979; Cadle, 1991), as well as a migration corridor for others (Quintana et al., 2017). This region lies along the Andes of southern Ecuador and northern Peru approximately between 4°S–7°S and consists of relatively low-elevation mountains that create a mixture of environments (Fig. 7). The Huancabamba Depression seems to have influenced the radiation of several Andean lizard clades, such as *Stenocercus* Duméril and Bibron, 1837, *Riama* Gray, 1858, *Macropholidus* and *Pholidobolus* (Doan, 2003; Torres-Carvajal, 2007; Torres-Carvajal and Mafla-Endara, 2013), of which *Macropholidus* is the only radiation restricted to this region. It is thought that the Huancabamba area was at sea level by the Eocene, when other parts of the Andes were already uplifted, and it was not until the Middle Miocene that the Andes had emerged to form the Huancabamba Depression (Quintana et al., 2017). In a recent large scale phylogenetic and biogeographic analysis of the Cercosaurinae, the age of *Macropholidus* was estimated at 15.60 (12.36–18.90) mya, which roughly corresponds to the Middle Miocene (Torres-Carvajal et al., 2016). Thus, it is reasonable to think that the rise of the Andes along the Huancabamba Depression had a great impact on the evolution and diversification of *Macropholidus* lizards. This suggests that in addition to a biogeographic barrier and a migration corridor, the Huancabamba Depression has acted as a center of origin and diversification.

ACKNOWLEDGMENTS

This research was financially supported by the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) under the ['Arca de Noé'Initiative] (PIs: S.R. Ron and O. Torres-Carvajal). PMSN is grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support (Fellowship #313622/2018-3). PJV field work was made possible by Nature & Culture International (NCI) in Peru. PJV is indebted to his field companions Diego Vasquez, Lourdes Echevarria and Michael Gulman. Specimens were collected under collection permits 008-09-IC-FAU-DNB/MA, 001-10-IC-FAU-DNB/MA, 005-14-IC-FAU-DNB/MA, 003-15-IC-FAU-DNB/MA, 002-16-IC-FAU-DNB/MA issued by Ministerio del Ambiente del Ecuador, and 110-2007-INRENA-IFFS-DCB, 118-2007-INRENA-IFFS-DCB and 0121-2012-AG-DGFFS-DGEFFS issued by INRENA and Dirección Forestal y Fauna Silvestre in Peru.

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APPENDIX

Additional specimens examined

Macropholidus annectens (*n* = 8).—ECUADOR: **Loja:** 12.8 km S Yangana on Loja-Zumba road, 4°25′38″S, 79°10′4.98″W, 2392 m, QCAZ 5539; 7.5 km Loja-Zamora, 3°59′3″S, 79°10′4.98″W, 2,502 m, QCAZ 5528; Loja, exit to Cuenca, 3°56′22.5″S, 79°15′11.2″W, 2,340 m, QCAZ 6019; La Esperanza, Reinaldo Espinoza Botanical Garden, 4°6′22″S, 79°11′56.98″W, 2,177 m, QCAZ 6126; 15 km Loja-Zamora, 3°59′9.72″S, 79°10′22.98″W, 2,355 m, QCAZ 11120, 11121; 5 km from San Lucas on old road to San Lucas, 3°48′40.68″S, 79°15′9.47″W, 2,064 m, QCAZ 11127; Loja, near Howard Johnson hotel, 3°59′38.22″S, 79°11′44.84″W, 2,115 m, QCAZ 13870.