

DESCRIPTION AND PHYLOGENETIC POSITION OF A NEW (SINGLETON) SPECIES OF
OREOBATES JIMÉNEZ DE LA ESPADA, 1872 (ANURA: CRAUGASTORIDAE)
 FROM THE YUNGAS OF COCHABAMBA, BOLIVIA

JÖRN KÖHLER

Hessisches Landesmuseum Darmstadt
 Friedensplatz 1, 64283 Darmstadt, Germany
 joern.koehler@hlmd.de

JOSÉ M. PADIAL

Assistant Curator, Section of Amphibians and Reptiles
 Carnegie Museum of Natural History,
 4400 Forbes Avenue, Pittsburgh, PA 15213, USA
 padialj@carnegiemn.org

ABSTRACT

We report the discovery of a new singleton species of *Oreobates* Jiménez de la Espada, 1872, from the Yungas forest of the Amazonian versant of the Andes in Bolivia, infer its phylogenetic position, revisit the phylogenetic relationships of *Oreobates*, and discuss the conditions that justify description of the species with a single specimen. The **new species**, *Oreobates yanucu*, differs from all other *Oreobates* in a combination of external conditions: granular dorsal skin with scattered warts, finger I longer than finger II, finger tips of fingers III and IV distinctly enlarged and truncate in outline, tips of toes II to V with unguis flaps, head longer than wide, basal webbing between toes I and II and toes II and III, foot length/snout–vent length = 50%, lack of orange, red, or scarlet flecks and blotches in life. Although similar in appearance to *O. amarakaeri* Padial et al., 2012, *O. choristolemma* (Harvey and Sheehy, 2005), *O. granulosis* (Boulenger, 1902), *O. sanctaerucis* (Harvey and Keck, 1995), and *O. sanderi* (Padial et al., 2005)—all of them species from the Yungas of Bolivia and southern Peru conforming a monophyletic group—the new species is nested within a clade, revealed by molecular phylogeny, in which all species share the condition of enlarged finger discs and is sister to *O. berdemenos* Pereyra et al., 2014, an allopatric species from the Yungas of Argentina. The new species is only known from a single specimen collected in 1999 at 1500 m above sea level within Carrasco National Park (Provincia Chapare, Departamento Cochabamba, Bolivia). *Oreobates* now includes 24 described species but seven other new species remain to be named formally and we expect the diversity of this group to increase considerably with the exploration of the Andean foothills of Bolivia and Peru.

KEY WORDS: 16S mtDNA, Amazon Basin, Amphibia, Andes, Brachycephaloidea, dynamic homology, Holoadeninae, tree-alignment.

INTRODUCTION

The genus *Oreobates* Jiménez de la Espada, 1872, represents a clade of New World direct-developing frogs or terraranas with 23 species distributed across a wide range of habitats in South America (Padial et al. 2012). Until very recently, species of *Oreobates* were considered part of a number of other taxa such as *Eleutherodactylus* Duméril and Bibron, 1841, *Ischnocnema* Reinhardt and Lütken, 1862, *Hypodactylus* Hedges et al., 2008, and *Phrynopis* Peters, 1873, but morphological analyses (Caramaschi and Canedo 2006; Padial et al. 2012) and molecular phylogenetic inferences led to re-arrangements that culminated in a redefined and monophyletic genus (Hedges et al. 2008; Padial et al. 2008a, 2012, 2014; Pyron and Wiens 2011). A combination of taxonomic approaches including molecular phylogenetics, bioacoustics, examination of historical material in museum collections, and the efforts of an increasing number of herpetologists to collect in the field has furthermore led to a proliferation of new species during the last years (Vaira and Ferrari 2008; Padial et al. 2012; Teixeira et al. 2012; Pereyra et al. 2014). As a result of this progress in taxonomic knowledge, our understanding of patterns of distribution and phenotypic variation in this clade has changed considerably.

Most species of *Oreobates* occur in the cloud forests

and montane forests of the Amazonian versant of the Andes of Bolivia and Peru (Harvey and Keck 1995; Harvey and Sheehy 2005; Padial et al. 2012), e.g., *O. choristolemma* (Harvey and Sheehy, 2005), *O. granulosis* (Boulenger, 1902), *O. sanctaerucis* (Harvey and Keck, 1995), and *O. saxatilis* (Duellman, 1990). A few species also occur along the adjacent Amazonian lowlands, e.g., *O. quixensis* Jiménez de la Espada, 1872, and *O. cruralis* (Boulenger, 1902), and three species, *O. crepitans* (Bokermann, 1965), *O. heterodactylus* (Miranda-Ribeiro, 1937), and *O. remotus* Teixeira et al., 2012, occur even farther east in the xeric environments of the Cerrado and dry Atlantic forests (Padial and De la Riva 2005; Teixeira et al. 2012). Within the Andes, some species occur in the highly seasonal forests of the inter-Andean dry valleys of Argentina and Bolivia (Reichle et al. 2001; Pereyra et al. 2014), and Peru (J.C. Chaparro pers. comm.), and one species colonized the Andean grasslands that occur above the tree line (Lynch 1975; Padial et al. 2012).

Paralleling the broad geographical and ecological distribution of *Oreobates*, species in this genus show a considerable variation in external morphology and mating calls. Structures of the skin such as granules, tubercles, warts, and folds (see Duellman and Lehr 2009) show different

TABLE 1. GenBank accession number for specimens sampled in this study

	12S	16S	CYTB	RAG-1	TYR
<i>Haddadus binotatus</i>	EF493361	EF493361	GQ345198	EF493397	DQ282918
<i>Lynchius flavomaculatus</i>	EU186667	EU186667	–	EU186745	EU186766
<i>Lynchius nebulanastes</i>	EU186704	EU186704	–	–	–
<i>Lynchius parkeri</i>	EU186705	EU186705	–	–	–
<i>Lynchius simmonsii</i>	JF809940	JF810004	–	JF809915	JF809894
<i>Oreobates amarakaeri</i>	JF809934	JF809996	–	JF809913	JF809891
<i>Oreobates ayacucho</i>	JF809933	FJ539069	–	JF809912	JF809890
<i>Oreobates barituensis</i>	JF809935	FJ539068	–	JF809914	JF809892
<i>Oreobates berdemenos</i>	–	KJ125507	KJ125511	–	–
<i>Oreobates CaEU192295</i>	–	EU192295	–	–	–
<i>Oreobates CaEU368903</i>	–	EU368903	–	–	–
<i>Oreobates CaJF809995</i>	–	JF809995	–	–	–
<i>Oreobates choristolemma</i>	JF809921	FJ539067	–	JF809900	JF809881
<i>Oreobates crepitans</i>	–	KJ125510	KJ125512	–	–
<i>Oreobates cruralis</i>	EU186666	EU186666	EU368881	EU186743	EU186764
<i>Oreobates discoidalis</i>	JF809925	FJ539068	EU368883	JF809914	JF809884
<i>Oreobates gemcare</i>	JF809930	FJ539069	–	JF809909	–
<i>Oreobates granulatus</i>	JF809929	FJ539067	–	JF809908	JF809887
<i>Oreobates heterodactylus</i>	JF809923	EU192296	EU368885	JF809902	JF809882
<i>Oreobates ibischi</i>	FJ438817	FJ438806	–	–	–
<i>Oreobates lehri</i>	JF809927	FJ539069	–	JF809906	–
<i>Oreobates lundbergi</i>	JF809928	JF809958	–	JF809907	JF809886
<i>Oreobates machiguenga</i>	JF809932	FJ539069	–	JF809911	JF809889
<i>Oreobates madidi</i>	JF809922	FJ539070	EU368887	JF809901	–
<i>Oreobates pereger</i>	JF809926	JF809958	–	JF809905	JF809885
<i>Oreobates quixensis</i>	EF493828	EF493662	EU368889	–	JF809893
<i>Oreobates remotus</i>	–	JN688275	JN688276	–	–
<i>Oreobates sanctaerucis</i>	JF809924	FJ539067	–	JF809903	JF809883
<i>Oreobates sanderi</i>	–	EU368904	EU368891	–	–
<i>Oreobates saxatilis</i>	JF809931	DQ283061	–	JF809910	JF809888
<i>Oreobates yanucu</i>	–	KY111322	–	–	–

degrees of development and similar conditions occur in different branches of the *Oreobates* tree, which suggests multiple independent origins and/or losses. Another feature that has been used for taxonomic analysis due to its pattern of variation is toepads. Most species are terrestrial and lack enlarged toepads, while in a clade of partially arboreal species outer fingers show enlarged toepads. Color variation is also considerable, and although most species are overall brown, they differ in pattern and shades, as well

as in the presence/absence of certain conspicuous marks such as dorsolateral bands, facial masks, or flash colors on the underside and hidden parts of limbs. Similarly, the mating calls of species of *Oreobates* are relatively similar in structure (multi-pulsed notes), but species differ in the number of notes emitted per call, the number of pulses per call, their duration, and frequency distribution (Padial et al. 2008b). This interspecific variation has been used as evidence for taxonomic (e.g., Reichle et al. 2001; Padial et

al. 2005, 2008b; Vaira and Ferrari 2008) and phylogenetic inferences (Goicoechea et al. 2010).

The patterns outlined above are nonetheless necessarily incomplete, as many areas of the Andes and the Amazon, where *Oreobates* are likely to occur, remain unexplored and the diversity of *Oreobates* is probably vastly underestimated. As an example, Padial et al. (2012) already reported three candidate species that remain to be named and recent fieldwork in the Andes revealed additional new species (J.M. Padial pers. obs.).

Here, we continue to fill gaps in our understanding of species diversity, distribution, and evolutionary history of *Oreobates* by describing and naming a new species and inferring its phylogenetic position using molecular data. The new species is only known from a single adult specimen collected in 1999 during a transect study within the Yungas forests of the Bolivian Andes (see Köhler 2000). This specimen was tentatively referred to as *O. sanctaerucis* by Köhler (2000) and later to *O. choristolemma* by Padial et al. (2008a, 2012). Comprehensive morphological comparisons revealed that it possesses a combination of conditions unknown in all other *Oreobates* known to date, and several traits of the external morphology of this specimen are conspicuously different from those shown by either *O. choristolemma* or *O. sanctaerucis*. Molecular phylogenetic inferences further support the new species. Consequently, we herein describe and name this singleton new species, describe the conditions that grant the description of the species with a single specimen, and provide an overview of species diversity and phylogenetic relationships of *Oreobates*.

MATERIAL AND METHODS

Morphology.—We follow Padial et al. (2008a) and Duellman and Lehr (2009) for morphological and color characteristics used in the diagnosis and description. Measurements were taken with a digital caliper to the nearest 0.01 mm and rounded to the nearest 0.1 mm to avoid pseudo precision. Abbreviations for measurements are as follows: snout–vent length, SVL; head length (from posterior margin of lower jaw to tip of snout), HL; head width (measured at level of rictus), HW; eye length (measured horizontally), EL; eye to nostril distance, EN; internarial distance, IND; tympanic membrane height, TYH; tympanic membrane length, TYL; arm length (from posterior margin of thenar tubercle to distal point of elbow), FA; tibia length (distance from knee to the distal end of the tibia), TL; thigh length, TH (from vent to knee); foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe), FL. We do not include values of interorbital distance (IOD) and upper eyelid width (EW); our experience indicates that these parameters are usually of limited value because the preservation condition of specimens highly influences the measurements and makes it difficult to have precise and comparable values.

Museum acronyms are those listed by Frost (2016), except that MHNC refers to Museo de Historia Natural de Cusco (MHNC in Frost 2016), and MCN-UNSA refers to Museo de Ciencias Naturales de la Universidad Nacional de Salta. JKSL refers to Jörn Köhler and Stefan Lötters field numbers. In the Appendix, we include a list of specimens examined.

The holotype specimen was euthanized using chlorobutanol solution, fixed in 98%, and preserved in 70% ethanol. We determined sexual condition and maturity by dissection and visual inspection of gonads.

The photo of the toe tip (Fig. 5) was produced with a Canon DSLR camera and a micro lens (Canon MP-E 65 mm) mounted on an automated macro rail. Sixteen images with different depths of focus were taken using the software Helicon Remote 3.8.1 and merged with the image stacking software Helicon Focus 6.7.1. (Helicon Soft Ltd.).

Molecular phylogenetics.—Our dataset includes 31 terminals representing 23 of the currently named species of *Oreobates* (Frost 2016), plus the new species, and three candidate species detected by Padial et al. (2012). We used four species of *Lynchius* Hedges et al., 2008, as outgroups following the results of more comprehensive phylogenetic analyses (Hedges et al. 2008; Pyron and Wiens 2011; Padial et al. 2014) and rooted the tree with *Haddadus binotatus* (Spix, 1824), a distant species of Craugastoridae (Padial et al. 2014). The final dataset is a supermatrix that includes legacy (GenBank) and a single newly produced sequence of the barcode fragment of the 16S gene for the new species (other loci failed to amplify probably because tissue was collected from the preserved holotype directly and though fixed in ethanol, it was stored in alcohol with residues of formalin at ZFMK). Protocols for DNA amplification, sequencing and editing are described in Padial et al. (2012). Legacy sequences from GenBank (non-coding mtDNA genes) include rRNA genes of the heavy strand transcription unit 1 fragment (12S, 16S, and the intervening tRNA^{valine}, and tRNA^{leucine} segments). Protein-coding mtDNA genes include cytochrome b (cytb). Nuclear protein-coding genes include the recombination-activating protein 1 (RAG1) and tyrosinase precursor (Tyr). We excluded other loci available for *Oreobates* when they were represented by less than three ingroup species (i.e., c-myc, HH3, POMC, Rhod, and SIA). Accession numbers for all sequences used in this study are listed in Table 1.

Sequences were aligned in MAFFT online version 7 using the G-INS-i strategy, which is considered appropriate for alignments that consist of large numbers of sequences (Kato and Standley 2013). The G-INS-i strategy performs global alignment with a Fast Fourier Transform (FFT) approximation progressively on a guide tree (modified UPGMA) followed by iterative edge refinement that evaluates the consistency between the multiple alignment and pairwise alignments. The iterative refinement is repeated until no improvement is observed in the weighted

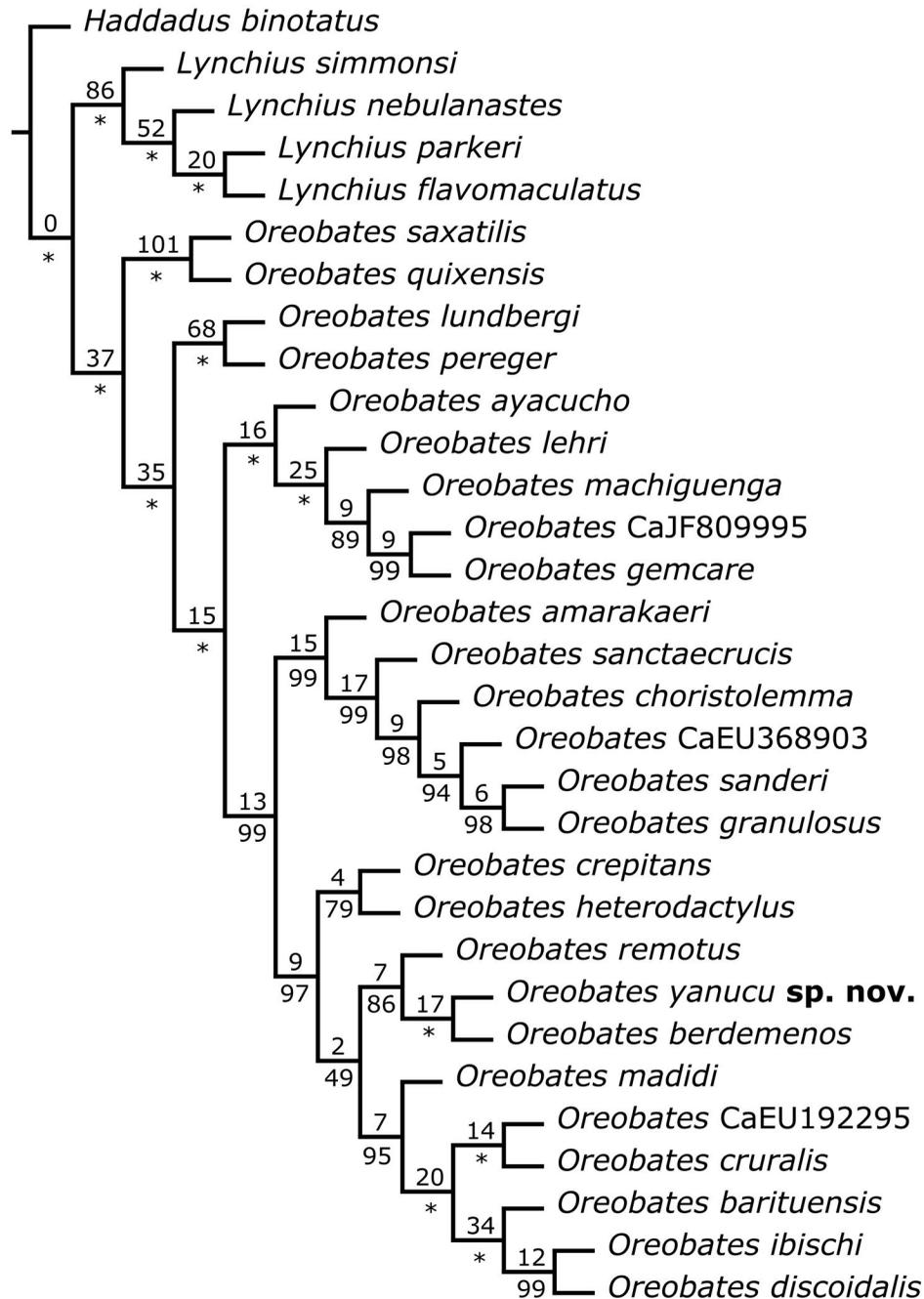


Fig. 1.—Phylogenetic relationships of *Oreobates* under tree-alignment and parsimony (5095 steps) for 23 nominal and three candidate species of *Oreobates* resulting from the analysis of fragments of the mitochondrial genes 12S, 16S, the intervening tRNA^{Val}, and tRNA^{Leu}, a fragment of the coding mtDNA gene cytb, and fragments of the nuclear protein-coding RAG1, and Tyr. Numbers above ancestral branches indicate Goodman-Bremer values, and those under branches are Jackknife proportions (asterisks represent Jackknife values of 100%).

sum-of-pairs score or 1000 cycles are completed (maxiterate = 1000). We applied the default transition/transversion cost ratio of 1:2 but changed the gap opening penalty from three times substitutions to one time substitutions to avoid penalizing insertions and deletions more than we did in the

parsimony analysis. Aligned sequences were partitioned into fragments of equal length separated by conserved regions with no gaps and few or no nucleotide substitutions. This strategy generated putatively homologous fragments where length variation among DNA sequences was

only due to insertions and/or deletions of nucleotides, a requisite for tree-alignment in POY (Wheeler et al. 2006). After the removal of gaps implied by MAFFT from sequence fragments, tree-alignment of unaligned sequences was performed under parsimony with equal weights for all classes of transformations using direct optimization (DO; Wheeler 1996; Wheeler et al. 2006) and iterative pass optimization (IPO; Wheeler 2003) algorithms in POY 5.1.1 (Varón et al. 2010). Tree searches were first conducted using DO under the command “search,” which implements an algorithm based on random addition sequence Wagner builds, subtree pruning and regrafting, and tree bisection and reconnection branch swapping (see Goloboff 1996, 1999), parsimony ratcheting (Nixon 1999), and tree fusing (Goloboff 1999); then, consecutive rounds of searches were done within a specified run-time, storing the shortest trees of each independent run and performing a final round of tree-fusing on the pooled trees. The optimal tree found during driven searches was swapped using iterative pass optimization. The optimal alignment resulting from iterative pass optimization was converted into a data matrix and additional driven searches were conducted in TNT (Goloboff et al. 2008) until a stable strict consensus was reached at least three times (see below for details of driven searches in TNT). We calculated Goodman-Bremer (GB) values for each supported clade in TNT using the optimal tree-alignment matrix and the parameters specified in the bremer.run macro (available at <http://www.zmuc.dk/public/phylogeny/tnt/>), which begins by searching for trees N steps longer than the optimum (ten random addition sequence Wagner builds and TBR swapping saving two trees per replicate), using inverse constraints for each node of the most parsimonious tree. Swapping of each constrained search was limited to 20 minutes and constrained searches were repeated three times as specified in the default settings of the bremer.run macro. We also calculated parsimony jackknife frequencies (Farris et al. 1996) for each supported clade by resampling the tree-alignment matrix. We caution that, as in analyses of similarity-alignment matrices, the resulting clade frequencies are conditional on this particular alignment and not the data themselves. Given that the tree-alignment matrix is derived from the optimal tree, the resulting clade frequencies are expected to be higher than would be obtained from matrices aligned according to different guide trees (e.g., a UPGMA or neighbor-joining tree, as in MAFFT and Clustal, respectively). We calculated jackknife frequencies from 1000 pseudoreplicates searches using driven searches (see below), gaps treated as fifth state, and removal probability of 0.36 ($\approx e^{-1}$), which purportedly renders jackknife and bootstrap values comparable (Farris et al. 1996).

RESULTS

Morphological analysis.—Comparisons of the single available specimen of the new species with type materi-

als of all other species of *Oreobates* reveals that the new species possesses a combination of qualitative character states that is unique within the genus: granular dorsal skin with scattered warts, finger I longer than finger II, finger tips of fingers III and IV distinctly enlarged and truncate in outline, tips of toes II to V with unguis flaps, supernumerary tubercles small and inconspicuous, head longer than wide, basal webbing between toes I and II and toes II and III, FL/SVL = 50%, lack of orange, red, or scarlet flecks and blotches in life. Other traits supporting the new species and comparisons with other species are listed in the diagnosis.

Molecular phylogenetics.—The final dataset included 31 terminals and 4230 aligned basepairs (under tree-alignment). The analyses performed 1979 rounds of tree building followed by TBR, 33817 rounds of fusing, and 1197 rounds of ratcheting. Character optimization resulted in a single most parsimonious tree of 5118 steps that was visited 11672 times. Swapping of the best tree under the “iterative pass” function resulted in a single tree with the same topology but with a better optimization of characters that required 5095 steps. Further searches of the tree-alignment in TNT rendered a single tree of the same length and topology. The resulting topology was completely resolved (29 nodes), and Goodman-Bremer and jackknife values are high for most nodes (Fig. 1).

Oreobates is recovered monophyletic and the new species is recovered as sister of *Oreobates berdemenos* in a clade that also includes *O. remotus*, and which is sister of a clade conformed by *O. barituensis* Vaira and Ferrari, 2008, *O. cruralis*, *O. discoidalis* (Peracca, 1895), *O. ibischi* (Reichle et al. 2001), *O. madidi* (Padial et al., 2005), and an undescribed species. This clade is in turn sister to the clade formed by *O. crepitans* and *O. heterodactylus*, all of them species with enlarged to slightly enlarged discs on fingers III and IV.

Four other major clades are recovered. A clade containing species from the Yungas of Bolivia and Peru is recovered as the sister group of that which includes *O. berdemenos* and the remaining species with enlarged finger discs. This Bolivian-Peruvian clade includes *O. amarakaeri*, *O. choristolemma*, *O. granulosus*, *O. sanctaerucis*, *O. sanderi*, and a candidate species from the Bolivian Yungas (*Oreobates* CaEU368903; see Padial et al. 2012). Sister to these two major clades is another clade that includes four species of cloud forest species from south-central Peru (Cusco-Ayacucho), namely, *O. ayacucho* (Lehr, 2007), *O. gemcare* Padial et al., 2012, *O. lehri* (Padial et al. 2007), *O. machigenga* Padial et al., 2012, and a candidate species (*Oreobates* CaJF809995; see Padial et al. 2012).

The sister group to the clade that includes all species mentioned above is formed by *O. lundbergi* (Lehr, 2005) and *O. pereger* (Lynch, 1975), both species from cloud forests of Peru, the former from central Peru (Pasco) and the latter from south-central Peru (Ayacucho).

Sister to the overall clade described so far, are the pair

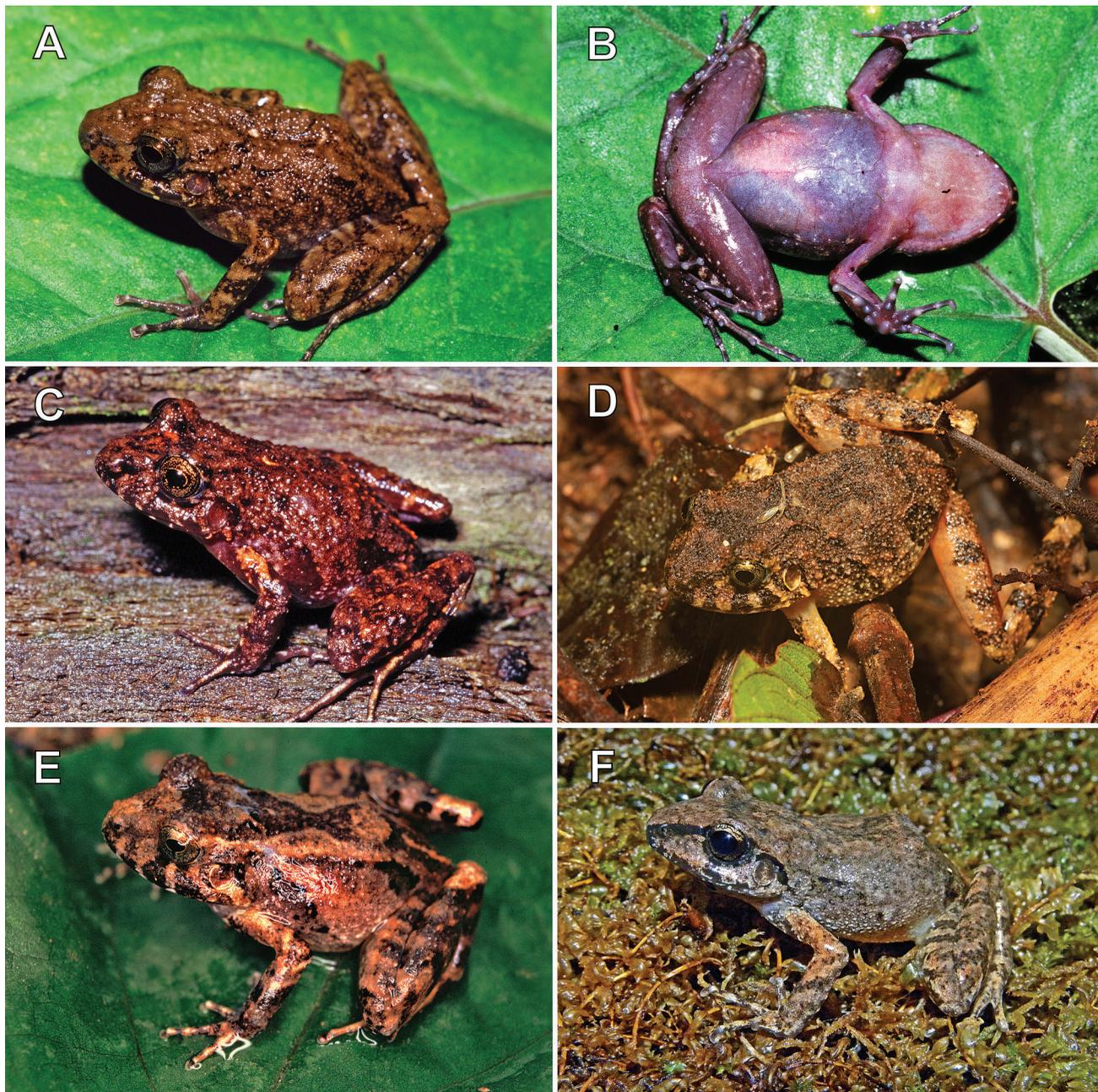


Fig. 2.—Living female holotype (ZFMK 72569) of *Oreobates yanucu*, new species: **A**, dorsolateral and **B**, ventral view. **C–F**, dorsolateral views of other *Oreobates* species in life for comparison either occurring in the Bolivian Yungas region (**C–E**) or are closely related to the new species (**F**): **C**, *O. sanctaerucis* (ZFMK 72647; Bolivia, Departamento Santa Cruz, near Karahuasi, 2200 m a.s.l.); **D**, *O. choristolemma* (not collected; Bolivia, Departamento La Paz, Río Camo, near Suapi, 1630 m a.s.l.); **E**, *O. sanderi* (NKA 6563; Departamento La Paz, north of Caranavi, 1300 m a.s.l.); and **F**, *O. berdemenos* (not collected; Argentina, Departamento Valle Grande, Abra de Cañas, 1700 m a.s.l.). Photos **A–C** by Jörn Köhler, **D** by Mauricio Pacheco. **E** by Andreas John, **F** by Laura Pereyra and Mauricio Akmentins.

of species conformed by *O. quixensis* and *O. saxatilis*, the former occurring along the Amazon lowlands of Ecuador, Peru, and Bolivia and adjacent Andean hills, and the later being restricted to the lower hills of the Andes of Peru.

Uncorrected-p distances evaluated for 18 species of

Oreobates sharing a 578 bp fragment of 16S with no missing data and gaps considered as absence of evidence range from 9.0–17.0%. *Oreobates berdemenos* shows 9% divergence to the new species, while *O. remotus*, sister to them, shows 12% divergence.

SYSTEMATICS

Class Amphibia Linnaeus, 1758

Order Anura Fischer von Waldheim, 1813

Family Craugastoridae Hedges, et al., 2008

Subfamily Holoadeninae Hedges, et al., 2008

Genus *Oreobates* Jiménez de la Espada, 1872*Oreobates yanucu*, new species

(Figs. 2–5)

Holotype.—ZFMK 72569 (field number JKSL 725), an adult female, from a point on “old Chapare road” that connected Villa Tunari and Cochabamba, 17°06'59" S, 65°34'43" W, 1500 m above sea level, Carrasco National Park, Provincia Chapare, Departamento Cochabamba, Bolivia, collected on 3 January 1999 by Jörn Köhler and Gandy Suárez.

Diagnosis.—A species of *Oreobates* (SVL of adult female 41.0 mm) characterized as follows: (1) skin of dorsum granular, granules conspicuous, keratinized, well defined, round to conical, heterogeneous in size, increasing in size toward flanks; small scattered warts on dorsum, larger and more numerous on flanks; indistinct occipital W-shaped fold on dorsum formed by interrupted rows of enlarged warts; venter smooth; posterior surfaces of limbs smooth, groin areolate; discoidal fold weak, barely recognizable; (2) tympanic membrane and annulus distinct, both with length slightly less than half of eye length; supratympanic fold short; postrictal tubercles conical; (3) head slightly longer than wide; snout short, broadly rounded in dorsal view, round in profile; canthus rostralis slightly sinuous in dorsal view, round in profile; (4) cranial crests absent; upper eyelid bearing small warts and granules; (5) dentigerous processes of vomers large, triangular, posteromedial to choanae, each with a single row of teeth; (6) hands with long and slender fingers, first slightly longer than second; subarticular tubercles large and prominent, round to conical; supernumerary tubercles small and inconspicuous; fingertips on fingers I and II round, enlarged, lacking circumferential grooves; fingertips on fingers III and IV truncate, distinctly enlarged, lacking circumferential grooves; lateral fringes and keels on fingers absent; (7) ulnar region lacking granules or tubercles; (8) no tubercles on heel or tarsus; (9) inner metatarsal tubercle conical, very prominent; outer metatarsal tubercle slightly larger in size, ovate, prominent; subarticular tubercles, conical, prominent; supernumerary tubercles smaller than subarticular tubercles, inconspicuous, small, round; (10) toes long and slender (foot length 50% SVL), basal webbing between toes I and II, II and III; tip of toe I slightly enlarged, rounded, unguis absent; tips of toes II, IV, and V enlarged, truncate, with distinct unguis flap; (11) axillary glands absent; (12) in life, dorsum brown with irregular black and dark brown flecks and blotches, and few scattered cream colored flecks; lips and extremities indistinctly barred, dark brown (-shaped mark in scapular region, venter with violet

tint; hidden and ventral surfaces of hind limbs, groin, and axillae purplish brown.

Oreobates yanucu differs from all other species of *Oreobates* by the unique combination of granular dorsal skin with scattered warts, finger I longer than finger II, finger tips of fingers III and IV distinctly enlarged and truncate in outline, tips of toes II to V with unguis flaps, supernumerary tubercles small and inconspicuous, head longer than wide, basal webbing between toes I and II and toes II and III, FL/SVL = 50%, lack of orange, red, or scarlet flecks and blotches in life.

In external morphology, *O. yanucu* superficially resembles *O. choristolemma*, *O. granulosus*, *O. sanctaerucis*, and *O. sanderi* in color pattern and dorsal skin texture (Fig. 2). The new species mainly differs from *O. choristolemma* (characters in parentheses) by head longer than wide (wider than long), indistinct dorsolateral fold (distinct), tips on fingers III and IV distinctly enlarged (slightly enlarged), basal webbing between toes I, II, and III (absent), supernumerary tubercles inconspicuous (conspicuous) (Fig. 3), and iris bronze with black reticulation in life (metallic green in life). The new species differs from *O. granulosus* (characters in parentheses) by larger size (max SVL in females 36.9 mm), weak discoidal fold (distinct); snout broadly rounded in dorsal view (rounded); dentigerous processes of vomers separated medially (almost in contact); first finger longer than second (equal in length or slightly shorter), tips of fingers III and IV distinctly enlarged, truncate (slightly enlarged, rounded), and basal webbing between toes I, II, and III (absent). *Oreobates yanucu* mainly differs from *O. sanctaerucis* (characters in parentheses) by head longer than wide (wider than long), snout broadly rounded in dorsal view (rounded), tips of fingers III and IV distinctly enlarged, truncate (not enlarged, round), basal webbing between toes I, II, and III (absent), and lack of scarlet flecks and blotches on dorsum and shanks (present). It mainly differs from *O. sanderi* (characters in parentheses) by head longer than wide (as wide as long), snout broadly rounded in dorsal view (rounded), tips of fingers III and IV distinctly enlarged, truncate (not enlarged, round), basal webbing between toes I, II, and III (absent), and small, inconspicuous supernumerary tubercles (conspicuous).

The new species is nested within a clade that includes other species having expanded discs on fingers III and IV (*O. barituensis*, *O. berdemenos*, *O. crepitans*, *O. cruralis*, *O. discoidalis*, *O. heterodactylus*, *O. ibischi*, and *O. remotus*; *O. madidi* is the only species in this clade having slightly expanded discs). From all of these it can be distinguished by having granular dorsal skin (versus smooth, shagreen, or coarsely shagreen). Similarly, it can be distinguished from other more distant species (*O. lehri*, *O. lundbergi*, *O. pereger*) by having granular dorsal skin (versus smooth, shagreen, or coarsely shagreen). Furthermore, *O. yanucu* differs from *O. ayacucho*, *O. gemcare*, *O. lehri*, *O. madidi*, *O. pereger*, *O. quixensis*, *O. saxatilis*, and *O. zongoensis* (Reichle and Köhler, 1997), by distinctly enlarged tips of fingers III and IV (versus not enlarged). The new

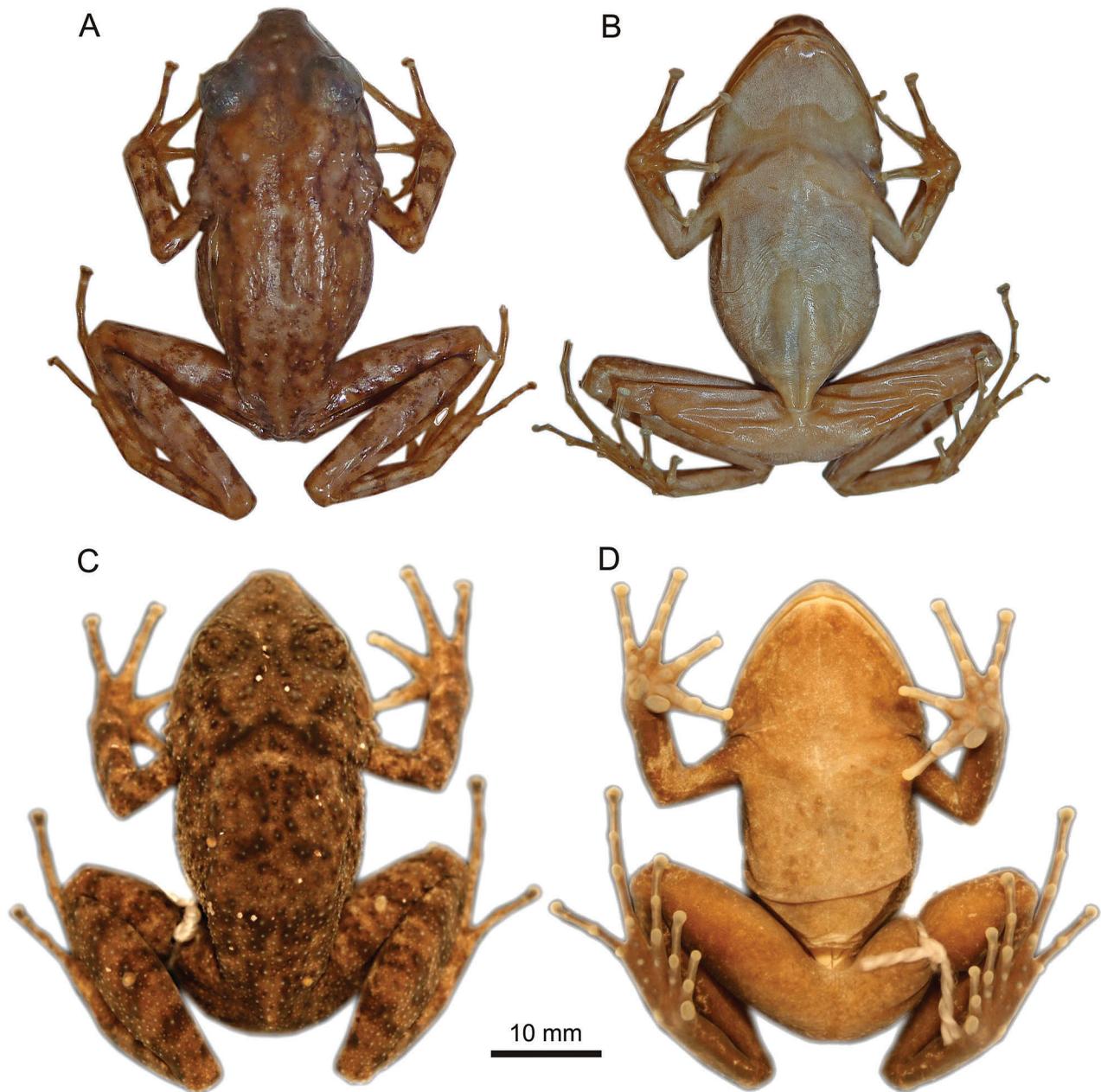


Fig. 3.—Dorsal and ventral views of preserved holotype specimens of (A–B) *Oreobates yanucu*, new species (ZFMK 72569), and (C–D) *Oreobates choristolemma* (CBF 5611).

species differs from *O. amarakaeri* and *O. machiguenga* by the presence of short supratympanic fold (absent), tips of fingers III and IV truncate (round), basal webbing between toes I, II, and III (absent), unguis flap on tips of toes II to V (absent), and throat without flecks or blotches (throat with white or orange blotches). From *O. crepitans*, the new species mainly differs by granular dorsal skin (warty) and a barely recognizable discoidal fold (distinct). Although genetically closest to *O. berdemenos* (Fig. 2F), *O. yanucu* differs from that species not only by granular

versus smooth/finely shagreen dorsal skin, but also by the upper eyelid covered with warts and granules (smooth, warts and tubercles absent), short supratympanic fold (well developed, long), and tips of fingers III and IV distinctly expanded, truncate (barely expanded, rounded).

Description of the holotype.—Adult female (body with lateral incision on left side). Head slightly wider than body, slightly longer than wide (HW/HL = 0.98); snout short, broadly rounded in dorsal view, round in lateral

view; nostrils slightly protuberant, oriented laterally; canthus rostralis slightly sinuous in dorsal view, round in frontal profile; loreal region slightly concave, sloping gradually to the lips; lips not flared; upper eyelid with numerous small granules and scattered small warts; cranial crests absent. Supratympanic fold short, extending to anterior level of insertion of arm; tympanic membrane and its annulus distinct; tympanic membrane nearly round, transparent, its length slightly less than half of eye length; postrictal tubercle large, single, conical. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae medium-sized, round, separated by distance equal to 6–7 times diameter of choana; dentigerous processes of vomers large, prominent, triangular in shape, situated posteromedial to choanae (anterior margin at posterior level of choanae), width about two times diameter of choanae, bearing four visible teeth arranged in a row each, separated medially by distance of approximately half the diameter of the process. Dorsal skin granular, granules conspicuous, keratinized, conical, heterogeneous in size on dorsum and flanks, smaller posteriorly, slightly increasing in size toward flanks; few small scattered warts present on dorsum, larger and more numerous on flanks; indistinct W-shaped occipital fold formed by interrupted row of flat warts; dorsolateral fold present, but indistinct, formed by interrupted row of flat warts; forelimbs with few small granules; ventral surfaces smooth; discoidal fold barely recognizable; skin in groin areolate. Ulnar region lacking tubercles; palmar tubercle divided in two ovate prominent subunits, inner larger than outer; thenar tubercle prominent, triangular in outline; subarticu-

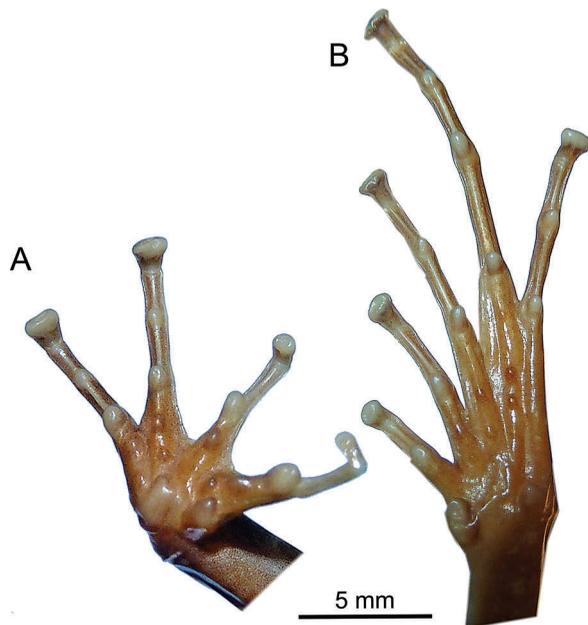


Fig. 4.—**A**, palmar surface of right hand and **B**, plantar surface of left foot of the preserved holotype of *Oreobates yanucu*, new species (ZFMK 72569).



Fig. 5.—Dorsal close-up view of the toe tip of fourth toe of left foot of the preserved holotype of *Oreobates yanucu*, new species (ZFMK 72569). Small red arrows indicate distal margin of unguis flap, being distinctly separated from the toepad. Photo by Arne Schulze.

lar tubercles prominent, round to conical; supernumerary tubercles small and inconspicuous; fingertips of fingers I and II round, slightly enlarged, fingertips of fingers III and IV truncate, distinctly enlarged, about two times the width of fingers; circumferential groove and unguis flap absent; lateral fringes and keels absent; relative length of fingers: III > IV > I > II (Fig. 4A). Toes long and slender (foot length 50% of SVL); heel and tarsus lacking tubercles or folds; inner metatarsal tubercle oval, prominent, about two times the size of outer metatarsal tubercle; outer metatarsal tubercle round, conical; lateral fringes absent, basal webbing between toes I and II, and toes II and III; tip of toe I slightly enlarged, round, without circumferential groove or unguis flap; tips of toes II, III, IV, and V truncate, enlarged (tip of toe IV on right foot missing), bearing distinct unguis



Fig. 6.—Montane rainforest formation (“old Chapare road” at app. 1600 m a.s.l.) close to the type locality of *Oreobates yanucu*, new species, photographed in January 1999 by Jörn Köhler.

flaps slightly indented (Fig. 5); relative length of toes $IV > V > III > II > I$; toe V reaching distal margin of second subarticular tubercle of toe IV, toe III reaching its proximal margin (Fig. 4B).

In preservative, dorsal surfaces of head and body brown, with dark brown, and pale brown irregular blotches and flecks; upper eyelids with greyish tint; granules of dorsum grayish brown; a dark brown)(-shaped interrupted occipital mark; lips barred with two dark brown subocular stripes; tympanic membrane transparent, annulus pale brown. Extremities brown, legs with diffusely defined dark brown transverse bars, arms with well-defined dark brown transverse bars; flanks brown with dark brown warts. Throat, chest, and belly cream with fine brown mottling; groin pale brown; posterior and anterior surfaces of hind limbs cream with dense brown mottling. In life, coloration generally had the same pattern but was much darker, with dorsum brown with irregular dark brown to black flecks and markings; violet tint at tympanic membrane and hidden surfaces of limbs; throat and chest white with

violet mottling; belly and ventral surfaces of limbs purplish brown; small, spaced cream flecks on lower lip; cream spot dorsally, median at posterior margin of scapular region; two small cream spots in interorbital region; two small cream spots on snout; iris bronze, reticulated with black, and with a narrow bronze ring around the pupil, which has a black ventral and smaller dorsal notch.

Measurements of the holotype.—SVL, 41.0; HL, 15.4; HW, 15.1; EL, 6.0; EN, 5.6; IND, 3.7; TYH, 2.8; TYL, 2.8; FIII, 1.5; FIV, 1.5; FA, 9.5, TL, 21.9; TH, 19.8; FL, 20.4; TIV, 1.48.

Distribution and ecology.—Only known from the type locality, but likely to occur at other locations in the central Bolivian Yungas. The single known female was collected at night during light rain at the edge of a small rivulet. It was sitting on a rock heavily covered with mosses. The habitat was undisturbed montane rainforest within the Carrasco National Park (Fig. 6), with steep slopes and numerous small- to medium-sized streams. Annual precipitation at the type locality supposedly ranges from 4500–5000 mm (see Köhler 2000). Other anuran species found in sympatry with the new species included *Atelopus tricolor*, *Rhinella justinianoi*, *R. leptoscelis*, *R. veraguensis*, *Nymphargus bejaranoi*, *Dendropsophus delarivai*, *Hypsiobas callipleura*, *H. riojanus*, *Pristimantis olivaceus*, *P. cf. platydactylus*, *P. reichlei*, *P. rhabdolaemus*, and *Yunganas tes mercedesae*.

Etymology.—The specific name is a composite of the Quechuan words *yan* (= road) and *rucu* (= old), with the ‘r’ of *rucu* being intentionally omitted for easier pronunciation and nicer sounding. The name refers to the type locality of the new species, located along the so-called ‘old road’ (carretera vieja) in the Chapare province, which connected the settlements of Villa Tunari and Cochabamba and which due to large landslides is no longer passable since the late 1990’s. The area represented by this road is known for remarkable amphibian species diversity and numerous discoveries (see Köhler 2000). The specific epithet is treated as a noun in apposition.

DISCUSSION

Singleton species.—The description of *O. yanucu* and the previous example of *O. zongoensis* raises the question of whether it is justified to name a species with a single known specimen—singletons in the terminology of Lim et al. (2012). Several examples of species from Bolivia described on the basis of a single specimen but using multiple lines of evidence simultaneously (e.g., mating calls and morphology; Köhler and Jungfer 1995; Reichle et al. 2001) have been corroborated by subsequent analyses with larger sample size and additional kinds of evidence such as DNA (Padial et al. 2009), but other singleton species named using morphology only were problematic (e.g.,

Gastrotheca lauzuricae, recently placed in synonymy; De la Riva 1992; Duellman 2015). The fact is that providing unambiguous evidence for the existence of a new species on the basis of a single specimen is challenging (for a discussion of the problem of limited sample size in taxonomy see Castroviejo-Fisher et al. 2011). We nonetheless consider that there are particular cases where the description of singleton species is justified and desirable. Often, the main limitation is a lack of good understanding of the intraspecific and interspecific variation of traits in the overall group, as this requires a thorough revision of the morphological diversity of the study group to be in place. In our case, the study of the bulk of specimens of *Oreobates* deposited in collections and the analysis of morphological, acoustic, and molecular divergences in species in this group (e.g., Padial et al. 2008a, 2008b, 2012) indicate that certain conditions such as expanded discs and granular or tuberculate skins are not variable intra-specifically even across specimens of different sex and age. Furthermore, the combination of expanded finger discs on fingers III and IV and the granular dorsum are not observed in any other species in this genus. Thus, and as Lim et al. (2012) put it, we consider that the new species has “a particularly distinct suite of morphological characters that renders it highly unlikely that it belongs to an already described species; that is, an implicit probability argument is used to justify the description.” Reinforcing this assumption, phylogenetic analyses of molecular data indicate that the new species falls within a clade of species with expanded finger discs but no granular dorsum, and it is furthermore not sister to any of the known species with granular dorsal skin, which represents strong evidence of a unique history of speciation and divergence. The new species also occurs in allopatry with respect to the other species forming this most inclusive clade, and it inhabits a different ecoregion—the humid Bolivian Yungas versus the Cerrado, inter-Andean dry valleys, and the more xeric Argentinian Yungas, which precludes the existence of a morphological cline that might be biasing our interpretation of character evidence (this scenario is nonetheless also rejected by the phylogenetic position of the new species). Other species of *Oreobates* from the Bolivian-Peruvian Yungas, such as *O. sanctaerucis*, *O. choristolemma*, *O. granulosus*, and *O. sanderi*, do have a granular dorsum but lack well-developed finger discs and they are distantly related. Finally, the question remains as to whether it will be problematic to differentiate *O. yanucu* from other species in the area that might be discovered in the future, such as the putative sister species of *O. yanucu*. In this case, molecular data will be key to provide evidence of divergence and fortunately the holotype of *O. yanucu* has proved to be useful for DNA sequencing. As methods for DNA sequencing even of formalin preserved samples are already in place (Taga et al. 2013), this problem will be marginal in the future, at least for amphibians and reptiles.

Evidently, the scenario outlined above is not optimal. Large series of specimens are always desirable, even if

establishing divergence with absolute certainty remains difficult statistically for realistically large samples (Wiens and Servedio 2000). But the need of naming singletons remains and it emerges for rather realistic reasons. As Lim et al. (2012) discussed, singletons are common in the recent taxonomic literature and this fact reveals the reality of rarity of many species. *Oreobates yanucu* and *O. zongoensis* are known from single specimens only collected more than 15 years ago despite their type localities being relatively well surveyed. Given that greatest species diversity occurs in poorly sampled tropical regions, and in face of the urgency and relevance of having good inventories of species diversity for the protection of areas and to monitor changes in species composition, naming rare yet well-supported species with single specimens will continue to be a common practice. In our opinion, the advantages of recognizing well-supported singleton species as targets for further research and conservation surpass the inconveniences of naming them (e.g., the risk of future synonymy). At the very least, we recommend that when evidence is not decisive (e.g., no DNA available, no clear qualitative differences, no pictures in life, imprecise type locality), putative new species known from a single specimen be flagged as candidate species. Fortunately, *O. yanucu* was collected in a well-preserved area and the type locality lies within a relatively well-protected area, which leaves hope for finding additional specimens in the future.

Species diversity.—Despite considerable progress in the taxonomy of *Oreobates*, species diversity in this genus remains underestimated. We are aware of seven additional putative new species of *Oreobates* that remain to be named. Three of these species correspond to two Bolivian and one Peruvian population highlighted as three different candidate species by Padial et al. (2012) using molecular data. Four other potential new species have been found along the Andean hills in south-central and northern Peru (J.M. Padial pers. obs.; J.C. Chaparro in litt.; G. Gagliardi in litt.). Given the recent rate of species discoveries (more than half of the named species have been described in the last ten years), the fact that most suitable areas for *Oreobates* remain unexplored—especially the sub-cordilleras that run parallel to the main Andean chain and that conform the divide between the Andes and the Amazon lowlands—and that some species appear to be extremely rare, it is justified to assert that we are far from having a good understanding of species diversity, distribution patterns, and morphological diversity in this genus.

IUCN Red List category.—Taking into account that the only other singleton species of *Oreobates* for which no additional specimens are known to date, *O. zongoensis*, is classified as “Critically Endangered” by the IUCN criteria Extent of Occurrence less than 100 km², Area of Occupancy less than 10km², all individuals are in a single location, and continuing decline in the extent and quality of its habitat (Cortez et al. 2004), it might seem plausible to propose

the same Red List category for the newly described singleton *O. yanucu*. However, forest at the type locality of *O. zongoensis* (Valle de Zongo, Departamento La Paz, Bolivia) has been largely cleared and intense searches to find additional individuals in that area failed. This situation is somehow different for *O. yanucu*, which was discovered in the vast montane rainforests of the fairly well-protected Carrasco National Park. Moreover, shortly after its discovery, the “old Chapare road” was blocked by huge landslides, largely impeding further scientific surveys of that area until today. We therefore propose the IUCN Red List category “Data Deficient” for *O. yanucu*.

Phylogenetic relationships of *Oreobates*.—With the discovery and description of *O. yanucu*, there are now 24 species recognized in the genus *Oreobates*. The position of 23 of these nominal species plus three candidate species has been inferred using DNA sequences and we can therefore consider that the monophyly of the genus is well supported. However, some of the internal relationships of *Oreobates* remain problematic. Below, we provide a summary of major conflicts among the relationships inferred in recent studies. These comparisons do not intend to favor some relationships over others but to underscore the most problematic relationships and identify thus future priorities for study. As not all analyses included the same species and characters, and the conditions and assumptions of analyses varied, differences among analyses can be attributed to multiple causes and need to be interpreted with caution. We restrict our comparisons to Pyron and Wiens (2011) who sampled 12 species of *Oreobates*, Pereyra et al. (2014) who sampled 22 nominal species and two unnamed species, Padial et al. (2014) who sampled 20 nominal species, and Motta et al. (2016) who sampled 22. We exclude from the comparisons analyses relying on only one marker (e.g., barcoding analyses of the 16S gene by Padial et al. 2012) or with scarce taxon sampling (e.g., Hedges et al. 2008 sampled three species and Teixeira et al. 2012 sampled six).

Our parsimony analyses using tree-alignment reveal a clade with *O. quixensis* and *O. saxatilis* as the sister group of the remaining *Oreobates*, a relationship that was recovered in maximum likelihood analyses but not in the parsimony analyses of Padial et al. (2014) or those of Pereyra et al. (2014). Alternatively, Motta et al. (2016) found this pair of species as the sister group of the remaining *Oreobates* in their parsimony analyses under tree-alignment but not in their maximum likelihood analyses. Pyron and Wiens (2011) did not recover this relationship either and found *O. lehri* as the sister group of the remaining *Oreobates*. *Oreobates lundbergi* and *O. pereger*, two species from central and south-central Peru, are recovered next as a clade in our analyses and as sister to the remaining *Oreobates*. These two species have been recovered as sister taxa in all other analyses but Pereyra et al. (2012), who surprisingly recovered *O. berdemenos* (a species from the Argentinian Yungas), as the sister group of *O. lundbergi*. Regardless, the

position of *O. lundbergi* + *O. pereger* varies across studies.

Our analyses also recover three major clades that are relatively stable across studies. These include a clade from south-central Peru (*O. ayacucho*, *O. gemcare*, *O. lehri*, *O. machiguenga*, and a candidate species from cloud forests in Departamento Cusco), and which, except for *O. ayacucho*, includes cloud forest species with relatively smooth skins, long fingers and toes and relatively low plantar tubercles (*O. ayacucho* inhabits Andean grasslands and has a distinctive and likely adaptive morphology). This clade was recovered by tree-alignment and parsimony in Padial et al. (2014) and Motta et al. (2016), but not by Pereyra et al. (2014), and maximum likelihood analyses in Padial et al. (2014) and Motta et al. (2016). Still, *O. gemcare*, *O. lehri*, and *O. machiguenga* were recovered as a clade in all studies but the relationships of *O. ayacucho*, *O. lundbergi*, and *O. pereger* vary.

The second relatively stable clade is formed by species with granular skins, relatively short fingers and toes, and conspicuous plantar tubercles, occurring through the Yungas of southern Peru and central to northern Bolivia (*O. amarakaeri*, *O. sanctaerucis*, *O. choristolemma*, *O. granulatus*, *O. sanderi*, and a candidate species from the Yungas of Cochabamba). Pyron and Wiens (2011) recovered part of this clade (they did not sample all species). Parsimony and tree-alignment and maximum likelihood analyses in Padial et al. (2014) and Motta et al. (2016) also recovered this clade but it was rejected by parsimony under similarity-alignment in the former study. Pereyra et al. (2014) found all but *O. amarakaeri* as part of this clade.

The third major clade includes more slender species with slightly to broadly expanded finger discs on fingers II and IV and generally soft skins (*O. barituensis*, *O. berdemenos*, *O. crepitans*, *O. cruralis*, *O. discoidalis*, *O. heterodactylus*, *O. ibischi*, *O. madidi*, *O. remotus*, *O. yanucu*, and a candidate species from the inter-Andean dry valleys of central Bolivia). This clade covers a substantial area of South America and includes species in a wide array of environments, from the dry Atlantic forest and the Cerrado to the Andean Yungas and dry inter-Andean forests of Argentina and Bolivia, and the Amazonian lowlands. The clade was supported by the analyses of Pyron and Wiens (2011) although with smaller sampling, and by all the analyses of Padial et al. (2014) and Motta et al. (2016). The clade was also recovered by Pereyra et al. (2014) except for the position of *O. berdemenos*, which was inferred as the sister group of high Andean species from central Peru. Within this clade, *O. barituensis*, *O. discoidalis*, and *O. ibischi*, three species from the Argentinian-Bolivian Yungas and inter-Andean dry valleys, are always found forming a monophyletic group. *Oreobates crepitans* and *O. heterodactylus*, the two species of the Cerrado domain, are also recurrently recovered as sister taxa (e.g., Teixeira et al. 2012). Pereyra et al. (2014) found *O. remotus* as sister to *O. crepitans*, while our analyses recover *O. remotus* as the sister group of *O. berdemenos* + *O. yanucu*.

Oreobates zongoensis, a species from the Bolivian Yun-

gas is the only known species of *Oreobates* that has not been analyzed phylogenetically using molecular data. Its generic position is based on potential synapomorphies such as the granular dorsal skin and smooth belly, the lack of expanded finger discs on fingers I and II, and the presence of well-developed conical plantar tubercles (Reichle and Köhler 1997), which allies this species to a clade composed of Bolivian and Peruvian species (*O. amarakaeri*, *O. choristolemma*, *O. granulosus*, *O. sanctaecrucis*, and *O. sanderi*) (Padial et al. 2008a). Unfortunately, this species is only known from the holotype and we failed both to find additional specimens at the type locality in the Bolivian Yungas (J.M. Padial pers. obs.) and to sequence DNA from a tissue sample from the holotype (likely fixed in formalin) using standard extraction and amplification procedures.

ACKNOWLEDGMENTS

We are grateful to the following persons for the loan of specimens, support, and/or space provided at their institutions: D. Frost and D. Kizirian, (AMNH); B. Clarke and M. Wilkinson (BM); J. Aparicio (CBF); R. Aguayo and A. Muñoz (CBG); W.E. Duellman, and L. Trueb (KU); J. Hanken and J. Rosado (MCZ); O. Aguilar and R. Orellana (MHNC); B. Álvarez, J.E. González-Fernández, and I. Rey (MNCN); L. González, A. Justiniano, R. Montaña, M. Suárez, and R. Vespa (MNK); J. Pombal (MNRJ); C. Aguilar (MUSM); P.E. Vanzolini and H. Zaher (MZUSP); D. Cannatella and T. LaDuc (TNHC); R.A. Nussbaum and G. Schneider (UMMZ); K. de Queiroz, W.R. Heyer, R. McDiarmid, and R.V. Wilson (USNM); J.A. Campbell (UTA); W. Böhme (ZFMK); M. Andersen and the late J.B. Rasmussen (ZMUC). L. Pereyra, M. Akmentins, A. John and M. Pacheco Suarez kindly provided photos of living specimens. The close-up image of the unequal flap was kindly produced by A. Schulze (HLMD), using stacked photography. We thank S. Castroviejo-Fisher and E. Lehr for critical review of the manuscript. Fieldwork of J.K. was funded by German Academic Exchange Service (DAAD) and grants of 'Graduiertenstipendium des Landes Nordrhein-Westfalen'. This work was partially funded by projects CGL2014-56160-P (I. De la Riva, principal investigator) and CGL2013-47547-P (Carles Vila, principal investigator) of the Spanish Ministry of Science and Innovation.

LITERATURE CITED

- CARAMASCHI, U., AND C. CANEDO. 2006. Reassessment of the taxonomic status of the genera *Ischnocnema* Reinhard and Lütken, 1862 and *Oreobates* Jiménez-de-la-Espada, 1872, with notes on the synonymy of *Leiuperus verrucosus* Reinhardt and Lütken, 1862 (Anura: Leptodactylidae). *Zootaxa*, 1116:43–54.
- CASTROVIEJO-FISHER, S., C. VILÀ, J. AYARZAGÜENA, M. BLANC, AND R. ERNST. 2011. Species diversity of *Hyalinobatrachium* glassfrogs (Amphibia: Centrolenidae) from the Guiana Shield, with the description of two new species. *Zootaxa*, 3132:1–55.
- CORTEZ, C., S. REICHLER, I. DE LA RIVA, AND J. KÖHLER. 2004. *Oreobates zongoensis*. The IUCN Red List of Threatened Species 2004: e.T57067A11576375. Downloaded on 02 June 2016.
- DE LA RIVA, I. 1992. Comentarios sobre el género *Gastrotheca* (Anura: Hylidae) en Bolivia y descripción de una nueva especie. *Revista Española de Herpetología*, 6:15–22.
- DUELLMAN, W.E. 2015. *Marsupial Frogs: Gastrotheca and Allied Genera*. The Johns Hopkins University Press, Baltimore, 432 pp.
- DUELLMAN, W.E., AND E. LEHR. 2009. Terrestrial-breeding Frogs (Strabomantidae) in Peru. *Natur und Tier Verlag*, Münster, 382 pp.
- FARRIS, J.S., V. A. ALBERT, M. KALLERSJO, D. LIPSCOMB, AND A.G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, 12:99–124.
- FROST, D.R. 2016. Amphibian species of the world: an online reference. Version 6.0 (27 May 2016). American Museum of Natural History, New York. Electronic database accessible online (<http://research.amnh.org/vz/herpetology/amphibia/>).
- GOICOECHEA, N., I. DE LA RIVA, AND J.M. PADIAL. 2010. Recovering phylogenetic signal from frog mating calls. *Zoologica Scripta*, 39:141–154.
- GOLOBOFF, P.A. 1996. Methods for faster parsimony analysis. *Cladistics*, 12:199–220.
- . 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics*, 15:415–428.
- GOLOBOFF, P.A., J.S. FARRIS, AND K.C. NIXON. 2008. TNT, a free program for phylogenetic analysis. *Cladistics*, 24:774–786.
- HARVEY, M.B., AND M.B. KECK. 1995. A new species of *Ischnocnema* (Anura: Leptodactylidae) from high elevations in the Andes of central Bolivia. *Herpetologica*, 51:56–66.
- HARVEY, M.B., AND C.M. SHEEHY III. 2005. A new species of *Ischnocnema* (Anura: Leptodactylidae) from La Paz, Bolivia. *Herpetologica*, 61:268–275.
- HEDGES, S.B., W.E. DUELLMAN, AND M.P. HEINICKE. 2008. New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa*, 1737:1–182.
- KATO, K., AND D.M. STANDLEY. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30:772–780.
- KÖHLER, J. 2000. Amphibian diversity in Bolivia: a study with special reference to montane forest regions. *Bonner zoologische Monographien*, 48:1–243.
- KÖHLER, J., AND K.-H. JUNGFER. 1995. Eine neue Art und ein Erstnachweis von Fröschen der Gattung *Eleutherodactylus* aus Bolivien. *Salamandra*, 31:149–156.
- LIM, G.S., M. BALKE, AND R. MEIER. 2012. Determining species boundaries in a world full of rarity: singletons, species delimitation methods. *Systematic Biology*, 61:165–169.
- LYNCH, J.D. 1975. A review of the Andean leptodactylid frog genus *Phrynos*. *Occasional Papers of the Museum of Natural History, University of Kansas*, 35:1–51.
- MOTTA, A.P., J.C. CHAPARRO, J.P. POMBAL, J.M. GUAYASAMIN, I. DE LA RIVA, AND J.M. PADIAL. 2016. Molecular phylogenetics and taxonomy of the Andean Genus *Lynchius* Hedges, Heinicke, and Duellman 2008 (Anura: Craugastoridae). *Herpetological Monographs*, 30:119–142.
- NIXON, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, 15:407–414.
- PADIAL, J.M., S. CASTROVIEJO-FISHER, J. KÖHLER, C. VILÀ, J.C. CHAPARRO, AND I. DE LA RIVA. 2009. Deciphering the products of evolution at the species level: the need for an integrative taxonomy. *Zoologica Scripta*, 38:431–447.
- PADIAL, J.M., J.C. CHAPARRO, S. CASTROVIEJO-FISHER, J.M. GUAYASAMIN, E. LEHR, A.J. DELGADO C., M. VAIRA, M. TEIXEIRA, JR., C.R. AGUAYO-VEDIA, AND I. DE LA RIVA. 2012. A revision of species diversity in the Neotropical genus *Oreobates* (Anura: Strabomantidae), with the description of three new species from the Amazonian slopes of the Andes. *American Museum Novitates*, 3752:1–55.
- PADIAL, J.M., J.C. CHAPARRO, AND I. DE LA RIVA. 2008a. Systematics of *Oreobates* and the *Eleutherodactylus discoidalis* species group (Amphibia, Anura) based on two mtDNA genes and external morphology. *Zoological Journal of the Linnean Society*, 152:737–773.
- PADIAL, J.M., AND I. DE LA RIVA. 2005. Rediscovery, redescription, and advertisement call of *Eleutherodactylus heterodactylus* (Miranda Ribeiro, 1937) (Anura: Leptodactylidae), and notes on other *Eleutherodactylus*. *Journal of Herpetology*, 39(3):372–379.
- PADIAL, J.M., L. GONZALEZ, AND I. DE LA RIVA. 2005. A new species of the *Eleutherodactylus discoidalis* group (Anura: Leptodactylidae) from Andean humid montane forest of Bolivia. *Herpetologica*, 61:318–325.
- PADIAL, J.M., T. GRANT, AND D.R. FROST. 2014. Molecular systematics

- of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa*, 3825:1–132.
- PADIAL, J.M., J. KÖHLER, A. MUÑOZ, AND I. DE LA RIVA. 2008b. Assessing the taxonomic status of tropical frogs through bioacoustics: geographical variation in the advertisement call in the *Eleutherodactylus discoidalis* species group (Anura: Brachycephalidae). *Zoological Journal of the Linnean Society*, 152:353–365.
- PEREYRA, M.O., D. CARDOZO, J.L. BALDO, AND D. BALDO. 2014. Description and phylogenetic position of a new species of *Oreobates* (Anura: Craugastoridae) from northwestern Argentina. *Herpetologica*, 70:211–227.
- PYRON, R.A., AND J.J. WIENS. 2011. A large-scale phylogeny of Amphibia including over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61:543–583.
- REICHLER, S., AND J. KÖHLER. 1997. A new species of *Eleutherodactylus* (Anura: Leptodactylidae) from the Andean slopes of Bolivia. *Amphibia-Reptilia*, 18: 333–337.
- REICHLER, S., S. LÖTTERS, AND I. DE LA RIVA. 2001. A new species of the *discoidalis* group of *Eleutherodactylus* (Anura, Leptodactylidae) from inner-Andean dry valleys of Bolivia. *Journal of Herpetology*, 35(1):21–26.
- TAGA, M., H., EGUCHI, T. SHINOHARA, K. TAKAHASHI, R. ITO, W. YASUI, K. NAKACHI, Y. KUSUNOKI, AND K. HAMATANI. 2013. Improved PCR amplification for molecular analysis using DNA from long-term preserved formalin-fixed, paraffin-embedded lung cancer tissue specimens. *International Journal of Clinical and Experimental Pathology*, 6:76–79.
- TEIXEIRA, M., JR., R.C. AMARO, R.S. RECODER, M.A. DE SENA, AND M.T. RODRIGUES. 2012. A relict new species of *Oreobates* (Anura, Strabomantidae) from the seasonally dry tropical forests of Minas Gerais, Brazil, and its implication to the biogeography of the genus and that of South American dry forests. *Zootaxa*, 3158:37–52.
- VAIRA, M., AND L. FERRARI. 2008. A new species of *Oreobates* (Anura: Strabomantidae) from the Andes of northern Argentina. *Zootaxa*, 1908:41–40.
- VARÓN, A., L.S. VINH, AND W.C. WHEELER. 2010. POY version 4, phylogenetic analysis using dynamic homologies. *Cladistics*, 26:72–85.
- WHEELER, W.C. 1996. Optimization alignment, the end of multiple sequence alignment in phylogenetics? *Cladistics*, 12:1–10.
- WHEELER, W.C. 2003. Implied alignment, a synapomorphy-based multiple sequence alignment method and its use in cladogram search. *Cladistics*, 19:261–268.
- WHEELER, W.C., C.P. ARANGO, T. GRANT, D. JANIES, A. VARÓN, L. AAGESEN, J. FAIVOVICH, C. D'HAESE, W.L. SMITH, AND G. GIRIBET. 2006. *Dynamic Homology and Phylogenetic Systematics, A Unified Approach Using POY*. American Museum of Natural History, New York, 365 pp.
- WIENS, J.J., AND M.R. SERVEDIO. 2000. Species delimitation in systematics: inferring diagnostic differences between species. *Proceedings of the Royal Society of London B: Biological Sciences*, 267:631–636.

APPENDIX
(continued on next page)

Specimens Examined

Oreobates amarakaeri (3 specimens): **PERU: Cusco:** Río Nusunscato, 685 m a.s.l. (13° 08' 13.1" S, 70° 51' 05.8" W), MHNC 6975 (holotype); Río Mabe, 1000 m a.s.l. (13° 06' 31.5" S, 70° 54' 56.0" W), MHNC 7017 (paratype), MHNC 7049 (paratype).

Oreobates ayacucho (3 specimens): **PERU: Ayacucho:** road between Punqui and Anco, 3 km before Anco, 3850 m a.s.l. (13.1038°S, 73.6983°W), MNCN 44484–5, MHNC 6840.

Oreobates crepitans (3 specimens): **BRAZIL: Mato Grosso:** São Vicente, Cuiabá, MZUSP 85628 (holotype), MZUSP 73671 (allotype), MNRJ 33985 (paratype).

Oreobates choristolemma (4 specimens): **BOLIVIA: La Paz:** Provincia Sud Yungas, Boquerón, CBG 765–68.

Oreobates cruralis (184 specimens): **BOLIVIA: Beni:** Areruta, CBG 1545–7; Asunción, Biosphere Reserve Pilón Lajas, MNK A 4074; Buena Vista, MZUM 66609–10; Huachi, MZUM 58987, 135343; Laguna Azul, Biosphere Reserve Pilón Lajas, MNK A 3975–76, 3979, 3985–87, 4003; Rurrenabaque, CBG 3667, MNCN 42558–9; San Luis Chico, MNK A 4027, 4030; Serranía del Pilón, Biosphere Reserve Pilón Lajas, MNK A 4182–83, 4209–13; **Cochabamba:** Bulo Bulo, TIPNIS, CBG 350, 380, 382–3, 393; Chaquisacha, 1300–1500 m a.s.l., Parque Nacional Carrasco, CBG 198, 199, 1140; between Paracti and El Palmar, ZFMK 66964, ZFMK 66971–2; El Palmar, 1300 m a.s.l., Parque Nacional Carrasco, CBG 276, 956, ZFMK 72570; between Paracti and El Palmar, ZFMK 66964 ZFMK 66971–2, ZFMK 72541–3; Los Guácharos (Chapare, 500 m a.s.l.), CBG 308, MNK A 6617–19, MNCN 43018–9, ZFMK 72532; on Villa Tunari road, USNM 146577; Puerto Villaroel, CBG 1337; Río Grande, 1000 m a.s.l., Parque Nacional Carrasco, CBG 275; San José de la Angostura, TIPNIS, CBG952–54; Santa Anita, TIPNIS, CBG 739; Santo Domingo, 800 m a.s.l., TIPNIS, CBG 596–7; Serranía de Mosetenes, 1580 m a.s.l., MNCN 43155; Valle de Sajta, MNK A 3633, 1489; Villa Tunari, MNK A 1492; **La Paz:** Arroyo Pico Plancha, San José y Apolo, ANMI-Parque Nacional Madidi, MNK A 7180; Aserradero San Francisco, Ixiamas, CBG 1133; Boquerón, Biosphere Reserve Pilón Lajas, CBG 789–93; Camino a Bella Vista, Sud Yungas, MNK A 3792; Camino maderero El Chaval, Arroyo Mikai, Biosphere Reserve Pilón Lajas, MNK A 3759–61; Chalalán, ANMI-Parque Nacional Madidi, MNCN 42560, 43021–2, 43058, 43222, 43223; Flor de Mayo, La Asunta, CBG 2936; Flor de Mayo, río Boopi, CBG 1791; Hornuni, Parque Nacional AMNI-Cotapata, CBG 4208; Irupana, CBG 529; La Paz (locality in error), BM 1947.2.15.70 (holotype); CBG 2484; Puerto Linares, 360 m a.s.l., USNM 281100–30; road from Caranavi to Palos Blancos, ZFMK 80599; Serranía Tequeje, CBG 5104–7; Serranía del Pilón, Biosphere Reserve Pilón Lajas MNK A 4182–6, 4201, 4208–13, 4218; Torno Azul, Biosphere Reserve Pilón Lajas, CBG 1004; Tumupassa, MZUM 58985–6, 58988; Tunquini, Parque Nacional AMNI-Cotapata, CBG 4224; 5 km W of San Buenaventura USNM 280617; **Pando:** Florida, Reserva Manuripi, MNK A 5086, 5122; Lago Bay, Reserva Manuripi, MNK A 6120–21; **Santa Cruz:** Buena Vista, USNM 118686; road to Bella Vista near road to Samaipata, MNCN 42557, 42977, MNK A 7171, ZFMK 71997; La Hoyada, 1800 m a.s.l., Parque Nacional Amboró, MNK A 5577, ZFMK 72644; Mataracú, Parque Nacional Amboró, MNK A 3950, 3952, 3954, 3968; Santa Cruz de la Sierra, BM 1904.10.29.102–107; south of Cuevas, 1100 m a.s.l., ZFMK 72644; Sara, MZUSP 119467; Víbora, CBG 3638. **PERU: Madre de Dios:** Colpa de Guacamayos, Zona Reservada Tambopata-Candamo, USNM 332436–37; Puerto Maldonado, 30 km SSW of Tambopata, USNM 284267, 343240, 342989–92; 15 km E of Puerto Maldonado, 200 m a.s.l., KU 207749, 215461–62, 215479–80; **Cusco:** Río los Amigos, MHNC 3170, 3172, 3177, 4959, 4966.

Oreobates discoidalis (114 specimens): **ARGENTINA: Tucumán:** El Indio, km 28 Tafí del Valle-Amaicha del Valle road, 960 m a.s.l., KU 182815; Horco Molle, “13 km W of Tucumán,” Sierra de San Javier, ca. 1200 m a.s.l., BM 1947.2.15.63–65 (syntypes), KU 154521–29; MCZ 35583, MZUSP 85542; 24 km W of Tucumán, KU 206434–38; without locality, MCZ 117097; **Jujuy:** Abra de las Cañas, Serranía de Calilegua, 1550 m a.s.l., KU 182813–14; Arroyo Agua Negra, near border Salta-Jujuy, 10 km up to Monolito and Valle Grande, from Yuto to Ledesma, TNHC 36776, 36778–93; San Lorenzo, BM 98.7.7.19.20. **BOLIVIA: Chuquisaca:** Río Pilipili, CBG 1396–7; Departamento Santa Cruz: Río Parabano, MNK A 5582; **Tarija:** Bajada de la Escalera, Reserva Nacional Tariquía, CBG 4978–9; Cambarí, Reserva Nacional de Tariquía, CBG 4889–90; Chiquiacá, MNK A 7262–3, MNCN 43133–35; Entre Ríos, MNK A 7257–59, MNCN 43132; La Planchada, CBG 3773, 3780–4; Potrerillas, Reserva Nacional Tariquía, CBG 4891; Serranía Aguarague, a 17 km de Villa Montes en dirección a Entre Ríos, MNK A 7246–56, MNCN 43123, 43125–31, 43136–9; Serranía Aguarague, a 40 km de Yacuiba, CBG 1411–17; subida a Palo Marcado, Reserva Nacional Tariquía, CBG 4970; 12.3 km NW of Entre Ríos on the road to Tarija, 1900 M, MNK A 3877–97, UTA 45645, 45648–50, 45652, 45658–62.

Oreobates gemcare (33 specimens): **PERU: Cusco:** Buenos Aires, 2400 m a.s.l., KU 173231; Esperanza, Kosñipata Valley, 2700 m a.s.l. (13.1824°S, 71.6036°W), MHNC 4557, 4564–4567, 4583–4586 (paratype), MHNC 4601–4602 (paratype), MHNC 6687 (holotype), MNCN 43740–41 (paratype), MNCN 44230 (paratypes); Paucartambo, AMNH 153087; Pillahuata, between Puente Kosñipata and road, 2430 m a.s.l., (13° 09' 52" S, 71° 35' 46" W), AMNH 11831 (paratype).

Oreobates granulosus (26 specimens): **PERU: Puno:** Agualani, Carabaya, 9000 ft, BM 1905.5.31.14.20 (four specimens); Limbani, Carabaya, 9000 ft, BM 1905.5.31.21 (formerly 1904.10.26.94–99); Ollachea, 11 km (airline NNE of), 1800 m a.s.l., USNM 299006–12; Santo Domingo, Carabaya, 1400–1500 m a.s.l., MHNC 5328, 5335; Santo Domingo, Carabaya, 6000 ft (1800 m a.s.l. aprox.), BM 1947.2.15.72 (holotype); Santo Domingo, Carabaya, 6500 ft (2000 m a.s.l. aprox.), BM 1907.5.7.17–18; Juliaca (locality in error), AMNH 6060–2, 6064.

Oreobates heterodactylus (10 specimens): **BOLIVIA: Santa Cruz:** Bella Boca, MNK A 6482; Cerro del Arco, Serranía de Santiago, MNK A 6356–7, 7175–6, MNCN 43055–6. **BRAZIL: Mato Grosso:** Gruta da Fazendinha, Cáceres, MNRJ 106 (holotype); Fazenda Santa Edwiges, MZUSP 71103–4.

Oreobates ibischi (4 specimens): **BOLIVIA: Santa Cruz:** km 68.5 on Santa Cruz de la Sierra-Samaipata road, 750 m a.s.l., CBF 3341 (holotype); km 60 on Santa Cruz de la Sierra-Samaipata road, MNK A 6612, MNCN 42959; El Fuerte de Samaipata, 1900 m a.s.l., ZFMK 60402 (paratype).

Oreobates lehri (32 specimens): **PERU: Cusco:** Apurimac River Valley, Camisea Natural Gas Pipeline, Wayrapata Camp, 2445 m a.s.l., (12°50'10" S, 73°29'43" W), USNM 537848 (holotype), USNM 537846–57 (paratopotypes); ca. 40 km from Vilcabamba, 2850 m a.s.l. (13°02'51.1"S, 072°55'29.4"W), MUSM 27616.

APPENDIX

(continued from previous page)

Oreobates lundbergi (2 specimens): **PERU: Pasco:** from Auquimarca to Uchuerta, 2760 m a.s.l., MTD 45902 (paratype); road Puagmaray Oxapampa km 77, 2550 m a.s.l., MUSM 19321 (paratype).

Oreobates machiguenga (1 specimen): **PERU: Cusco:** Maguireni (12°34'42"S/73°40'21"W), Río Kimbiri, Apurímac River Basin, MHNC 6809 (holotype).

Oreobates madidi (6 specimens): **BOLIVIA: La Paz:** Arroyo Huacataya, Serranía Eslabón, Área Natural de Manejo Integrado Madidi, MNK A 7856 (holotype), 7197 (paratype), MNCN 42014–15 (paratypes); La Cascada, Biosphere Reserve Pílon Lajas, MNK A 4137–38 (paratypes).

Oreobates pereger (40 specimens): **PERU: Ayacucho:** Provincia La Mar, Yanamonte (12°47'25.0"S, 74°00'02.4"W), 2600 m a.s.l., MUSM 19982–19984, MTD 46807–09, FMNH 39747–65, 39768–70, 39773–78, 39780, 39782, 39837; Mitupucuru on Tambo-Valle del Apurímac path, KU 151908-09 (paratypes), KU 196595.

Oreobates quixensis (69 specimens): **BOLIVIA: Pando:** Luz de América, Reserva Nacional de Vida Silvestre Manuripi-Heath, MNK A 6525; Murden, 4591; Reserva Nacional de Vida Silvestre Manuripi-Heath, MNK A 3640–1, 6099; Reserva Nacional de Vida Silvestre Tahuamanu, NKA 4586–90; Río Negro, MNK A 6525–27, 6900; San Antonio, Reserva Nacional de Vida Silvestre Manuripi-Heath, MNK A 6526–27. **COLOMBIA: Amazonas:** Puerto Nariño MCZ 93777–80; Río Amaca–Yacu, MCZ 95734. **ECUADOR: Morona-Santiago:** Sucua, MCZ 91361; **Napo:** AMO-II drill site, MCZ 111776–7; Archidona, MNCN 1709–21 (paralectotypes); Coca, MCZ 105868, 106017–19, 124825; Hacienda Primavera, MCZ 97881; La Cruz Blanca, South side of Río Napo, MCZ 109213–14, 119093; Limón Cocha MCZ 56307; near Tarapoa, MCZ 101262; Santa Cecilia, MCZ 57793–97, 57799; San José de Moti, MNCN 1708 (lectotype); **Oriente:** Canelos, MCZ 19734–6; **Zamora:** surroundings of Villano, 430 m a.s.l., QCAZ 38670, 38693, 39215, 39217, 39244. **PERU: Amazonas:** MCZ 125896–7; **Loreto:** Arboretum de la UNAM, camino hacia Puerto Almendras, 103 m a.s.l., MHNC 8805, 8809; Camino a Zungarococha y Arboreto de la Universidad de Iquitos, Camino a Puerto Almendras, 103 m a.s.l., MHNC 8832; Pucaurquillo, Río Ampiyacu, 99 m a.s.l., MHNC 8997; Río Ampiyacu, 92 m a.s.l., MHNC 8918.

Oreobates remotus (24 specimens): **BRAZIL: Minas Gerais:** Januária, Parque Nacional Cavernas do Peruaçu, 624 m a.s.l., MZUSP 141708 (holotype), MZUSP 141709–724, MZUFV 5005–10 (paratypes); Rio Pandeiros, MZUSP 23306.

Oreobates sanctaerucis (13 specimens): **BOLIVIA: Cochabamba:** Chaquisacha, 1300–1500 m a.s.l., Parque Nacional Carrasco, CBG 152–3; Diampampa, Parque Nacional Carrasco, CBG 634; Karahuasi, 2200 m a.s.l., ZFMK 72647; Río Grande, 1000 m a.s.l., Parque Nacional Carrasco, CBG 291–292; **Santa Cruz:** El Bibosi, Parque Nacional Amboró, MNK A 6697; El Chapé, Parque Nacional Amboró, 2060 m a.s.l., MNK A 1198 (holotype), 7158, MNCN 42010–13.

Oreobates sanderi (19 specimens): **BOLIVIA: La Paz:** Arroyo Bilunto, Churirumi Valley, Bilunto Mountains, Area Natural de Manejo Integrado Madidi, near Santa Cruz de Valle Ameno, CBF 5385 (holotype), MNCN 42016–7 (paratypes), CBF 5383–4 (paratypes); Bajo Hornuni, CBF 4119–22, 4218–19, 4223 (paratypes); Colonia Eduardo Avaroa, ca. 30 km north of Caranavi on the road from Caranavi to Yucumo, ZFMK 80600–1 (paratypes), MNK A 6563 (paratypes); road from Apolo to Sarayo, MNK A 6695–6 (paratypes). **PERU: Puno:** Abra de Maruncuna, 10 km SW of San Juan del Oro, 1650 m a.s.l., KU 206101; Juliaca (locality in error), AMNH 6063.

Oreobates saxatilis (14 specimens): **PERU: Cusco:** Río Kimbiri, 1000 m a.s.l., MHNC 6775–77; Río Kimbiri, Comunidad Machiguenga Pomoreni, 1100 m a.s.l., MNCN 44412, 44426–28; **San Martín:** Pongo de Shilcayo, about 4 km NNW of Tarapoto, 470 m, KU 212556 (holotype), MUSM 8431 (paratype); San Roque de Cumbasa, 609 m a.s.l., MHNC 9200; 2 Km al Este de Roque, 1138 m a.s.l., MHNC 9202; 6 Km S of Roque, on Río Sisa, Catarata de Pueblo Nuevo, 1086 m a.s.l., MHNC 9219–21.

Oreobates zongoensis (1 specimen): **BOLIVIA: La Paz:** Valle de Zongo, 1250 m a.s.l., CBF 2503 (holotype).