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Systematics of *Pholidobolus* from southern Ecuador, with description of three new species (Squamata, Gymnophthalmidae)

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ROMMY VANESSA PARRA SOTALÍN

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A mis padres,

por enseñarme que se puede tener raíces y a la vez alas.

Systematics of *Pholidobolus* from southern Ecuador, with description of three new species (Squamata, Gymnophthalmidae)

Vanessa Parra S. (vaneparra2510@hotmail.es). Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca. Quito, Ecuador.

Omar Torres-Carvajal. (omartorcar@gmail.com). Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca. Quito, Ecuador.

Abstract

We describe three new species of *Pholidobolus* from southern Ecuador based on morphological and genetic evidence. We infer the phylogenetic position using DNA sequences of mitochondrial and nuclear genes. The phylogeny shows that the new species, *Pholidobolus samek* **sp. n.**, *P. elquimi* **sp. n.** and *P. oni* **sp. n.**, are nested within the "*P. macbrydei"* clade. *Pholidobolus samek* and *P. elquimi* differ from their congeners by having bright green dorsolateral stripes on the head; whereas *P. oni* in having small scales between the posterior corner of the orbit and the anterior edge of the external auditory meatus. *Pholidobolus samek* and *P. elquimi* occur in Cordillera del Cóndor, a region with few lizard collections. Our results allowed us to confirm that *P. macbrydei,* as currently defined, is a species complex that includes three new species described herein and four candidate species. These discoveries reveal high levels of diversity within *Pholidobolus* in southern Ecuador.

Keywords: Andes, Diversity, Ecuador, Phylogeny, *Pholidobolus elquimi* **sp. n.**, *"Pholidobolus macbrydei"*, *Pholidobolus oni* **sp. n.**, *Pholidobolus samek* **sp. n.**

Introduction

The uplift of the Andes Mountains was one of the most important geological events for countries like Ecuador, Colombia and Peru. It created habitats and microclimates that became important centers of biodiversity and species endemism (Pérez-Escobar et al. 2017). Therefore, the study of the evolutionary relationships of diverse Andean taxa, such as gymnophthalmid lizards, is an interesting research focus (Torres-Carvajal et al. 2016). With 253 species, Gymnophthalmidae is one of the most diverse lizard in the Neotropics. The uplift of the Andes facilitated the radiation of this clade, which resulted in high endemism levels (Torres-Carvajal et al. 2016).

Ecuador hosts the largest number of species of the gymnophthalmid lizard genus *Pholidobolus* as currently defined (Torres-Carvajal and Mafla-Endara 2013). Recently, three species of *Pholidobolus* were described, based on morphological and phylogenetic

evidence (Hurtado-Gómez et al. 2018; Torres-Carvajal et al. 2014; Venegas et al. 2016). Thus, ten species of *Pholidobolus* are currently recognized: *P. affinis, P. anomalus, P. dicrus, P. hillisi, P. macbrydei, P. montium, P. paramuno, P. prefrontalis, P. ulisesi* and *P. vertebralis.* They occur between 1800 and 4100 m in the southern portion of the northern Andes between the southern Andes of Colombia and the Huancabamba Depression in northern Peru (Hurtado-Gómez et al. 2018; Torres-Carvajal et al. 2014; Venegas et al. 2016), except for *P. anomalus* Müller 1923 from Cusco (southern Peru). Nonetheless, the placement of *P. anomalus* within *Pholidobolus* has not been tested phylogenetically due to the lack of tissue samples.

The study of *Pholidobolus* and other gymnophthalmid genera has been oftenly hampered by the paucity of specimens in collections. The recent description of *P. paramuno* from Colombia reveals the importance of increased sampling effort. Recent collections in poorly explored areas of the southern Andes of Ecuador yielded new specimens, which we were unable to assign to any of the currently recognized species. Based on these specimens, in this paper, we combine evidence from morphology and DNA sequences data to describe three new species of *Pholidobolus*, and infer their phylogenetic affinities to other species of *Pholidobolus.*

Materials and Methods

Specimens and Morphological Data

We examined 23 specimens, 11 specimens from "*Pholidobolus macbrydei"* and 12 from unidentified species. All type specimens and additional specimens examined (Appendix I) were deposited in the herpetological collections at Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ). The six following measurements were taken with a digital caliper (to the nearest 0.01 mm), except for tail length, which was taken with a ruler: head length (HL), head width (HW), shank length (ShL), axilla-groin distance (AGD), snout-vent length (SVL), and tail length (TL). Sex was determined by dissection or by noting the presence of everted hemipenes. We followed the terminology of Montanucci (1973) and Kizirian (1996) for description of the holotype and scale counts.

Because the new species are similar in morphology to *Pholidobolus macbrydei*, we assessed the degree of differentiation among these species with a Principal Components Analysis (PCA) in JMP 9 (SAS Institute 2010). The PCA was based on 15 quantitative morphological characters: (1) number of supraoculars (NSO), (2) number of scales along margin of upper jaw (SUJ), (3) number of scales along margin of lower jaw (SLJ), (4) number

of gular and jaw scales (SGJ), (5) number of ventrals (SGV), (6) number of dorsals (DEL), (7) number of temporals (NTS), (8) number of scales around the body (SAB), (9) number of scales around the tail (SAT), (10) number of supradigital scales of the third finger (SF3), (11) number of supradigital scales of the fifth finger (SF5), (12) number of supradigital scales of the third toe (ST3), (13) number of supradigital scales of the fourth toe (ST4), (14) number of supradigital scales of the fifth toe (ST5), and (15) lower eyelid scales (LES) (Montanucci 1973).

Genetic Data

We analyzed 58 sequences from *Anadia, Pholidobolus* and *Macropholidus* genus. Total genomic DNA was digested and extracted from liver or muscle tissue using a guanidinium isothiocyanate extraction protocol. Tissue samples were first mixed with Proteinase K and a lysis buffer and digested overnight prior to extraction. DNA samples were quantified using a Nanodrop® ND-1000 (NanoDrop Technologies, Inc), re-suspended and diluted to 25 ng/µl in ddH2O prior to amplification.

Using primers and amplification protocols from the literature (Pellegrino et al. 2001; Torres-Carvajal and Mafla-Endara 2013), we obtained 1,492 nucleotides (nt) encompassing three mitochondrial genes, 12S (338 nt), 16S (533 nt), and ND4 (621 nt) from 12 unidentified individuals and 9 individuals of *Pholidobolus macbrydei* from several localities. Mitochondrial ribosomal genes 12S and 16S were amplified using the primers 12Sa, 12Sb, 16SL, 16SH, 16SF.0, and 16SR.0, whereas the ND4 gene was amplified with the primers ND4F and ND4R (Pellegrino et al. 2001; Torres-Carvajal and Mafla-Endara 2013). The amplification protocol consisted of 1 cycle of initial denaturation for 3 min at 96 °C, 40–45 cycles of denaturation for 30 s at 95 °C, annealing for 1 min at 52 °C, and extension for 1 min at 72 °C, as well as a final extension for 10 min at 72 °C (Torres-Carvajal et al. 2014). Furthermore, we obtained 411 nucleotides of the Dynein Axonemal Heavy Chain 3 (DNAH3) nuclear gene from 49 individuals of *Anadia rhombifera, Macropholidus annectens, M. huancabambae, M. labiopunctatus, M. ruthveni, Pholidobolus affinis, P. dicrus, P. hillisi, P. macbrydei P. montium, P. prefrontalis, P. ulisesi, P. vertebralis* and the three new species. DNAH3 was amplified using the primers DNAH3_f1 (GGTAAAATGATAGAAGAYYTACTG) and DNAH3_r6 (CTKGAGTTRGAHACAATKATGCCAT). The amplification protocol consisted of 1 cycle of initial denaturation for 5 min at 95 °C, 40 cycles of denaturation for 35 s at 94 °C, annealing for 1 min at 72 °C, and extension for 1 min at 72 °C, as well as a final extension for 10 min at 72 °C (Townsend et al. 2008). Positive PCR products were visualized in agarose electrophoretic gels and treated with ExoSAP-it to remove unincorporated

primers and dNTPs. Cycle sequencing reactions were carried out by Macrogen Inc. Genbank accession numbers of sequences generated in this study and localities are shown in Table 1.

Additionally, we incorporated 37 sequences of *Pholidobolus, Macropholidus* and *Anadia rhombifera* (outgroup) species from the GenBank published by Torres-Carvajal et al. (2015) and Hurtado-gómez et al. (2018).

Phylogenetic analyses

Data were assembled and aligned in Geneious v5.4.6. (Kearse et al. 2012) under default settings for MAFFT Multiple Alignment (Katoh and Toh 2010). ND4 and DNAH3 sequences were translated into amino acids for confirmation of alignment. The best-fit nucleotide substitution models and partitioning scheme were chosen simultaneously using PartitionFinder v2.1.1 (Lanfear et al. 2012) under the Bayesian Information Criterion (BIC). Genes were combined into a single dataset with six partitions: (i) 1st codon position of ND4 [HKY + I + G]; (ii) 2nd codon positions of ND4 and DNAH3 [HKY + I]; (iii) 3rd codon position of ND4 [GTR + G]; (iv) 12S and 16S [GTR + I + G]; (v) 1st codon position of DNAH3 [K80 + I]; and (vi) 3rd codon position of DNAH3 [K80 + G]. Also, a single nuclear gene phylogeny was inferred with two partitions: (i) 1st codon and 2nd codon positions of DNAH3 [K80 + I]; and (ii) 3rd codon position of DNAH3 [K80 + I]. Both maximum likelihood (ML) and Bayesian inference (BI) methods were used to obtain the optimal tree topology of the combined, partitioned dataset using the programs GARLI v.2.0 (Zwickl 2006) and MrBayes v3.2.6 (Ronquist et al. 2012), respectively. The ML analysis was conducted using GARLI 2.0 with default settings. Analysis were terminated after 10 000 generations without an improvement in tree topology. We ran a total of 20 independent searches and used random starting addition (streefname = random) to reduce the probability of inferring a suboptimal likelihood solution. Support was evaluated using 100 bootstrap replicates, with each replicate terminated after 5000 replications without improvement in topology. For BI analysis, all parameters were unlinked between partitions (except topology and branch lengths), and rate variation (prset ratepr = variable) was invoked. Four independent runs, each with four MCMC chains, were run for ten million generations, sampling every 1,000 generations. Results were analyzed in Tracer 1.6 (Rambaut and Drummond 2007) to assess convergence and effective sample sizes (ESS) for all parameters. The remaining trees were used to calculate posterior probabilities (PP) for each bipartition in a 50% majority-rule consensus tree. The phylogenetic tree was visualized and edited using FigTree v1.4.2 (Rambaut 2014).

Uncorrected genetic distances were assessed in MEGA 7 (Kumar et al. 2016).

Systematics

The taxonomic conclusions of this study are based on the observation of morphological features and color pattern, as well as inferred phylogenetic relationships. We consider this information as species delimitation criteria following a general lineage or unified species concept (de Queiroz 1998; 2007).

The new species share with all other species of *Pholidobolus* the presence of a ventrolateral fold between fore and hind limbs and the absence of a single transparent palpebral disc (Montanucci 1973).

Results

Pholidobolus samek sp. n.

Proposed standard English name: Green striped Cuilán
Proposed standard Spanish name: Cuilán de franjas verdes

Holotype: QCAZ 14955 (Figs 1, 2), adult male, Ecuador, Provincia Zamora-Chinchipe, Cerro Plateado Biological Reserve, plateau Cerro Plateado, 4.6159S, 78.7870W, WGS84, 2844 m, 23 September 2016, collected by Diego Almeida, Eloy Nusirquia, Fernando Ayala, Javier Pinto, Alex Achig and Malki Bustos.

Paratypes (2): ECUADOR: Provincia Zamora-Chinchipe: QCAZ 14954 (adult female), same data as holotype; QCAZ 14956 (adult female), Cerro Plateado Biological Reserve, 4.6159S, 78.7870W, WGS84, 2320 m, 28 September 2016, same collectors as holotype.

Diagnosis

The new species is unique among species of *Pholidobolus* in having bright green dorsolateral stripes on the head, living on sandstone plateaus of Zamora-Chinchipe. In addition, *P. affinis*, *P. prefrontalis*, *P. macbrydei, P. oni* sp. n. and *P. montium* differ from *P. samek* sp. n. (character states in parentheses), in having a loreal scale frequently in contact with the supralabials (loreal scale not in contact with supralabials), and dorsal scales finely wrinkled. *Pholidobolus ulisesi* and *P. hillisi* differ from *P. samek* sp. n. in having a diagonal white bar along the rictal region (white rictal bar absent). *Pholidobolus samek* sp. n. can be distinguished from *P. dicrus* by lacking a bifurcating vertebral stripe at midbody. *Pholidobolus affinis*, *P. prefrontalis*, *P. dicrus, P. hillisi and P. vertebralis* differ from *P. samek* sp. n. in having well-defined prefrontal scales (if present, prefrontal scales poorly differentiated). Additionally, the new species has fewer dorsal scales (27−28 $\bar{x}$ = 27.7) than

P. affinis (45−55), *P. montium* (35−50), *P. prefrontalis* (37−46), and *P. macbrydei* (31−43), and *P. oni* sp. n. (35−40, $\bar{x}$ = 36.8).

Characterization: (1) Two (rarely three) supraoculars, anteriormost larger than posterior one; (2) prefrontals present or absent; (3) femoral pores absent in both sexes; (4) four to five opaque lower eyelid scales; (5) scales on dorsal surface of neck striated, becoming slightly keeled from forelimbs to tail; (6) two or three rows of lateral granules at midbody; (7) 27-28 dorsal scales between occipital and posterior margin of hindlimb; (8) lateral body fold present; (9) keeled ventrolateral scales on each side absent; (10) dorsum dark brown with a distinct pale brown middorsal stripe, slender at midbody, becoming gray towards the tail; (11) labial stripe white or orange; (12) flanks of body dark brown.

Description of holotype

Adult male (QCAZ 14955) (Figs 1, 2); snout-vent length 46.72 mm; tail length 80.91 mm; dorsal and lateral head scales juxtaposed, finely wrinkled; rostral hexagonal, 2.06 times as wide as high; frontonasal irregularly quadrangular, wider than long, laterally in contact with nasal, loreal and first superciliary, smaller than frontal; prefrontal scales absent; frontal pentagonal, longer than wide, slightly wider anteriorly, in contact with one supraocular on the left side, and two on the right side; frontoparietals pentagonal, longer than wide, slightly wider posteriorly, each in contact laterally with supraocular II; interparietal roughly heptagonal, lateral borders nearly parallel to each other; parietals slightly smaller than interparietal, hexagonal, and positioned anterolaterally to interparietal, each in contact laterally with supraocular II (and supraocular III on right side) and dorsalmost postocular; postparietals three, medial scale smaller than laterals; seven supralabials, fourth one longest and below the center of eye; six infralabials, fourth one below the center of eye; temporals enlarged, irregularly hexagonal, juxtaposed, smooth; two large supratemporal scales, smooth; nasal slightly divided, irregularly pentagonal, longer than high, in contact with rostral anteriorly, first and second supralabials ventrally, frontonasal dorsally, loreal posterodorsally and frenocular posteroventrally; nostril on ventral aspect of nasal, directed lateroposteriorly; loreal rectangular, wider dorsally; frenocular higher than long, higher anteriorly, in contact with nasal, separating loreal from supralabials; two supraoculars on the left side, three supraoculars on the right side (posterior most much smaller), with the first one being the largest; four elongate superciliaries, first one enlarged, in contact with loreal; palpebral disk divided into four scales, pigmented; suboculars three (on the left side the medial subocular is fragmented), elongated and homogeneous in size; two postoculars, the dorsalmost wider than the other; ear opening vertically oval, without denticulate margins;

tympanum recessed into a shallow auditory meatus; mental semicircular, wider than long; postmental pentagonal, slightly wider than long, followed posteriorly by three pairs of genials, the anterior two in contact medially and the posterior two separated by postgenials; all genials in contact with infralabials; gulars imbricate, smooth, widened in four longitudinal rows; posterior row of gulars (collar) with four scales, the medial two distinctly widened.

Scales on nape similar in size to dorsals, except for the anteriormost that are widened; scales on sides of neck small and granular; dorsal scales hexagonal, elongate, imbricate, arranged in transverse rows; scales on dorsal surface of neck striated, becoming progressively keeled from forelimbs to the tail; number of dorsal scales between occipital and posterior margin of hindlimbs 27; dorsal scale rows in a transverse line at midbody 26; one longitudinal row of smooth, enlarged ventrolateral scales on each side; dorsals separated from ventrals by two rows of small scales at the level of the 13th row of ventrals; lateral body fold between fore and hindlimbs present; ventrals smooth, wider than long, arranged in 20 transverse rows between the collar fold and preanals; six ventral scales in a transverse row at midbody; subcaudals smooth; axillary region composed of granular scales; scales on dorsal surface of forelimb striated, imbricate; scales on ventral surface of forelimb granular; two thick, smooth thenar scales; supradigitals (left/right) 3/3 on finger I, 6/7 on II, 8/8 on III, 9/9 on IV, 6/6 on V; supradigitals 3/4 on toe I, 6/6 on II, 10/9 on III, 11/12 on IV, 7/7 on V; subdigital lamellae of finger I and II single, on finger III all paired, except the four distalmost, on finger IV paired at the base, on finger V all single; number of subdigital lamellae 5/5 on finger I, 11/12 on II, 15/16 on III, 17/16 on IV, 9/10 on V; subdigital lamellae on toes I and II single, on toe III, IV and V all paired, except for the three distalmost subdigitals; number of subdigital lamellae 6/6 on toe I, 11/10 on II, 16/15 on III, 21/21 on IV, 14/14 on V; groin region with small, imbricate scales; scales on dorsal surface of hindlimbs smooth and imbricate; scales on ventral surface of hindlimbs smooth; scales on posterior surface of hindlimbs granular; femoral pores absent; preanal pores absent; cloacal plate paired, bordered by four scales anteriorly, of which the two medialmost are enlarged.

Additional measurements (mm) and proportions of the holotype: HL 11.44; HW 7.41; ShL 7.03; AGD 23.91; TL/SVL 1.54; HL/SVL 0.24; HW/SVL 0.16; ShL/SVL 0.15; AGD/SVL 0.51.

Color in preservative of the holotype

Dorsal background uniformly brown with a grayish light brown vertebral stripe extending from occiput onto tail; vertebral stripe wider anteriorly, becoming slightly slender at the most posterior part of body; dorsal surface of head light brown (rostral, frontonasal, frontal,

frontoparietals and supraoculars) and brown laterally; white longitudinal stripe extending from the first supralabial to the shoulder and fading away on the flanks; ventrolateral aspect of neck dark brown with a dorsolateral light brown stripe that extends posteriorly along the flanks to the hindlimbs; forelimbs with scattered ocelli (black with white center); flanks brown with two dorsolateral stripes on each side, the dorsal one dark brown and the most ventral one brown diffuse with dark brown spots; tail brown dorsally; ventral surface of head gray, chest and venter dark gray, ventral surface of tail slightly brown, with scattered dark brown marks (Fig. 1).

Variation

Measurements and scale counts of *Pholidobolus samek* are presented in Table 2. Supralabials 8/7 (left/ right) and temporals five in specimen QCAZ 14956; small and separated prefrontals on both sides in specimen QCAZ 14954 and one prefrontal on right side in QCAZ 14956; little intrusive scales between parietal and postparietal in specimen QCAZ 14954; frontal hexagonal in specimen QCAZ 14956; roughly decagonal interparietal in specimen QCAZ 14954. Usually two scales on posterior cloacal plate, four posterior cloacal scales in specimens QCAZ 14954 and 14956. Male is larger (SVL 46.7 mm, n=1) than females (maximum SVL 45.4 mm, n=2)

The male (holotype) can be distinguished from the two female paratypes by color pattern. Coloration in life of male holotype (QCAZ 14955) was different to the females paratypes's coloration. The females paratypes (QCAZ 14954 and 14956) have an orange-brown longitudinal stripe extending from third supralabial to the shoulder and fading away on the flanks (Fig. 3). The ventral zone is gray without spots nearly to the cloacal plate. No variation was observed in color pattern in preservative among females paratypes (QCAZ 14954, 14956).

Distribution and natural history

Pholidobolus samek inhabits cloud forests in the eastern slopes of the Andes of southern Ecuador in Cordillera del Cóndor (Fig. 4). The new species is known only from Provincia Zamora-Chinchipe (La Canela), at 2324−2844 m, on the sandstone plateaus of Cerro Plateado Biological Reserve. The ground is cover by mosses, roots and bromeliads. This type ground cover is locally known as bamba. All specimens were found active at 11h30-17h00, mostly under stones or terrestrial bromeliads (Fig. 5). *Pholidobolus* receive solar heat through basking and substrate absorption. The principal basking sites are rocks, agave

leaves and bromeliads, furthermore, *Pholidobolus* uses these sites as hiding places (Montanucci 1973).

Conservation status

Pholidobolus samek is only known from Cordillera del Cóndor. The population size for this species is unknown, but our sampling suggests low abundances. Nevertheless, Valencia et al. (2017) and Ron et al. (2018) mentioned that Cordillera del Cóndor is threatened by mining activities. Because of the small known distribution, habitat destruction and mining activities, we suggest to assign *P. samek* to the Critically Endangered category under criteria B1a, b(iii), according to IUCN (2001) guidelines.

Etymology

The specific epithet “samek” means “green” in Shuar language in allusion to the green dorsolateral head stripes distinguishing the new species from other congeners. The type locality of *Pholidobolus samek* lies within the territory belonging to the Shuar indigenous people, who inhabit the Amazonian rainforest in Ecuador and Peru.

***Pholidobolus elquimi* sp. n.**

Proposed standard English name: El Quimi Cuilán
Proposed standard Spanish name: Cuilán del Quimi

Holotype: QCAZ 15844 (Figs 6, 7, 8), adult male, Ecuador, Provincia Morona Santiago, buffer zone in El Quimi Biological Reserve, plateau on the eastern side of El Quimi river valley, 3.5189S, 78.3690W, WGS84, 2209 m, 11 July 2017, collected by Diego Almeida, Darwin Núñez, Eloy Nusirquia, Alex Achig and Ricardo Gavilanes.

Paratypes (3): ECUADOR: Provincia Morona Santiago: QCAZ 16790 (hatchling), El Quimi Biological Reserve, base camp towards the old heliport (high zone), 3.51894S, 78.36897W, WGS84, 2226 m, 17 April 2018; QCAZ 16788 (hatchling), 16789 (hatchling), El Quimi Biological Reserve, near to base camp, 3.5182S, 78.3913W, WGS84, 1994 m, 12 April 2018, collected by Diego Almeida, Darwin Núñez, Eloy Nusirquia, Alex Achig and María del Mar Moretta.

Diagnosis

The new species is unique among species of *Pholidobolus* in having adult males with slightly greenish cream dorsolateral stripes on the head, orange red color on the flanks and under the tail, and orange red spots on the ventral surface of the hindlimbs. In addition, *P. ulisesi*, *P. dicrus*, *P. hillisi*, and *P. vertebralis* differs from *P. elquimi* sp. n. (character states in parentheses), in having a conspicuous light vertebral stripe (light vertebral stripe absent). *Pholidobolus* affinis, *P. prefrontalis*, *P. dicrus*, *P. hillisi* and *P. vertebralis* differs from *P. elquimi* sp. n. in having prefrontal scales (prefrontal scales absent). Despite *P. elquimi* sp. n. shares with *P. samek* the green dorsolateral stripes on the head, *P. samek* differs from the new species in distribution. *Pholidobolus samek* is in Zamora Chinchipe and *P. elquimi* sp. n. in Morona Santiago

Characterization: (1) Two or (rarely three) supraoculars, anteriormost larger than posterior one; (2) prefrontals absent; (3) femoral pores absent; (4) four opaque lower eyelid scales; (5) scales on dorsal surface of neck striated or smooth, becoming striated from forelimbs to tail; (6) two rows of lateral granules at midbody; (7) 27−31 dorsal scales between occipital and posterior margin of hindlimb; (8) lateral body fold present; (9) keeled ventrolateral scales on each side absent; (10) dorsum dark brown with a not conspicuous narrow, pale brown; (11) labial stripe white; (12) flanks of body dark brown or gray.

Description of holotype

Adult male (QCAZ 15844) (Figs 6, 7, 8); snout-vent length 42.71 mm; tail length 74.76 mm; dorsal and lateral head scales juxtaposed, finely wrinkled; rostral hexagonal, 1.67 times as wide as high; frontonasal quadrangular, laterally in contact with nasal, loreal and first superciliary, smaller than frontal; prefrontal scales absent; frontal pentagonal, longer than wide, wider anteriorly, in contact with the first superciliary and supraocular; frontoparietals hexagonal, longer than wide, slightly wider in the middle, each in contact laterally with supraocular II; interparietal octagonal, with a medial suture posteriorly, lateral borders nearly parallel to each other; parietals higher than interparietal, hexagonal and positioned anterolaterally to interparietal, each in contact laterally with supraocular II and dorsalmost postocular; postparietals three, medial scale smaller than laterals; eight supralabials, fourth one longest and below the center of eye; six infralabials, fourth one below the center of eye; temporals enlarged, irregularly hexagonal, smooth; two large supratemporal scales, smooth; nasal shield slightly divided above the nostril, irregularly pentagonal, longer than high, in contact with rostral anteriorly, first and second supralabials ventrally, frontonasal dorsally, loreal posterodorsally and frenocular posteroventrally; nostril on ventral aspect of nasal, directed laterally; loreal quadrangular, slightly wider dorsally, not in contact with

supralabials; frenocular, higher than long, in contact with nasal; nasal separating loreal from supralabials; two supraoculars, with the first one being the widest; four elongate superciliaries, first one enlarged, in contact with loreal; palpebral disk divided into five scales, pigmented; four suboculars, three elongated and homogeneous in size, the last one widest; two postoculars, the dorsalmost wider than the other; ear opening vertically oval, without denticulate margins; tympanum recessed into a shallow auditory meatus; mental semirectangular, wider than long; postmental pentagonal, slightly wider than long, followed posteriorly by three pairs of genials, the anterior two pair in contact medially and the third pair separated by postgenials; all genials in contact with infralabials; gulars imbricate, smooth, widened in five longitudinal rows; posterior row of gulars (collar) with four scales, the medial two distinctly widened.

Scales on nape slightly smaller than the dorsals, except for the anteriormost that are widened; scales on sides of neck small and granular; dorsal scales elongate, imbricate, arranged in transverse rows; scales on dorsal surface of neck striated, becoming progressively keeled from forelimbs to the tail; number of dorsal scales between occipital and posterior margin of hindlimbs 27;dorsal scale rows in a transverse line at midbody 27; one longitudinal row of smooth, enlarged ventrolateral scales on each side; dorsals separated from ventrals by two rows of small scales at the level of the 13th row of ventrals; lateral body fold between fore and hindlimbs present; ventrals smooth, wider than long, arranged in 20 transverse rows between the collar fold and preanals; six ventral scales in a transverse row at midbody; subcaudals smooth; axillary region composed of granular scales; scales on dorsal surface of forelimb striated, imbricate; scales on ventral surface of forelimb granular; two thick, smooth thenar scales; supradigitals (left/right) 3/3 on finger I, 6/6 on II, 8/8 on III, 9/9 on IV, 6/6 on V; supradigitals 3/3 on toe I, 6/6 on II, 9/9 on III, 12/12 on IV, 7/7 on V; subdigital lamellae of forelimb finger I, II and III single, on finger IV few scales paired at the middle, on finger V all single; number of subdigital lamellae 6/6 on finger I, 11/11 on II, 15/15 on III, 17/16 on IV, 10/10 on V; subdigital lamellae on toes I and II single, on toe III, IV and V all paired , except for the two or three distalmost subdigitals; number of subdigital lamellae 7/6 on toe I, 12/12 on II, 15/16 on III, 22/22 on IV, 12/12 on V; groin region with small, juxtaposed scales; scales on dorsal surface of hindlimbs striated and imbricate; scales on ventral surface of hindlimbs smooth; scales on posterior surface of hindlimbs granular; femoral pores absent; preanal pores absent; cloacal plate paired, bordered by four scales anteriorly, of which the two medialmost are enlarged.

Additional measurements (mm) and proportions of the holotype: HL 11.01; HW 6.62; ShL 5.80; AGD 20.39; TL/SVL 1.75; HL/SVL 0.26; HW/SVL 0.16; ShL/SVL 0.14; AGD/SVL 0.48.

Color of holotype in preservative

Dorsal background uniformly dark brown with a grayish brown middorsal stripe extending from occiput onto tail; dorsolateral stripe distinct, pale gray, extending from snout to near base of tail; dorsal surface of head brown (rostral, frontonasal, frontal, frontoparietals and supraoculars) and dark brown laterally; white longitudinal stripe extending from first supralabial to forelimb; lateral aspect of neck dark brown with a dorsolateral light brown stripe that extends posteriorly along the flanks to the hindlimbs; flanks grayish brown; tail dark brown dorsally and bronze on the sides, anteriorly; ventral surface of head gray, with dirty cream genials and scattered black marks; chest, belly and ventral surface of tail light gray with light red spots; ventral surface of limbs dark gray (Fig. 8).

Variation

Measurements and scale counts of *Pholidobolus elquimi* are presented in Table 2. Supraoculars three on the left side of QCAZ 16789; supralabials six in QCAZ 16789 and 16790, and seven on specimen QCAZ 16788; two cuadrangular frontonasals in specimen QCAZ 16788; 18 ventral scales in transverse row at midbody in QCAZ 16788 and 19 in QCAZ 16790.

Variation was observed in color pattern in live among adult male and hatched specimens. The hatched lizard did not present the reddish color on the tail.

Distribution and natural history

Pholidobolus elquimi occurs in the southeastern portion of the northern Andes in Cordillera del Cóndor, Amazon basin, at elevations between 1994–2226 m. The new species is known from Provincia Morona Santiago, on the plateau of El Quimi Biological Reserve (Fig. 4). Specimen QCAZ 15844 was found on a sandstone plateau of shrub vegetation (1.50m); active at 21h14, at the base of bromeliad leaf (Fig. 9).

The eggs of specimens QCAZ 16788, 16789 and 16790 were found at the base of bromeliad leaf, exposed to the air, and under a trunk and were born in captivity at PUCE herpetology lab. These were incubated for 3 months approximately, in sphagnum, at 21ºC and 75% humidity.

Conservation status

Pholidobolus elquimi is only known from Cordillera del Cóndor. The population size for this species is unknown, but our sampling suggests low abundances. Nevertheless, Valencia et al. (2017) and Ron et al. (2018) mentioned that Cordillera del Cóndor is threatened by mining activities; According to Mazanda et al. (2018) large part of concessions for open pit copper mining exploitation overlap the Cordillera del Cóndor. Habitat destruction and fragmentation is evident at a distance of ~11 km from the collection sites (according to Monitoring of the Andean Amazon Project, MAAP #89: Impacts of Mining Project "Mirador" in the Ecuadorian Amazon). Because of the small known distribution, habitat destruction and mining activities, we suggest to assign *P. samek* to the Critically Endangered category under criteria B1a, b(iii), according to IUCN (2001) guidelines.

Etymology

The specific name is a noun in apposition and refers to the type locality, el Quimi Biological Reserve. This 9071 ha reserve in southern Ecuador has tepuy montane forest, on which several eggs of the new species were found.

***Pholidobolus oni* sp. n.**

Proposed standard English name: Foothill Cuilán
Proposed standard Spanish name: Cuilán de pie de colina

Holotype: QCAZ 16353 (Figs 10, 11), adult male, Ecuador, Provincia Azuay, San Felipe de Oña, 3.4292S, 79.2364W, WGS84, 2672 m, 16 March 2018, collected by Diego Almeida, Darwin Núñez, Eloy Nusirquia, Alex Achig and Katherine Nicolalde.

Paratypes (5): ECUADOR: Provincia Azuay: QCAZ 16352 (adult female), 16349 (adult female), San Felipe de Oña, Susudel-Poetate road, 3.4322S, 79.2369W, WGS84, 2506 m, 16 March 2018; QCAZ 16351 (juvenile), CQAZ 16350 (juvenile), San Felipe de Oña, 3.4275S, 79.2339W, WGS84, 2675 m, 16 March 2018, same collectors as holotype (Fig. 10).

Diagnosis

Pholidobolus oni sp. n. is unique among species of *Pholidobolus* in having small scales between the posterior corner of the orbit and the anterior edge of the external auditory meatus and a ventrolateral fold between fore and hindlimbs not well differentiated. It further differs from all species of *Pholidobolus*, except *P. affinis, P. vertebralis* and *P. hillisi*, in having three supraoculars (two in *P. macbrydei, P. montium, P. ulisesi, P. dicrus, P. prefrontalis, P. samek* and *P. elquimi*). *Pholidobolus oni* sp. n. differs from *P. affinis* by the absence of ocelli on flanks (ocelli flanks present in the last species), and by distribution, *P. affinis* is in Chimborazo, Tungurahua and Cotopaxi provinces, while *P. oni* sp. n. is restricted to Azuay province.

The new species further differs from *P. macbrydei* and *P. montium* in having prefrontal scales (prefrontals absent in the other two species).

Characterization: (1) Three supraoculars, anteriormost larger than posteriors; (2) prefrontals present; (3) femoral pores present in both sexes; (4) four to six opaque lower eyelid scales; (5) scales on dorsal surface of neck smooth, becoming slightly keeled from forelimbs to tail; (6) two or three rows of lateral granules at midbody; (7) 35−20 dorsal scales between occipital and posterior margin of hindlimb; (8) lateral body fold present but fairly distinguished; (9) keeled ventrolateral scales on each side absent; (10) dorsum dark brown with a slightly narrow, pale brown, vertebral stripe that becomes grayish brown towards the tail; (11) labial stripe white; (12) flanks of body gray brown; (13) white stripe along forelimb present.

Description of holotype

Adult male (QCAZ 16353) (Figs 10, 11); snout-vent length 41.08 mm; tail length 96.32 mm; dorsal and lateral head scales imbricated, smooth; rostral hexagonal, 1.75 times as wide as high; frontonasal heptagonal, slightly wider than long, laterally in contact with nasal, smaller than frontal; prefrontals present, in wide contact medially and in laterally contact with loreal and first supercilary scale; frontal hexagonal, longer than wide, wider anteriorly, in contact with first and second supraoculars; frontoparietals pentagonal, longer than wide, slightly wider posteriorly, each in contact with second and third supraoculars, parietals and interparietal; interparietal heptagonal, lateral borders nearly parallel to each other; parietals higher than interparietal, hexagonal, and positioned anterolaterally to interparietal, each in contact with third supraocular and dorsalmost postocular; postparietals three, medial scale smaller than laterals; seven supralabials, fourth one longest and below the center of eye; five infralabials, fourth one below the center of eye; temporals small, irregularly, smooth;

supratemporal scales not well differentiated, smooth; nasal shield divided above the nostril, irregularly tetragonal, longer than high, in contact with rostral anteriorly, first and second supralabials ventrally, frontonasal dorsally, loreal posterodorsally and frenocular posteroventrally; loreal pentagonal, slightly wider dorsally, in contact with supralabials; frenocular longer than high, in contact with loreal; three supraoculars, with the first one being the widest; four elongate superciliaries, first one enlarged, in contact with loreal; palpebral disk oval, pigmented, divided into four scales; four suboculars, two elongated and similar in size, the first and the most posteriorly scale widest; three postoculars, the dorsalmost wider than the others; ear opening vertically oval, without denticulate margins; tympanum recessed into a shallow auditory meatus; mental semirectangular, wider than long; postmental pentagonal, slightly wider than long, followed posteriorly by three pairs of genials, the anterior two pair in contact medially and the third pair separated by postgenials; all genials in contact with infralabials; gulars imbricate, smooth, widened in four longitudinal rows; gular fold complete, posterior row of gulars (collar) with four scales, the medial two distinctly widened.

Scales on nape slightly smaller than the dorsals, except for the anteriormost that are widened; scales on sides of neck small and granular; dorsal scales elongate, juxtaposed, arranged in transverse rows; scales on dorsal surface of neck striated, becoming slightly keeled from forelimbs to the tail; number of dorsal scales between occipital and posterior margin of hindlimbs 35; dorsal scale rows in a transverse line at midbody 32; one longitudinal row of smooth, enlarged ventrolateral scales on each side; dorsals separated from ventrals by three rows of small scales at the level of the 13th row of ventrals; lateral body fold between fore and hindlimbs present but not well defined; ventrals smooth, arranged in 26 transverse rows between the collar fold and preanals; six ventral scales in a transverse row at midbody; subcaudals smooth; axillary region composed of granular scales; scales on dorsal surface of forelimb smooth, imbricate; scales on ventral surface of forelimb granular; two thick, smooth thenar scales; supradigitals (left/right) 3/0 on finger I, 7/7 on II, 9/8 on III, 10/10 on IV, 5/5 on V; supradigitals 4/4 on toe I, 7/7 on II, 11/11 on III, 12/11 on IV, 9/8 on V; subdigital lamellae of forelimb finger I single, finger II one paired scale at the middle, III and IV few paired scales at the base and the middle, on finger V all single; number of subdigital lamellae 5/0 on finger I, 10/10 on II, 14/14 on III, 14/14 on IV, 9/9 on V; subdigital lamellae on toes I single, on toe II few scales paired at the middle, on toe III and IV paired scales from the base to the middle, and on toe V paired scales at the base; number of subdigital lamellae 5/5 on toe I, 10/10 on II, 14/14 on III, 18/19 on IV, 11/11 on V; groin region with small, imbricate scales; scales on dorsal surface of hindlimbs striated and imbricate; scales on ventral surface of hindlimbs smooth; scales on posterior surface

of hindlimbs granular; femoral pores present, three on left leg and five on right leg; preanal pores absent; cloacal plate paired, bordered by four scales anteriorly, of which the two medialmost are enlarged.

Additional measurements (mm) and proportions of the holotype: HL 9.83; HW 6.21; ShL 5.41; AGD 20.74; TL/SVL 2.43; HL/SVL 0.24; HW/SVL 0.15; ShL/SVL 0.13; AGD/SVL 0.50.

Color of holotype in preservative

Dorsal background uniformly brown with a light brown vertebral stripe extending from occiput onto tail; vertebral stripe fading at the posterior end of body; dorsal and ventral surface of head brown; sides of body light brown, with scattered dark brown spots; white longitudinal stripe extending from first supralabial to forelimb; lateral aspect of neck brown with a dorsolateral light brown stripe that extends posteriorly along the flanks to the hindlimbs; flanks light brown without well differentiated dorsolateral stripes; tail grayish brown; gular, chest and venter region pale gray; ventral surface of tail gray; ventral surface of limbs gray (Fig. 12).

No variation was observed in color pattern in preservative between adult and juveniles, except by the specimen QCAZ 16349, it has a pale gray vertebral stripe (Fig. 13).

Variation

Measurements and scutellation data of *Pholidobolus oni* are presented in Table 2. Superciliary 4/5 (left / right) in specimen QCAZ 16350; palpebral disk divided into 5/6 scales in QCAZ 16352 and 3/5 in QCAZ 16351. Frontonasal pentagonal in QCAZ 16349, 16350, 16351 and 16352; prefrontals pentagonal in QCAZ 16349, 16350 and 16352. Rows of lateral granules at midbody two in QCAZ 16439, 16350 and 16351 to three in QCAZ 16350. Gular scales (22) in QCAZ 16351; temporals 7 in QCAZ 16351 and 9 in QCAZ 16350. Male is smaller (SVL 41.08 mm, n=1) than females (maximum SVL 48.08 mm, n=2).

Variation was observed in color pattern between life male holotype (QCAZ 16353), female paratypes (QCAZ 16349 and 16352) and juveniles (QCAZ 16350 and 16351) (Fig. 14). Adult females differ from the holotype in having vertebral stripe, which is disappearing at the most posterior part of body, and flanks pale brown. Juvenile QCAZ 16350 differs from holotype in having sides of body light brown, without scattered dark brown spots; juvenile QCAZ 16351 is unique by having withe spots on the flanks and over forelimbs.

Distribution and natural history

Pholidobolus oni is known to occur between 2506−2675 m on the southwest of Azuay Province, in San Felipe de Oña (Fig. 4), a locality within a vast geography rich landscapes, with small valleys, desert areas and wet paramo. The localities where *P. oni* was collected are not within protected areas, indicating that at least some populations of this species are not protected. Most specimens were found active at day (10h26-15h30), mostly on the ground or achupallas.

Conservation status

Pholidobolus oni is only known from localities around Oña. The population size for this species is unknown, but our sampling suggests low abundances. Because of the small known distribution, we suggest to assign *P. oni* to the Data Deficient category, according to IUCN (2001) guidelines, until more information become available.

Etymology

The specific epithet “oni” means “foot” (of the hill) in Celtic language in allusion to the geographical distribution to the Oña locality, where the holotype of this species was collected.

Morphological comparisons among species

Two components with eigenvalues > 1.0 were extracted from the PCA (Table 3). The two components accounted for 44.76% of the total variation. The highest loadings for the PCA were number of supradigital scales of the fifth toe (ST5) and scales around the body (SAB) for PC I, and number of supradigital scales of the third finger (SF3) and supradigital scales of the fifth finger (SF5) for PC II (Table 3). In general, there is wide overlap in morphological space among species for “*P. macbrydei*” (Fig. 15). The only exception is for the three new species.

Phylogenetic relationships and genetic distances

Tree topologies under ML and Bayesian approaches were similar. The phylogenetic tree inferred from the analysis of the concatenated mitochondrial and nuclear DNA sequences (Fig. 16A) supports the monophyly of *Pholidobolus* (BS= 66, PP = 0.96). Our tree is congruent with previous molecular phylogenies in that *P. ulisesi* and *P. hillisi* form a clade

sister to all other congeners (Torres-Carvajal et al. 2015, 2016; Hurtado Gomez et al. 2018). Currently recognized species of *Pholidobolus* are monophyletic, except for *P. macbrydei*, which is paraphyletic upon recognition of the species described herein. All three new species are nested within the "*P. macbrydei"* clade, which is sister (PP = 1) to the (*P. prefrontalis*, (*P. paramuno*, (*P. dicrus*, *P. vertebralis*))) clade. The "*P. macbrydei"* clade is divided into two clades, one (BS = 100, PP = 1) containing *P. elquimi* sp. n. as sister to the subclade (*P. samek* sp. n., *P. "macbrydei"* Clade A [southern Loja province]), and the other (BS = 54, PP = 0.83) composed of *P. "macbrydei"* Clade B (Cañar province) as sister to subclade (*P. "macbrydei"* Clade C [Azuay and Cañar provinces], (*P. "macbrydei"* Clade D [El Oro province], *P. oni* sp. n.)). The new species are strongly supported as monophyletic (PP ≥ 0.95, BS = ≥ 77). The nuclear phylogenetic tree (Fig. 16B) is poorly resolved; it only includes a few well-supported clades that are congruent with the concatenated tree (e.g., *P. oni* sp. n. and *P. "macbrydei"* are in the same clade [BS = 71, PP = 0.98]).

Uncorrected genetic distances for 16S, 12S and ND4 are presented in Tables 4, 5 and 6, respectively. Distance values among all recognized species of *Pholidobolus*, the new species described in this paper, and the four *P. "macbrydei*" Clades range between 0.01–0.10 for 12S, 0.02–0.06 for 16S, and 0.04–0.19 for ND4. Distance values between all "*Pholidobolus macbrydei*" clades and the three new species range between 0.01–0.05 for 12S, 0.02–0.04 for 16S and 0.04–0.13 for ND4. For 12S, the genetic distance between *P. elquimi* sp. n. and *P. samek* sp. n. (0.01) is slightly lower than all other interspecific distance values within *Pholidobolus* (0.02–0.10). The genetic distances for nuclear gene NDH3 are generally short (0–0.02).

Discussion

Phylogenetic relationships and genetic distances

The systematics of *Pholidobolus* has been controversial partly because morphological evidence has been misinterpreted. Nonetheless, the recent use of DNA sequences has helped to characterize intraspecific morphological variation and phylogeny of this genus (Torres-Carvajal and Mafla-Endara 2013; Torres-Carvajal et al. 2015). In addition, recent collections in poorly explored areas along the Andes of Colombia, Ecuador, and Peru has resulted in the discovery and description of new species (Hurtado-gómez et al. 2018; Torres-Carvajal et al. 2014; Venegas et al. 2016).

The phylogeny (Fig. 16A) supports strongly the monophyly of *Pholidobolus* and its sister taxon relationship with *Macropholidus*, as suggested by previous authors (Montanucci 1973; Torres-Carvajal et al. 2014). In her undergraduate thesis, Mafla-Endara (2011) found that the widely distributed *P. macbrydei* could represent a species complex. In the present

study, the integrative analysis of mtDNA, nDNA and morphology allowed us to confirm that *P. "macbrydei"* as currently defined is indeed a species complex that includes the three species described herein and four candidate species. Moreover, the three new species are easily diagnosable with morphological evidence.

An analysis of the remaining taxa within the *P. "macbrydei"* species complex is underway. However, here we assign the name *P. macbrydei* to Clade C because it includes a specimen (QCAZ 10050, from Juncal, Cañar) that was collected 18.25 km west from the type locality of this species. Moreover, specimens from Clade C match the description of the holotype of *P. macbrydei* (Montanucci 1973); specimens of Clade C share with *P. macbrydei* sensu stricto the dorsolateral pale stripe not extending to tip of snout, sides of body with several brown stripes alternating with cream stripes and sides of neck and tail with bright red stripe in males. Interestingly, specimens of Clade B are from the same locality as QCAZ 10050, but they differ in morphology and color pattern; the males of Clade B differ from males of Clade C in lacking the underside of the tail blue-black. In her systematic study of *Pholidobolus* from Ecuador, Mafla-Endara (2011) commented that Cañar populations (Clade B) presented greater morphological variation in relation to other populations of *P. macbrydei*, and were similar in morphology to *P. prefrontalis*. Mafla-Endara suggested that Cañar populations are the result of introgression events between *P. prefrontalis* and *P. macbrydei.* Nevertheless, our nuclear phylogenetic tree does not suggest hybridization. The combined mitochondrial and nuclear phylogeny (Fig. 16A) shows that Clade B is strongly supported (PP=1, bootstraps value= 1); therefore, this clade could be considered as an unconfirmed candidate species (UCS), until more data become available. If Clade B and Clade C are different species, they would be sympatricy in Cañar province. Nonetheless, the relationship between Clade B and Clade C is still unresolved and Clade C is only moderately supported by mL bootstrapping (bootstraps value= 56). Further studies should include more nDNA genes and specimens from localities where UCS occur in sympatry.

Mafla-Endara (2011) considered the Jimbura and Guanazán populations (i.e., our Clades A and D, respectively) as evolutionary significant units (ESU). Our results suggest that Clade A is sister to *P. samek* sp. n., although they can be easily distinguished from each other (specimens from Jimbura lack the bright green dorsolateral stripes on the head characteristic of *P. samek* sp. n.). Moreover, Clade A and *P. samek* sp. n. are allopatrically distributed and geographically isolated by the Andes (Fig. 4). A similar pattern is true for sister taxa Clade D and *P. oni* sp. n. Additionally, Clades A and D are highly supported by the phylogeny (Fig. 16A), which further supports our conclusion that they represent candidate species.

According to the genetic distances (Tables 4, 5 and 6), ND4 is the most variable of all genes included in this study. Interspecific ND4 genetic distances within *Pholidobolus* range between 0.04–0.19; distances between *P. samek* and other species range between 0.08–0.17; and between 0.08–0.17 for *P. elquimi* sp. n. and 0.04–0.17 for *P. oni* sp. n. According to Brown and Su (2002) a divergence threshold of 2.6%, for the 16S mitochondrial gene in lizards could be enough to separate populations in different species. This threshold further supports the recognition of the three new species herein described. Within the clade (*P. elquimi* sp. n., (*P. samek* sp. n., *P. "macbrydei"* Clade A)), 16S distances vary between 0.02–0.03. Similarly, the 16S distance between *P. oni* sp. n. and its sister clade (*P. "macbrydei"* Clade D) is 4%.

According to the PCA (Fig. 15), the three new species are morphologically different from other "*Pholidobolus macbrydei".* This is especially true for *P. oni* sp. n., which further segregates from the other species along PC II. However, we recognize that a larger sample size is necessary to confirm this differentiation. We suspect that the difficulty in defining species boundaries based on morphology arises from the high intraspecific polymorphism in coloration (Fig. 17) characteristic of most groups of gymnophthalmid lizards (Torres-Carvajal et al. 2015, 2016). The components I and II in the PCA (Table 3) explain less than 50% of the variation of the new species and *"P. macbrydei"* clade. It is necessary to include more morphological characters and increase the sample size to better elucidate the morphological differences.

The Cordillera del Cóndor is a sub-Andean mountain chain geologically similar to the Tepuis in the Guianan region (Neill 2005). In the last ten years a large number of unknown species of amphibians, plants, mammals and lizards has been discovered (Brito et al. 2017; Huamantupa-Chuquimaco and Neill 2015; Ron et al. 2018; Torres-Carvajal et al. 2009; Valencia et al. 2017). The discovery of *P. samek* and *P. elquimi*, both presumably threatened, reflects the importance of the conservation of Cordillera del Cóndor.

The discovery of new species and four additional clades within "*P. macbrydei*" reveals high levels of diversity within *Pholidobolus*. This suggests that the Andean herpetofauna in southern Ecuador is possibly more diverse in species numbers than previously thought, especially for poorly explored areas like Cordillera del Cóndor. Future studies should include larger samples and other types of evidence (e.g., environmental variables) that might prove useful for species delimitation.

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Maximum likelihood phylogram depicting relationships within *Pholidobolus.* The phylogram was derived from the analysis of 411 bp of nuclear DNA. Bootstrap values are shown above and Bayesian posterior probabilities below the branches; asterisk (*) indicate maximum values. The outgroup taxon is not shown (*Anadia rhombifera*). Abbreviation are: **EC** Ecuador, **PE** Peru (B).

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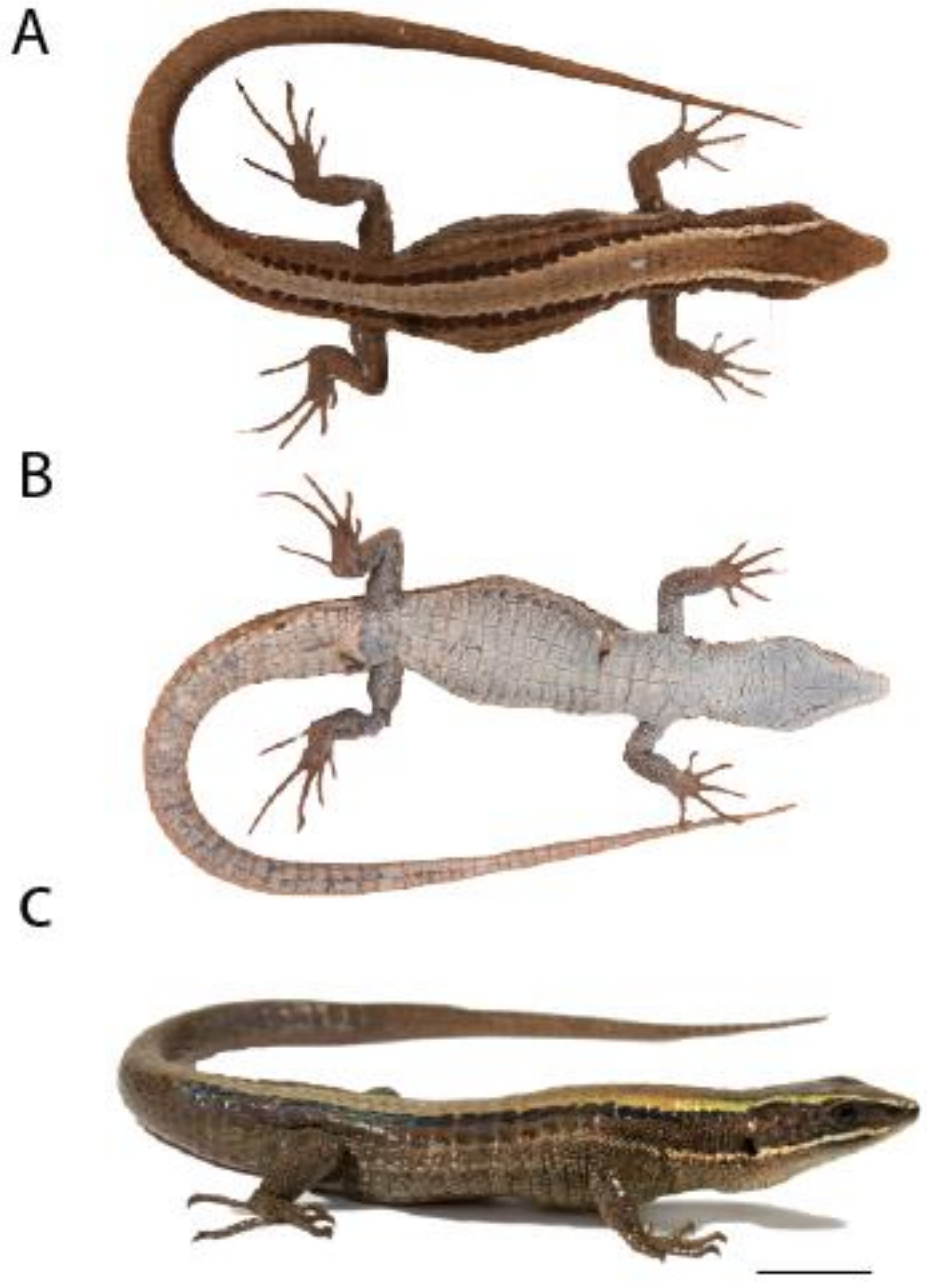
Figure 1.



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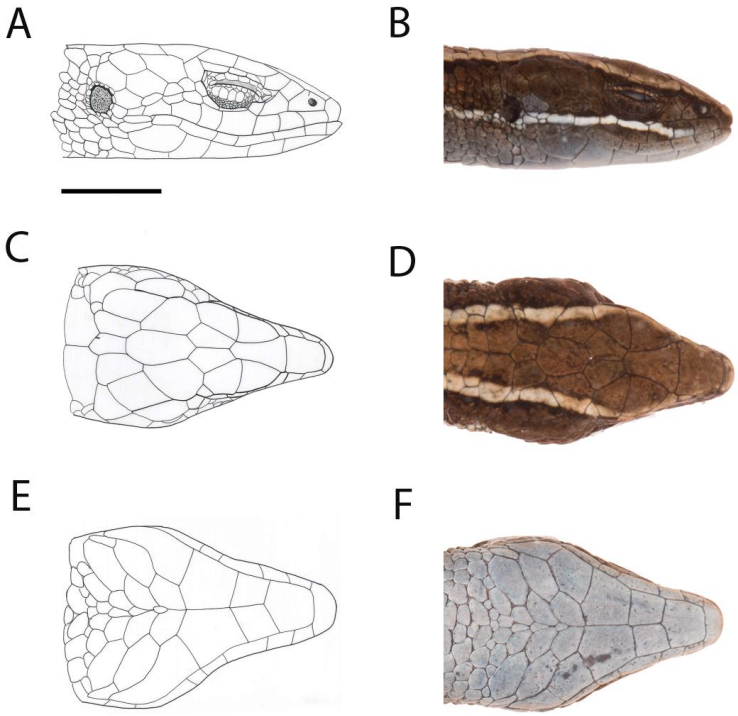

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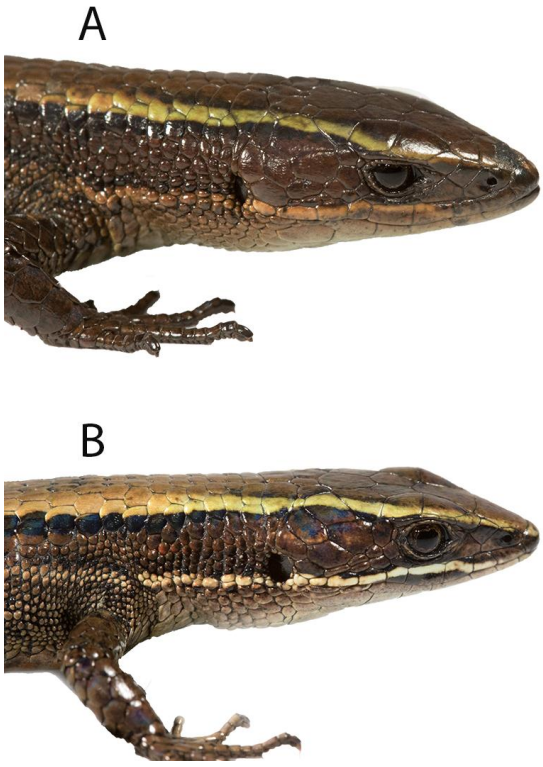

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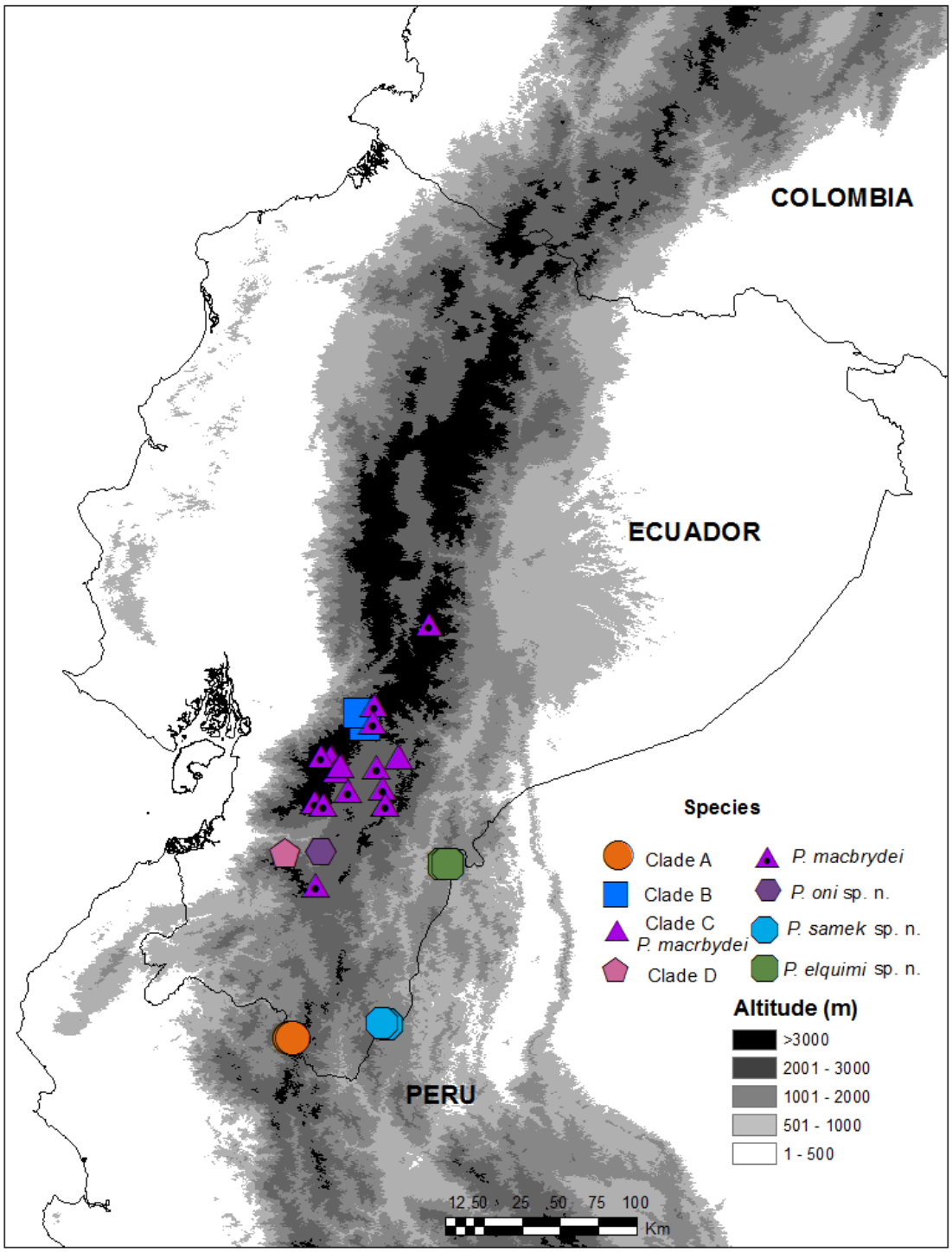
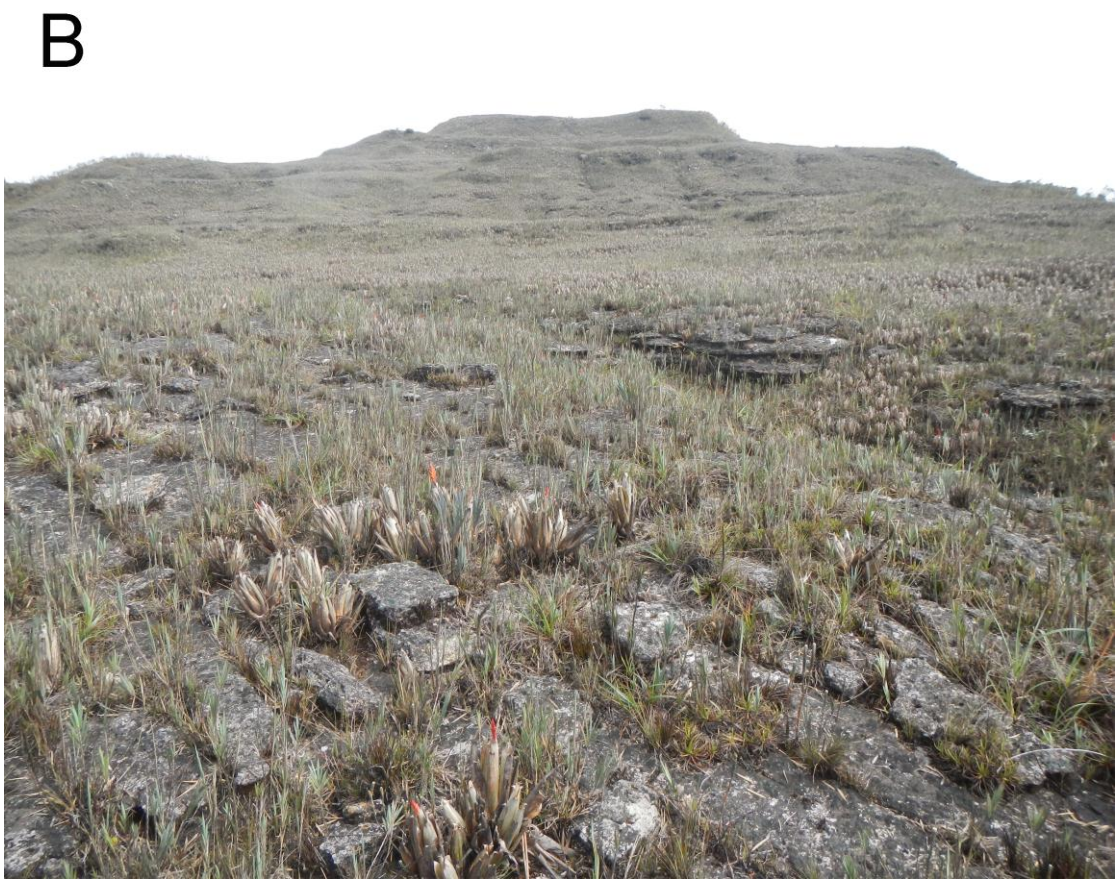

COLOMBIA
ECUADOR
PERU
Species
Clade A
Clade B
Clade C
P. macrbydei
Clade D
P. macbrydei
P. oni sp. n.
P. samek sp. n.
P. elquimi sp. n.
Altitude (m)
>3000
2001 - 3000
1001 - 2000
501 - 1000
1 - 500
12.50 25 50 75 100
Km

Figure 5.



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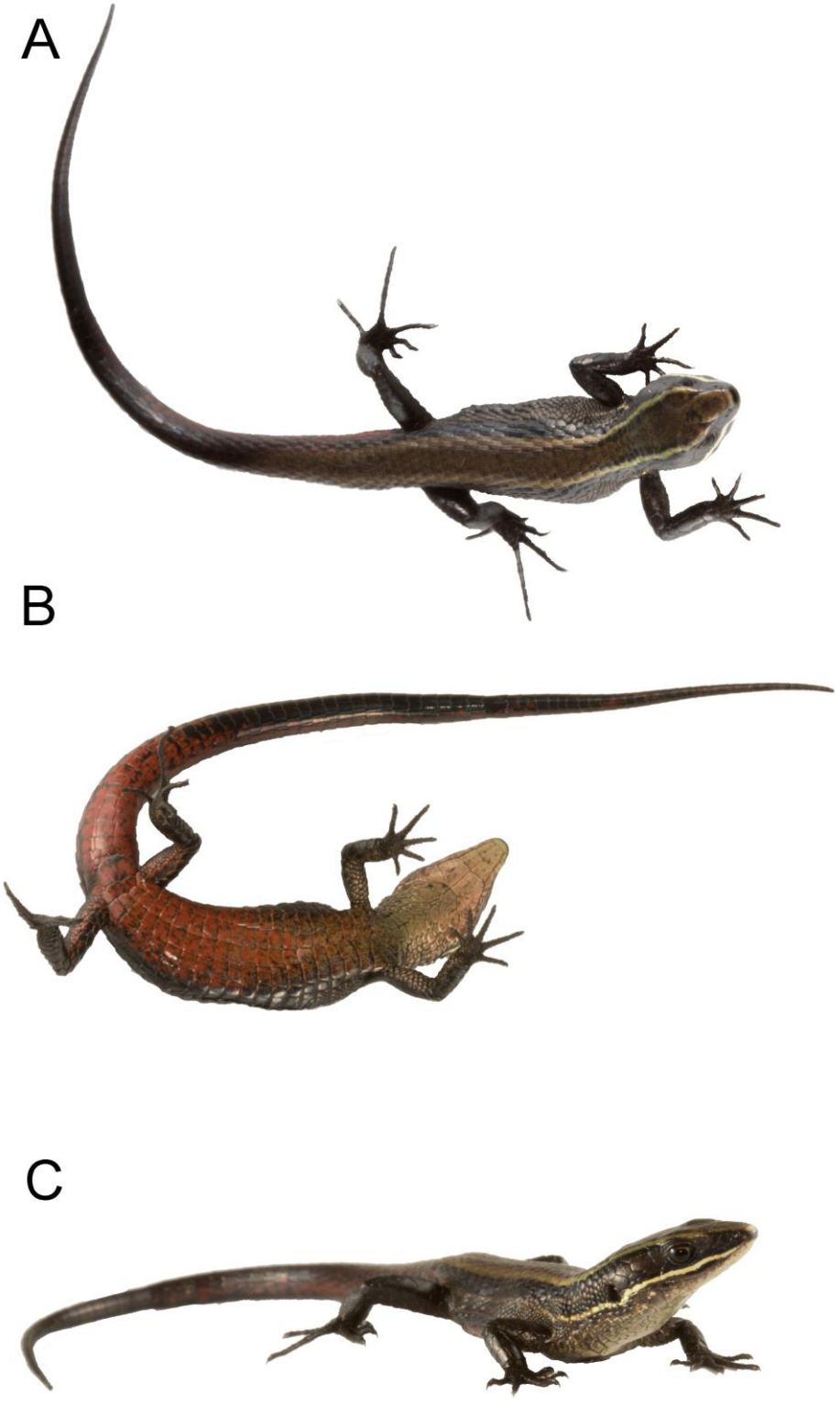

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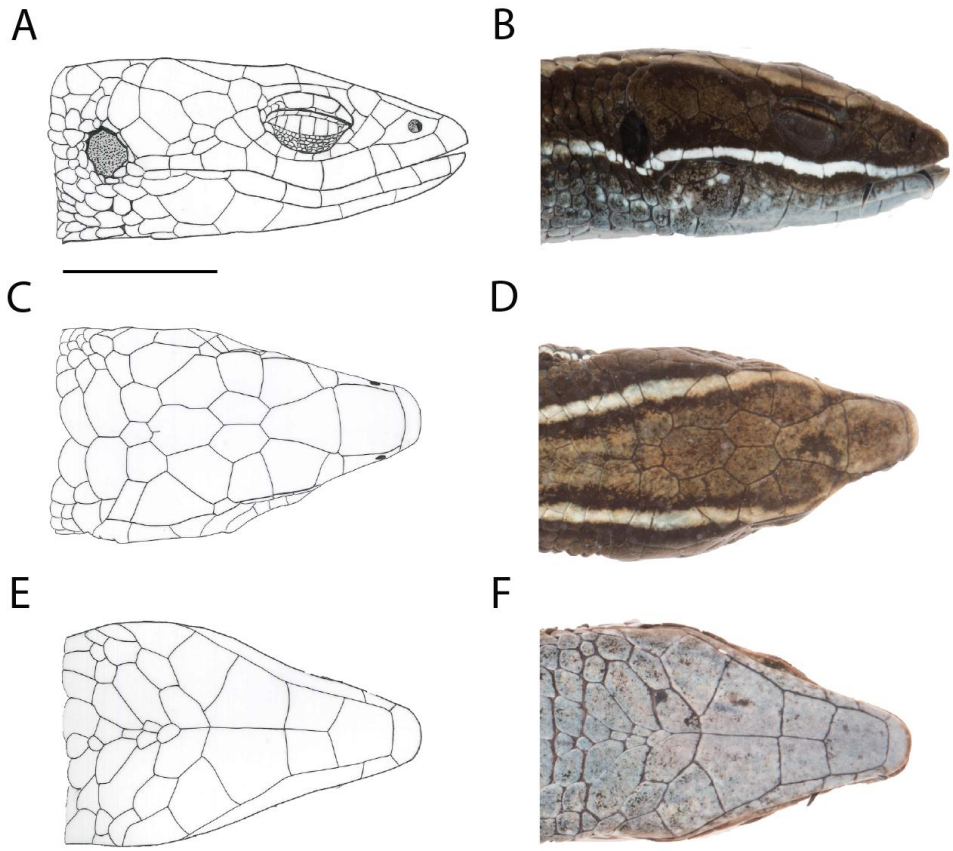

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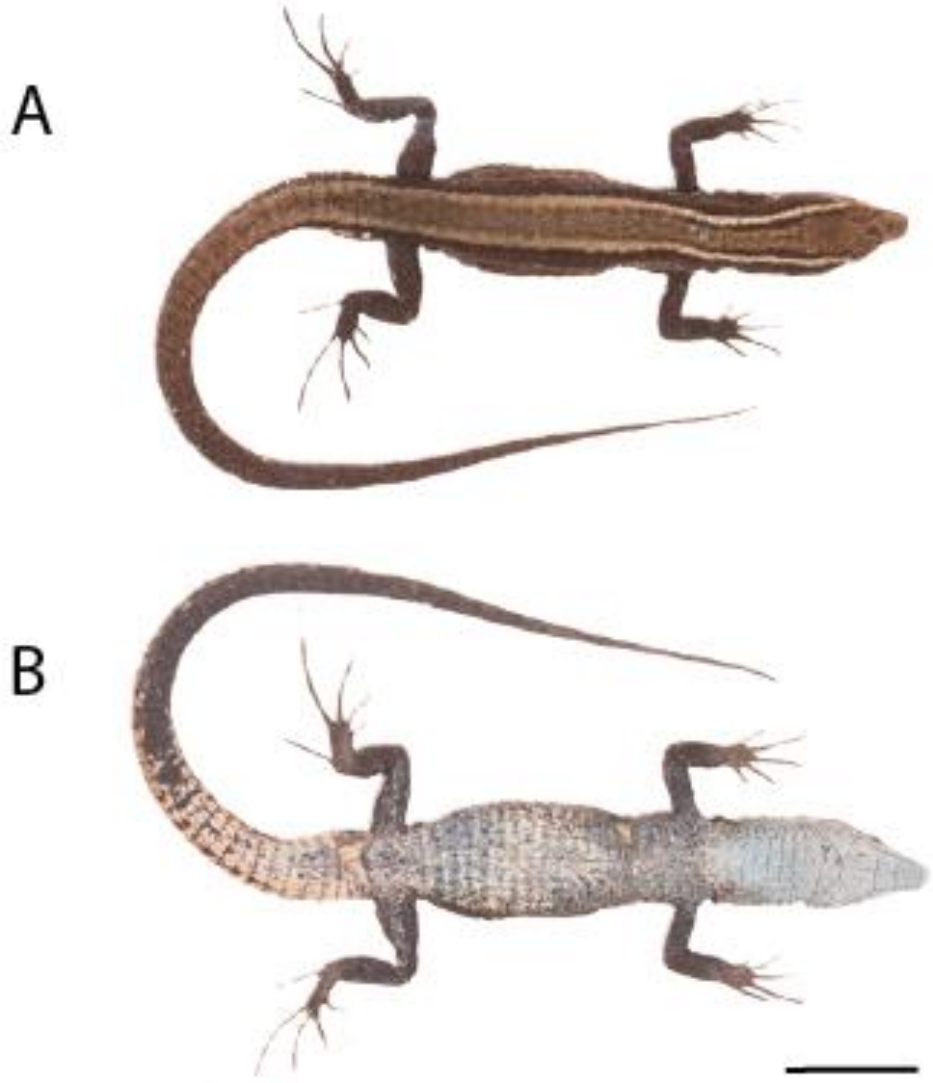
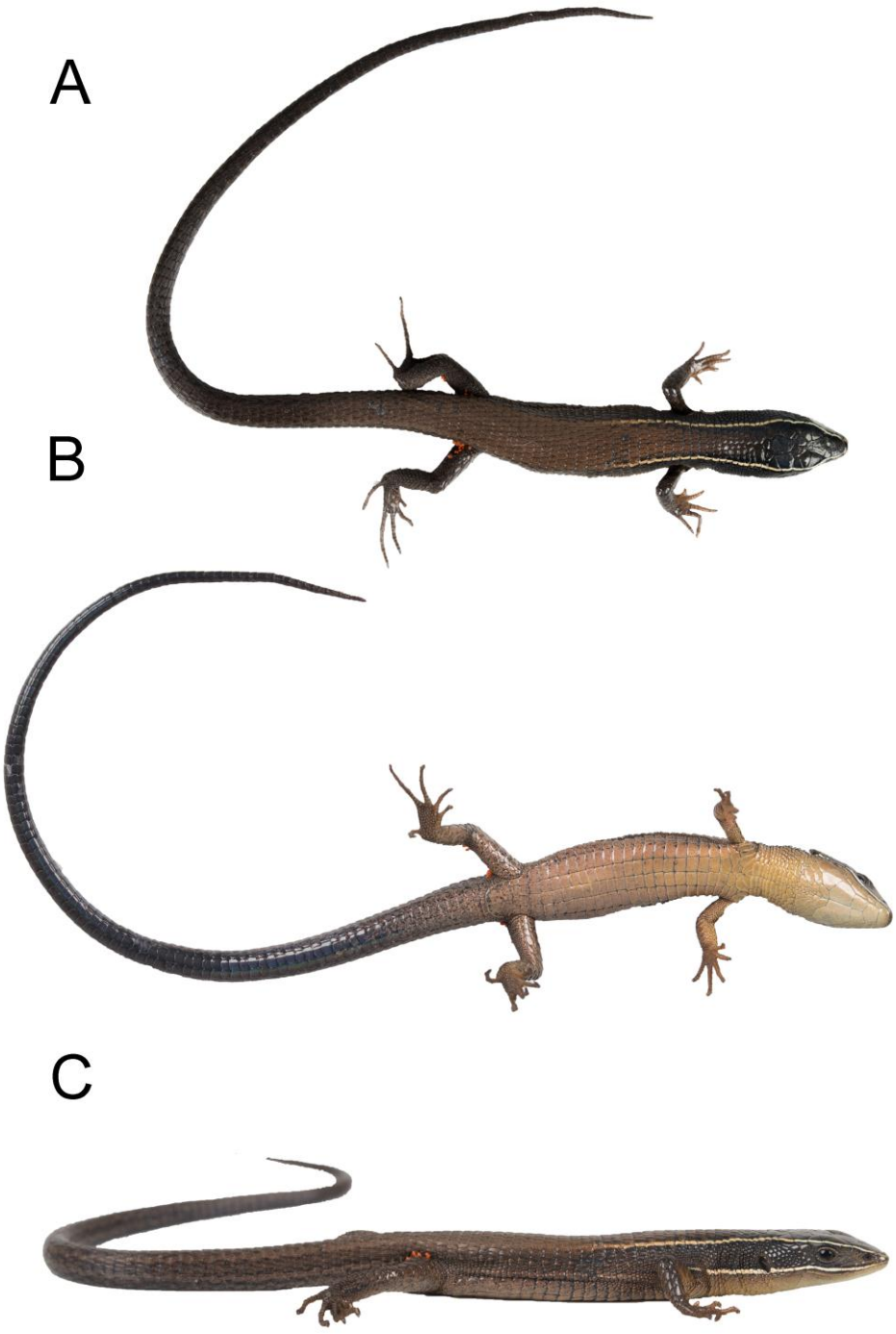


Figure 9.

Figure 10.



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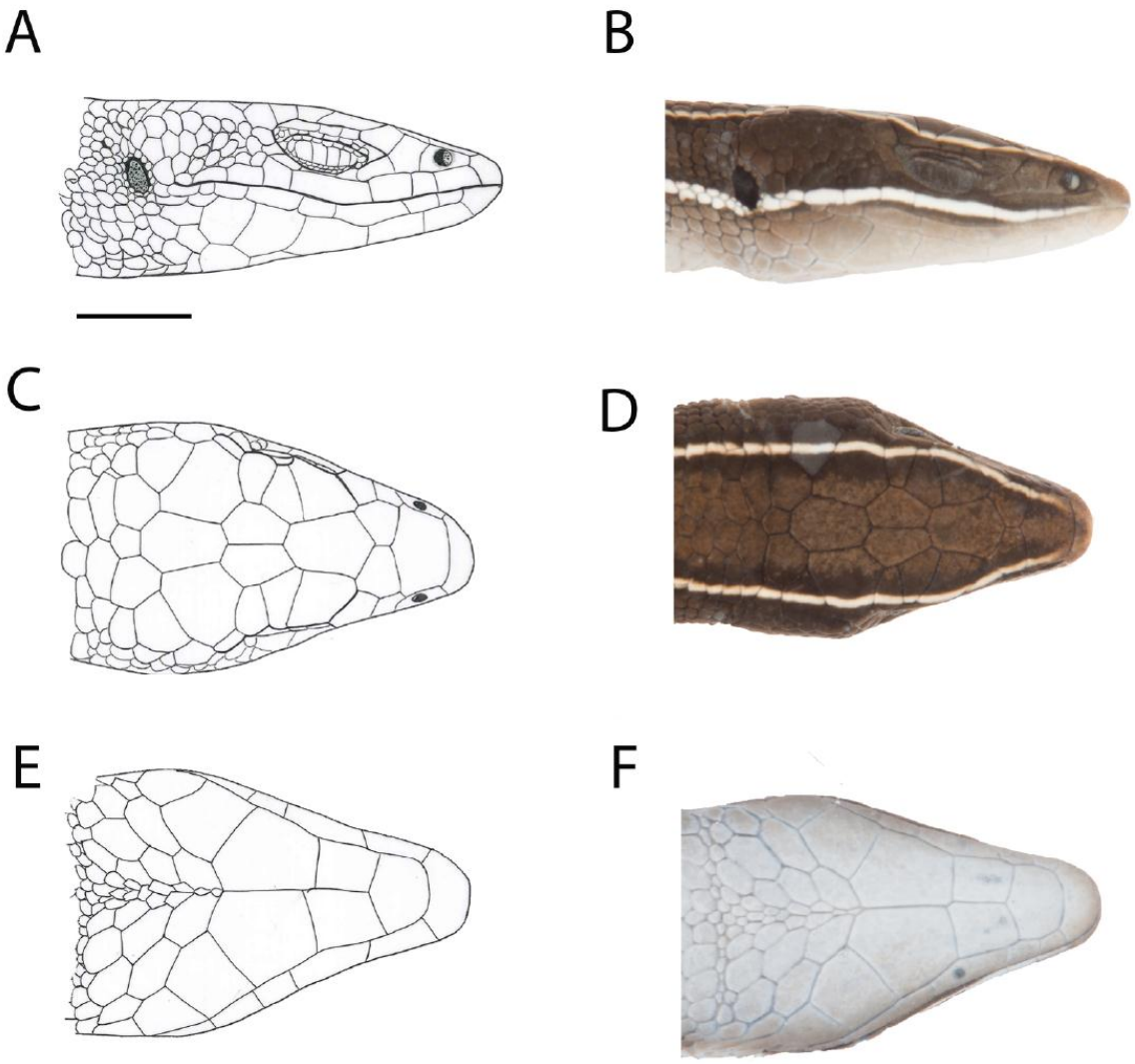

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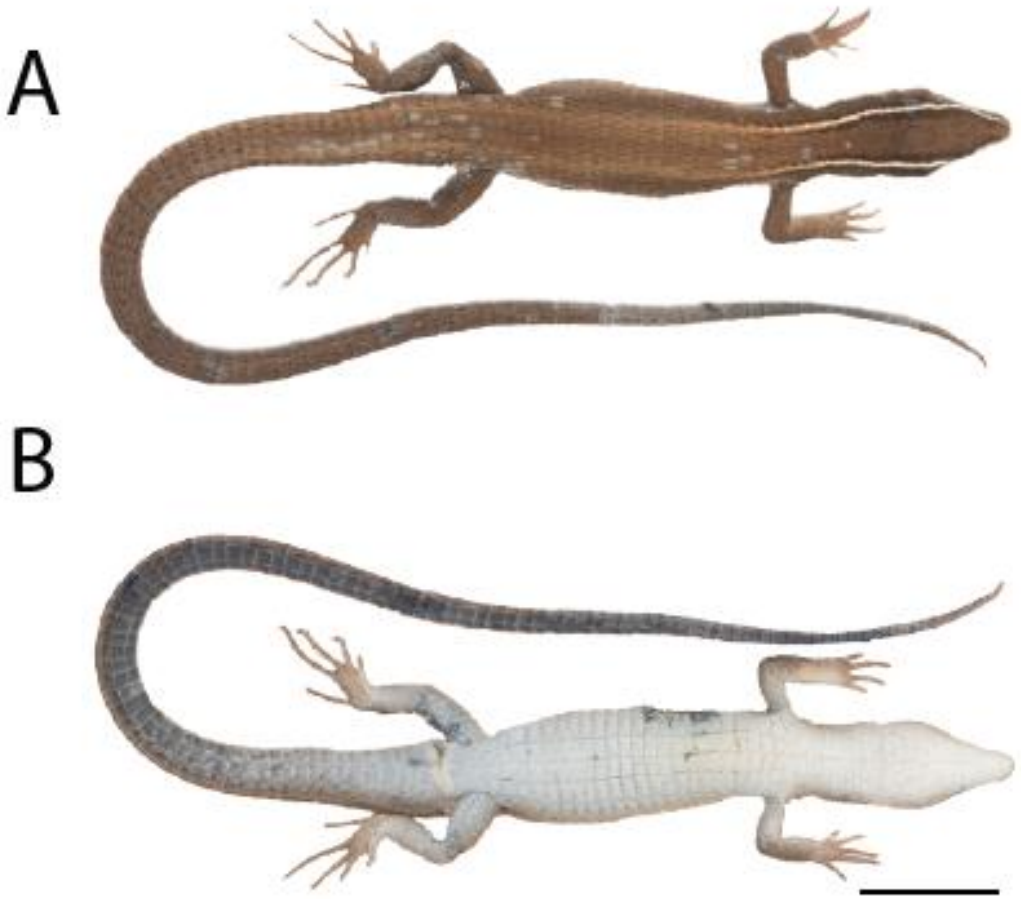
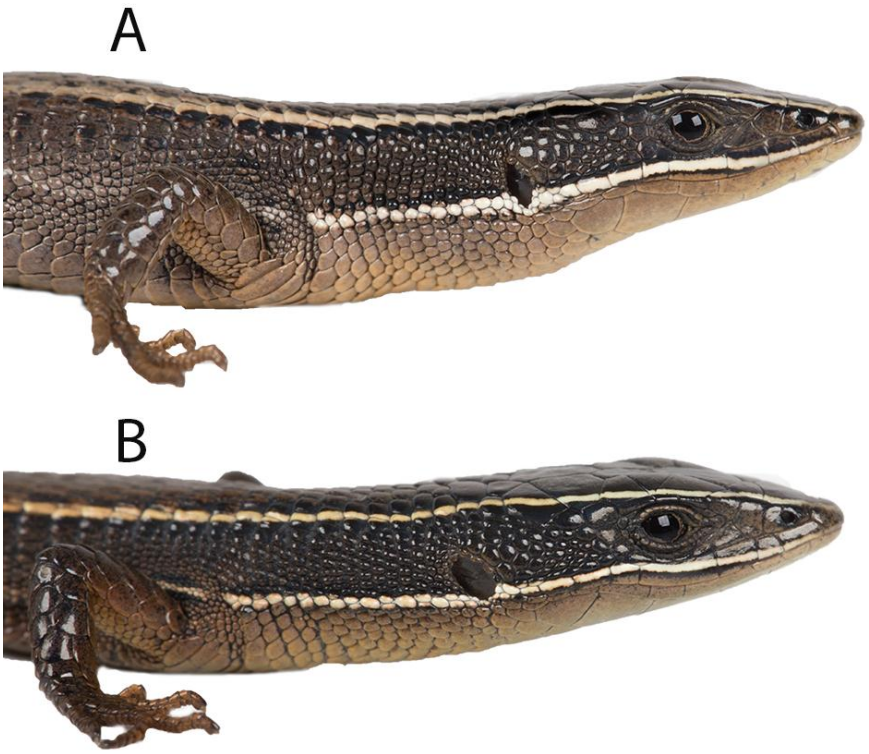


Figure 13.

Figure 14.



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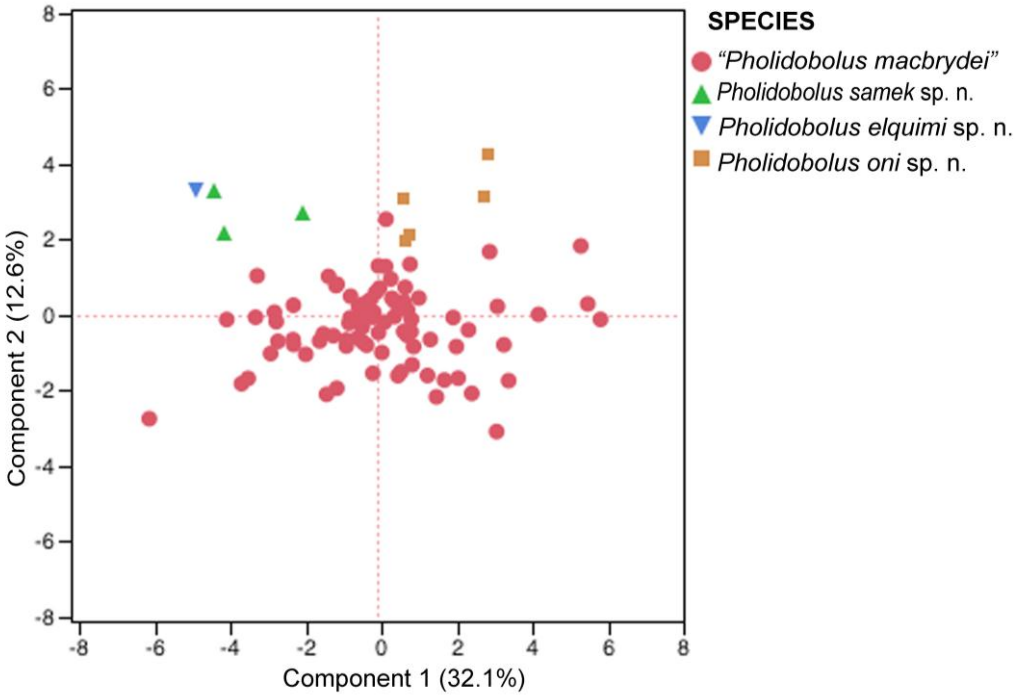

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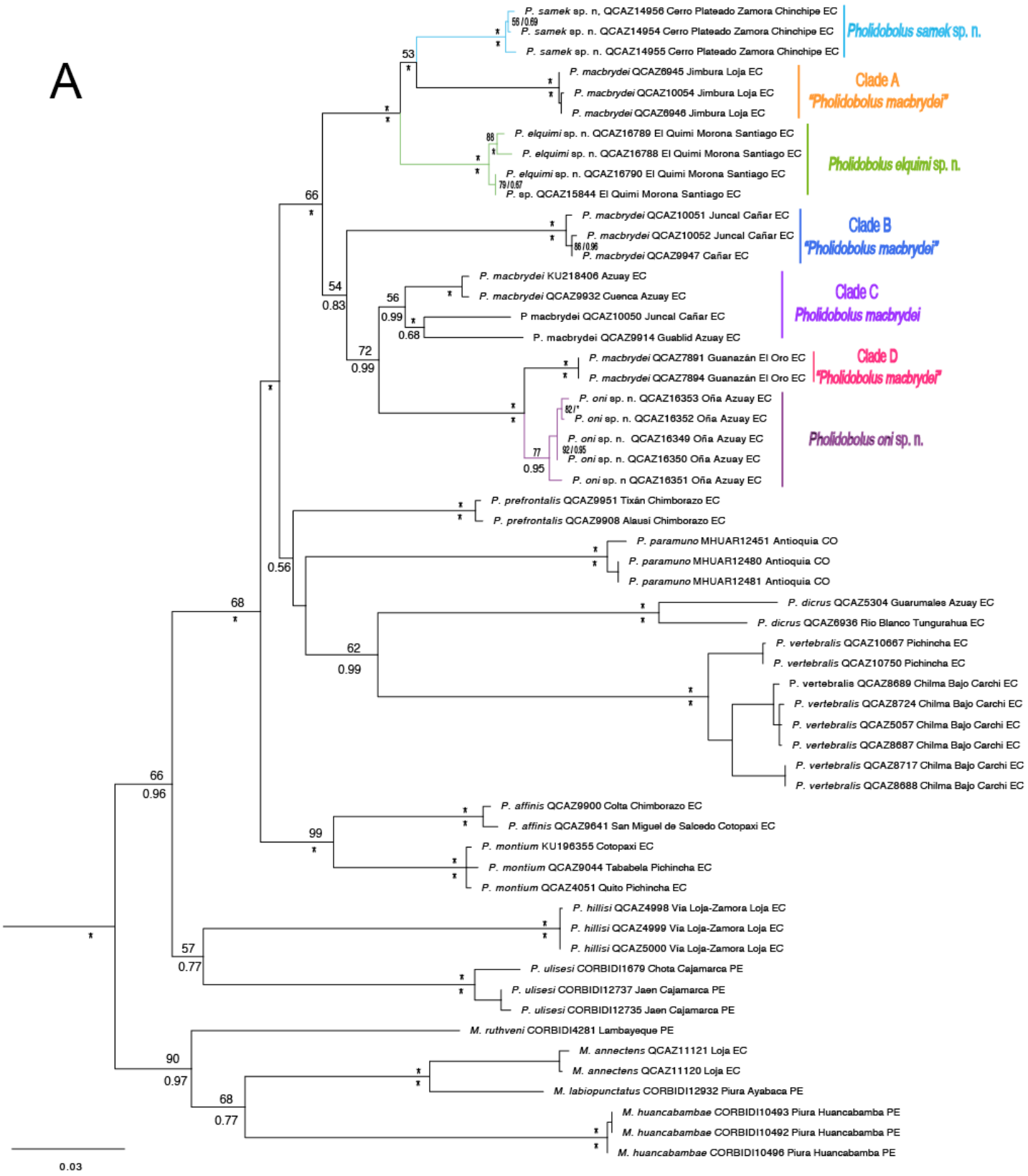
A
P. samek sp. n, QCAZ14956 Cerro Plateado Zamora Chinchipe EC
P. samek sp. n. QCAZ14954 Cerro Plateado Zamora Chinchipe EC
P. samek sp. n. QCAZ14955 Cerro Plateado Zamora Chinchipe EC
Pholidobolus samek sp. n.
P. macbrydei QCAZ6945 Jimbura Loja EC
P. macbrydei QCAZ10054 Jimbura Loja EC
P. macbrydei QCAZ6946 Jimbura Loja EC
Clade A
"Pholidobolus macbrydei"
P. elquimi sp. n. QCAZ16789 El Quimi Morona Santiago EC
P. elquimi sp. n. QCAZ16788 El Quimi Morona Santiago EC
P. elquimi sp. n. QCAZ16790 El Quimi Morona Santiago EC
P. sp. QCAZ15844 El Quimi Morona Santiago EC
Pholidobolus elquimi sp. n.
P. macbrydei QCAZ10051 Juncal Cañar EC
P. macbrydei QCAZ10052 Juncal Cañar EC
P. macbrydei QCAZ9947 Cañar EC
Clade B
"Pholidobolus macbrydei"
P. macbrydei KU218406 Azuay EC
P. macbrydei QCAZ9932 Cuenca Azuay EC
P macbrydei QCAZ10050 Juncal Cañar EC
P. macbrydei QCAZ9914 Guablid Azuay EC
Clade C
Pholidobolus macbrydei
P. macbrydei QCAZ7891 Guanazán El Oro EC
P. macbrydei QCAZ7894 Guanazán El Oro EC
Clade D
"Pholidobolus macbrydei"
P. oni sp. n. QCAZ16353 Oña Azuay EC
P. oni sp. n. QCAZ16352 Oña Azuay EC
P. oni sp. n. QCAZ16349 Oña Azuay EC
P. oni sp. n. QCAZ16350 Oña Azuay EC
P. oni sp. n QCAZ16351 Oña Azuay EC
Pholidobolus oni sp. n.
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P. prefrontalis QCAZ9908 Alausí Chimborazo EC
P. paramuno MHUAR12451 Antioquia CO
P. paramuno MHUAR12480 Antioquia CO
P. paramuno MHUAR12481 Antioquia CO
P. dicrus QCAZ5304 Guarumales Azuay EC
P. dicrus QCAZ6936 Río Blanco Tungurahua EC
P. vertebralis QCAZ10667 Pichincha EC
P. vertebralis QCAZ10750 Pichincha EC
P. vertebralis QCAZ8689 Chilma Bajo Carchi EC
P. vertebralis QCAZ8724 Chilma Bajo Carchi EC
P. vertebralis QCAZ5057 Chilma Bajo Carchi EC
P. vertebralis QCAZ8687 Chilma Bajo Carchi EC
P. vertebralis QCAZ8717 Chilma Bajo Carchi EC
P. vertebralis QCAZ8688 Chilma Bajo Carchi EC
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P. affinis QCAZ9641 San Miguel de Salcedo Cotopaxi EC
P. montium KU196355 Cotopaxi EC
P. montium QCAZ9044 Tababela Pichincha EC
P. montium QCAZ4051 Quito Pichincha EC
P. hillisi QCAZ4998 Vía Loja-Zamora Loja EC
P. hillisi QCAZ4999 Vía Loja-Zamora Loja EC
P. hillisi QCAZ5000 Vía Loja-Zamora Loja EC
P. ulisesi CORBIDI1679 Chota Cajamarca PE
P. ulisesi CORBIDI12737 Jaen Cajamarca PE
P. ulisesi CORBIDI12735 Jaen Cajamarca PE
M. ruthveni CORBIDI4281 Lambayeque PE
M. annectens QCAZ11121 Loja EC
M. annectens QCAZ11120 Loja EC
M. labiopunctatus CORBIDI12932 Piura Ayabaca PE
M. huancabambae CORBIDI10493 Piura Huancabamba PE
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M. huancabambae CORBIDI10496 Piura Huancabamba PE
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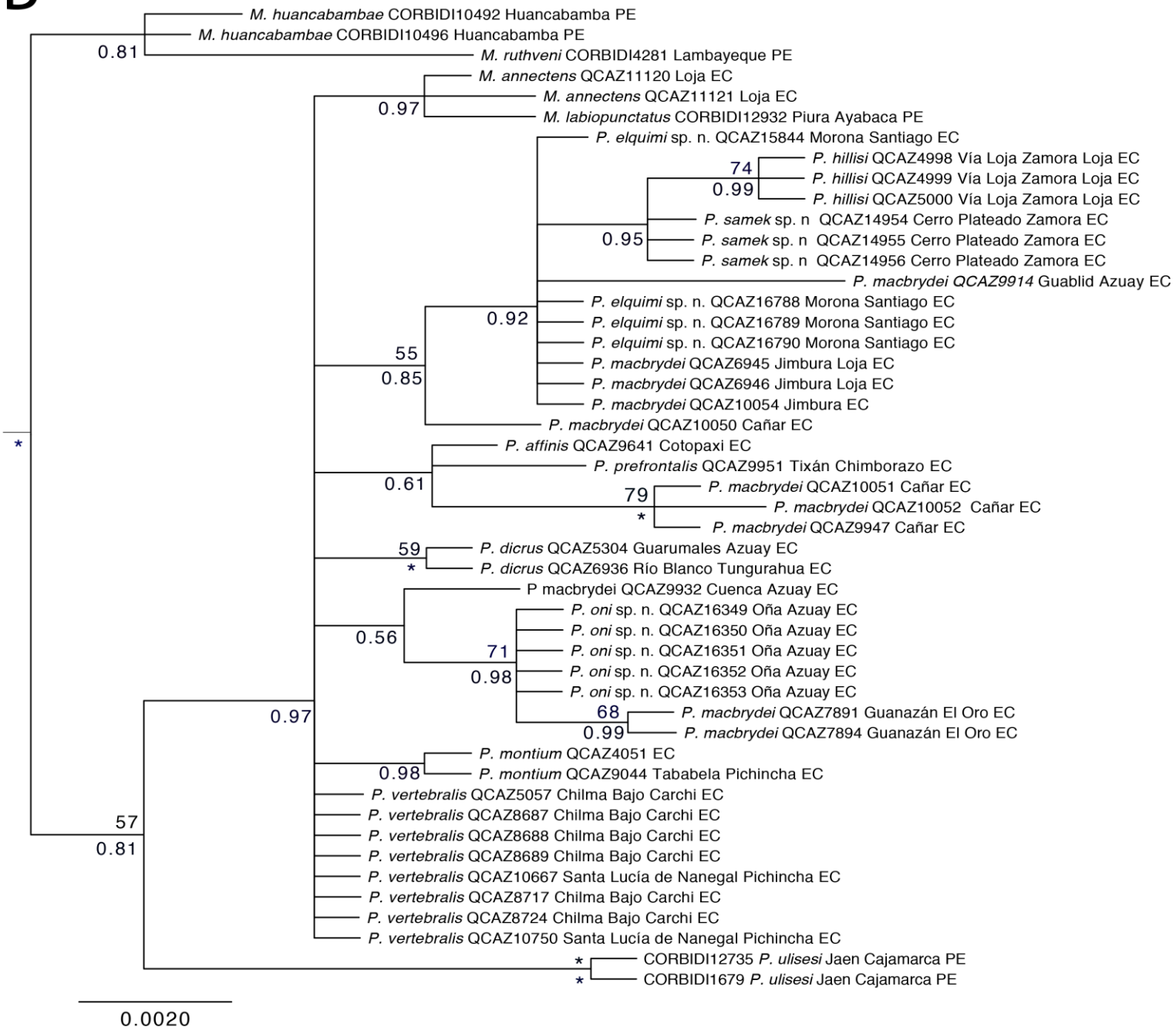
B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M. annectens QCAZ11121 Loja EC
0.97
M. labiopunctatus CORBIDI12932 Piura Ayabaca PE
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74
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P. hillisi QCAZ5000 Vía Loja Zamora Loja EC
P. samek sp. n QCAZ14954 Cerro Plateado Zamora EC
0.95
P. samek sp. n QCAZ14955 Cerro Plateado Zamora EC
P. samek sp. n QCAZ14956 Cerro Plateado Zamora EC
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P. elquimi sp. n. QCAZ16788 Morona Santiago EC
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P. elquimi sp. n. QCAZ16789 Morona Santiago EC
P. elquimi sp. n. QCAZ16790 Morona Santiago EC
55
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P. macbrydei QCAZ10054 Jimbura EC
P. macbrydei QCAZ10050 Cañar EC
*
P. affinis QCAZ9641 Cotopaxi EC
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P. macbrydei QCAZ10051 Cañar EC
79
P. macbrydei QCAZ10052 Cañar EC
*
P. macbrydei QCAZ9947 Cañar EC
59
P. dicrus QCAZ5304 Guarumales Azuay EC
*
P. dicrus QCAZ6936 Río Blanco Tungurahua EC
P macbrydei QCAZ9932 Cuenca Azuay EC
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0.98
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P. oni sp. n. QCAZ16353 Oña Azuay EC
0.97
68
P. macbrydei QCAZ7891 Guanazán El Oro EC
0.99
P. macbrydei QCAZ7894 Guanazán El Oro EC
P. montium QCAZ4051 EC
0.98
P. montium QCAZ9044 Tababela Pichincha EC
P. vertebralis QCAZ5057 Chilma Bajo Carchi EC
57
P. vertebralis QCAZ8687 Chilma Bajo Carchi EC
0.81
P. vertebralis QCAZ8688 Chilma Bajo Carchi EC
P. vertebralis QCAZ8689 Chilma Bajo Carchi EC
P. vertebralis QCAZ10667 Santa Lucía de Nanegal Pichincha EC
P. vertebralis QCAZ8717 Chilma Bajo Carchi EC
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P. vertebralis QCAZ10750 Santa Lucía de Nanegal Pichincha EC
*
CORBIDI12735 P. ulisesi Jaen Cajamarca PE
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Figure 17.

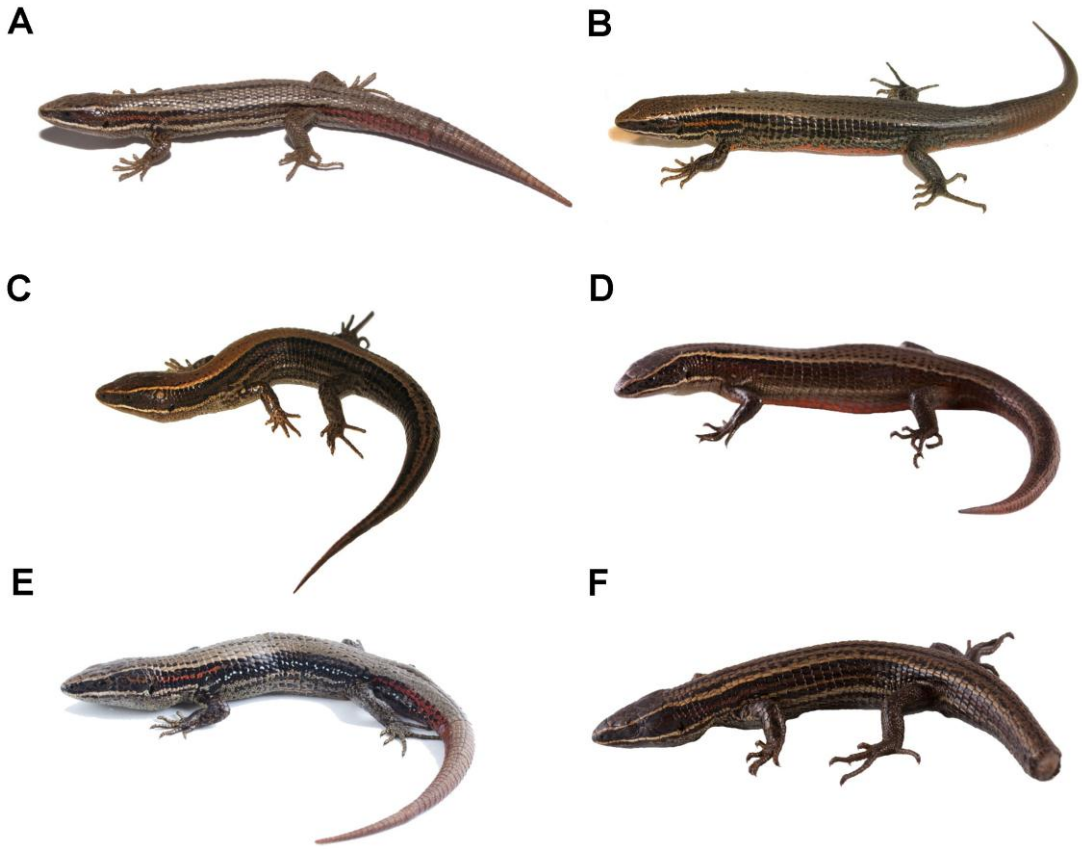

Table 1. Vouchers, locality data, and GenBank accession numbers of taxa included in this study. * represents sequences to be added in GenBank.

Taxon	Voucher	Locality	GenBank number			
			12S	16S	ND4	DNAH3
Anadia rhombifera	QCAZ 11862	QCAZ 11862; Ecuador: Cotopaxi: San Francisco de Las Pampas	KU902135	KU902216	KU902291	*
Macropholidus annectens	QCAZ 11120	Ecuador: Loja: 15 km E Loja	KC894341	KC894355	KC894369	*
Macropholidus annectens	QCAZ 11121	Ecuador: Loja: 15 km E Loja	KC894342	KC894356	KC894370	*
Macropholidus huancabambae	CORBIDI 10492	Peru: Piura: Huancabamba: Las Pozas	KC894343	KC894357	KC894371	*
Macropholidus huancabambae	CORBIDI 10493	Peru: Piura: Huancabamba: Las Pozas	KC894344	KC894358	KC894372	*
Macropholidus huancabambae	CORBIDI 10496	Peru: Piura: Huancabamba: Las Pozas	KC894345	KC894359	KC894373	*
Macropholidus labiopunctatus	CORBIDI 12932	Peru: Piura: Ayabaca	KP874774	KP874826	KP874936	*
Macropholidus ruthveni	CORBIDI 4281	Peru: Lambayeque: El Totora	KC894354	KC894368	KC894382	*
Pholidobolus affinis	QCAZ 9641	Ecuador: Cotopaxi: San Miguel de Salcedo, Cutuchi River	KC894348	KC894362	KC894376	*
Pholidobolus affinis	QCAZ 9900	Ecuador: Chimborazo: Colta	KC894349	KC894363	KC894377	*
***Pholidobolus samek* sp. n.**	QCAZ14954	Ecuador: Zamora Chinchipe: Cerro Plateado	*	*	*	*
***Pholidobolus samek* sp. n.**	QCAZ 14955	Ecuador: Zamora Chinchipe: Cerro Plateado	*	*	*	*
***Pholidobolus samek* sp. n.**	QCAZ 14956	Ecuador: Zamora Chinchipe: Cerro Plateado	*	*	*	*
Pholidobolus dicrus	QCAZ 5304	Ecuador: Morona-Santiago: Guarumales	KP874776	KP874828	KP874938	*
Pholidobolus dicrus	QCAZ 6936	Ecuador: Tungurahua: Río Blanco		KP874829	KP874939	*
***Pholidobolus elquimi* sp. n.**	QCAZ 16788	Ecuador: Morona-Santiago: el Quimi	*	*	*	*
***Pholidobolus elquimi* sp. n.**	QCAZ 16789	Ecuador: Morona-Santiago: el Quimi	*	*	*	*
***Pholidobolus elquimi* sp. n.**	QCAZ 16790	Ecuador: Morona-Santiago: el Quimi	*	*	*	*

***Pholidobolus elquimi* sp. n.**	QCAZ 15844	Ecuador: Morona-Santiago: el Quimi	*	*	*	*
Pholidobolus hillisi	QCAZ 4998	Ecuador: Zamora-Chinchipe: near San Francisco Research Station	KP090167	KP090170	KP090173	*
Pholidobolus hillisi	QCAZ 4999	Ecuador: Zamora-Chinchipe: near San Francisco Research Station	KP090169	KP090172	KP090175	*
Pholidobolus hillisi	QCAZ 5000	Ecuador: Zamora-Chinchipe: near San Francisco Research Station	KP090168	KP090171	KP090174	*
"Pholidobolus macbrydei"	KU 218406	Ecuador: Azuay: Cuenca	AY507848	AY507867	AY507886	*
"Pholidobolus macbrydei"	QCAZ 9914	Ecuador: Azuay: Guablid	KC894352	KC894366	KC894380	*
"Pholidobolus macbrydei"	QCAZ 9932	Ecuador: Azuay: 20 km on road Cuenca-El Cajas	KC894353	KC894367	KC894381	*
"Pholidobolus macbrydei"	QCAZ 9947	Ecuadro: Cañar: Cañar	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 10051	Ecuador: Cañar: Río Guallicanga, quebrada Juncal	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 10052	Ecuador: Cañar: Río Guallicanga, quebrada Juncal	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 10050	Ecuador: Cañar: A 1000 m de la Panamericana Juncal	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 6945	Ecuador: Loja: Jimbura	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 6946	Ecuador: Loja: Jimbura	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 10054	Ecuadro: Loja: Colambo Yacuri Forest	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 7894	Ecuador: El Oro: Guanazán	*	*	*	*
"Pholidobolus macbrydei"	QCAZ 7891	Ecuador: El Oro: Guanazán	*	*	*	*
Pholidobolus montium	QCAZ 4051	Ecuador: Pichincha: Quito	KC894346	KC894360	KC894374	*
Pholidobolus montium	QCAZ 9044	Ecuador: Pichincha: Tababela	KC894347	KC894361	KC894375	*
***Pholidobolus oni* sp. n.**	QCAZ 16349	Ecuador: Cañar: Oña	*	*	*	*
***Pholidobolus oni* sp. n.**	QCAZ 16350	Ecuador: Cañar: Oña	*	*	*	*
***Pholidobolus oni* sp. n.**	QCAZ 16351	Ecuador: Cañar: Oña	*	*	*	*
***Pholidobolus oni* sp. n.**	QCAZ 16352	Ecuador: Cañar: Oña	*	*	*	*
***Pholidobolus oni* sp. n.**	QCAZ 16353	Ecuador: Cañar: Oña	*	*	*	*

Pholidobolus paramuno	MHUAR 12451	Colombia: Antoquia	MK215018	MK215032	MK215046	---
Pholidobolus paramuno	MHUAR 12480	Colombia: Antoquia	MK215019	MK215033	MK215047	---
Pholidobolus paramuno	MHUAR 12481	Colombia: Antoquia	MK215020	MK215034	MK215048	---
Pholidobolus prefrontalis	QCAZ 9908	Ecuador: Chimborazo: Alausí	KC894350	KC894364	KC894378	*
Pholidobolus prefrontalis	QCAZ 9951	Ecuador: Chimborazo: Tixán	KC894351	KC894365	KC894379	*
Pholidobolus ulisesi	CORBIDI 12735	Peru: Cajamarca: Jaen: Huamantanga Forest	KP874787	KP874839	KP874948	*
Pholidobolus ulisesi	CORBIDI 12737	Peru: Cajamarca: Jaen: Huamantanga Forest	KP874788	KP874840	KP874949	*
Pholidobolus ulisesi	CORBIDI 1679	Perú: Chota: La Granja	KP874786	KP874838	KP874947	*
Pholidobolus vertebralis	QCAZ 10667	Ecuador: Pichincha: Santa Lucía de Nanegal	KP874784	KP874836	KP874946	*
Pholidobolus vertebralis	QCAZ 10750	Ecuador: Pichincha: Santa Lucía de Nanegal	KP874785	KP874837	KP874947	*
Pholidobolus vertebralis	QCAZ 5057	Ecuador: Carchi: Chilma Bajo	KP874778	KP874830	KP874940	*
Pholidobolus vertebralis	QCAZ 8687	Ecuador: Carchi: Chilma Bajo	KP874779	KP874831	KP874941	*
Pholidobolus vertebralis	QCAZ 8688	Ecuador: Carchi: Chilma Bajo	KP874780	KP874832	KP874942	*
Pholidobolus vertebralis	QCAZ 8689	Ecuador: Carchi: Chilma Bajo	KP874781	KP874833	KP874943	*
Pholidobolus vertebralis	QCAZ 8717	Ecuador: Carchi: next to Chilma Bajo	KP874782	KP874834	KP874944	*
Pholidobolus vertebralis	QCAZ 8724	Ecuador: Carchi: next to Chilma Bajo	KP874783	KP874835	KP874945	*

Table 2. Summary of morphological characters and measurements (mm) of *Pholidobolus samek* sp. n., *P. elquimi* sp. n. and *P. oni* sp. n. Range (first line) and mean ± standard deviation (second line) are given for quantitative characters, except when there was no variation.

Character	*P. samek* sp. n. **N**=3	*P. elquimi* sp. n. **N**=4	*P. oni* sp. n. **N**=5
Scales along margin of upper jaw	10	8–9 8.75 ± 0.5	10–11 10.2 ± 0.48
Gulars	16–18 17 ± 1	15	22–23 22.8 ± 0.48
Ventrals in transverse row at midbody	20–21 20.3 ± 0.58	18–20 19.25 ± 0.96	25–27 25.8 ±0.84
Dorsals from the occiput to base of tail	27–28 27.7 ± 0.58	27–31 28.25 ± 1.89	35–40 36.8 ± 2.04
Temporals	4-5 4.33 ± 0.58	5	7–9 8 ± 0.70
Scales around midbody	26–32 29.67 ± 3.21	27–28 27.5 ± 0.58	31–33 32.2 ± 0.84
Lower eyelid scales	4	4–5 4.25 ± 0.5	4–6 4.8 ± 0.84
Head length	9.92–10.92 10.76 ± 0.77	11.01	8.51–10.58 9.46 ± 0.88
Head width	6.46–7.41 6.93 ± 0.48	6.62	4.93–6.30 5.74 ± 0.88
Snout-vent length	41.65–49.29 45.89 ± 3.89	42.71	35.48–50.55 41.77 ± 6.40

Table 3. Character loadings, eigenvalues, and percentage of variance explained by Principal Components (PC) I and II. The analysis was based on fifteen morphological characters of adult specimens of *"Pholidobolus macbrydei"*, *Pholidobolus samek* sp. n., *Pholidobolus elquimi* sp. n. and *Pholidobolus oni* sp. n. Highest loadings are in bold.

Variable	**PCA**	
	PC I	**PC II**
NSO	0.01	0.20
SUJ	0.26	-0.01
SLJ	0.17	-0.24
SGJ	0.28	0.14
SGV	0.31	-0.22
DEL	0.30	-0.38
NTS	0.29	0.25
SAB	**0.32**	-0.29
SAT	0.24	-0.42
SF3	0.26	**0.38**
SF5	0.26	**0.33**
ST3	0.30	0.16
ST4	0.24	0.30
ST5	**0.32**	-0.00
LES	-0.02	-0.02
Eigenvalue	4.82	1.90
%	32.12	12.64

Table 4. Pairwise genetic distances (uncorrected *p*) of 16S DNA sequences among samples of *Pholidobolus* included in this study.

	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Pholidobolus. elquimi* sp. n.														
2	*Pholidobolus samek* sp. n.	0.03													
3	*Pholidobolus oni* sp. n.	0.04	0.03												
4	Clade A	0.03	0.02	0.04											
5	Clade B	0.03	0.03	0.03	0.02										
6	Clade C	0.03	0.02	0.03	0.03	0.03									
7	Clade D	0.04	0.03	0.04	0.04	0.02	0.03								
8	*Pholidobolus affinis*	0.04	0.03	0.03	0.03	0.03	0.03	0.03							
9	*Pholidobolus dicrus*	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05						
10	*Pholidobolus hillisi*	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.05					
11	*Pholidobolus montium*	0.03	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.04	0.04				
12	*Pholidobolus paramuno*	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.04	0.04	0.04	0.03			
13	*Pholidobolus prefrontalis*	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.04	0.04	0.03	0.03		
14	*Pholidobolus ulisesi*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.04	0.05	0.05	0.05	
15	*Pholidobolus vertebralis*	0.05	0.04	0.04	0.05	0.04	0.04	0.04	0.05	0.05	0.05	0.04	0.04	0.04	0.06

Table 5. Pairwise genetic distances (uncorrected *p*) of 12S DNA sequences among samples of *Pholidobolus* included in this study.

	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Pholidobolus. elquimi* sp. n.														
2	*Pholidobolus samek* sp. n.	0.01													
3	*Pholidobolus oni* sp. n.	0.04	0.04												
4	Clade A	0.03	0.03	0.05											
5	Clade B	0.03	0.04	0.03	0.03										
6	Clade C	0.02	0.02	0.03	0.03	0.02									
7	Clade D	0.04	0.04	0.05	0.05	0.04	0.03								
8	*Pholidobolus affinis*	0.05	0.04	0.06	0.05	0.05	0.05	0.06							
9	*Pholidobolus dicrus*	0.07	0.07	0.08	0.07	0.07	0.08	0.08	0.08						
10	*Pholidobolus hillisi*	0.05	0.04	0.05	0.06	0.05	0.04	0.05	0.06	0.08					
11	*Pholidobolus montium*	0.03	0.04	0.05	0.04	0.04	0.04	0.05	0.02	0.07	0.05				
12	*Pholidobolus paramuno*	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.06	0.09	0.07	0.06			
13	*Pholidobolus prefrontalis*	0.02	0.02	0.04	0.03	0.03	0.03	0.05	0.03	0.05	0.04	0.03	0.05		
14	*Pholidobolus ulisesi*	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.07	0.04	0.03	0.05	0.03	
15	*Pholidobolus vertebralis*	0.08	0.08	0.09	0.08	0.07	0.08	0.08	0.08	0.06	0.10	0.08	0.09	0.06	0.08

Table 6. Pairwise genetic distances (uncorrected *p*) of ND4 DNA sequences among samples of *Pholidobolus* included in this study.

	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Pholidobolus. elquimi* sp. n.														
2	*Pholidobolus samek* sp. n.	0.08													
3	*Pholidobolus oni* sp. n.	0.13	0.12												
4	Clade A	0.09	0.09	0.13											
5	Clade B	0.12	0.12	0.12	0.07										
6	Clade C	0.10	0.11	0.10	0.11	0.11									
7	Clade D	0.12	0.12	0.04	0.13	0.12	0.09								
8	*Pholidobolus affinis*	0.13	0.14	0.15	0.14	0.15	0.12	0.15							
9	*Pholidobolus dicrus*	0.15	0.15	0.14	0.16	0.15	0.15	0.15	0.16						
10	*Pholidobolus hillisi*	0.17	0.17	0.17	0.17	0.18	0.15	0.16	0.17	0.19					
11	*Pholidobolus montium*	0.13	0.14	0.15	0.14	0.17	0.13	0.14	0.11	0.17	0.17				
12	*Pholidobolus paramuno*	0.14	0.15	0.16	0.15	0.16	0.14	0.16	0.14	0.17	0.17	0.15			
13	*Pholidobolus prefrontalis*	0.13	0.12	0.15	0.13	0.14	0.13	0.14	0.13	0.16	0.17	0.13	0.13		
14	*Pholidobolus ulisesi*	0.15	0.15	0.15	0.16	0.16	0.15	0.15	0.15	0.17	0.16	0.16	0.17	0.16	
15	*Pholidobolus vertebralis*	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.16	0.19	0.18	0.18	0.16	0.17	0.17

Appendix I.

Pholidobolus macbrydei.—ECUADOR: Provincia Azuay: 20 km Cuenca-El Cajas, 2°46’39”S, 79°10’12”W, 3,508 m, QCAZ 9932; Guablid, 2°46’30”S, 78°41’51”W, 2,453 m, QCAZ 9914. Provincia Cañar: Cañar, 2°33’39”S, 78°55’51”W, QCAZ 9947; Guallicanga river, 2°28’24”S, 78°58’22”W, 3,048 m, QCAZ 10051–52; Juncal, 2°28’24”S, 78°58’22”W, 3,048 m, QCAZ 10050. Provincia El Oro: Guanazán, 3°26’24”S, 79°29’13”W, 2,638 m, QCAZ 7891, 7894. Provincia Loja: Jimbura, Jimbura lake, 4°42’32”S, 79°26’48”W, 3,036 m, QCAZ 6945–46; Jimbura, path to Jimbura lake, 4°42’34”S, 79°26’8”W, 3,348 m, QCAZ 10054.