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A bizarre new species of *Lynchius* (Amphibia, Anura, Strabomantidae) from the Andes of Ecuador and first report of *Lynchius parkeri* in Ecuador

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Abstract

We describe a new species of *Lynchius* from the eastern montane forest of southern Ecuador. We also report the occurrence of *L. parkeri* in Ecuador, on paramos of Yacuri National Park, near the border with Peru. We used morphological and genetic evidence for the description of the new species and the new report of *L. parkeri*. Phylogenetic analyses were carried out using both maximum likelihood and Bayesian inference on DNA sequences for mitochondrial and nuclear genes. The phylogeny shows that *L. simmonsi* is sister to a clade composed of the remaining species of *Lynchius* and that the new species is sister to *L. flavomaculatus*. The new species has a prominent and heavily ossified head with noticeably spiculate cranial exostosis that easily distinguishes it from all its congeners. The dorsal region and limbs present several reduced subconical and rounded tubercles and pronounced dermal ridges on the dorsum. We also describe the osteology of the new species based on x-rays of the holotype. Only six species of *Lynchius*, two known to occur in Ecuador (*L. flavomaculatus* and *L. simmonsi*). The description of *L. megacephalus* **sp. n.** and new record of *L. parkeri* double the number of known *Lynchius* in Ecuador and suggest that the diversity of Ecuadorian and Peruvian *Lynchius* is still underestimated.

Key words: Systematics, osteology, morphology, co-ossified skull, natural history, Morona Santiago, new record

Introduction

The genus *Lynchius* was proposed by Hedges *et al.* (2008) to include three species: *L. flavomaculatus*, *L. nebulanastes*, and *L. parkeri*. The genus definition included characteristics such as a narrow head less wide than the body, tympanic membrane and annulus present, cranial crest absent (except in *L. flavomaculatus*), dentigerous processes of vomers prominent, oblique, adductor muscle in "S" condition, Finger I longer than Finger II, and dorsum and venter smooth. A very morphologically dissimilar species (*L. simmonsi*) was later included in the genus (Padial *et al.* 2012), rendering the characterization given by Hedges *et al.* (2008) incomplete. Recently, Motta *et al.* (2016) presented a systematic review of the genus and describe two new species of *Lynchius* (*L. oblitus* and *L. tabaconas*). They also presented a new diagnosis of *Lynchius* and identified a wide variation in the external characteristics of species of the genus.

The genus *Lynchius* is, thus, composed of six species, all distributed in the Andes southern Ecuador and northern Peru: *L. flavomaculatus* (Parker 1938), *L. nebulanastes* (Cannatella 1984), *L. oblitus* Motta, Chaparro, Pombal, Guayasamín, De la Riva & Padial 2016, *L. parkeri* (Lynch 1975), *L. simmonsi* (Lynch 1974) and *L. tabaconas* Motta, Chaparro, Pombal, Guayasamín, De la Riva & Padial 2016. Only *L. flavomaculatus* and *L.*

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simmonsi are known to occur in Ecuador. They occur in the montane forests, provinces of Loja, Morona Santiago, and Zamora Chinchipe (Ron *et al.* 2018). Recent field work at the Ecological Conservation Area Tinajillas-Río Gualaceño, Morona Santiago province, resulted in the discovery of a singular new species of *Lynchius* with prominent skull ossification. Herein we describe the new species based on genetic and morphological evidence (Harvey *et al.* 2013, Heinicke *et al.* 2015). We also report for the first time the occurrence of *L. parkeri* in Ecuador.

Material and methods

Collected specimens were deposited in the herpetology collection of the Museo de Zoología de la Universidad del Azuay (MZUA) and Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ). Morphology, osteology, and color characters for comparisons were obtained from specimens deposited at MZUA, ZQCAZ, and Instituto Nacional de Biodiversidad del Ecuador (INABIO). We also used the diagnoses from Motta *et al.* (2016) and Duellman & Lehr (2009) for the comparisons. Examined specimens are listed in Appendix 1.

Morphological data. Morphological terminology is based on Duellman & Lehr (2009) and Motta *et al.* (2016). Osteological description follows Duellman & Trueb (2015) and Laloy *et al.* (2013). The illustrations of osteology are based on x-rays and examination of the holotype. Photographs of the specimen in life were used to describe coloration.

Morphometric measurements follow Motta *et al.* (2016). All measurements were taken three times with a digital caliper to the nearest 0.1 mm. We analyzed the average value between measurements. Measured morphometric variables were: snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), interorbital distance (IOD), upper eyelid width (UEW), eye to nostril distance (EN), internarial distance (IND), eye to eye distance (EE), tympanic membrane height (TyH), tympanic membrane length (TyL), width of disc of Finger III (F3), width of disc of Finger IV (F4), arm length (FA), tibia length (TL), thigh length (TH), foot length (FL), and width of disc of Toe IV (T4). Sex was determined by direct examination of gonads in a lateral dissection.

Amplification and DNA sequencing. The specimens were euthanized with benzocaine (2%), fixed with formaldehyde (10%) and preserved in ethanol (75%). Tissue samples of the liver were placed in cryovials with ethanol (95%).

Total DNA was extracted from muscle or liver tissue preserved in 95% ethanol using Fujita's guanidine thiocyanate protocol (Esselstyn *et al.* 2008). We used Polymerase Chain Reaction (PCR) to amplify three mitochondrial genes: 12SrRNA (12S), 16S rRNA (16S), NADH dehydrogenase subunit 1 (ND1), and adjacent tRNAs ($tRNA^{Val}$, $tRNA^{Leu}$ and $tRNA^{Ile}$). Additionally, the nuclear gene for the recombination-activating 1 protein (RAG-1) was amplified. PCR amplifications were carried out under standard protocols. PCR primers are listed in Table 2. Amplicons were sequenced in both directions by Macrogen Inc. (Seoul, Republic of Korea).

Phylogenetic analysis and genetic distances. Genetic analyses were based on newly generated sequences of three individuals from three populations: one individual from Morona Santiago province and two individuals from two different populations from Loja province. A summary of localities, coordinates, GenBank accession numbers, and museum ID for new vouchers is given in Appendix 2. Our matrix also included available sequences of *Lynchius* from GenBank (http://www.ncbi.nlm.nih.gov/genbank) for the 12S, 16S, ND1, RAG1, and Tyrosinase precursor genes. Data for sequences downloaded from GenBank are detailed in Motta *et al.* (2016). We defined as outgroup species of *Bryophryne* (1 species), *Holoaden* (2 species), *Hypodactylus* (2 species), *Noblella* (3 species), *Oreobates* (13 species), *Phrynopus*, *Pristimantis*, and *Psychrophrynella*.

Data were assembled and aligned in GeneiousPro 5.4.6 (Biomatters Ltd.) under default settings for MAFFT (Katoh and Standley 2013). The matrix was then visually inspected to correct misaligned bases manually using Mesquite v2.75 (Maddison & Maddison 2011). The aligned matrix is available at https://zenodo.org under DOI 10.5281/zenodo.1477905. Prior to the concatenated analyses, we obtained single gene phylogenies (12S-16S, RAG-1, and Tyrosinase) to detect laboratory or alignment errors. Maximum likelihood (ML) phylogenetic analyses were carried out in Garli v2.0 (Zwickl 2006) starting from a stepwise tree and using GTR + I + G as substitution model. Other search parameters were set to default values. The resulting gene trees only differed in weakly supported nodes (Bayesian posterior probability < 0.95, non-parametric bootstrap < 70) and therefore we concatenated the matrices to conduct phylogenetic analyses based on all genes.

For the phylogenetic analyses of the concatenated matrix, we used PartitionFinder v1.1.1 (Lanfear *et al.* 2012) to determine the best-fitting partition scheme and models of molecular evolution according to the Bayesian

Information Criterion (BIC). We defined fourteen *a priori* partitions: one partition for each codon position in protein coding loci (ND1, RAG1, and Tyr, total = 9) plus one for each non-coding loci (12S, 16S, $tRNA^{leu}$, $tRNA^{val}$, $tRNA^{ile}$, total = 5). Both maximum likelihood (ML) and Bayesian inference methods were used to obtain the optimal tree of the combined and partitioned dataset using software Garli v2.0 (Zwickl 2006) and MrBayes v3.2.1 (Ronquist *et al.* 2012), respectively.

The maximum likelihood search consisted of 40 independent runs: 20 starting from stepwise-addition trees (streefname = stepwise) and 20 starting from random trees (streefname = random). Analyses were terminated when all searches resulted in similar likelihood values (within 2-ln units of each other). To estimate support values for the nodes, we performed non-parametric bootstrapping with 200 pseudoreplicates. Each search was performed with random starting trees (streefname = random). The search was set to stop after a maximum of 5000000 of generations (stopgen = 5000000). Other parameters were set to default values. The 50% majority-rule consensus tree was obtained in Mesquite v2.75 (Maddison & Maddison 2011).

Bayesian phylogenetic inference was carried out in MrBayes v.3.1.2 (Ronquist *et al.* 2012) under Markov chain Monte Carlo (MCMC) sampling. We conducted 6 parallel and independent runs. Each analysis consisted of 3×10^7 generations and four Markov chains with default heating settings. The prior of the rate matrix was a uniform dirichlet and we assumed equal probabilities for all topologies. The first 50% of generations were discarded as burn-in. Each run was considered finished when the average standard deviation of split frequencies was < 0.05 and effective sample size (ESS) was > 200 for all parameters. Values for ESS were obtained in Tracer v1.5 (Rambaut and Drummond 2007).

We calculated the uncorrected pairwise *p*-distances for the aligned 16S fragment (1681 bp) with 500 bootstrap pseudoreplicates to estimate distance variance and pairwise deletion in software MEGA 6 (Tamura *et al.* 2013). We excluded one specimen of *L. parkeri* (QCAZ 31466) and one of *L. oblitus* (MHNC 8676) from genetic distance calculations because their 16S fragment were too short (less than 500 bp) and they were not comparable in length to other samples.

Results

Phylogeny. The concatenated matrix included a total of 5143 bp (1045 bp of 12S, 71 bp of $tRNA^{val}$, 1679 bp of 16S, 74 bp of $tRNA^{leu}$, 961 bp of ND1, 118 bp of $tRNA^{ile}$, 652 bp of RAG-1, and 543 bp of Tyr). PartitionFinder selected a scheme of five partitions: (i) 3rd codon position of RAG-1 and 3rd codon position of Tyr under model K80 + G; (ii) 1st and 2nd positions of RAG-1, and 1st and 2nd position of Tyr under model HKY + I + G; (iii) 12S, 16S, $tRNA^{val}$, $tRNA^{leu}$, and $tRNA^{ile}$ and 1st position of ND1 under GTR + I + G; (iv) 2nd position of ND1 under model HKY + G; and (v) 3rd codon position of ND1 under GTR + G. Within *Lynchius*, the ML analysis (best topology log likelihood = –50449.2; Fig. 1) yielded a tree topologically identical to the Bayesian consensus tree except for intrapopulation relationships of *L. oblitus*. There are strong support values for the monophyly of *Lynchius* (BB = 100, PP = 1) and also for the relationships among species except for the position of *L. nebulanastes*. Within *Lynchius*, the earliest divergence occurs between *L. simmonsi* and the remaining species, which include the new species (Fig. 1).

Our analyses show that the new species is sister to *L. flavomaculatus*, and together they are sister to the recently described Peruvian species, *L. tabaconas* (Motta *et al.* 2016). Our phylogeny also supports the monophyly of *L. parkeri* (BB = 100, PP = 1) including sequences from populations of Peru and southern Ecuador (uncorrected *p*-distance between populations = 1.6%). This is the first record of *L. parkeri* from Ecuador. *Lynchius parkeri* is sister to a clade comprising *L. nebulanastes* and *L. oblitus*. Genetic distances between species ranged from 5% to 19% for 16S (Table 1). Intraspecific genetic distances were generally low (0.1–1.9%) except for the distance between two populations of *L. oblitus* (4.6%).

The genetic distance between the new species and *L. flavomaculatus*, its closest relative, is 4.7%. The unique morphology (see below), phylogenetic position, and large genetic distances relative to all described species of *Lynchius* demonstrate unequivocally that the specimen from Tinajillas-Río Gualaceño represents an undescribed species which we describe in the following section.

TABLE 1. Pairwise uncorrected p-genetic distances among *Lynchius* species. The data under the diagonal are the mean, standard error, and range of the distances between clades. The number of individuals for each comparison is shown above the diagonal. Bold numbers correspond to intraspecific genetic distances.

	L. flavomaculatus	*L. nebulanastes*	*L. parkeri*	*L. tabaconas*	*L. oblitus*	*L. megacephalus* sp. nov.	*L. simmonsi*
L. flavomaculatus	–	n = 2	n = 3	n = 3	n = 5	n = 2	n = 2
L. nebulanastes	0.079± 0.008	–	n = 3	n = 3	n = 5	n = 2	n = 2
L. parkeri	0.077± 0.008 0.073–0.081	0.076± 0.008 0.072–0.081	**0.019**	n = 4	n = 6	n = 3	n = 3
L. tabaconas	0.056± 0.006 0.056–0.057	0.087± 0.008 0.087	0.078± 0.008 0.076–0.081	**0.001**	n = 6	n = 3	n = 3
L. oblitus	0.07± 0.006 0.065–0.075	0.079± 0.007 0.075–0.082	0.071± 0.006 0.064–0.075	0.07± 0.006 0.061–0.082	**0.001– 0.046**	n = 5	n = 5
L. megacephalus	0.049± 0.007	0.083± 0.010	0.069± 0.010 0.068–0.071	0.055± 0.008 0.055	0.066± 0.008 0.038–0.071	–	n = 2
*L. **simm**onsi*	0.165± 0.013	0.166± 0.013	0.152± 0.013 0.146–0.158	0.158± 0.013 0.158	0.145± 0.013 0.085–0.173	0.159± 0.013	–

TABLE 2. List of primers used in the present study for amplification and sequencing of DNA.

Gene	Primer name	Sequence (5'–3')	Direction	Source
12S Mitochondrial	12Sh	AAAGGTTTGGTCCTAGCCTT	F	Cannatella *et al.* (1998)
	12SKH	GGGAACTACGAGCAAAGCTT	R	Goebel *et al.* (1999)
	12S-tRNAphe	ATAGCRCTGAARAYGCTRAGATG	F	Wiens *et al.* (2005)
	12S- tRNAval	TGTAAGCGARAGGCTTTKGTTAAGCT	R	Wiens *et al.* (2005)
16S Mitochondrial	16L19	AATACCTAACGAACTTAGCGATAGCTGGTT	F	Heinicke *et al.* (2007)
	16H36E	AAGCTCCAWAGGGTCTTCTCGTC	R	Heinicke *et al.* (2007)
	16L34	TTTAACGGCCGCGGTATCCTAACCG	F	Heinicke *et al.* (2007)
	16H47	AAAGRGCTTAGRTCTTTYGCA	R	Heinicke *et al.* (2007)
	12Sm	GGCAAGTCGTAACATGGTAAG	F	Goebel *et al.* (1999)
	16H13	CCGGTCTGAACTCAGATCACGTA	R	Goebel *et al.* (1999)
ND1 Mitochondrial	16S_Frog	TTACCCTRGGGATAACAGCGCAA	F	Wiens *et al.* (2005)
	tMet_frog	TTGGGGTATGGGCCCAAAAGCT	R	Wiens *et al.* (2005)
RAG1 Nuclear	R182	GCCATAACTGCTGGAGCATYAT	F	Heinicke *et al.* (2007)
	R270	AGYAGATGTTGCCTGGGTCTTC	R	Heinicke *et al.* (2007)

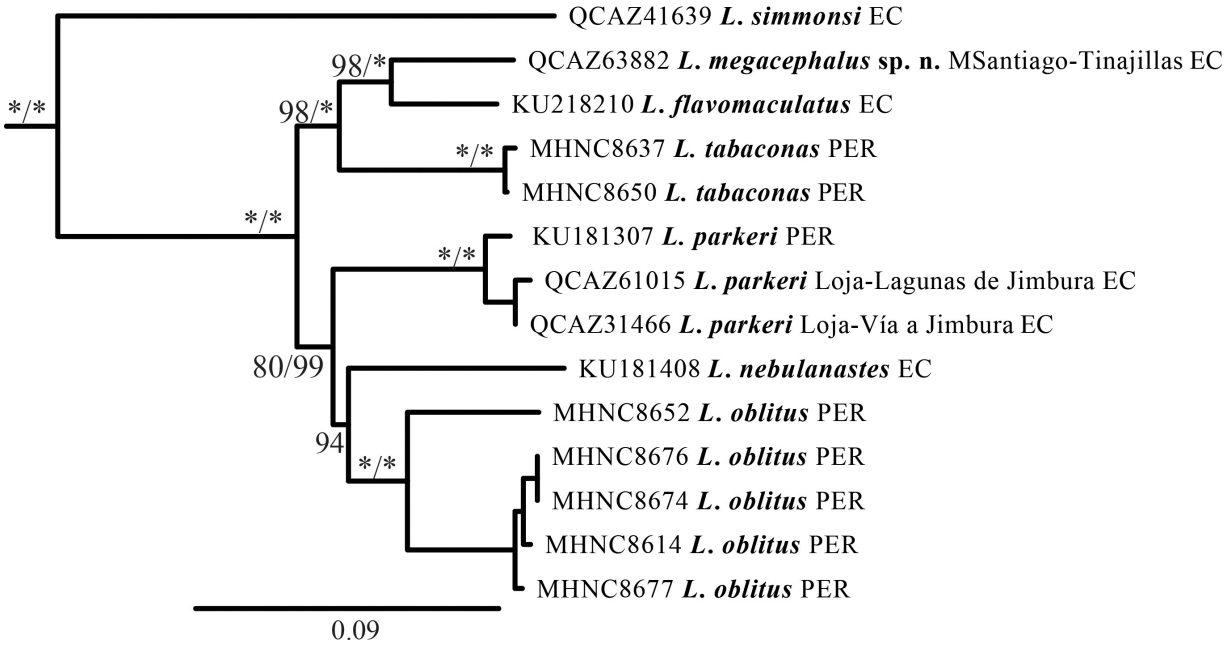

FIGURE 1. Phylogenetic relationships of *Lynchius* species. Maximum-likelihood tree obtained from analyses of mitochondrial (12S, 16S, and ND1) and nuclear (RAG and Tyr) DNA sequences from 14 specimens of *Lynchius* (excluding outgroup taxa). Numbers before slashes correspond to Bayesian posterior probabilities (PP); asterisks (*) represent PP = 1.0. Numbers after slashes are ML bootstrap support (BB) values; asterisks (*) represent BB = 100. PP and BB values < 0.50 or 50, respectively are not shown. Museum number and country are shown for each specimen. Locality is also shown for *L. megacephalus* **sp. n.** and *L. parkeri*. Locality details, as well as voucher, GenBank accession numbers, and literature references are provided in Appendix 2.

Lynchius megacephalus new species

Figs. 3–7|

Holotype. An adult female (MZUA.AN.0633) collected in September 2013 by Christian Nieves and Danny Villalta at the Ecological Conservation Area Tinajillas-Río Gualaceño (3.011677° S, 78.614464° W [WGS84], elevation: 2770 m), Morona Santiago province, Ecuador (Fig. 2).

Diagnosis. A member of the genus *Lynchius*, as defined by Motta *et al.* (2016). We base its assignment to *Lynchius* on the phylogeny (Fig. 1). *Lynchius megacephalus* is a large species (adult female SVL = 41.8 mm; males unknown) diagnosed by having: (1) skin on dorsum of head, body, flanks and limbs with many low subconical and rounded tubercles; with pronounced and irregular dermal ridges (more noticeable in life); postocular fold ›‹-shaped; dorsolateral fold absent; middorsal fold present, low; skin of venter smooth; discoidal and thoracic folds present; (2) tympanic membrane and annulus distinct, its diameter 64% of eye diameter, upper edge of tympanum obscured by supratympanic fold; three postrictal tubercles; (3) head wider than long (HW/HL=1.3) and wider than the body; snout rounded in dorsal and lateral views; nostrils laterally oriented; *canthus rostralis* slightly concave; upper eyelid covered by small scattered tubercles; (4) cranium heavily ossified, with spiculate dermal bones completely covering external surface of cranium; cranial crests present; (5) dentigerous processes of vomers prominent, oblique; choana rounded; tongue cordiform, attached to the floor of the mouth along 73% of its length; (7) digits long and slender; tips of digits narrow, circumferential groves absent and lateral fringes narrow, interdigital webbing absent; terminal phalanges knob-shaped; (8) finger I as long as II; subarticular tubercles prominent; few supernumerary tubercles, low and smaller than subarticular tubercles; single palmar tubercle; thenar tubercle prominent, almost the same size of the palmar tubercle; (9) ulnar tubercle absent or hidden by dermal tubercles in arms; (10) toe III as long as toe V; subarticular tubercles prominent toes III and V reaching proximal border of second subarticular tubercle of Toe IV; supernumerary tubercles absent; (11) inner metatarsal tubercle elongate, prominent, as the outer metatarsal tubercle; (12) heel and tarsal tubercles absent or hidden by dermal tubercles in legs.

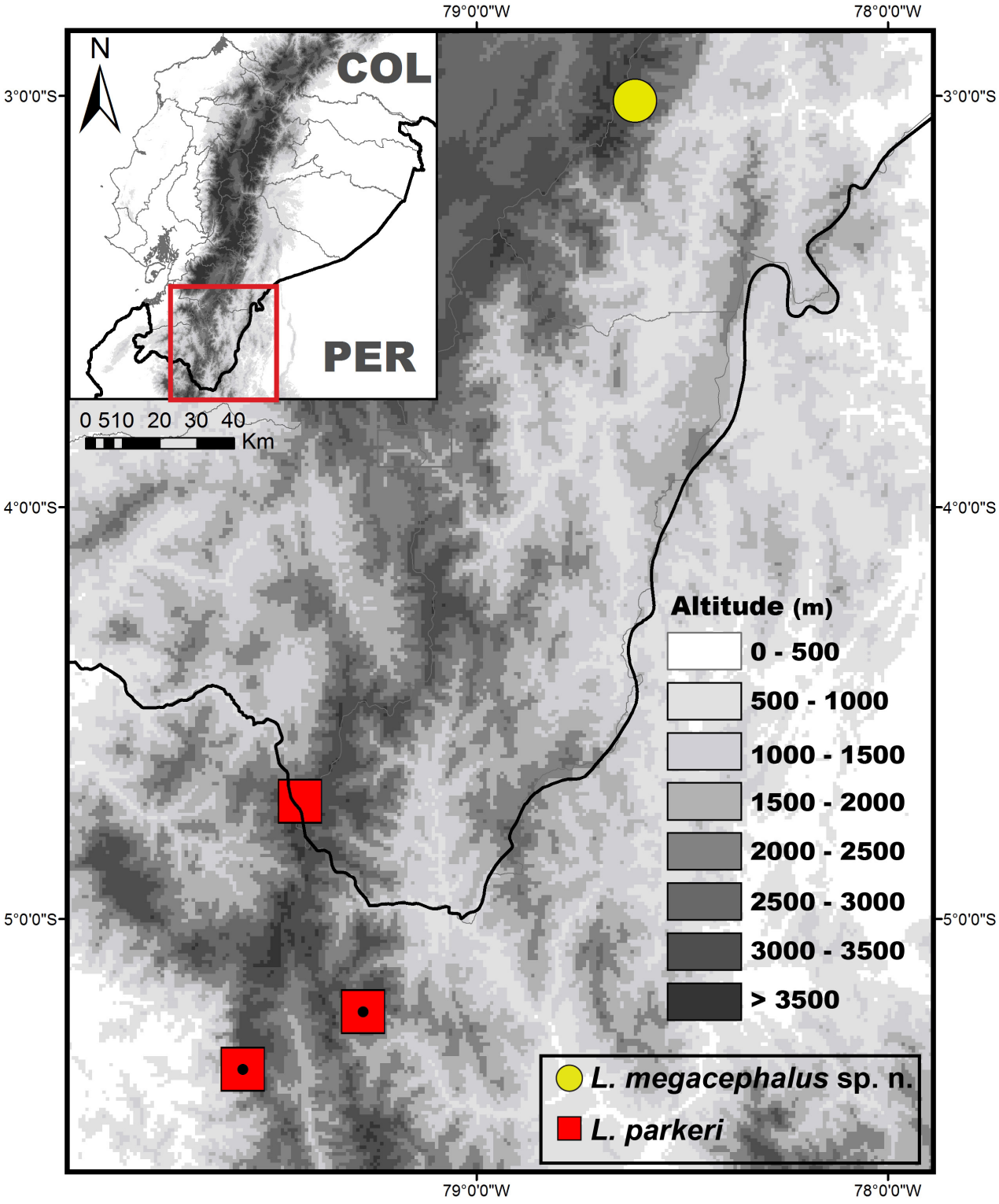

FIGURE 2. Map showing the type locality of the new species *Lynchius megacephalus* (yellow dot) from Ecological Conservation Area Tinajillas Río Gualaceño-Morona Santiago province, Ecuador (elevation: 2800 m), and localities of *Lynchius parkeri* (red squares) from Yacuri National Park, Laguna Jimbura-Loja province, Ecuador (elevation: 2990–3397 m) and previously known localities from Peru (red squares with a black point). The bold black line represents the boundary between Ecuador, Peru, and Colombia. Locality details are shown in Appendix 2. Abbreviations are Colombia = COL, Ecuador = ECU, Peru = PER.

FIGURE 3. Holotype of *Lynchius megacephalus* **sp. n.**, female MZUA.AN.0633 (SLV 41.8 mm) in life.

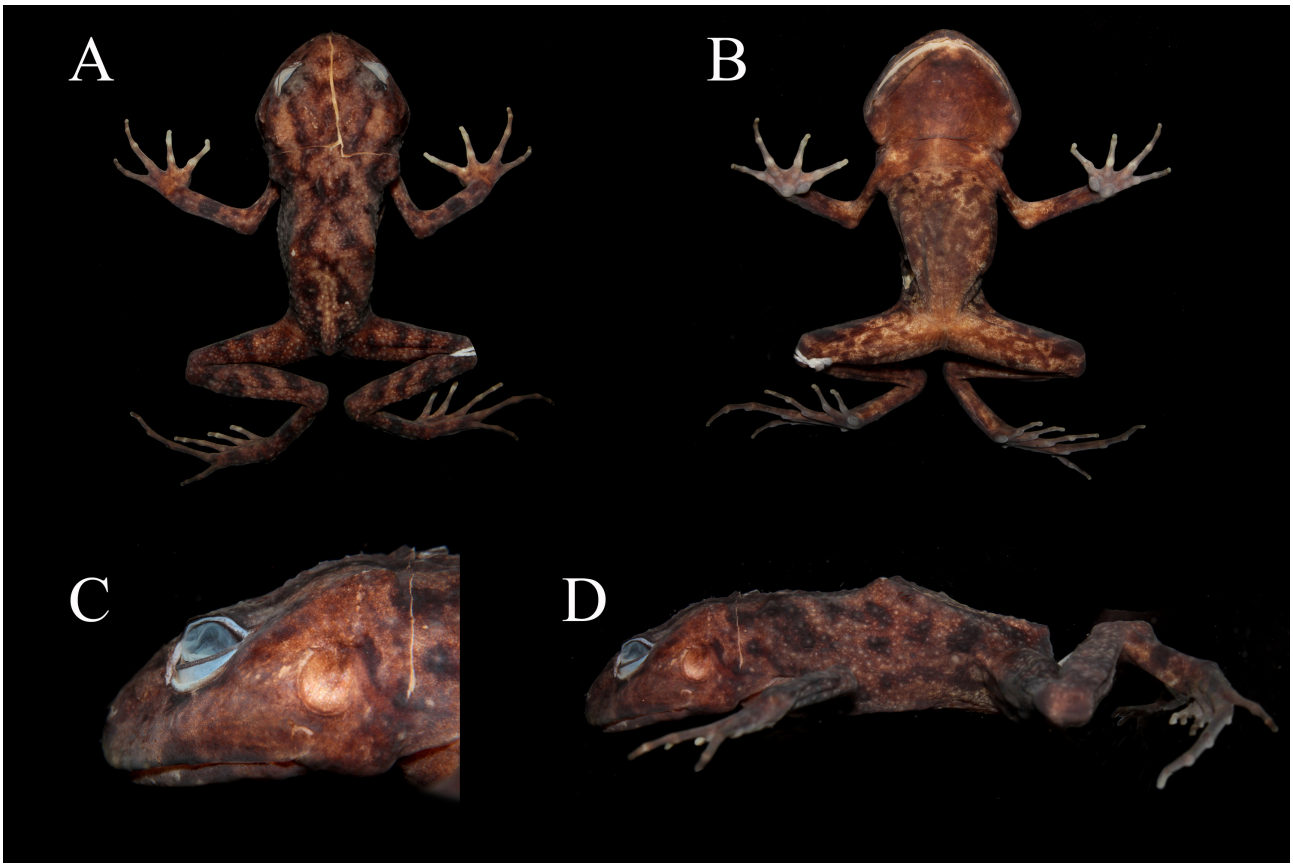

FIGURE 4. Holotype of *Lynchius megacephalus* **sp. n.** in preservative. (A) Dorsal view, (B) ventral view, (C) profile view of the head, (D) lateral view.

FIGURE 5. Ventral view of A) hand and B) foot of the holotype of *Lynchius megacephalus* **sp. n.** (hand length 5.2 mm, foot length 13.4 mm).

Comparison with similar species. *Lynchius megacephalus* differs from other species of *Lynchius* by having extensive exostosis on all the external surface of skull and head wider than body. *Lynchius flavomaculatus* and *L. parkeri* are the only other congeneric species with exostosis (condition described as rugosity in *L. parkeri*)

but it differs in extent (circumscribed to the frontoparietals vs. present in the frontoparietals, squamosal, nasals and *pars facialis* in *L. megacephalus*). The new species differs from *L. nebulanastes* and *L. parkeri* by having a distinct tympanic membrane and larger size, from *L. oblitus* by having a head wider than body and by lacking dorsolateral folds, and from *L. simmonsi* and *L. tabaconas* by having cranial crests. *Lynchius megacephalus* can be further distinguished from *L. flavomaculatus, L. nebulanastes, L. oblitus, L. parkeri,* and *L. tabaconas* by lacking, in life, bright coloration (yellow, green, or orange) in dorsum, venter or flanks. *Lynchius megacephalus* is very similar to *L. simmonsi* because both species are black or dark brown. However, in *L. simmonsi* the dorsum has spicule-like warts (skin of dorsum with tubercles and projected dermal ridges in *L. megacephalus*). Additionally, *L. simmonsi* lacks an occipital fold (present in *L. megacephalus*) and Finger I longer than Finger II (Finger I = Finger II in *L. megacephalus*).

Description of the holotype morphology. Adult female (Fig 3−5). Head wider than long, and wider than the body. Snout short, rounded in dorsal and lateral view. *Canthus rostralis* slightly convex. Nostrils in anterolateral direction. Upper eyelid tubercles small and rounded. Tympanum and tympanic annulus evident; 64% of eye

diameter. Dorsal skin shagreened with small subconical and rounded tubercles; dorsal and postocular folds with pronounced and irregular dermal ridges; middorsal fold low. Skin on venter smooth; discoidal and thoracic folds present. Thin arms; upper arm 1/3 length of the lower arm. Subconical and rounded tubercles in dorsal surface of arms and legs. Supernumerary palmar tubercles present. Fingers long and slender, tips narrow; lateral fringes narrow; subarticular tubercles projecting. Finger I as long as II; few supernumerary tubercles, low and smaller than subarticular tubercles; thenar tubercle prominent, almost the same size of palmar tubercle. Relative length of fingers: I=II=IV<III. Toe III almost as long as toe V, without supernumerary tubercles. Relative length of toes: I<II<III<V<IV.

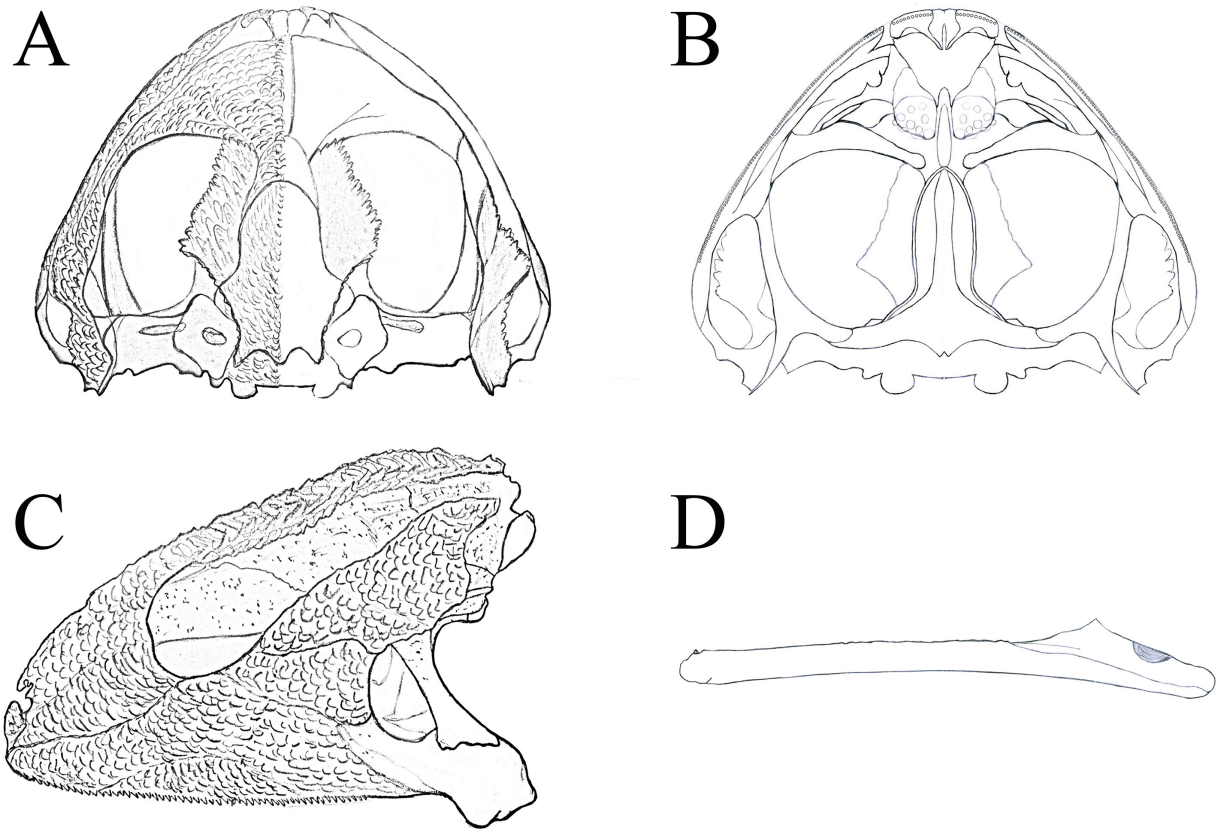

FIGURE 6. Skull of holotype of *Lynchius megacephalus* **sp. n.** (adult female) in (A) dorsal (B) ventral, and (C) lateral view, with visible exostosis and ornamentation (with wrinkled appearance), and (D) lateral view of the mandible (grey region represents the site of the articulation of the maxilla with the quadratojugal).

Description of the holotype osteology. The cranium (Fig. 6) is robust, 5/4 times wider than long and its maximum height is > 65% of the length of the skull. The frontoparietals are wide, fused in the medial region and expanded laterally posteriorly. Integumentary-cranial co-ossification is evident. The frontoparietal fontanelle, nasals, maxilla, sphenethmoid, premaxillae, *lamella alaris*, and squamosal are covered by dermal bone. The frontoparietals, nasals, *pars facialis* of the maxillae, the zygomatic, and otic ramus of the squamosal have exostosis. All dermal bones have a spiculate ornamentation, which is most notorious in the frontoparietal region, forming a crown that reaches ¼ of the eye orbit. This crown is projected towards the anterior region of the cranium. The size of the spicules decreases towards the posterior region, forming a W-shaped crest. The nasals and sphenethmoids are ossified and large. The nasals cover extensively the olfactory capsule. The alary process of the premaxilla is well developed. The zygomatic ramus of the squamosal articulates with the maxilla and quadratojugal. The oto-occipital is well ossified with coarse epiotic eminences. The *crista parotica* are long and coarse. The operculum is present. The occipital condyles are pedunculated. The *lamella alaris* is long, extending to the dorsal edge of the maxilla, behind the eye orbit, posteriorly forming an acuminated projection at the end of the squamosal. The co-ossification, exostosis, and the extent of the nasals, and quadratojugal form a sturdy skull. The maxillary teeth are curved, spreading along the maxillary arcade, but not reaching the pterygoid. The prevomers are

large. The dentigerous processes of vomers are prominent and narrow, with seven to nine pointed teeth. The neopalatines are wide, extending from the maxilla to the sphenethmoid and with a notorious thickening in the middle-anterior region. The cultriform process of the parasphenoid is long, ending in the mid-posterior portion of the sphenethmoids, between the neopalatines. Two posteromedial ossified process can be distinguished posteriorly on the hyoid.

The vertebral column (Fig. 7A) has eight procelic presacral vertebrae, I and II fused. All vertebrae show transversal processes: III = sacral diapophyses > IV > V = VI = VII = VIII > II. The orientation of these transversal processes is slightly perpendicular in vertebrae III and VIII, anterolateral in II and posterolateral in IV, V, VI, and VII. The neural spines are evident in all the presacral vertebrae. Diapophyses sacra without sesamoid cartilage at the edge, extending distally and posterolaterally. Sacrococcygeal articulation with a double condyle. The urostyle (Fig. 7A) is shorter than the presacral spine and have a long middorsal crest. The ilium is long. The ilia are thin, U-shaped in dorsal and ventral view. The pubis is ossified.

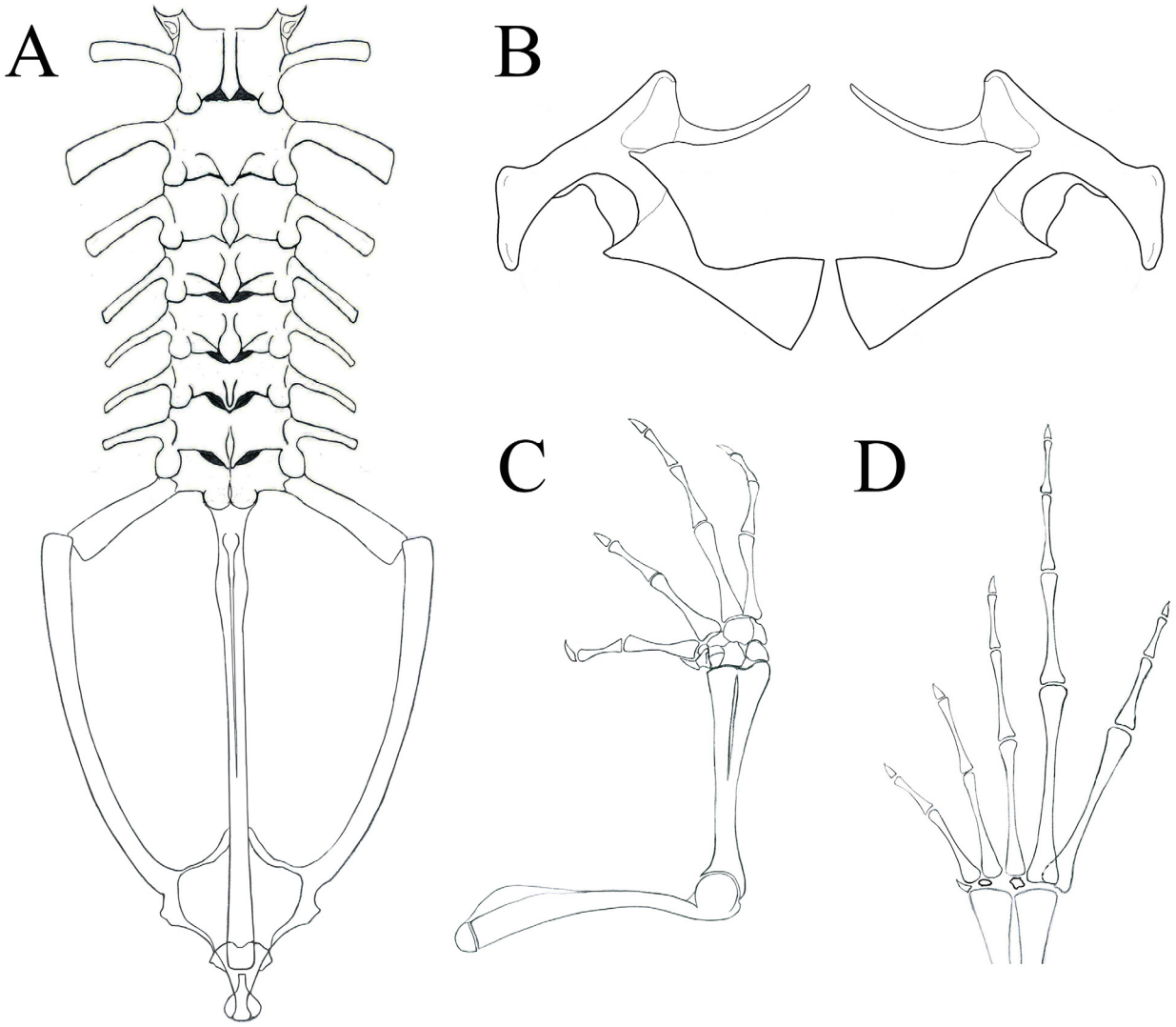

FIGURE 7. (A) Vertebral column and pelvic griddle, (B) pectoral grid, C) arm and D) foot of the holotype of *Lynchius megacephalus,* sp. n (adult female).

The pectoral girdle (Fig. 7B) is arciferal and formed by strong and ossified coracoids, scapulae, suprascapulae, and thin clavicles. The clavicles are concave with medial tips distinctly separated from one another. The lateral edge of the clavicle articulates with the *pars acromialis* of the scapulae. The coracoids are coarse and heavily concave at the anterior edge and slightly concave at the posterior edge. The glenoid fossa is wider than the union with the epicoracoids.

The humerus is thin, about the same length as the radioulna (Fig. 7C). The deltoid crest is well developed,

reaching the middle of the humerus. The radioulna is fused from the middle to the posterior region. The manual phalange formula is 2-2-3-3, the terminal phalanges have an acute apical knob. The prepollex is short, ¼ the length of the proximal phalange of finger II. The femur is 1/3 longer than the tibiofibula. The tibia and fibula are fused in their proximal and distal edges. The phalange formula in the foot is 2-2-3-4-3. A small prehallux is present (Fig. 7D).

FIGURE 8. *Lynchius parkeri* in life (QCAZ 61015, adult female, SVL 34.9 mm) from Laguna de Jimbura, Provincia de Loja, Ecuador.

Coloration of the holotype. In life (Fig. 3), dorsolaterally, the head, body, and limbs are brown with irregular dark brown spots, in the back, these spots cover the dermal ridges and, in the limbs, form a series of transverse bars. In ventral view, the belly, and limbs are grayish-brown with irregular spots brown. Throat is dark brown. Fingers I and II are cream from the most proximal subarticular tubercle to their distal end. The iris is golden with reticulated black lines, a median horizontal black streak, and a light-blue sclera can be distinguished at the upper edge of the eye.

In preservation (Fig. 4), dorsolaterally, the head, back, flanks, limbs, throat, and fingers I and II have the same color as in life. Ventrally, belly and limbs have a light brown coloration, with darker brown irregular spots.

Etymology. The name *megacephalus* is derived from the Greek *mega* meaning big and *cephala* meaning head. The name refers to the wide and robust head of this new species. The epithet is used as a noun in apposition.

Distribution and Natural history. *Lynchius megacephalus* is only known from one specimen collected at the type locality in the Tinajillas-Río Gualaceño Ecological Conservation Area, Morona Santiago province, 2770 m of elevation. The holotype was collected during the night (approximately 22h00) on the forest floor, between leaflitter in the middle of a heavy rainy week. The ecosystem is Eastern Montane Forest (according to Ron *et al.* 2018 natural regions). The forest at the type locality is characterized by small streams and leaflitter >10 cm thick (Urgilés & Nieves 2014). The dense canopy can exceed 20 m height. The vegetation includes species of the genus *Oreopanax, Weinmannia, Cinchona, Chusquea*, and *Baccharis* (Baez *et al.* 2013). The new species was recorded in sympatry with *Pristimantis versicolor, P. andinognomus, P. atratus, P. cryophilius, P. gualacenio, P. proserpens, P. spinosus*, and an undescribed *Lynchius* species similar to *L. flavomaculatus*.

Conservation Status. The scarcity of records of *L. megacephalus* at the type locality, a natural reserve that has been thoroughly surveyed by herpetologists since 2013, could indicate either that the population is small or that capture probabilities are low as result of its habitat preferences. Because nothing is known about its natural history and population status, we suggest assigning the new species to the Data Deficient category of the Red List (according to IUCN 2001 criteria).

First report of *Lynchius parkeri* (Lynch 1975) from Ecuador

Lynchius parkeri was previously known from two Peruvian localities: El Tambo, Cordillera de Huancabamba, Departamento de Piura and Tabaconas-Namballe National Sanctuary, Cajamarca, at elevations of 2770–3100 m (Lehr 2008, Duellman & Lehr 2009) (Fig. 2). We report this species for one locality in southern Ecuador, Laguna Jimbura, Yacuri National Park, Provincia de Loja. Three individuals, QCAZ 31466–468, were found on March 14, 2006 by Elicio Tapia. One additional specimen QCAZ 61015 (female) was found at the same locality, on April 24, 2015 by Darwin Nuñez (4.7098° S, 79.4448° W, 2990–3397 m). The localities correspond to two types of vegetation: Eastern Montane Forest and Páramo (Ron *et al.* 2018). The new records are approximately 60 km north from the nearest record in Peru, at Tabaconas National Park.

Our identification was based on DNA data (Fig. 1) and morphological examination of specimens through the diagnosis proposed by Lynch (1975) and Motta *et al.* (2016). *Lynchius parkeri* can be easily distinguished from similar species by having transversal folds on shanks and by the presence of discoidal and thoracic folds. It can also be distinguished from other *Lynchius* by having indistinct tympanic membrane and annulus and the first finger as long as or shorter than second. The genetic distance (uncorrected *p*) between the Ecuadorian specimens and *L. parkeri* from Peru (KU 181307) is < 2% (gene 16S) (Table 1).

Discussion

Our findings, based on phylogenetic and morphological evidence, strongly support the species status for *Lynchius megacephalus.* Morphologically, the most recognizable differences of the new species are the extensive ossification and exostosis in the skull, the proportionally large head, and the articulation of the maxilla with the zygomatic ramus of the squamosal.

Several osteological traits identified in *L. megacephalus* are also present in other *Lynchius*: well ossified skulls, head usually wider than long, large nasals extensively covering the olfactory capsule, long prevomers, and the

anterior ramus of the parasphenoid in contact with the palatines (Trueb & Lehr 2008). Remarkably, the cranium of *L. megacephalus* has extensive exostosis and highly ornamented dermal bones. *Lynchius flavomaculatus* and *L. parkeri* also have cranium exostosis. Additionally, *L. megacephalus* has a unique articulation between the zygomatic ramus of the squamosal and the maxillae. This condition is absent in the other member species of *Lynchius* but is characteristic on frogs with robust and hyper-ossified craniums, such as *Gastrotheca* (Duellman *et al.* 2006) and *Strabomantis* (Ospina 2012).

Similar craniums, with heavy ossification, are present in burrowing amphibians such as species of the genera *Ceratophrys, Calyptocephala*, and *Pyxicephalu*s (Duellman & Lizana 1994, Evans *et al.* 2008, Gómez *et al.* 2011, Evans *et al.* 2014). The absence of an enlarged inner metatarsal tubercle, an expanded humeral crest, a poorly developed prehallux, heavy ossification of the cranium, and a tibiofibula equal in size to the femur, (the last one absent in *L. megacephalus*) are characteristics of head-first burrower species (Hall 2008, Emerson 1976). However, first-head burrower species have reductions in the proportion of limbs with significant reductions in the length and stiffness of the digits (Emerson 1976); these conditions are absent in *L. megacephalus*, since its limbs and fingers are long and slender. Although the adaptive value of the unusual skull morphology of *L. megacephalus* is still unknown, the osteological characteristics of its head are suggestive of fossoriality. Fieldwork is needed to assess the population status of this species and to document its natural history.

The discovery of *L. megacephalus,* the revision of the taxonomic status of L. *simmonsi* in addition to the description of *L. oblitus* and *L. tabaconas* has increased the number of *Lynchius* from three to seven species in the last ten years. Moreover, the 4.6% genetic distance between *L. oblitus* MHNC 8652 and other *L. oblitus* is close to the distance between *L. megacephalus* and *L. flavomaculatus* (4.9%) and, therefore, suggest that they may represent separate species. This conclusion is reinforced by the short geographic distance between the locality of MHNC 8652 and the other known locality of *L. oblitus*, just 12 km straight-line distance, suggesting the existence of reproductive barriers in sympatry. Studies of Almendariz *et al.* 2014 in Cordillera del Condor and recent field expeditions to Yacuri National Park (southern Ecuador) suggest that more *Lynchius* species are yet to be named in the following years. As more collection efforts are taking place in high elevation ecosystems between southern Ecuador and northern Peru, we believe that the diversity of the genus is far from being completely known.

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APPENDIX 1. Specimens used for morphological comparison and museum specimens of *Lynchius* genus housed in Ecuador. Museum number, species name, locality, elevation, and sex are given for each individual. For species we did not physically examine for comparison, we present the reference source. Museum abbreviations are as follows: KU (University of Kansas Natural History Museum, USA), MECN (Instituto Nacional de Biodiversidad-Museo Ecuatoriano de Ciencias Naturales, Ecuador), MHNC (Museo de Historia Natural- Universidad Nacional de San Antonio Abad del Cusco, Peru), MZUA (Museo de Zoología-Universidad del Azuay, Ecuador), QCAZ (Museo de Zoología-Pontificia Universidad Católica del Ecuador, Ecuador). The locality abbreviations are: EC (Ecuador), PER (Peru).

Museum number	Species	Locality	Alt (m)	Sex	Source
QCAZ 20980	*Lynchius* sp.	Loja and Zamora Chinchipe, Vilcabamba-Valladolid road, EC	2680	Male	This study
QCAZ 24869	*Lynchius* sp.	Loja, Cerro Toledo, EC	3171	Female	This study
QCAZ 25221	*Lynchius* sp.	Loja, Cordillera Lagunillas, EC	3450	Male	This study
QCAZ 25263	*Lynchius* sp.	Loja, Cordillera Lagunillas, EC	3450	Male	This study
QCAZ 25264	*Lynchius* sp.	Loja, Cordillera Lagunillas, EC	3450	Female	This study
QCAZ 25265	*Lynchius* sp.	Loja, Cordillera Lagunillas, EC	3450	Female	This study
QCAZ 25266	*Lynchius* sp.	Loja, Cordillera Lagunillas, EC	3450	Female	This study
QCAZ 31454	*Lynchius* sp.	Loja, Fierro urcu, EC	-	Female	This study
QCAZ 63267	*Lynchius* sp.	Loja, Huashapamba - Saraguro, EC	2904	Female	This study
QCAZ 63268	*Lynchius* sp.	Loja, Huashapamba - Saraguro, EC	2906	Female	This study
QCAZ 63269	*Lynchius* sp.	Loja, Huashapamba - Saraguro, EC	2917	Female	This study
QCAZ 40805	*Lynchius* sp.	Loja, Huashapamba, EC	2894	Female	This study
MECN 02155	*Lynchius* sp.	Loja, Laguna Negra, EC	-	Juvenil	This study
MECN 02156	*Lynchius* sp.	Loja, Laguna Negra, EC	-	Juvenil	This study
MECN12738	*Lynchius* sp.	Loja, Loja, EC		Female	This study
QCAZ 30704	*Lynchius* sp.	Loja, Yangana, EC	2730	Female	This study
QCAZ 4528	*Lynchius* sp.	Loja, Yangana, EC	2760	Male	This study
MZUA AN0637	*Lynchius* sp.	Morona Santiago, Tinajillas, EC	-	Female	This study
MZUA AN0958	*Lynchius* sp.	Morona Santiago, Tinajillas, EC	-	Female	This study
MZUA AN0945	*Lynchius* sp.	Morona Santiago, Tinajillas, EC	-	Female	This study
MZUA AN0633	*Lynchius megacephalus*	Morona Santiago, Tinajillas, EC	-	Female	This study
QCAZ 55507	*Lynchius simmonsi*	Zamora Chinchipe, Campo Maicu, EC	1356	Female	This study
QCAZ 61035	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3333	Female	This study
QCAZ 61032	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3333	Juvenil	This study

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APPENDIX 1. (Continued)

Museum number	Species	Locality	Alt (m)	Sex	Source
QCAZ 61033	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3333	Female	This study
QCAZ 61034	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3333	Female	This study
QCAZ 61031	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3411	Female	This study
QCAZ 61036	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3411	Female	This study
QCAZ 61025	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3240	Female	This study
QCAZ 63324	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3274	Female	This study
QCAZ 63325	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3275	Female	This study
QCAZ 63326	*Lynchius* sp.	Zamora Chinchipe, Colambo Yacuri, EC	3137	Juvenile	This study
QCAZ 30828	*Lynchius* sp.	Zamora Chinchipe, Cóndor Mirador, EC	1850	Female	This study
QCAZ 30829	*Lynchius* sp.	Zamora Chinchipe, Cóndor Mirador, EC	1850	Female	This study
QCAZ 61015	*Lynchius parkeri*	Zamora Chinchipe, Jimbura, EC	2990	Female	This study
QCAZ 61024	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3240	Female	This study
QCAZ 61023	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3240	Female	This study
QCAZ 61017	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3049	Female	This study
QCAZ 61014	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3215	Female	This study
QCAZ 61018	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3240	Female	This study
QCAZ 61019	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	2990	Female	This study
QCAZ 61012	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3240	Male	This study
QCAZ 61016	*Lynchius parkeri*	Zamora Chinchipe, Jimbura, EC	3117	Female	This study
QCAZ 61022	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3240	Male	This study
QCAZ 61021	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3318	Male	This study
QCAZ 61020	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3318	Male	This study
QCAZ 61013	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3240	Juvenile	This study
QCAZ 31467	*Lynchius parkeri*	Zamora Chinchipe, Jimbura, EC	-	Female	This study
QCAZ 31466	*Lynchius parkeri*	Zamora Chinchipe, Jimbura, EC	-	Female	This study
QCAZ 31468	*Lynchius parkeri*	Zamora Chinchipe, Jimbura, EC	-	Male	This study
QCAZ 61009	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	2597	Female	This study

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APPENDIX 1. (Continued)

Museum number	Species	Locality	Alt (m)	Sex	Source
QCAZ 61010	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3506	Female	This study
QCAZ 61011	*Lynchius* sp.	Zamora Chinchipe, Jimbura, EC	3506	Male	This study
QCAZ 63265	*Lynchius* sp.	Zamora Chinchipe, La Canela, EC	1933	Female	This study
QCAZ 63266	*Lynchius* sp.	Zamora Chinchipe, La Canela, EC	1908	Juvenil	This study
QCAZ 61027	*Lynchius* sp.	Zamora Chinchipe, Laguna de los Patos, EC	3247	Female	This study
QCAZ 61028	*Lynchius* sp.	Zamora Chinchipe, Laguna de los Patos, EC	3247	Female	This study
QCAZ 61029	*Lynchius* sp.	Zamora Chinchipe, Laguna de los Patos, EC	3247	Female	This study
QCAZ 61026	*Lynchius* sp.	Zamora Chinchipe, Laguna Negra, EC	3186	Female	This study
QCAZ 61030	*Lynchius* sp.	Zamora Chinchipe, Lagunilla vía Jimbura-San Andrés, EC	3411	Female	This study
QCAZ 41639	*Lynchius simmonsi*	Zamora Chinchipe, Miasi Alto, EC	1300	Female	This study
QCAZ 41640	*Lynchius simmonsi*	Zamora Chinchipe, Miasi Alto, EC	1300	Male	This study
QCAZ 63361	*Lynchius* sp.	Zamora Chinchipe, Podocarpus National Park, EC	3152	Female	This study
QCAZ 63362	*Lynchius* sp.	Zamora Chinchipe, Podocarpus National Park, EC	3134	Male	This study
QCAZ 63363	*Lynchius* sp.	Zamora Chinchipe, Podocarpus National Park, EC	3137	Female	This study
KU 165955	*Lynchius flavomaculatus*	Loja, 15km E Loja, EC	3000	Female	Duellman & Lehr (2009); Motta *et al.* (2016)
KU 181407	*Lynchius nebulanastes*	Piura, El Tambo, 31.5km E Canchaque, PE	2770	Female	Duellman & Lehr (2009); Motta *et al.* (2016)
KU 135278	*Lynchius parkeri*	Piura, Huancabamba, between Canchaque and Huancabamba, PE	3100	Male	Duellman & Lehr (2009); Motta *et al.* (2016)
MHNC 8674	*Lynchius oblitus*	Cajamarca, Tabaconas-Namballe National Sanctuary, PE	3270	Female	Motta *et al.* (2016)
KU 147068	*Lynchius simmonsi*	Morona Santiago, Río Piuntza, Cordillera del Cóndor, EC	1830	Female	Lynch (1974); Motta *et al.* (2016)
MHNC 8637	*Lynchius tabaconas*	Cajamarca, Tabaconas District, San Ignacio Province, PE	2745	Female	Motta *et al.* (2016)

APPENDIX 2. Species of *Lynchius* and outgroup taxa included in phylogenetic analyses. The outgroup taxa are specified with a label under the species name. For each individual, museum voucher, source, locality, and GenBank accession number are given in detail when data was available. Museum abbreviations are as follows: AMNH (American Museum of Natural History, USA), CBG (Centro de Biodiversidad y Genética-Universidad Mayor de San Simón, Bolivia), LGE (Laboratorio de Genética Evolutiva de Tucumán, Argentina), IDlR (Ignacio De la Riva), MHNC (Museo de Historia Natural- Universidad Nacional de San Antonio Abad del Cusco, Perú), MNCN (Museo Nacional de Ciencias Naturales, Spain), MNK (Museo de Historia Natural Noel Kempff Mercado, Bolivia), MUSM (Museo de Historia Natural-Universidad Mayor de San Marcos, Perú), MTD (Museum für Tierkunde Dresden, Germany), MZUA (Museo de Zoología-Universidad del Azuay, Ecuador), MZUSP (Museu de Zoologia-Universidade de São Paulo, Brazil), KU (University of Kansas Natural History Museum, USA), QCAZ (Museo de Zoología-Pontificia Universidad Católica del Ecuador, Ecuador), USNM (U.S. National Museum of Natural History-Smithsonian Institution, USA), ZUEC (Museu de Zoologia- Universidade Estadual de Campinas ''Adão José Cardoso'', Brazil). The collection locality abbreviations are: EC (Ecuador), PER (Peru), BRA (Brazil), Prov. (Province), and Dept. (Department).

Museum number	**Species**	**Locality**	**Alt (m)**	**Latitude**	**Longitude**	**Genbank Accession numbers**				**Source**
						12S	**16S-ND1-tRNA**	**RAG**	**TYR**	
						EU186727	EU186727	EU186744	EU186765	Hedges *et al.* (2008)
KU173497	*Bryophryne cophites* (outgroup)	Cusco, Tres Cruces, PER	-	-	-	EF493537	EF493537	EF493423	EF493508	Heinicke *et al.* (2007)
-	*Euparkerella brasilinesis* (outgroup)	-	-	-	-	JX267390	JX267390	JX267545	JX267682	Canedo & Haddad (2012)
USNM 303077	*Haddadus binotatus* (outgroup)	São Paulo, Estacão Biologica de Borgceia, BRA	-	-	-	EF493361	EF493361	EF493397	-	Heinicke *et al.* (2007)
USNM 207945	*Holoaden bradei* (outgroup)	Minas Gerias, Brejo de Lapa, BRA	-	-	-	EF493378	EF493378	EF493449	EU186779	Heinicke *et al.* (2007); Hedges *et al.* (2008)
MZUSP 131872	*Holoaden luederwaldti* (outgroup)	São Paulo, Estacão do Bananal, BRA	-	-	-	EU186728	EU186728	-	EU186768	Hedges *et al.* (2008)
KU178258	*Hypodactylus brunneus* (outgroup)	Carchi, 14.6 km NW Carchi, EC	-	-	-	EF493357	EF493357	EF493422	EF493484	Heinicke *et al.* (2007)
-	*Hypodactylus dolops* (outgroup)	-	-	-	-	EF493394	EF493394	EF493414	EF493483	Heinicke *et al.* (2007)
KU218210	*Lynchius flavomaculatus*	Loja, 19.4 km S Yangana, EC	-	-	-	EU186667	EU186667	EU186745	EU186766	Hedges *et al.* (2008)
MZUA633 (QCAZ 63882)	*Lynchius megacephalus* sp. n.	Morona Santiago, Tinajillas, EC	2773	-3.011676	-78.61446	-	MK423938	-	-	This study

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APPENDIX 2. (Continued)

Museum number	Species	Locality	Alt (m)	Latitude	Longitude	Genbank Accession numbers				Source
						12S	16S-ND1-tRNA	RAG	TYR	
KU181408	*Lynchius nebulanastes*	Piura, El Tambo-31.5 km E Canchaque, PER	-	-	-	EU186704	EU186704	-	-	Hedges *et al.* (2008)
MHNC8652	*Lynchius oblitus*	Cajamarca, Tabaconas NS-Namballe, PER	2950	-5.1648083	-79.20126	KX470775	KX470782	KX470791	KX470798	Motta *et al.* (2016)
MHNC8674	*Lynchius oblitus*	Cajamarca, Tabaconas NS-Namballe, PER	3270	-5.2375278	-79.280180	KX470777	KX470784	KX470793	KX470800	Motta *et al.* (2016)
MHNC8676	*Lynchius oblitus*	Cajamarca, Tabaconas NS-Namballe, PER	3270	-5.2375278	-79.280180	KX470778	KX470785	KX470794	KX470801	Motta *et al.* (2016)
MHNC8614	*Lynchius oblitus*	Cajamarca, Tabaconas-Laguna Victoria, PER	3297	-5.2292361	-79.287102	KX470776	KX470783	KX470792	KX470799	Motta *et al.* (2016)
MHNC8677	*Lynchius oblitus*	Cajamarca, District Namballe-Quebrada del Vino, PER	3291	-5.2375278	-79.280180	KX470779	KX470786	KX470795	KX470802	Motta *et al.* (2016)
KU181307	*Lynchius parkeri*	Piura, 31 km SW of Huancabamba, PER	-	-	-	EU186705	EU186705	-	-	Hedges *et al.* (2008)
QCAZ 31466	*Lynchius parkeri*	Loja, Laguna de Jimbura, EC	-	-4.714000	-79.42799	MK423935	-	MK423933	-	This study
QCAZ 61015	*Lynchius parkeri*	Loja, PN Yacuri-Vía a Jimbura, EC	2990	-4.709810	-79.44476	MK423936	MK423937	MK423934	-	This study
QCAZ 41639	*Lynchius simmonsi*	Zamora Chinchipe. Alto Miazi. EC	1315	-4.256560	-78.62246	JF809940	JF810004	JF809915	JF809894	Padial *et al.* (2012)
MHNC8650	*Lynchius tabaconas*	Cajamarca, District Tabaconas-Quebrada del Vino, PER	3115	-5.1693472	-79.20746	KX470774	KX470781	KX470790	KX470797	Motta *et al.* (2016)
MHNC8637	*Lynchius tabaconas*	Cajamarca, District Tabaconas-Quebrada del Vino, PER	2745	-5.1648083	-79.20126	KX470773	KX470780	KX470789	KX470796	Motta *et al.* (2016)
QCAZ 31471	*Noblella heyeri* (outgroup)	Loja, Vía a Zamora, EC	-	-	-	JX267463	JX267463	-	-	Canedo and Haddad (2012)

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APPENDIX 2. (Continued)

Museum number	Species	Locality	Alt (m)	Latitude	Longitude	Genbank Accession numbers				Source
						12S	16S-ND1-tRNA	RAG	TYR	
KU177356	*Noblella lochites* (outgroup)	Mera, Pastaza, Ecuador	-	-	-	EU186699	EU186699	EU186756	EU186777	Hedges *et al.* (2008)
QCAZ 40180	*Noblella myrmecoides* (outgroup)	Morona Santiago, Macuma Wisiu, EC	-	-	-	JX267464	JX267464	-	-	Canedo and Haddad (2012)
MHNC6975	*Oreobates amarakaeri* (outgroup)	Cuzco, Distrito Camanti, Prov. Quispicanchi, Rio Nusiniscato, PER	685	-	-	JF809934	JF809934	JF809913	JF809891	Unpub.
IDlR5024	*Oreobates ayacucho* (outgroup)	Ayacucho, Prov. La Mar, between Punqui y Anco, PER	3850	-	-	JF809933	JF809933	JF809912	JF809890	Unpub.
MNCN1359	*Oreobates barituensis* (outgroup)	Dept. Ledesma, Jujuy, Caimancito, ARG	556	-	-	JF809935	JF809935	JF809914	JF809892	Unpub.
LGE 2530	*Oreobates berdemenos* (outgroup)	Dept. Ledesma, Jujuy, Abra Colorada, ARG	-	-	-	-	KJ125507	-	-	Pereyra *et al.* (2014)
CBG765	*Oreobates choristolemma* (outgroup)	La Paz, Sud Yungas-Boqueron, Pilon Lajas, BOL	1000	-	-	JF809921	FJ539067	JF809900	JF809881	Unpub.
ZUEC14119	*Oreobates crepitans* (outgroup)	Mato Grosso, Cuiaba, São Vicente, BRA	-	-	-	-	KJ125510	-	-	Pereyra *et al.* (2014)
KU215462	*Oreobates cruralis* (outgroup)	Madre de Dios, Cuzco Amazonico, 15 km E of Puerto Maldonado, PER	-	-	-	EU186666	EU186666	EU186743	EU186764	Hedges *et al.* (2008)
MNCN43133	*Oreobates discoidalis* (outgroup)	Prov. Oconnor, Tarija, BOL	1763	-	-	JF809925	FJ539068	JF809904	JF809884	Unpub.

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APPENDIX 2. (Continued)

Museum number	Species	Locality	Alt (m)	Latitude	Longitude	Genbank Accession numbers				Source
						12S	16S-ND1-tRNA	RAG	TYR	
MNK-A6612	*Oreobates ibischi* (outgroup)	Santa Cruz, km 60 on Santa Cruz de la Sierra-Samaipata road, BOL	-	-	-	FJ438817	FJ438817	-	-	Padial *et al.* (2009)
MHNC6687	*Oreobates gemcare* (outgroup)	Cuzco, Provincia Paucartambo, Esperanza, Valle de Kosnipata, PER	2700	-	-	JF809930	-	JF809909	-	Unpub.
MHNC3396	*Oroebates granulosus* (outgroup)	Puno, Provincia Carabaya, Santo Domingo de Carabaya, PER	1800	-	-	JF809929	EU368897	JF809908	JF809887	Padial *et al.* (2008)
MNK-A7175	*Oreobates heterodactylus* (outgroup)	Santa Cruz, Prov. Chiquitos, Cerro del Arco, BOL	800	-	-	JF809923	FJ438805	JF809902	JF809882	Unpub.
MUSM27616	*Oreobates lehri* (outgroup)	Cuzco, Prov. Grau, cloud forest next to road leading to Vilcabamba, PER	2850	-	-	JF809927	JF809957	JF809927		Unpub.
MTD45902	*Oreobates lundbergi* (outgroup)	Pasco, Prov. Huanchon, from Auquimarca to Uchuerta, PER	2760	-	-	JF809928	JF809958	JF809907	JF809886	Unpub.
MHNC6809	*Oreobates machiguenga* (outgroup)	Cuzco, Prov. La Convencion, Rio Kimbiri, PER	1300	-	-	JF809932	JF809969	JF809911	JF809889	Unpub.
MNK-A7856	*Oreobates madidi* (outgroup)	La Paz, Franz Tamayo, Arroyo Huacataya, BOL	1300	-	-	JF809922	FJ539070	Jf809901	-	Padial *et al.* (2009)
MTD46808	*Oreobates pereger* (outgroup)	Ayacucho, Prov. Lamar, Yanamonte, BOL	1600	-	-	JF809926	JF809955	JF809905	JF809885	Unpub.

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APPENDIX 2. (Continued)

Museum number	Species	Locality	Alt (m)	Latitude	Longitude	Genbank Accession numbers				Source
						12S	16S-ND1-tRNA	RAG	TYR	
KU178249	*Oreobates quixensis* (outgroup)	Sucumbíos, Limoncocha, EC	-	-	-	EF493828	EF493662			Heinicke *et al.* (2007)
QCAZ 25520	*Oreobates quixensis* (outgroup)	Pastaza, Kapawi Lodge,EC	250	-	-	-	JF810002		-	Padial *et al.* (2012), this study
QCAZ 31186	*Oreobates quixensis* (outgroup)	Pastaza, Parroquia Teniente Hugo Ortiz, EC	951	-	-	-	-	-	JF809893	Unpub.
MNK-A5507	*Oreobates sanctaecrucis* (outgroup)	Santa Cruz, Prov. Florida, La Yunga de Mairana, BOL	2060	-	-	JF809924	JF809924	JF809903	JF809883	Unpub.
MHNC6775	*Oreobates saxatilis* (outgroup)	Cuzco, Prov. La Convencion, Rio Kimbiri, PER	1000	-	-	JF809931	JF809962	JF809910	JF809888	Unpub.
KU291634	*Phrynopus auriculatus* (outgroup)	Pasco, 2.9 km N, 5.5 km E Oxapampa, PER	-	-	-	EF493708	EF493708	-	-	Heinicke *et al.* (2007)
n/a	*Phrynopus barthlenae* (outgroup)	Huanuco, PER	-	-	-	AM039721	-	-	-	Lehr *et al.* (2005)
USNM 286919	*Phrynopus bracki* (outgroup)	Pasco, 2.9 km N, 5.5 km E Oxapampa, PER				EF493709	EF493709	EF493421	EF493507	Heinicke *et al.* (2007)
n/a	*Phrynopus bufoides* (outgroup)	Pasco, PER	-	-	-	AM039713	-	-	-	Lehr *et al.* (2005)
n/a	*Phrynopus heimorum* (outgroup)	Huanuco, PER	-	-	-	AM039704	-	-	-	Lehr *et al.* (2005)
n/a	*Phrynopus horstpauli* (outgroup)	Huanuco, PER	-	-	-	AM039715	-	-	-	Lehr et *al.* (2005)
n/a	*Phrynopus juninensis* (outgroup)	Pasco, PER	-	-	-	AM039725	-	-	-	Lehr *et al.* (2005)

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APPENDIX 2. (Continued)

Museum number	Species	Locality	Alt (m)	Latitude	Longitude	Genbank Accession numbers				Source
						12S	16S-ND1-tRNA	RAG	TYR	
n/a	*Phrynopus kauneorum* (outgroup)	Huanuco, PER	-	-	-	AM039718	-	-	-	Lehr *et al.* (2005)
n/a	*Phrynopus pesantesi* (outgroup)	Pasco, PER	-	-	-	AM039724	-	-	-	Lehr *et al.* (2005)
n/a	*Phrynopus tautzorum* (outgroup)	Huanuco, PER	-	-	-	AM039720	-	-	-	Lehr *et al.* (2005)
KU291630	*Phrynopus tribulosus* (outgroup)	Pasco, 2.9 km N, 5.5 km E Oxapampa, PER	-	-	-	EU186725	EU186725	-	-	Hedges *et al.* (2008)
AMNH-A 165108	*Psychrophrynella guillei* (outgroup)	La Paz, Bautista Saavedra, Canton Charazani, BOL	-	-	-	AY843720	AY843720	-	DQ282995	Faivovich *et al.* (2005); Frost *et al.* (2006)
KU173495	*Psychrophrynella usurpator* (outgroup)	Cusco, Abra Acanacu, 25 km NNE Paucartabo, PER	-	-	-	EF493714	EF493714	EU186762	EU186780	Heinicke *et al.* (2007); Hedges *et al.* (2008)
KU183049	*Psychrophrynella wettsteini* (outgroup)	La Paz, 2.3 km S Unduavi, BOL	-	-	-	EU186696	EU186696	EU186755	EU186776	Hedges *et al.* (2008)