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Underestimated anuran radiations in the high Andes: five new species and a new genus of Holoadeninae, and their phylogenetic relationships (Anura: Craugastoridae)

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Despite recent efforts to accelerate exploration and species description, the diversity of high Andean frogs remains highly underestimated. We report high levels of species diversity in direct-developing frogs or terraranas inhabiting the wet puna and adjacent cloud forests of the Amazonian versant of the Andes in Bolivia and Peru. Descriptive evidence of external morphology, distribution patterns and molecular phylogenetic analyses support the existence of nine unnamed species in two clades, which represents a 30% increase in species diversity for those clades. The relationships of these species and their relatives in Holoadeninae are tested using nuclear and mitochondrial genes for 159 terminals representing the 11 genera in this subfamily and 25 species of previously unknown relationships. Our results corroborate species monophyly in all but three cases and support the monophyly of all Holoadeninae genera, albeit the position of some differs between analyses. We propose a new genus (***Microkayla* gen. nov.**) for the clade containing all Bolivian species formerly in *Psychrophrynella* plus five species from southern Peru. The new genus is monophyletic and supported by anatomical synapomorphies. *Psychrophrynella* is re-diagnosed and redefined to include three species from the Andes of southern Peru. We discuss the taxonomic instability associated with *Noblella* and *Psychrophrynella* due to the fact that the type species of both genera share a number of traits that support a close relationship. We also name and describe three new species of *Bryophryne* and two of *Microkayla* from Peru, provide baseline data for the future description of four Bolivian species of *Microkayla*, and describe the unknown mating calls of two species. Our results support that the grasslands of the Amazonian versant of the Andes harbour a large diversity of species with small altitudinal and horizontal distributions that replace each other along a latitudinal axis. These species belong to different lineages whose closest relatives are forest species, often from distant parts of the continent. These patterns suggest that high Andean environments were colonized several times independently by species with forest ancestors and which radiated into a multitude of species with remarkably similar ecomorphologies. The extent of these radiations remains obscured by a still rudimentary knowledge of species diversity due to insufficient fieldwork and taxonomic research.

ADDITIONAL KEYWORDS: adaptive radiation – Amazon basin – *Bryophryne* – grasslands – integrative taxonomy – *Microkayla* gen. nov. – *Noblella* – *Psychrophrynella* – tree alignment – wet puna.

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INTRODUCTION

Terrarana frogs (Brachycephaloidea Günther, 1858) form a large clade of over 1000 species that occur in almost every environment of the Americas from southern USA to northern Argentina. Although the majority of terraranas live in humid tropical forests, taxonomic research during the present century has revealed that the high humid grasslands and adjacent elfin forests of the Amazonian versant of the Andes in Bolivia and Peru are also inhabited by a highly diverse fauna of terraranas (e.g. Lehr, Aguilar & Köhler, 2002a; De la Riva, 2007; Duellman & Hedges, 2008; Lehr & Catenazzi, 2008, 2009, 2010; Lehr, Moravec & Cusi 2012; Chaparro *et al.*, 2015). Based on the similar morphology of terraranas inhabiting the cold and wet conditions of these habitats (small and robust bodies, short extremities and digits with pointed or knobbed terminal phalanges and rounded, non-expanded tips), Lynch (1975) deduced that most species were part of a single natural group with a broad distribution spanning the high Andes from Colombia to Bolivia; this group was the genus *Phrynopus* Peters, 1873, at that time containing 14 species.

Molecular phylogenetics challenged this view to reveal several geographically disjunct and phylogenetically distant clades of Andean terraranas that converge in their ecological distribution and external morphology (Lehr, Fritzsch & Müller, 2005; Heinicke, Duellman & Hedges, 2007; Hedges, Duellman & Heinicke, 2008; Padial *et al.*, 2012; Padial, Grant & Frost, 2014). Most interestingly, several of these primarily Andean clades are more closely related to clades or species from the Atlantic Forest of Brazil than to geographically closer Andean or Amazonian taxa (Heinicke *et al.*, 2007; Hedges *et al.*, 2008; Canedo & Haddad, 2012; Padial *et al.*, 2014). This new perspective of the genealogy of terraranas constituted the backbone of a new taxonomy (Hedges *et al.*, 2008) that reflects the geographic and phylogenetic independence of high Andean radiations of terraranas. Thus, the once widespread genus *Phrynopus*, as delimited by Lynch (1975), is now restricted to a clade mostly endemic to a relatively small region of the central part of the Cordillera Oriental in Peru (the species *P. thompsoni* Duellman, 2000 from the north-western part of the country being the only outlier). Other genera (*Bryophryne* Hedges, Duellman & Heinicke, 2008; *Hypodactylus* Hedges, Duellman & Heinicke, 2008; *Lynchius* Hedges, Duellman & Heinicke, 2008; *Niceforonia* Goin & Cochran, 1963; and *Psychrophrynella* Hedges, Duellman & Heinicke, 2008) were erected or recovered for different radiations with non-overlapping distributions along the Andes (Hedges *et al.*, 2008). These and some other genera with representatives from the Amazon lowlands to the Brazilian Atlantic Forest (*Barycholos* Heyer, 1969; *Euparkerella* Griffiths, 1959; *Holoaden* de Miranda-Ribeiro, 1920; and *Oreobates* Jiménez de la Espada, 1872) constitute the subfamily Holoadeninae Hedges, Duellman & Heinicke, 2008, one of the three subfamilies in Craugastoridae Hedges, Duellman & Heinicke, 2008 (Padial *et al.*, 2014).

Progress in our understanding of the relationships of high Andean terraranas relies, nonetheless, on a scarce sampling of species. As such, support for inferences of species relationships and hypotheses about the extent of independence and origin of Andean radiations is limited. For example, Padial *et al.*, (2014) reported that only 30% of species of *Bryophryne*, *Phrynopus* and *Psychrophrynella* have been sampled for phylogenetic studies. But limited taxon sampling is just part of a more general problem, as our knowledge of species diversity in the high Andes is far from satisfactory. Most suitable areas for terraranas in the high Andes of Peru and Bolivia have yet to be explored, and there are good reasons to suspect that many new species will be found in those areas. This supposition is supported by the discovery of 42 species of high Andean terraranas in the last 10 years during explorations in Bolivia and Peru (e.g. De la Riva, 2007; Lehr & Catenazzi, 2009; Lehr *et al.*, 2012; De la Riva & Burrowes, 2014; Chaparro *et al.*, 2007, 2015). Guided by the absence of species records for the dozens of unexplored glacial valleys in the cordilleras Apolobamba (Peruvian section) and Carabaya and intermediate areas in southern Peru – in strong contrast with our findings of many new species in Bolivia (De la Riva, 2007) – we surveyed several of those valleys and found multiple unknown species of terraranas.

In this study, we use descriptive analyses of the external morphology and phylogenetic analyses of nuclear and mitochondrial gene sequences to assess the phylogenetic relationships and divergence of these newly discovered populations as well as many species described by us and colleagues over the years that had not been analysed phylogenetically. We describe and name five new species from Peru, flag an additional four new species from Bolivia, erect a new genus for a diverse clade of Bolivian and Peruvian species, and provide new insights into the overall phylogenetic relationships and biogeographic history of high Andean species in Holoadeninae.

MATERIAL AND METHODS

General procedures

After collecting specimens in the field, they were photographed alive, euthanized with lidocaine, fixed in 10% formalin or in 70% ethanol, and preserved in

70% ethanol. For the majority of specimens, tissues (either liver, muscle or toes) were preserved in 96% ethanol for subsequent molecular studies. Unless stated otherwise, these tissues were deposited at the Museo de Biodiversidad del Peru, Cusco, Peru (MUBI), and the Collection of Tissues and DNA of the Museo Nacional de Ciencias Naturales (MNCN, Madrid, Spain). Sex was determined by secondary sexual traits (i.e. vocal sacs) or by the examination of gonads. Coordinates were obtained by means of a Garmin 12XL Global Positioning System (GPS), and further assessments and geographical data using Google Earth. Specimens studied belong to the following institutions (acronyms in parentheses): American Museum of Natural History, New York, USA (AMNH); Colección Boliviana de Fauna, La Paz, Bolivia (CBF); Estación Biológica de Doñana, Sevilla, Spain (EBD); Museum of Natural History, The University of Kansas, Lawrence, USA (KU); Museo de la Universidad Mayor de San Marcos, Lima, Peru (MUSM); Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); Museo de Historia Natural de Cochabamba, Cochabamba, Bolivia (MHNC-B); Museo de Historia Natural 'Noel Kempff Mercado', Santa Cruz de la Sierra, Bolivia (MNK); Museo de Biodiversidad del Peru, Cusco, Peru (MUBI; this collection was part of Museo de Historia Natural, Universidad Nacional deSan Antonio Abad, Cusco, Peru); and Naturhistorisches Museum, Zoologische Abtheilung, Wien, Austria (NHMW) (Appendix 1).

Molecular phylogenetics

The main targets of our analyses are species of high Andean terraranas from Peru and Bolivia of unknown phylogenetic position. According to molecular phylogenetic relationships of some species as well as the distribution and external appearance of others, high Andean species from Bolivia and Peru are currently considered part of *Bryophryne* (restricted to Cordillera Oriental of southern Peru), *Noblella* Barbour, 1930 (with a broad distribution from the Andes of Ecuador to central Bolivia and the Amazon lowlands), *Oreobates* (two high Andean species inhabiting central Peru), *Phrynopus* (restricted, except for *P. thompsoni,* to the Cordillera Oriental in central Peru) and *Psychrophrynella* (known from the Cordillera Oriental, from southern Peru to central Bolivia). However, generic assignation based only on morphology and distribution has been problematic in some cases. For example, two former *Phrynopus*, *Oreobates lundbergi* (Lehr, 2005) and *Oreobates ayacucho* (Lehr, 2007), were unexpectedly inferred as part of *Oreobates* in molecular phylogenetic analyses (Padial *et al.*, 2012, 2014). As such, the analysis of the affinities of our target species requires sampling of representative species of all genera that are part of Holoadeninae (we failed to include the Colombian genus *Niceforonia* because there are no available sequences). Sampled genera in this study are: *Barycholos*, *Bryophryne*, '*Eleutherodactylus bilineatus*' Bokermann, 1975 [*incertae sedis* within Holoadeninae awaiting generic allocation; Canedo & Haddad (2012)], *Euparkerella*, *Holoaden*, *Hypodactylus*, *Lynchius*, *Noblella*, *Oreobates*, *Phrynopus* and *Psychrophrynella*. We rooted all our analyses with the distant craugastorid species *Haddadus binotatus* (Spix, 1824).

We produced sequences of two complementary strands for 100 specimens that represent species of *Bryophryne*, *Phrynopus* and *Psychrophrynella*, as well as specimens of previously unknown affinities. Non-coding mtDNA genes sequenced 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 a fragment of the cytochrome *c* oxidase subunit I (*COI*). Nuclear protein-coding genes include the two exons of the cellular myelocytomatosis (*c-myc*), a fragment of the propiomelanocortin A (*POMC*), a fragment of the recombination-activating protein 1 (*RAG1*), and a fragment of tyrosinase precursor (*Tyr*). Non-coding nuclear genes include the intron region of the cellular myelocytomatosis gene (*c-myc*). Laboratory protocols for newly produced sequences follow standard procedures as described in Padial *et al.* (2012). Primers are listed in Appendix 2.

Our gene sampling strategy consists of (1) a dense sampling of the 16S mtDNA gene (including the barcode fragment; Vences *et al.*, 2005) that covered 98 out of 100 specimens available (only two samples failed to produce sequences for this fragment); and (2) sampling of additional genes for at least one terminal of each species or putative new species, as guided by preliminary 16S analyses. In this way, we built a supermatrix where most terminals are sampled for 16S, while each species or putative new species is also sampled for the other loci mentioned above. Specimen vouchers sequenced, GenBank accession numbers and locality data are listed in Appendix 3. In addition to these loci, we sampled all additional legacy sequences available in GenBank for both ingroup and outgroup species: 28S, chemokine receptor 4 (*CXCR4*), histone H3 (*HH3*), sodium–calcium exchanger 1 (*NCX1*), rhodopsin (*Rhod*) and seven-in-absentia (*SIA*) (Supporting Information, Appendix S1). The identity of ingroup sequences and outgroup sequences in *Hypodactylus*, *Oreobates* and *Lynchius* was verified by direct or photographic examination of voucher specimens. Vouchers of the root (*H. binotatus*) and outgroup sequences of *Holoaden* and *Euparkerella* were not examined but the identification of these species is straightforward and

problems are not expected. Blast searches in GenBank did not reveal any problems with sequences.

Maximum likelihood analyses

Sequence alignments were performed in MAFFT online version 7 using the G-INS-i strategy, which is considered appropriate for large numbers of sequences (Katoh & Standley, 2013). 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. PartitionFinder V1.0.1 (Lanfear *et al.*, 2012) was used to select the optimal partition scheme and substitution models for our dataset under the Akaike Information Criterion (AIC). Compared partition schemes were: (1) all data combined, (2) a 2-partition, mtDNA/nuDNA, scheme, (3) by locus (each partition corresponding to individual loci mentioned above) and (4) by locus and codon position (for protein coding genes). Maximum-likelihood tree searches and 1000 bootstrap replicates were performed using random addition sequence replicates in GARLI 2.0 (Zwickl, 2006) with gaps treated as absence of evidence. For more details about how tree searches were performed in GARLI, see Padial *et al.* (2014).

Maximum parsimony analyses

Sequences were partitioned into fragments of equal length separated by conserved regions with no gaps and few or no nucleotide substitutions and tree searches were performed under parsimony with equal weights for all classes of transformations using direct optimization and iterative pass optimization algorithms in POY 5.1.1 (Wheeler *et al.*, 2015). Tree searches were first conducted using DO under the command 'search'. Initial searches implemented the command 'auto_static_approx'. The optimal tree found during driven searches was swapped using iterative pass optimization. The implied tree-alignment resulting from iterative pass optimization was exported and driven searches were conducted in TNT (Tree analysis using New Technology; Goloboff, Farris & Nixon, 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. Swapping of each constrained search was limited to 20 min and constrained searches were repeated three times as specified in the default settings of the bremer.run macro. We also calculated parsimony jackknife frequencies from 1000 pseudoreplicates searches using driven searches, 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).

Morphology and morphometrics

We measured eight standard variables: snout–vent length (SVL), head length (from rictus to tip of snout) (HL), head width (at level of rictus) (HW), internarial distance (IND), distance from eye to nostril (END), eye diameter (ED), tibia length (TL) and foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe) (FL). We omitted two characters traditionally used in craugastorid morphometric studies, eyelid width and interorbital distance, because they are soft structures prone to alteration in preservative. For character state definitions we followed Duellman & Lehr (2009). Lengths of digits were compared by adpressing them against each other. We took measurements with a digital calliper to the nearest 0.01 mm, but rounded all measurements to only one decimal to avoid pseudoprecision. Diagnosis format for species and the new genus follows Duellman & Lehr (2009), while holotype descriptions follow De la Riva (2007). Comparisons of diagnostic characters among species aim to provide evidence of divergence and are therefore restricted to sister and closely related species, morphologically similar species and geographically close species.

Bioacoustics

Vocalizations were recorded with a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone; recordings were processed with a digital signal analysis system based on an Apple Macintosh computer. The sounds were digitized and edited at a sampling frequency of 44.1 kHz and 16 bit resolution with Peak 4.1 software. Frequency information was obtained through fast Fourier transform (FFT) (width, 1024 points). We used Praat 4.5.02 software for MacOS X (Boersma & Weenink, 2006) to obtain numerical information and to generate audiospectrograms and oscillograms; calls were edited with Audacity 1.2.6 for MacOS X (Free Software Foundation, Inc., 1991). A total of seven bioacoustic variables were considered: notes per call group, call group duration, note duration, dominant frequency, notes per minute and call groups per minute. Recordings were deposited at the Fonoteca Zoológica Digital (www.fonozoo.com) of MNCN.

RESULTS

Molecular phylogenetics

Parsimony tree searches identified an optimal tree of 16 161 steps that was visited 1193 times during

1857 rounds of build + TBR, 16 824 of fusing and 892 of ratchet. A round of swapping under iterative pass optimization recovered a single tree of 16 111 steps and a tree-alignment (i.e. the alignment implied by the optimal topology) of 9966 characters. Additional searches of the tree-alignment in TNT rendered 167 trees of the same length (strict consensus shown in Fig. 1A–C). Alternatively, the optimal MAFFT similarity-alignment comprised 9118 character columns. Partition Finder selected the 2-partition (mtDNA/nuDNA) scheme with GTR + I + G substitution model for mtDNA and TIMef + I + G for nuDNA. Under this partition scheme and model, the best maximum likelihood was found once during 10 adaptive searches in GARLI (maximum likelihood score = –78 046.256; Fig. 1A–C). Note that differences in the number of characters (positions) in alignments are not due to differences in the amount of data used but to the way the different methods represent the alignment in matrix format. In tree-alignment, the resulting alignment is implied by the optimal topology (the alignment is the graphical representation of the way character states change along tree branches). Alternatively, the MAFFT alignment is not implied by the optimal topology. Instead, the alignment is built on UPGM trees and maximum likelihood trees are inferred from this fixed matrix. If we were to represent the optimization of the MAFFT alignment implied by the optimal maximum likelihood tree in matrix format, the resulting alignment would also differ from the original MAFFT alignment. However, the fact that relationships recovered by both methods are almost identical, with differences affecting a few, moderately supported clades (bootstrap values ranging from 70% to 81%), indicate that both methods converge in spite of their underlying assumptions. Convergence is largely related to the way parsimony operates under tree-alignment, in which tree-searches are able to minimize the number of transformations while minimizing homoplasy (Wheeler, 1996). Our findings coincide with those of two other recent studies comparing tree-alignment and maximum likelihood that found higher convergence between parsimony under tree-alignment and maximum likelihood analyses than between parsimony under tree-alignment and standard parsimony for exactly the same reasons (Padial *et al.*, 2014; Goicoechea *et al.*, 2016).

Both sets of analyses recover all testable genera of Holoadeninae as monophyletic with the highest support for resampling frequencies, but differ in the relative placement of a few clades. Parsimony analyses recover with high support a clade composed of *Euparkerella* and *Holoaden* as the sister group of a clade that includes the remaining species of Holoadeninae. Also with high support, *Hypodactylus* is inferred as part of a clade that also includes *Phrynopus* as the sister group of *Lynchius* + *Oreobates*; *Bryophryne* is the sister group of a clade that includes *Barycholos*, '*E. bilineatus*', and *Noblella*, and this clade is, in turn, the sister group of *Psychrophrynella usurpator* and *Microkayla* gen. nov. (Fig. 1A–C).

In maximum likelihood analyses the relationships just mentioned differ in that *Euparkerella* and *Holoaden* are the sister group of a clade including *Barycholos*, '*E. bilineatus*' and *Noblella*, and this clade is in turn the sister group of the clade including *Bryophryne* and *Psychrophrynella*. Thus, *Bryophryne* and *Psychrophrynella* + *Microkayla* are sister groups. These relationships are moderately supported, with resampling proportions ranging from 70% to 81%.

Except for two nominal species, the remaining species sampled for more than one terminal were inferred as monophyletic with high support (Fig. 1A–C). *Phrynopus nicoleae* Chaparro, Padial & De la Riva, 2008 was found embedded within a paraphyletic *P. tribulosus* Duellman & Hedges 2008 (suggesting a possible synonymy) (Fig. 1A); terminals assigned to *Microkayla iatamasi* (Aguayo-Vedia & Harvey, 2001) appeared in two non-sister clades (Fig. 1C). Several nominal species that had so far not been analysed phylogenetically are corroborated as part of *Microkayla* sp. nov. in all analyses (Fig. 1C).

Several populations identified as new species during field surveys in Peru and Bolivia were either inferred as part of *Bryophryne* or *Microkayla*. Within *Bryophryne*, *B. quellokunka* sp. nov. from the Marcapata Valley in Peru was found as sister to *B. cophites*. *Bryophryne wilakunka* sp. nov. and *B. tocra* sp. nov., two allopatric species from southern Peru, were inferred as sister groups in maximum likelihood analyses but their relationships collapsed in parsimony analyses. Interspecific genetic distances in *Bryophryne* ranged between 3.3 and 8.4 (Table 1).

Psychrophrynella usurpator De la Riva, Chaparro & Padial, 2008 was found as the sister group of the remaining species that formed this genus and that are now assigned to *Microkayla* gen. nov. *Psychrophrynella usurpator* has a remarkably long branch and high genetic distances with respect to species of *Microkayla* (14.4%–18.7 %) (Table 2). A Bolivian population from Coscapa was found as the sister group of *M. teqta*, the two forming the sister group of *M. wettsteini* (Parker, 1932). Also in Bolivia, a population from Khatu River currently considered part of *M. quimsacruzis* was recovered as the sister group of *M. illimani* in maximum likelihood, and a population from Utururo was found as the sister of one of the clades of *M. iatamasi* in parsimony analyses. In southern Peru, *M. chapi* sp. nov. was found as the sister group of a clade including *M. boettgeri* and a population from the confluence of the rivers Sayaco and Huacuy, *M. chilina* sp. nov.

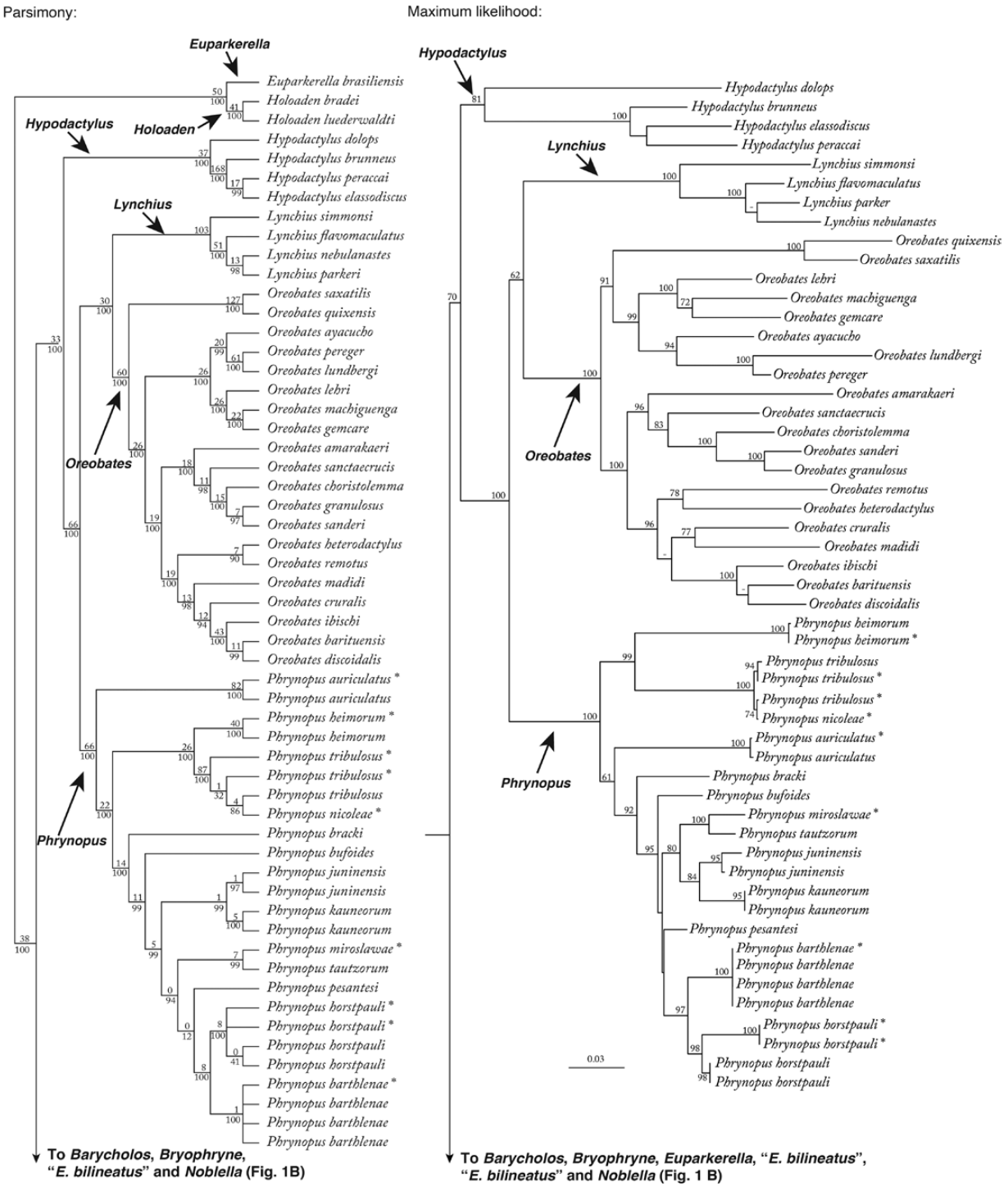

Figure 1. (A–C) Phylogenetic trees resulting from the analysis of a dataset of 9118 aligned bp (MAFFT) and 9966 bp (POY) of the mitochondrial genes 12S, 16S and the intervening $tRNA_{valine}$ and $tRNA_{leucine}$ segments, a fragment of the mtDNA gene *COI*, and fragments of the nuclear protein-coding genes 28S, *c-myc, CXCR4, HH3, NCX1, POMC, RAG1, Rhod, SIA* and *Tyr*. Left, maximum parsimony optimal tree of 16 111 transformations depicting the relationships of ten genera within Holoadeninae (numbers above branches are Goodman–Bremer values, and those under branches are Jackknife proportions). Right, maximum likelihood optimal tree with bootstrap node values. Terminals for which new sequences were generated for this study are marked with an asterisk.

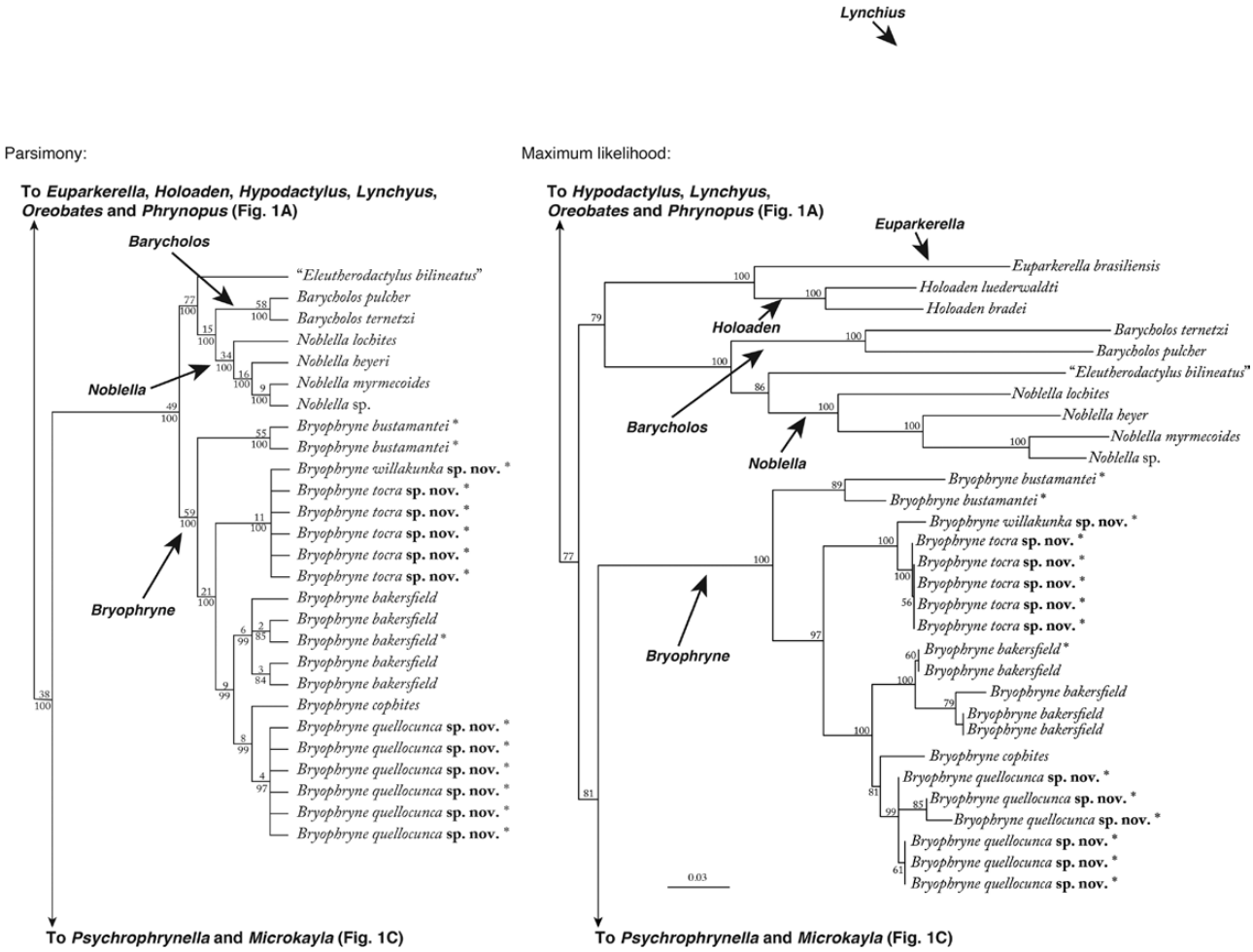

Figure 1. *Continued*

SPECIES DELIMITATION

Bryophryne

Two allopatric species from the Carabaya mountains of Puno, *B. wilakunka* sp. nov., from the Ayapata Valley at *c.* 3940 m, and *B. tocra* sp. nov., from Ollachea valley at *c.* 3840 m, are found as sister groups in maximum likelihood analyses with maximum support while they collapse into a polytomy in parsimony analyses (Fig. 1B). The polytomy might result from partial overlap in homologous sequences between these two species, leading to inconclusive phylogenetic signal (optimal trees placed *B. wilakunka* sp. nov. as either the sister to or embedded within *B. tocra*). Still, the two species are morphologically distinct and reciprocally diagnosable (see diagnoses), and genetic distances are 3.5% (Table 1). The two species occur isolated from each other in the headwaters of their, respectively, glacial valleys, which run in parallel and are separated by the highest parts of the Carabaya massif.

Bryophryne quellokunka sp. nov. from the Marcapata Valley of the Vilcanota (Willkanuta) mountains in department Cusco at *c.* 3960 m was inferred as the sister group of *B. cophites* and they have genetic distances of 3.8%–4.0% (Fig. 1B). *Bryophryne cophites* occurs in the Paucartambo valley, *c.* 70 km north of the Marcapata Valley. These two species show qualitative differences in external morphology (see Diagnosis of the new species). They are allopatric and are separated by a long series of steep crests, mountains and deep valleys that run from west to east along the eastern versant of the Vilcanota range.

Microkayla

Six putative new species are supported by our results, two from Peru and four from Bolivia (Fig. 1C). In Peru, *M. chapi* sp. nov. from the Hirigache Valley of the Apolobamba mountains near the village of Sina (Department Puno) was found as the sister group of a clade composed of *M. boettgeri* from the eastern Carabaya mountains (the northern and westernmost

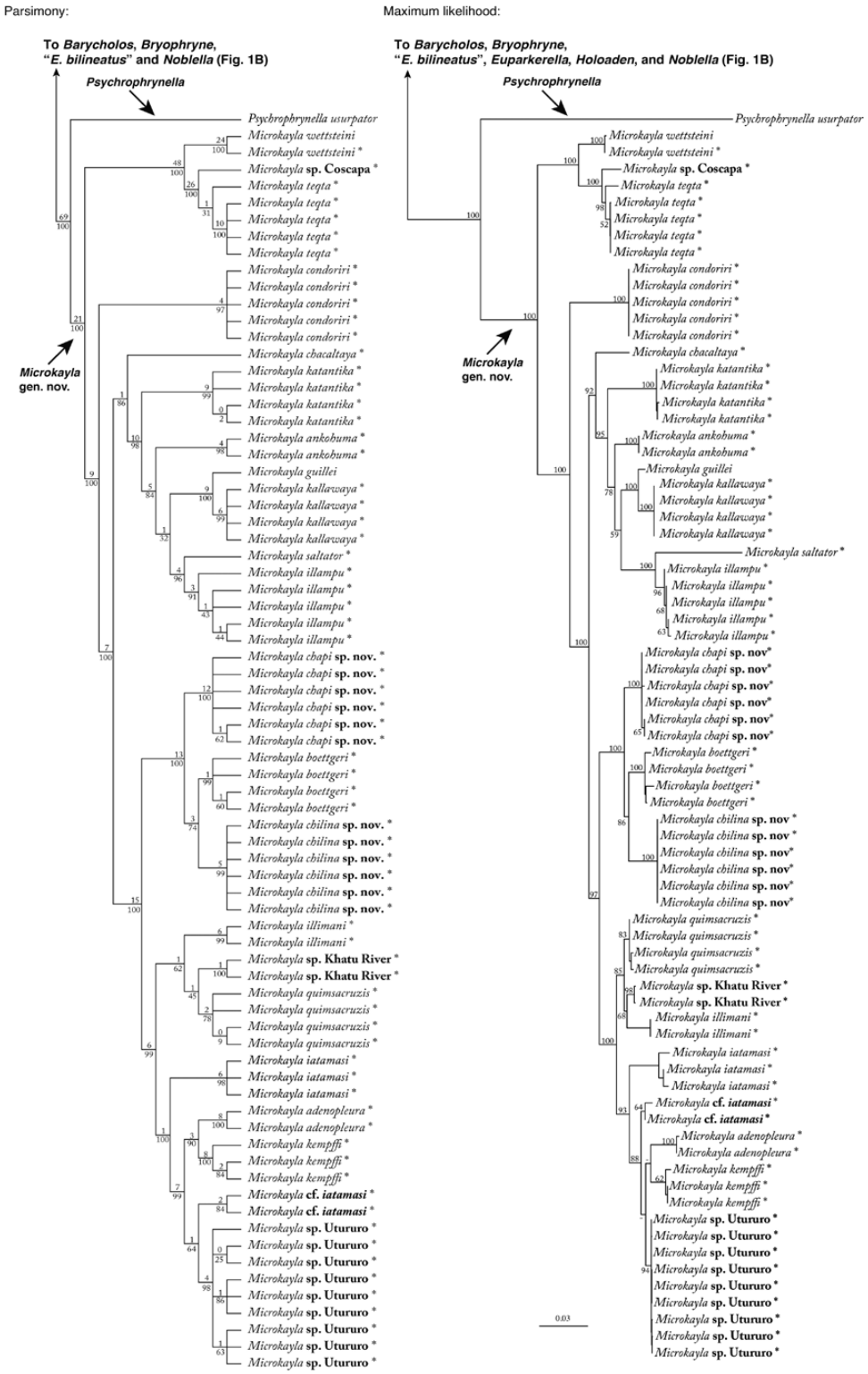

Figure 1. *Continued*

known species of *Microkayla*) and *P. chilina* sp. nov. from the Peruvian part of the Apolobamba mountains in the valley of Sandia (Fig. 1C). All three species are allopatric and occur in distant valleys separated by high Andean slopes, are diagnosable morphologically, and have genetic distances of 2.5%–5.8% (Table 2).

Table 1. Uncorrected pairwise genetic distances among species of *Bryophryne*

	B. bakersfield	*B. bustamantei*	*B. cophites*	*B. quellokunka*	*B. tocra*
B. bakersfield (5)	0.0–0.2				
B. bustamantei (1)	5.4	–			
B. cophites (1)	4.0–4.2	6.8	–		
B. quellokunka (4)	3.2–3.5	5.9–6.1	3.8–4.0	0.0–0.2	
B. tocra (5)	5.7–6.0	8.4	6.6	6.3–6.5	0.0
B. wilakunka (1)	–	–	–	–	3.5

Genetic distances were calculated for a 518 bp fragment of the 16S gene. For *B. wilakunka*, which lacked the homologous fragment, we report genetic distances with its sister species (*B. tocra*) based on a similarity alignment of 519 nucleotides of a different fragment of 16S. Sample size for each species is in parentheses.

In Bolivia, a population from Coscapa at 3550 m in the eastern slopes of the Condoriri (Kunturiri) massif of the Cordillera Real of La Paz was recovered as the sister group of *Microkayla teqta* (Fig. 1C), from the Pablo Amaya Valley (La Paz), further northwest in the Cordillera Real. These populations are allopatric and morphologically distinct (see diagnosis), with a genetic distance of 1.3%–1.7% (for distances to other species, see Table 2).

A Bolivian population formerly considered part of *Microkayla quimsacruzis*, from the Khatu River of the cordillera Quimsa Cruz in La Paz, at 3730 m, is reciprocally monophyletic to nominal *M. quimsacruzis* in parsimony analyses, and sister to *M. illimani*, from the slopes of the Illimani mountain in the Cordillera Real, in maximum likelihood analyses with a resampling frequency of 68% (Fig. 1C). While nominal *M. quimsacruzis* is restricted to the Choquetanga Valley on the northern versant of the cordillera, the population of the new species is restricted to a southern valley that belongs to another river drainage, the Khatu River of the Quime-Inquisivi Valley. Both populations, reciprocally monophyletic in parsimony and non-sister in maximum likelihood, show distinct morphological differences and have genetic distances of 2.5%–2.6% (for distances to other species, see Table 2).

A population from Utururo, on the Andean slopes that conform the upper basin of the Chapare river system in Cochabamba, at 3800 m, is found as the sister group of a clade associated with the name *M. iatamasi* (non-monophyletic) in parsimony analyses, and as the sister group of a clade composed of *M. adenopleura* (Aguayo-Vedia & Harvey, 2001) and *M. kempffi* (De la Riva, 1992) in maximum likelihood analyses (Fig. 1C). This population is morphologically diagnosable and differs from its closest relatives by genetic distances of 1.9%–2.7% (Table 2).

Potentially sympatric specimens of *M. iatamasi* from the Andean slopes of the Chapare river basin in Cochabamba are found in two different clades (Fig. 1C). The clade with nominal *P. iatamasi* ranges from 3000 to 4192 m in the upper basin of the Chapare river and is the sister group of a larger clade that includes other species from that part of the Andes (*M. adenopleura*, *M.* cf. *iatamasi*, *M. kempffi* and *Microkayla* sp. from Utururo). *Microkayla* cf. *iatamasi* is found as the sister group of *Microkayla* sp. from Utururo in parsimony analyses, and as the sister group of a larger clade (*M. adenopleura*, *M. kempffi* and *Microkayla* sp. from Utururo) in maximum likelihood analysis with a resampling frequency of 88%. Accordingly, we consider *M.* cf. *iatamasi* a different species. Morphological differences are unknown because specimens were not available for study. Genetic distances between this species and nominal *M. iatamasi* are 4.0%–4.8 % (for distances to other species, see Table 2).

SYSTEMATICS

In this section, we name and describe as new species the populations of *Bryophryne* from Marcapata, Ollachea and Ayapata. We also propose a new genus (*Microkayla* gen. nov.) for the clade of Bolivia and Peruvian species that forms the sister group of *P. usurpator*, and name and describe as new species in the new genus populations from Sandia and Sina, in the Peruvian section of the cordillera de Apolobamba. Bolivian populations of *Microkayla* gen. nov. identified herein as new species will be described in forthcoming publications.

Superfamily Brachycephaloidea Günther, 1858

Family Craugastoridae Hedges, Duellman & Heinicke, 2008

Subfamily Holoadeninae Hedges, Duellman & Heinicke, 2008

Bryophryne Hedges, Duellman & Heinicke, 2008

Type species: Phrynopus cophites Lynch, 1975.

Included species: Bryophryne abramalagae Lehr and Catenazzi, 2010; *B. bakersfield* Chaparro, Padial, Gutiérrez, and De la Riva, 2015; *B. bustamantei* (Chaparro, De la Riva, Padial, Ochoa, and Lehr,

Table 2. Intra (diagonal) and inter (below diagonal) uncorrected pairwise genetic distances among representatives of *Psychrophrynella usurpator* and *Microkayla* sp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *M. adenopleura* (2)	0																					
2 *M. boettgeri* (2)	7.1–7.9	1																				
3 *M.* cf. *iatamasi* (2)	2.3–2.5	5.6–6.9	0.6																			
4 *M. chacaltaya* (1)	8.2	6.7–7.7	6.9–7.5	–																		
5 *M. condoriri* (4)	8.1	7.9–8.9	7.9–8.3	8.5	0																	
6 *M. guillei* (1)	8.3	7.9–8.8	7.5–8.1	5.5	8.5	–																
7 *M. iatamasi* (3)	3.2–3.6	5.4–6.4	4.0–4.8	6.9–7.5	7.9–8.8	7.5–8.1	0.0–0.4															
8 *M. illampu* (5)	8.0–8.4	8.1–9.4	7.5–8.6	5.4–5.6	7.5–7.9	2.7–2.9	7.1–8.3	0.0–0.6														
9 *M. illimani* (2)	5.6–5.8	5.7–6.7	4.0–4.8	6.7	7.9–8.0	7.9–8.1	3.8–4.2	7.7–8.3	0													
10 *M. kallawaya* (4)	7.9	8.7–9.6	7.3–7.5	6.3	8.7	2.1	7.5	2.7–3.1	7.9–8.1	0												
11 *M. katantika* (3)	8.1	6.9–7.9	7.1–7.7	6.3	7.7	6	6.7–7.7	5.9–6.3	6.3–6.7	6.1	0											
12 *M. kempffi* (2)	3.6–4.0	6.3–7.3	2.5–3.1	6.9–7.5	8.1–8.3	7.9–8.4	3.4–4.0	7.3–8.4	4.8–5.3	7.7–8.0	7.9	0.8										
13 *M. quimsacruzis* (2)	4.8–5.0	4.6–5.8	3.6–4.4	6.0–6.1	7.7–7.9	7.9–8.1	3.8–4.3	7.3–7.9	2.1–2.4	7.9–8.1	6.5–6.7	4.0–4.4	0.2									
14 *M. saltator* (1)	9.2	10.4–11.3	8.6–9.2	9.6	10.2	10.7	9.3–10.0	10.2–10.5	9.7–9.8	10.9	10.4	8.7–9.2	8.8–9.0	–								
15 *M. chilina* (5)	6.9	2.5–3.5	5.4–5.9	6.3	8.5	8.6	5.6–5.9	7.9–8.3	5.2	8.7	7.9	5.4–6.0	4.0–4.2	9.6	0							
16 *M. chapi* (6)	6.6–6.7	4.6–5.8	5.0–5.4	7.3–7.5	9.1–9.3	9.3–9.4	6.0–7.4	9.2–9.8	5.5–6.1	9.3–9.5	8.1–8.3	6.1–6.9	5.2–5.6	10.2–10.4	4.6–4.8	0.0–0.2						
17 *M.* sp. Khatu River (2)	4.6	5.2–6.2	3.5–4.1	6.2	8.3–8.4	7.5	4.4–4.7	7.1–7.5	2.5–2.6	7.5	7.1–7.2	4.4	1.9–2.1	8.9	4.4	6.2–6.3	0					
18 *M.* sp. Coscapa (1)	10.4	11.7–12.1	10.0–10.6	10	10.4–10.5	10.9	10.0–10.4	10.4–10.8	10.2–10.3	11.1	12.1	10.9–11.0	9.8–10.0	13.2	11	12.2–12.4	9.5	–				
19 *M.* sp. Utururo (9)	2.3–2.5	6.3–7.3	1.1–1.9	7.9–8.0	8.5–8.7	8.1	3.2–3.8	7.8–8.4	4.4–4.8	7.3	7.9–8.1	2.1–2.7	4.0–4.4	8.6–8.8	5.8–5.9	6.2–6.5	3.3–3.7	10.0–10.2	0.0–0.4			
20 *M. teqta* (5)	9.4–9.6	11.4–12.3	9.0–10.2	9.6–9.8	10.2	10.7–10.8	9.0–10.2	9.8–10.2	8.9–9.9	10.9	11.1–11.3	10.0–10.2	8.9–9.6	12.7–13.2	10.4–10.6	11.9–12.2	8.5–9.1	1.3–1.7	9.0–9.8	0.0–0.8		
21 *P. usurpator* (1)	15.9	17.7–18.7	15.4	15.8	15.3–15.4	16.4	15.9–16.8	16.8–17.2	15.9–16.3	16.6	16.7	16.6–16.9	16.7–16.9	17.5	17.9	17.5–17.7	17.3–17.4	15.1	15.8–16.0	14.4–14.8	–	
22 *M. wettsteini* (2)	9.4	11.5–11.9	8.8–9.4	10.2	11	11.1	9.1–9.5	10.2–10.7	9.9–10.0	11.5	12.5	10.3	9.2–9.4	12.3	10.6	12.1–12.3	9.0–9.1	3.7	9.4–9.6	2.9–3.5	15.4	0
23 *M. ankohuma* (2)	–	–	–	–	–	7.5	–	12.7 (1)	–	7.7 (1)	–	–	–	–	–	–	–	–	–	–	–	–

Genetic distances were calculated based on 532 nucleotides of a similarity alignment of the gene 16S except for *M. ankohuma*, which lacked the homologous fragment. For the latter species, we report genetic distances with its sister taxon (a clade including *M. guillei*, *M. illampu* and *M. kallawaya*) based on a similarity alignment of 485 nucleotides of a different and non-overlapping fragment of 16S. Sample size for each species is in parentheses.

2007); *B. cophites* (Lynch, 1975); *B. flammiventris* Lehr and Catenazzi, 2010; *B. gymnotis* Lehr and Catenazzi, 2009; *B. hanssaueri* Lehr and Catenazzi, 2009; *B. quellokunka* sp. nov.; *B. nubilosus* Lehr and Catenazzi, 2008; *B. tocra* sp. nov.; *B. wilakunka* sp. nov.; *B. zonalis* Lehr and Catenazzi, 2009.

Diagnosis: see Hedges et al. (2008).

BRYOPHRYNE QUELLOKUNKA SP. NOV.

(FIG. 2)

urn:lsid:zoobank.org:act:0396BD77-2426-4541-93DE-D22D858AD292

Holotype: MUBI 5380 (field number 4626), adult female from Qorpinte, 2 km from Tambopampa towards Marcapata, Palquilla river valley, province Quispicanchis, department Cusco, Peru, 13°36′18.8″S, 71°03′8.8″W, 3964 m (Fig. 3), collected on 20 February 2006 by I. De la Riva, J. M. Padial, S. Castroviejo-Fisher, and J. C. Chaparro.

Paratopotypes: MUBI 5374, 5375, 5377 (field numbers 4617, 4618, 4620), and MNCN 43780, 43782 (field numbers 4616, 4622) (adult males); MNCN 43784 (field number 4627) (adult female); MUBI 5376, 5378, 5379 (field numbers 4619, 4624, 4625) and MNCN 43799, 43781, 43783 (field numbers 4615, 4621, 4623) (juveniles), same data as the holotype.

Diagnosis: *Bryophryne quellokunka* is characterized by: (1) skin on dorsum uniformly warty, warts round to conical and low, with two incomplete dorsolateral folds barely reaching midbody and continuing sometimes as an irregular row of warts; skin of head shagreen to smooth, warty dorsally; belly and chest areolate, throat smooth; (2) tympanic membrane and tympanic annulus slightly perceptible beneath skin, smaller than 2/3 of EL, supratympanic fold composed of a row of warts; (3) snout short, round in dorsal view, blunt in lateral view; (4) upper eyelid lacking tubercles, bearing small conical warts; (5) dentigerous process of vomers absent; (6) vocal slits and sac present, nuptial pads absent; (7) Finger I shorter than Finger II, tips of digits rounded, lacking ungual flap and circumferential grooves; (8) fingers lacking lateral fringes; (9) ulnar region bearing warts; (10) heel lacking tubercles, tarsus lacking tubercles and folds; (11) two metatarsal tubercles, inner slightly larger than outer; supernumerary tubercles inconspicuous; (12) toes lacking lateral fringes; webbing absent; Toe III longer than V, tips of digits rounded, lacking ungual flap and circumferential grooves; (13) dorsal coloration reddish-brown to dark brown, sometimes with a blackish-grey interorbital and/or middorsal mark; ventral coloration variable, from greyish-purple with diffuse black blotches to brown, throat and plantar surfaces orange to yellow, axillae and groins without flash marks; (14) females larger than males, SVL 27.6–28.2 in adult females (n = 2), 18.0–20.3 mm in adult males (n = 5) (Table 3).

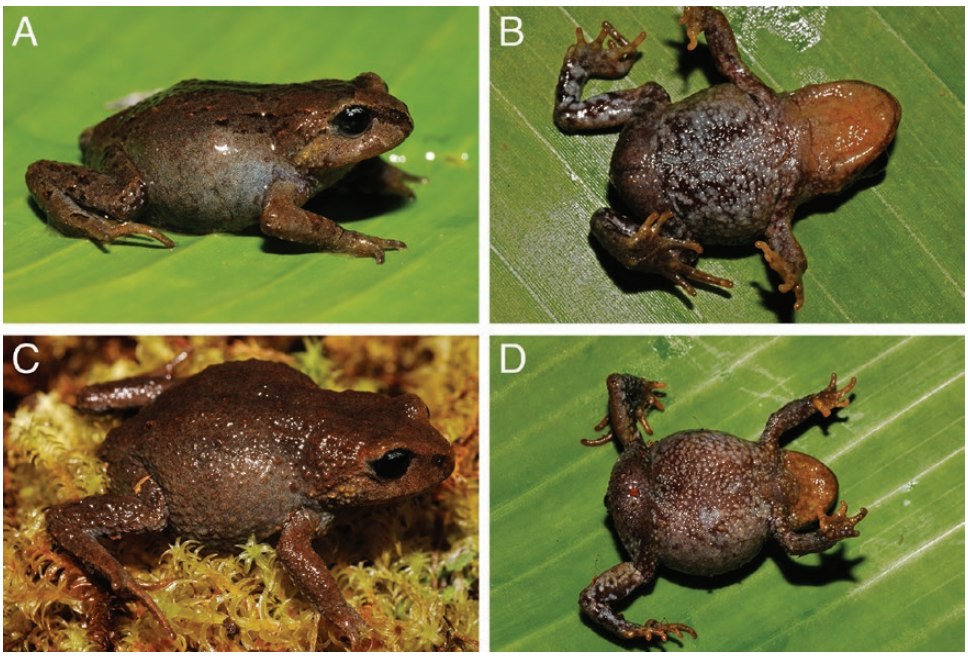

Figure 2. Live specimens of ***Bryophryne quellokunka* sp. nov.** from Qorpinte, Marcapata, Peru. (A–B) Adult female holotype (MUBI 5380, SVL 28.2 mm). (C–D) Adult male (MNCN 43782, SVL 20.1 mm), from the type locality.

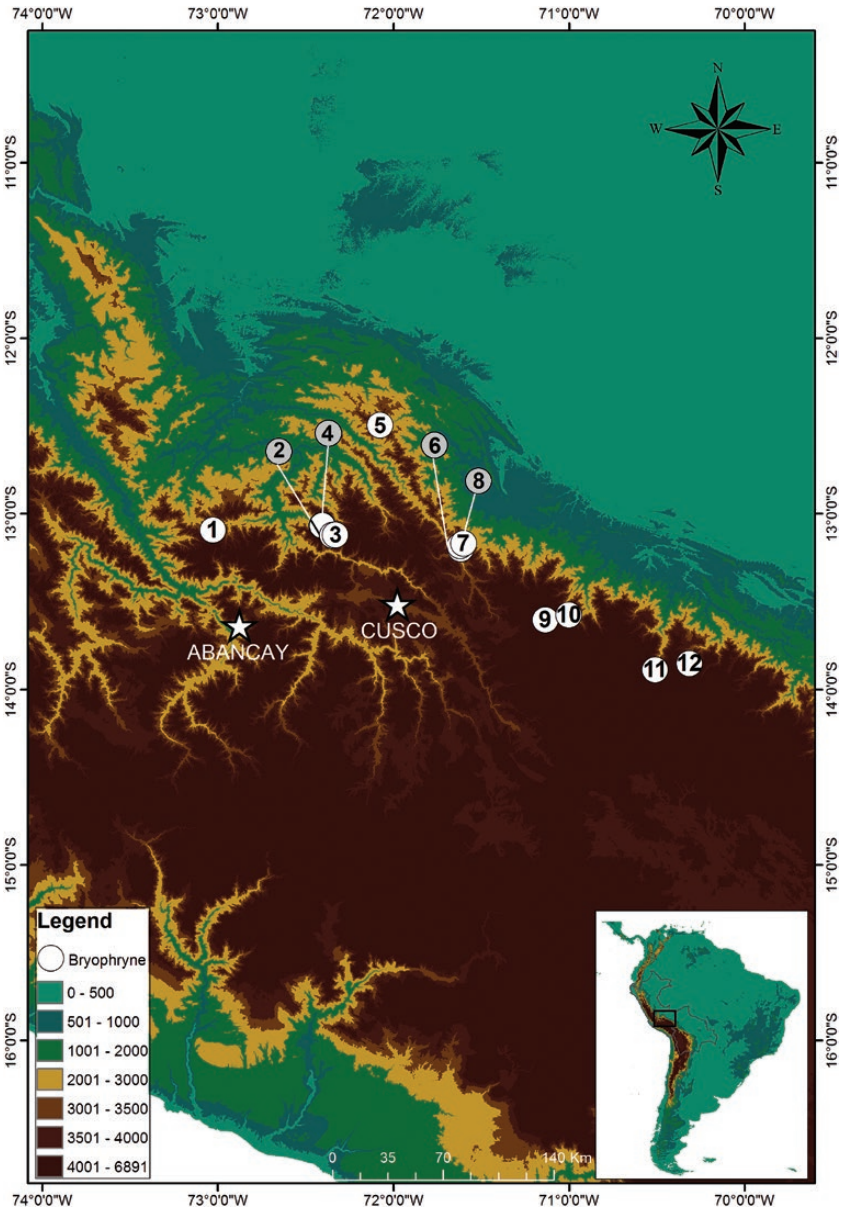

Figure 3. Map of the Andes of southern Peru around department of Cusco showing the distribution (type localities only) of the 12 nominal species of *Bryophryne* described hitherto: (1) *B. flammiventris*; (2) *B. abramalagae*; (3) *B. bustamantei*; (4) *B. bakersfield*; (5) *B. cophites*; (6) *B. hanssaueri*; (7) *B. nubilosus*; (8) *B. gymnotis*; (9) ***B. quellokunka* sp. nov.**; (10) *B. zonalis*; (11) ***B. tocra* sp. nov.**; (12) ***B. wilakunka* sp. nov.**

Bryophryne quellokunka is sister to *B. cophites*, and this clade is in turn sister to *B. bakersfield*. The three species in this clade are similar but have some morphological differences. *Bryophryne bakersfield* has complete dorsolateral folds and short but conspicuous dorsal and occipital fold, and the dorsal skin is less homogeneously warty. Also, while the coloration of *B. quellokunka* is mostly homogeneously brown, colour patterns in *B. bakersfield* are diverse (orange, yellow, black, olive green, etc.). *Bryophryne cophites* has a less warty dorsal skin, almost smooth or with low warts, while the skin of *B. quellokunka* is conspicuously and homogeneously warty and has two incomplete dorsolateral folds sometimes shown as an irregular row of warts. Another species, *B. zonalis*, is known from near the type locality of *B. quellokunka* but at a lower elevation (Kusillochayoc, 3129 m; Lehr & Catenazzi, 2009), and both species have marked differences. *Bryophryne zonalis* has metallic blue to metallic orange spots surrounded by bold black in the lower part of the belly

Table 3. Morphometrics for new Peruvian species of *Bryophryne* and *Microkayla* (range, in parenthesis, follows mean; AM, adult males; AF, adult females)

	B. wilakunka		*B. quellokunka*		*B. tocra*		*M. chilina*		*M. chapi*	
	AM	AF	AM	AF	AM	AF	AM	AF	AM	AF
	MNCN 43788	MUBI 5425	$n = 5$	$n = 2$	$n = 5$	$n = 2$	$n = 4$	MUBI 5350	$n = 9$	$n = 3$
SVL	17.9	24.6	19.3 (18.0–20.3)	27.9 (27.6–28.2)	19.3 (18.4–20.0)	27.4 (27.2–27.6)	23.8 (23.2–24.3)	25.5	17.8 (16.3–19.1)	20.8 (19.9–21.6)
HL	6.1	7.7	6.2 (5.3–6.8)	8.8 (8.7–9.0)	6.0 (5.4–6.4)	8.1 (8.1–8.1)	7.8 (7.5–8.0)	7.4	5.9 (5.4–6.4)	6.6 (6.2–6.8)
HW	6.1	8.5	6.3 (5.7–6.8)	9.1 (8.9–9.3)	6.6 (5.8–7.1)	9.0 (8.4–9.5)	8.1 (8.0–8.2)	7.7	6.2 (5.7–6.9)	6.9 (6.8–7.0)
IND	1.9	2.0	1.9 (1.7–2.0)	2.1 (2.1–2.2)	2.0 (1.8–2.2)	2.5 (2.4–2.5)	2.3 (2.0–2.5)	2.3	1.7 (1.4–1.9)	1.6 (1.5–1.8)
END	1.5	1.9	1.6 (1.5–1.9)	2.1 (2.1–2.2)	1.6 (1.5–1.9)	2.3 (2.1–2.5)	1.9 (1.7–2.3)	2.1	1.5 (1.3–1.7)	1.5 (1.4–1.6)
ED	2.6	3.3	2.6 (2.4–2.8)	3.7 (3.7–3.7)	2.6 (2.3–2.9)	3.5 (3.4–3.6)	3.0 (2.8–3.2)	3.4	2.4 (2.0–2.7)	2.5 (2.4–2.7)
TL	6.9	8.3	6.8 (6.3–7.5)	9.2 (9.0–9.3)	7.2 (6.6–7.8)	9.9 (9.7–10.0)	7.3 (6.9–7.7)	7.7	5.6 (4.9–6.0)	6.5 (6.0–7.0)
FL	7.6	10.2	7.9 (7.2–8.6)	10.5 (9.9–11.0)	8.2 (7.9–8.5)	10.9 (10.9–10.9)	8.8 (7.6–9.4)	9.5	6.6 (6.0–7.0)	7.4 (7.2–7.6)
HL/SVL	0.3	0.3	0.3 (0.3–0.3)	0.3 (0.3–0.3)	0.3 (0.3–0.3)	0.3 (0.3–0.3)	0.3 (0.3–0.3)	0.3	0.3 (0.3–0.4)	0.3 (0.3–0.3)
HW/SVL	0.3	0.3	0.3 (0.3–0.3)	0.3 (0.3–0.3)	0.3 (0.3–0.4)	0.3 (0.3–0.4)	0.3 (0.3–0.3)	0.3	0.3 (0.3–0.4)	0.3 (0.3–0.3)
END/ED	0.6	0.6	0.6 (0.6–0.7)	0.6 (0.6–0.6)	0.6 (0.5–0.7)	0.6 (0.6–0.7)	0.6 (0.6–0.7)	0.6	0.6 (0.5–0.7)	0.6 (0.6–0.6)
TL/SVL	0.4	0.3	0.4 (0.3–0.4)	0.3 (0.3–0.3)	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.3 (0.3–0.3)	0.3	0.3 (0.3–0.3)	0.3 (0.3–0.3)
FL/SVL	0.4	0.4	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.4 (0.4–0.5)	0.4 (0.4–0.4)	0.4 (0.3–0.4)	0.4	0.4 (0.3–0.4)	0.4 (0.3–0.4)

SVL, snout-vent length; HL, head length; HW, head width; IND, internarial distance; END, distance from eye to nostril; ED, eye diameter; TL, tibia length; FL, foot length.

and ventral parts of shanks, lacking in *B. quellokunka*. It has a shagreen to smooth dorsal skin, and the upper third of iris is golden with fine black reticulations (bluish-grey in *B. quellokunka*). Also, *B. quellokunka* is larger in size (maximum SVL of females 28.2 mm vs. 24.4 mm).

Description of the holotype: An adult female, 28.2 mm SVL. Body robust; dorsal skin homogeneously warty; ventral skin areolate; dorsolateral folds present, incomplete; pectoral fold absent; head wider than long; HW 32.9% of SVL, HL 31.9% of SVL; snout moderately short, rounded in dorsal view and in profile; nostrils not prominent, closer to snout than to eyes; canthus rostralis barely marked; eye–nostril distance 59.4% of eye length; loreal region slightly concave; cranial crests absent; tympanic membrane and tympanic annulus small, slightly evident beneath the skin; supratympanic fold absent; tongue large, oval; choanae small, oval, broadly separated; dentigerous processes of vomers absent; limbs short; tips of digits round, not expanded laterally; ulnar tubercle and fold absent; inner palmar tubercle oval, flattened, poorly defined, the same size as round outer; fingers moderately short, not fringed, tips rounded and lacking circumferential grooves and ungual flap; subarticular tubercles at the base of fingers round, large; supernumerary tubercles round, poorly marked; first finger slightly shorter than second, relative length of fingers 1 < 2 < 4 < 3; tibia length 32.9% of SVL; tarsal fold absent; two round metatarsal tubercles, inner approximately the same size as outer; supernumerary tubercles flat, not well marked; subarticular tubercles round, moderately swollen; toes lacking basal webbing or lateral fringes, toe tips round, lacking circumferential groves and ungual flap; relative length of toes 1 < 2 < 3 < 5 < 4; foot length 39.0% of SVL.

In preservative, dorsum uniformly brown, venter and throat pale brown with a uniform, fine marbled cream pattern; digits cream. In life, the dorsum was uniformly brown with some reddish-brown warts, the venter was grey with irregular brown markings, the throat was yellowish orange, and the digits were orange; there were small orange irregular blotches on axillae and groins; the venter was greyish-brown with irregular dirty-yellow patterns; the digits were yellowish-orange; the two inferior thirds of the iris were dark brown while the upper third was metallic bluish-grey.

Measurements (in mm) of the holotype: SVL, 28.2; HL, 9.0; HW, 9.3; IND, 2.2; END, 2.2; ED, 3.7; TL, 9.3; FL, 11.0.

Variation: Dorsal colour pattern is similar in all specimens; some of them (e.g. MUBI 5375, 5377) have irregular, feeble dark brown markings on the sides of the scapular region, above the groins, on limbs and on the canthal region; the venter and throat vary from almost uniformly brown (MUBI 5376) to almost uniformly cream (MUBI 5375). Only two specimens out of 13 did not have the bluish-grey upper third of the iris, having it brown as the rest of the eye. For morphometric variation, see Table 3.

Distribution and natural history: Known only from the type locality (Fig. 3). Individuals were found during the day under stones in wet puna.

Etymology: The species epithet is used as a name in apposition, and derives from the Quechua word Q'ello Kunka meaning yellow throat (q'ello yellow, kunka throat), and refers to the yellowish throat of the species. Q'ello Kunka is also the name of a mountain (5100 m) in the Quispicanchis province, Marcapata district, that belongs to the Vilcanota (Willkamayu) mountain range.

BRYOPHRYNE TOCRA SP. NOV.

(FIG. 4)

urn:lsid:zoobank.org:act:22B3787A-AC6A-4936-A1AF-338AA34FE6DA

Holotype: MUBI 5420 (field number 4697), adult female from a point between Ollachea and the junction to Corani on the Ollachea–Macusani road, province Carabaya, department Puno, Peru, 13°50′31.2″S, 70°29′51.7″W, 3213 m (Fig. 3), collected on 24 February 2006 by I. De la Riva, J. M. Padial, S. Castroviejo-Fisher and J. C. Chaparro.

Paratopotypes: MUBI 5418–19, (field numbers 4693, 4695) and MNCN 43785–87 (field numbers 4692, 4694, 4696) (males), same data as the holotype; MNCN 44214 (field number 4783) (female) and MUBI 5696 (field number 4784) (male) from the type locality, collected on 4 February 2007 by I. De la Riva, J. M. Padial, S. Castroviejo-Fisher, and J. C. Chaparro.

Diagnosis: *Bryophryne tocra* is characterized by: (1) skin on dorsum coarsely shagreen with scattered warts to warty (warts small, round to elongate); flanks coarsely warty, with some enlarged conical warts; head and forearms smooth to slightly shagreen; dorsal folds absent, a row of large warts from behind the eye to sacral region in some specimens; ventral skin coarsely areolate, throat areolate, chest smooth; (2) tympanic membrane and tympanic annulus small, differentiable beneath the skin; supratympanic fold short, conspicuous; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid lacking tubercles, cranial crests absent; (5) dentigerous process of vomers absent; (6) vocal slits present, nuptial pads absent; (7) Finger I slightly shorter than

Figure 4. Live specimens of ***Bryophryne tocra* sp. nov.** from near Ollachea, Peru. (A–B) Adult female (MNCN 44214, SVL 27.6 mm), from the type locality. (C–D) Adult female holotype (MUBI 5420, SVL 27.2 mm). (E–F) Adult male (MNCN 43786, SVL 19.3 mm), from the type locality.

Finger II, tips of digits rounded, lacking circumferential grooves and ungual flap; (8) fingers lacking lateral fringes; (9) ulnar region bearing warts; (10) heel lacking tubercles, tarsus lacking tubercles and folds; (11) plantar surfaces of feet bearing two metatarsal tubercles, inner slightly larger than outer; supernumerary plantar tubercles low, weakly defined; (12) toes lacking lateral fringes; webbing absent; Toe III equal to V, tips of digits rounded, lacking circumferential grooves and ungual flap; (13) dorsal coloration dark brown to grey, with metallic tones; ventral coloration white with black spots or marbled with black stripes; groins, axillae and posterior surfaces of thighs with yellow flash marks surrounded by bold black; (14) females larger than males, SVL 27.2–27.6 in adult females (n = 2), 18.4–20.0 mm in adult males (n = 5) (Table 3).

The sister and geographically closest species to *B. tocra* is *B. wilakunka* (type localities separated by 19.8 km straight line distance). Differences between them are listed below under *B. wilakunka*. Two species, *B. quellokunka* and *B. zonalis* occur at the Marcapata Valley, 65 km northwest of *B. tocra*. From *B. quellokunka*, *B. tocra* differs by having throat areolate (smooth in *B. quellokunka*), chest smooth (areolate), yellow blotches surrounded by black on groins, axillae and posterior surfaces of thighs (absent), and venter white with black spots or stripes (variable from greyish-purple with diffuse black blotches to brown); additionally, the iris of *B. tocra* in life is brown with fine black reticulations (two inferior thirds of iris dark brown and upper third metallic bluish-grey in *B. quellokunka*). From *B. zonalis*, *B. tocra* differs by having tympanum and tympanic annulus visible (absent in *B. zonalis*), and groins, axillae and posterior surfaces of thighs with yellow flash marks surrounded by bold black (yellow marks absent).

Description of the holotype: An adult female, 27.2 mm SVL. Body moderately robust; dorsal skin coarsely

shagreen with scattered warts of different sizes; ventral skin areolate; dorsolateral folds absent; pectoral fold present; head slightly wider than long; HW 30.8% of SVL, HL 29.8% of SVL; snout moderately short, rounded in dorsal view and in profile; nostrils slightly prominent, closer to snout than to eyes; canthus rostralis straight in dorsal view and in profile; eye–nostril distance 62.0% of eye length; loreal region concave; cranial crests absent; tympanic membrane and tympanic annulus small, barely perceptible beneath the skin; skin of tympanic area covered by large subconical warts; supratympanic fold well marked, short; tongue large, oval; choanae small, rounded, broadly separated; dentigerous process of vomers absent; ulnar tubercle and fold absent; inner palmar tubercle single, oval, slightly smaller than outer; fingers moderately short, not fringed, lacking circumferential grooves and ungual flap; subarticular tubercles round, poorly marked; supernumerary tubercles irregular and poorly defined; first finger slightly shorter than second, relative length of fingers 1 < 2 < 4 < 3; tibia length 35.6% of SVL; tarsal fold absent; two metatarsal tubercles, oval inner slightly larger than rounded outer; supernumerary and subarticular tubercles low, poorly defined; toe tips round, not expanded laterally, lacking circumferential grooves and ungual flap, toes lacking basal webbing or lateral fringes; relative length of toes 1 < 2 < 3 = 5 < 4; foot length 40.0% of SVL.

In preservative, dorsum greyish-brown, venter pale cream with brown, small, irregular blotches; throat cream; large cream blotches on groin surrounded by dark brown; palmar and plantar surfaces cream. In life, the dorsum of the holotype was mostly uniformly brown above; there were large pale yellow blotches surrounded by black in axillae, flanks and posterior surface of thighs, with a similar, more attenuated pattern on flanks and lower surfaces of hind limbs; the venter was grey with irregular brown dots and the throat was yellowish-cream; palmar and plantar surfaces were dirty orange; the iris was brown with fine black reticulation.

Measurements (in mm) of the holotype: SVL, 27.2; HL, 8.1; HW, 8.4; IND, 2.4; END, 2.1; ED, 3.4; TL, 9.7; FL, 10.9.

Variation: Males are smaller than females (Table 3), and have vocal slits but lack nuptial pads. In preservative, they are grey–brown above with a pale grey dorsal triangle between eyes and snout, and a brown canthal stripe that in MUBI 5419 and MUBI 5696 extends to the tympanic region; ventral coloration is variable, from finely dotted with brown (MUBI 5418, MNCN 43785, 43787) to brown with a marbled cream pattern (MUBI 5696 and MNCN 43786) to mostly uniformly brown (MUBI 5419); MNCN 43785 has irregular dark grey blotches on dorsum, outlined by pale grey margins; males have vocal slits and lack nuptial pads. In life, these males were uniformly brown with small orange blotches on groin, which can be present on the lower surface of the shanks and the posterior surfaces of thighs too. In preservative, female MNCN 44214 is similar to the holotype but with a marbled venter forming a brown and cream pattern, including the throat; the palmar and plantar surfaces are pale brown instead of cream, and the pale blotches on groin, axillae, lower belly and flanks are smaller; in life, these blotches were yellow, and the venter consisted of a reticulated pattern of dark brown and greenish-cream.

Distribution and natural history: Known only from the type locality. Individuals were found during the day under stones in open wet puna (Fig. 5). The holotype bears mature unpigmented eggs. When disturbed, individuals were able to make small leaps (something unusual in other species of this genus).

Etymology: The species epithet is used as a name in apposition, and derives from the Quechua word T'uqra for faded, discoloured, pale, and we use it to refer to the white belly of the new species. T'uqra is also the name of a mountain (5000 m) in the Willkanuta mountain range in the Andes of Puno, Peru.

BRYOPHRYNE WILAKUNKA SP. NOV.

(FIG. 6)

http://zoobank.org/urn:lsid:zoobank.org:act:F655DAB0-847F-4612-8803-30980E3D688A

Holotype: MUBI 5425 (field number 4704), adult female from Ayapata valley, province Carabaya, department Puno, Peru, 13°51′10.6″S, 70°18′52.2″W, 3947 m (Fig. 3), collected on 24 February 2006 by I. De la Riva, J. M. Padial, S. Castroviejo-Fisher, and J. C. Chaparro.

Paratopotype: MNCN 43788 (field number 4705) (adult male), same data as the holotype.

Diagnosis: *Bryophryne wilakunka* is characterized by: (1) skin on dorsal surfaces, including extremities and head, densely and uniformly warty (warts irregular in shape, low and flat to conical); flanks more densely warty and with larger and sharper warts than dorsum; dorsal folds absent; skin on belly and throat areolate (apparently smooth in preservative); (2) tympanic membrane and annulus small, slightly differentiated, tympanic fold conspicuous; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid covered with small low warts, cranial crests absent; (5) dentigerous process of vomers absent; (6) vocal slits present, nuptial pads absent; (7) Finger I equal to Finger II, tips of digits rounded, lacking circumferential grooves, ungual flaps and pads; (8)

Figure 5. Habitat at the type locality of ***Bryophryne tocra* sp. nov.** along the Ollachea-Macusani road, province Carabaya, department Puno, Peru, 3213 m a.s.l.

fingers lacking lateral fringes; (9) ulnar region bearing warts; (10) heel lacking tubercles, tarsus lacking tubercles and folds; (11) plantar surfaces of feet bearing two metatarsal tubercles, inner slightly larger than outer; supernumerary plantar tubercles low, weakly defined; (12) toes short and broad, lacking lateral fringes; feet webbing absent; Toe III equal to V, tips of digits rounded, lacking ungual flap and pads; (13) dorsal coloration dark grey to dark brown and black; ventral coloration orange to bright dark red with irregular orange spots on shanks, groins, and throat; palmar and plantar surfaces, and inner dorsal surfaces the same colour as belly; (14) females larger than males, SVL 17.9 (one adult male) to 24.6 (one adult female) (Table 3).

The sister species and also the geographically closest species to *B. wilakunka* is *B. tocra* sp. nov. (type localities separated by 19.8 km straight line distance), from which it differs by having slightly areolate belly (coarsely areolate in *B. tocra*), densely warty head and extremities (slightly warty to smooth), a dark grey to black dorsal coloration (dark brown with metallic hues), bright dark red to orange ventral coloration (white with black spots or marbled with black stripes), and reddish-orange blotches on flanks, groins, and axillae (groins, axillae and posterior surfaces of thighs with yellow flash marks surrounded by bold black). Two other species, *B. quellokunka* and *B. zonalis* occur in the Marcapata Valley, 80 km northwest of *B. wilakunka.* From *B. quellokunka, B. wilakunka* differs by having skin on head densely and uniformly warty (shagreen to smooth in *B. quellokunka*), ventral coloration orange to bright dark red (variable, from greyish-purple with diffuse black blotches to brown), and iris dark brown (two inferior thirds dark brown and upper third metallic bluish-grey). From *B. zonalis, B. wilakunka* differs by lacking dorsolateral folds (present in *B. zonalis*), having tympanic membrane and annulus slightly differentiated (absent), vocal slits present in males (absent) and ventral coloration orange to bright dark red (dark grey with white flecks).

Description of the holotype: An adult female, 24.6 mm SVL. Body moderately robust; dorsal skin warty, especially in posterior third of body and flanks; ventral skin slightly areolate, but with large and flat glandular warts; complete dorsolateral folds absent, faintly visible folds in the anterior third of body; pectoral fold absent; head wider than long; HW 34.5% of SVL, HL 31.3% of SVL; snout short, rounded in dorsal view and in profile; nostrils prominent, closer to snout than to eyes; canthus rostralis straight in dorsal view, rounded in profile; eye–nostril distance 57.6% of eye length; loreal region concave; cranial crests absent; tympanic membrane and tympanic annulus small, differentiated beneath the skin; supratympanic fold conspicuous in life; tongue large, oval; choanae small, rounded, broadly separated; dentigerous processes of vomers absent; limbs moderately short; tips of digits round, not expanded laterally, lacking circumferential groves and ungual flap; ulnar tubercle and fold absent; inner palmar tubercle single, round, slightly smaller than oval outer; fingers moderately short, not fringed;

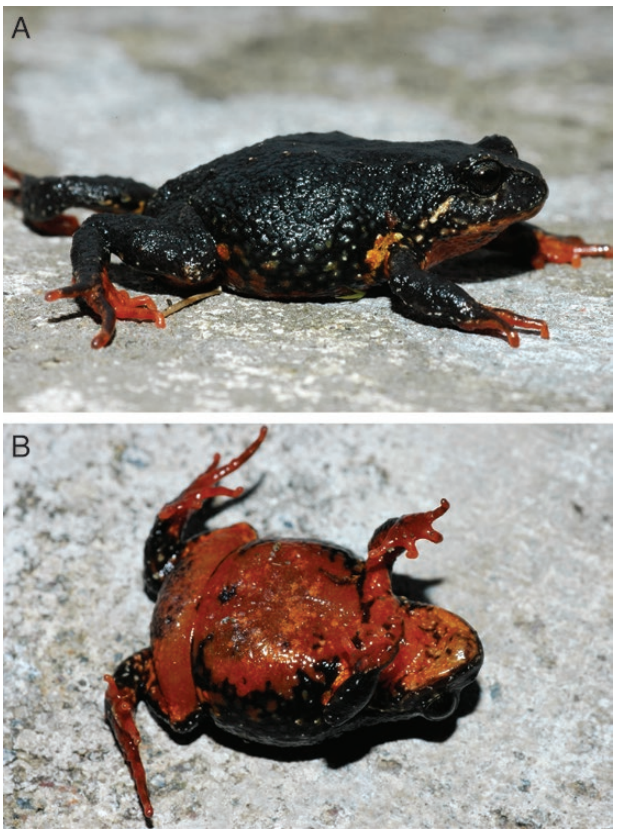

Figure 6. Live specimen of ***Bryophryne wilakunka* sp. nov.** from the type locality in Ayapata, Peru. (A–B) Dorsal and ventral views of adult female holotype (MUBI 5425, SVL 24.6 mm).

subarticular tubercles round, poorly marked; supernumerary tubercles irregular and weakly defined; first finger the same length as second, relative length of fingers 1 = 2 < 4 < 3; tibia length 33.7% of SVL; tarsal fold absent; two metatarsal tubercles, oval inner slightly larger than rounded outer; supernumerary and subarticular tubercles low, poorly defined; toes lacking basal webbing or lateral fringes; relative length of toes 1 < 2 < 3 = 5 < 4; foot length 41.5% of SVL.

In preservative, dorsum grey, venter and throat cream with diffuse brown small blotches; flanks and groins with small pale grey blotches; palmar and plantar surfaces cream. In life, the dorsum of the holotype was mostly dark brown, with faint dorsolateral folds formed by small tubercles; a creamy-yellow line run from the eye to the insertion of the forelimb and across the tympanic region; reddish-orange blotches on flanks, groins and axillae; all ventral surfaces were reddish-orange, paler on throat; the iris was dark brown.

Measurements (in mm) of the holotype: SVL, 24.6; HL, 7.7; HW, 8.5; IND, 2.0; END, 1.9; ED, 3.3; TL, 8.3; FL, 10.2.

Variation: The male MNCN 43788 is smaller than the holotype (see Table 3), but otherwise highly similar in all other respects; it lacks nuptial pads. In life, the paratype was greenish-brown, with ventral surfaces cream instead of reddish-orange.

Distribution and natural history: Known only from the type locality. Individuals were found during the day under rocks in open wet puna at almost 4000 m (Fig. 7); they moved quite fast and were able to make short leaps (something unusual in other species of this genus).

Etymology: The species epithet is used as a name in apposition and derives from the Aymara 'Wila Kunka', meaning red throat (wila = red, kunka = throat), which we use to refer to the bright dark red to orange ventral coloration of this species. Wila Kunka is also the name of a mountain (5350 m) in the Kallawaya mountain range of Puno, Peru.

MICROKAYLA GEN. NOV.

urn:lsid:zoobank.org:act:F7221ACB-FD97-4DFE-85F9-4CEFE5F6F058

Type species: *Psychrophrynella teqta* De la Riva & Burrowes, 2014

Included species: *Microkayla adenopleura* (Aguayo-Vedia & Harvey, 2001), comb. nov.; *M. ankohuma* (Padial & De la Riva, 2007), comb. nov.; *M. boettgeri* (Lehr, 2006), comb. nov.; *M. chacaltaya* (De la Riva, Padial & Cortéz, 2007), comb. nov.; *M. chapi* sp. nov.; *M. chaupi* (De la Riva & Aparicio, 2016), comb. nov.; *M. chilina* sp. nov.; *M. colla* (De la Riva, Aparicio, Soto & Ríos, 2016), comb. nov.; *M. condoriri* (De la Riva, Aguayo & Padial, 2007), comb. nov.; *M. guillei* (De la Riva, 2007), comb. nov.; *M. harveyi* (Muñoz, Aguayo & De la Riva, 2007), comb. nov.; *M. iani* (De la Riva, Reichle & Cortéz, 2007), comb. nov.; *M. iatamasi* (Aguayo-Vedia & Harvey, 2001), comb. nov.; *M. illampu* (De la Riva, Reichle & Padial, 2007), comb. nov.; *M. illimani* (De la Riva & Padial, 2007), comb. nov.; *M. kallawaya* (De la Riva & Martínez-Solano, 2007), comb. nov.; *M. katantika* (De la Riva & Martínez-Solano, 2007), comb. nov.; *M. kempffi* (De la Riva, 1992), comb. nov.; *M. melanocheira* (De la Riva, Ríos & Aparicio, 2016), comb. nov.; *M. pinguis* (Harvey & Ergueta, 1998), comb. nov.; *M. quimsacruzis* (De la Riva, Reichle & Bosch, 2007), comb. nov.; *M. saltator* (De la Riva, Reichle & Bosch, 2007), comb. nov.; *M. teqta* (De la Riva & Burrowes, 2014), comb. nov.; and *M. wettsteini* (Parker, 1932) comb. nov.

Diagnosis: (1) head wider than long, not as wide as body, body robust, extremities short; (2) tympanic membrane and annulus present (either visible beneath skin

Figure 7. Habitat at the type locality of ***Bryophryne wilakunka* sp. nov.** in the Ayapata valley, province Carabaya, department Puno, Peru, 3947 m a.s.l.

or hidden under the skin); (3) cranial crests absent; (4) prevomerine teeth and dentigerous process of vomers absent; pterygoid not in contact with parasphenoid; anterior parasphenoid ramus not reaching palatines; ear fully developed; (5) pectoral girdle functionally arciferal; (6) nasal bones narrowly separated medially; (7) tongue ovate, longer than wide; (8) tips of digits rounded, not pointed or expanded, lacking circumferential groves and pads; (9) terminal phalanges T-shaped to knobbed; (10) Finger I shorter or equal to Finger II; (11) two subarticular tubercles on Finger IV; (12) Toe V slightly longer than Toe III; (13) lateral fringes and webbing absent on fingers, basal webbing in toes of some species; (14) two metatarsal tubercles both prominent and subconical; tarsal fold or fold-like tubercle absent (Fig. 8); (15) dorsum tuberculate; belly areolate (apparently smooth in preservative); (16) trigeminal nerve passing external to *m. adductor mandibulae externus* ('S' condition; Lynch, 1986); (17) eggs large, not pigmented; (18) males with median subgular vocal sac and vocal slits, nuptial pads absent; (19) advertisement call usually composed of a single tonal note.

Character states for this diagnosis are based on our own examination of the type series of all the species in the genus except for *M. wettsteini* (of which we examined topotypic material). The clade of *Microkayla* is supported by putative synapomorphies: a rounded tongue (elongated in its sister group), areolate belly (smooth in *Psychrophrynella*), and lack of a pair of prominent metatarsal tubercles and the conspicuous tarsal fold or tubercle that are present in its sister group (Fig. 8; see also figures by Lynch [1975: 25; 1986: 425] and De la Riva *et al.* [2008b: 46]). *Microkayla* is externally similar to *Bryophryne*, but they are not

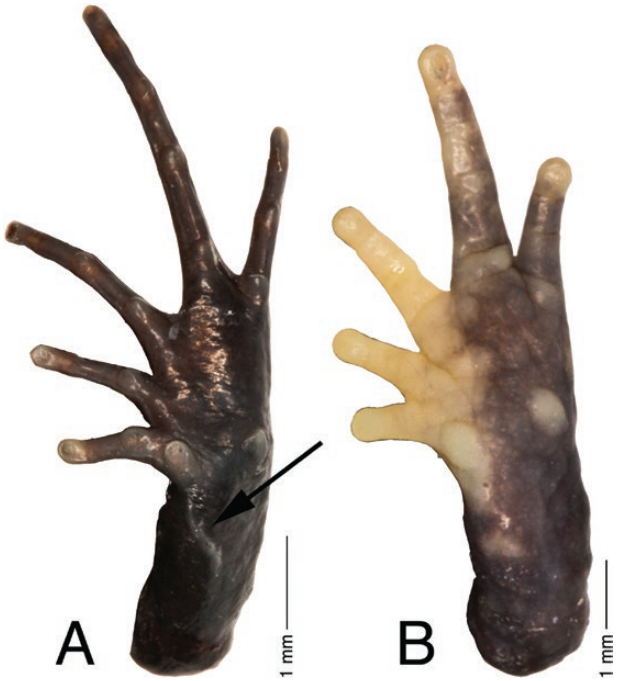

Figure 8. Plantar surfaces of (A) *Psychrophrynella usurpator* (MUBI 4643; SVL 24.4 mm) and (B) *Microkayla boettgeri* (MUBI 5365; SVL 18.8 mm) showing, respectively, the presence and absence of tarsal tubercle.

sister taxa in molecular phylogenies (Hedges *et al.*, 2008; Padial *et al.*, 2012, 2014; Chaparro *et al.*, 2015; this study, Fig. 1B). Mating calls are known for 11 species of *Microkayla* (including those of *M. boettgeri* and *M. chapi*, described herein). The call usually consists of a single, isolated, tonal, whistle-like, short note, but there are some exceptions: *M. teqta* has a pulsed call (De la Riva & Burrowes, 2014) while *M. wettsteini* and *M. saltator* emit several notes per call in a rapid series (De la Riva, 2007). *Microkayla saltator* is the most peculiar species in the genus; it lives at relatively low elevations (*c.* 2550 m a.s.l.) in comparison to other species in the genus (see below), in semi-humid forests (most species inhabit grasslands and elfin forest), has saltatorial locomotion — even arboreal — has slightly swollen tips of digits, and males have a large vocal sac.

Etymology: The name is a composite of the Greek word 'mikrós', meaning small, and the Quechua word for frog, 'k'ayla'. The entire name thus describes what these animals are, small frogs.

Distribution: *Microkayla* frogs inhabit cloud forests, elfin forests and humid puna of the Amazonian versant of the Cordillera Oriental of the Andes, from the eastern part of Cordillera of Carabaya (Department of Puno) in southern Peru to the western limits of department Santa Cruz in central Bolivia (Serranía Siberia, on the boundaries of Carrasco National Park – Department of Cochabamba – and Amboró National Park – Department of Santa Cruz), between 2466 and *c.* 4000 m a.s.l., encompassing a straight line distance of *c.* 670 km. Most species occur in humid puna and adjacent elfin forests above 3500 m, and only *M. colla, M. kempffi* and *M. saltator* are known to occur below 3000 m (De la Riva, 2007; De la Riva & Aparicio, 2016). Only three species are known from Peru, although more species are expected to be discovered when unexplored valleys and ridges of the eastern part of the Cordillera of Carabaya and western Cordillera of Apolobamba are surveyed. How many still unnamed species of *Microkayla* occur in Bolivia is difficult to know but, as indicated by this study, the number of described species highly underestimates the actual diversity.

Remarks: The new genus constitutes a diverse (24 species, including the two new ones described herein), well-supported and phenotypically diagnosable clade. As shown in the present study and others (Heinicke *et al.*, 2007; Hedges *et al.*, 2008; Padial *et al.*, 2014), *P. usurpator* is the sister group to *Microkayla* gen. nov. (Fig. 1C). Species of *Psychrophrynella* (redefined below) are markedly different from of *Microkayla* gen. nov., from which they can be externally distinguished by having two conspicuous metatarsal tubercles, a conspicuous tarsal fold-like tubercle (Fig. 8), a smooth belly, and a long and slender tongue. Our proposal of a new genus takes into consideration taxonomic stability (ICZN, 1999; Guayasamin *et al.*, 2009) and follows three main criteria for naming taxa (Vences *et al.* 2013): monophyly, phenotypic diagnosability, and clade stability—supported by evidence at hand. We interpret that our phylogenetic hypothesis is supported insofar as it is not refuted by critical evidence (it is the optimal solution according to optimality criteria) or contradicted by other equally optimal hypotheses. The inferred relationships are also well supported according to the Goodman–Bremer index, which measures the relative amount of evidence supporting clades (Grant & Kluge 2008), jackknife frequencies (Farris *et al.* 1996), which measure the relative amount of favourable and contradictory evidence for each clade, and bootstrapping frequencies (Felsenstein, 1985). Furthermore, the clade represents a radiation restricted to the high Andes of Bolivia and southern Peru, and such a pattern of distribution is consistent with phylogenetic inferences (a criterion considered important when naming taxa; see Vences *et al.*, 2013).

Part of this clade was first inferred by Lehr *et al.* (2005) in the first molecular study of frogs formerly grouped under *Phrynopus*, which showed a phylogeny with Bolivian species grouped separately from Peruvian species. The monophyly of this group was subsequently corroborated by other studies (e.g. Hedges *et al.*, 2008; Padial *et al.*, 2014; Chaparro *et al.*, 2015).

MICROKAYLA CHAPI SP. NOV.

(FIG. 9)

urn:lsid:zoobank.org:act:A11DB6F2-E959-4570-A369-00A7D64C8E13

Holotype: MUBI 5326 (field number 4516), adult female from 3.7 km from Sina, Hirigache River valley, province Sandia, department Puno, Peru, 14°30′09.7″S, 69°15′44.3″W, 3504 m (Fig. 10), collected on 10 February 2006 by I. De la Riva, J. M. Padial, S. Castroviejo-Fisher, J. C. Chaparro, and J. Bosch.

Paratopotypes: MUBI 5325, 5327, 5330, 5331 (field numbers 4514, 4519, 4524, 4527), MNCN 43763–65 and 43767–69 (males) (field numbers 4515, 4517, 4518, 4522, 4525, 4526); MNCN 43762 and 43766 (field numbers 5183 and 5191) (females); and MUBI 5328, 5329 (field numbers 4520, 4523) (juveniles), same data as the holotype.

Diagnosis: *Microkayla chapi* is characterized by: (1) skin on dorsum shagreen with large scattered sharp warts and short folds, sometimes coalescing into a pair of dorsolateral folds and/or incomplete middorsal and occipital folds; dorsal surface of extremities warty; flanks uniformly warty; ventral skin areolate,

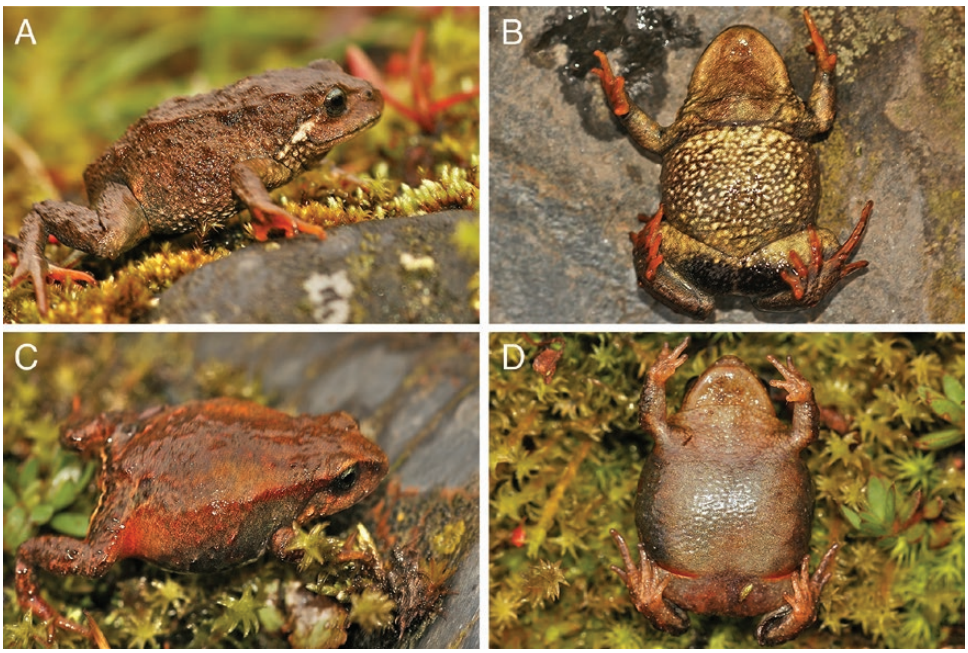

Figure 9. Live specimens of ***Microkayla chapi* sp. nov.** from the type locality in Sina, Peru. (A–B) Adult female holotype (MUBI 5326, SVL 19.9 mm). (C–D) Adult male (MNCN 43767, SVL 17.3 mm).

throat areolate; (2) tympanic membrane and tympanic annulus evident beneath the skin, supratympanic fold conspicuous; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid lacking tubercles, bearing small conical warts; (5) dentigerous process of vomers absent; (6) vocal slits and sac present, subgular; nuptial pads absent; (7) Finger I slightly shorter than Finger II; tips of digits rounded, lacking circumferential grooves and ungual flap; (8) fingers lacking lateral fringes; (9) ulnar region bearing warts, sometimes coalescing into a sharp ridge; (10) heel lacking tubercles, tarsus lacking tubercles and folds; (11) plantar surfaces of feet bearing two metatarsal tubercles, inner slightly larger than outer; supernumerary plantar tubercles low, inconspicuous; (12) toes lacking lateral fringes; webbing absent; Toe III slightly longer than V, tips of digits rounded, lacking circumferential grooves and ungual flap; (13) dorsal coloration with various shades of reddish-brown to dark brown or grey with metallic tones; ventral coloration variable, from grey with shades of red to dark grey with yellow spots; distal portions of hands and feet orange to red; groin with orange or red flash marks; (14) females slightly larger than males, SVL 19.9–21.6 in adult females (n = 3), 16.3–19.1 mm in adult males (n = 9) (Table 3).

Microkayla chapi sp. nov. is readily distinguished from both *M. boettgeri* and *M. chilina* sp. nov. (the two other Peruvian species) by having sharp and well-developed dorsolateral folds, occipital and sacral sharp warts and folds, a large and conspicuous tympanic membrane that is longer than 50% of eye length, and longer toes, less areolate belly, and smooth to granular skin. On the Bolivian side of the Cordillera of Apolobamba, the species *M. chaupi* and *M. katantika* occur 37.6 and 39.8 km straight line distance, respectively, from *M. chapi*. *Microkayla chapi* differs from *M. chaupi* mostly by having conspicuous dorsolateral folds (absent in *P. chaupi*), ventral coloration variable from grey with shades of red to dark grey with yellow spots (uniformly greyish-brown) and ventral skin areolate (finely granular). *Microkayla chapi* differs from *M. katantika* by being smaller (maximum SVL in *M. chapi* 21.6 mm, 27.7 mm in *M. katantika*), having dorsolateral folds (absent) and dorsal and ventral coloration variable (uniformly dark brown or grey). In addition, *M. chapi* can be distinguished from all other species of *Microkayla* by its sharp dorsal ridges and warts, a conspicuous tympanic membrane, and red flash marks in the groin.

Description of the holotype: An adult female, 19.9 mm SVL. Body robust; dorsal skin shagreen, with irregular warts scattered all over, and a pair of dorsolateral folds becoming inconspicuous ridges at level of mid-body; ventral skin areolate pectoral fold absent; head wider than long, HW 34.7% of SVL, HL 31.1% of SVL; snout moderately short, rounded in dorsal view and in profile; nostrils not prominent, slightly closer to snout than to eyes; canthus rostralis sharp, straight in dorsal view and lateral profile; eye-nostril distance 58.3% of eye length; loreal region faintly concave; cranial crests absent; tympanic membrane and tympanic annulus perceptible beneath skin; supratympanic fold

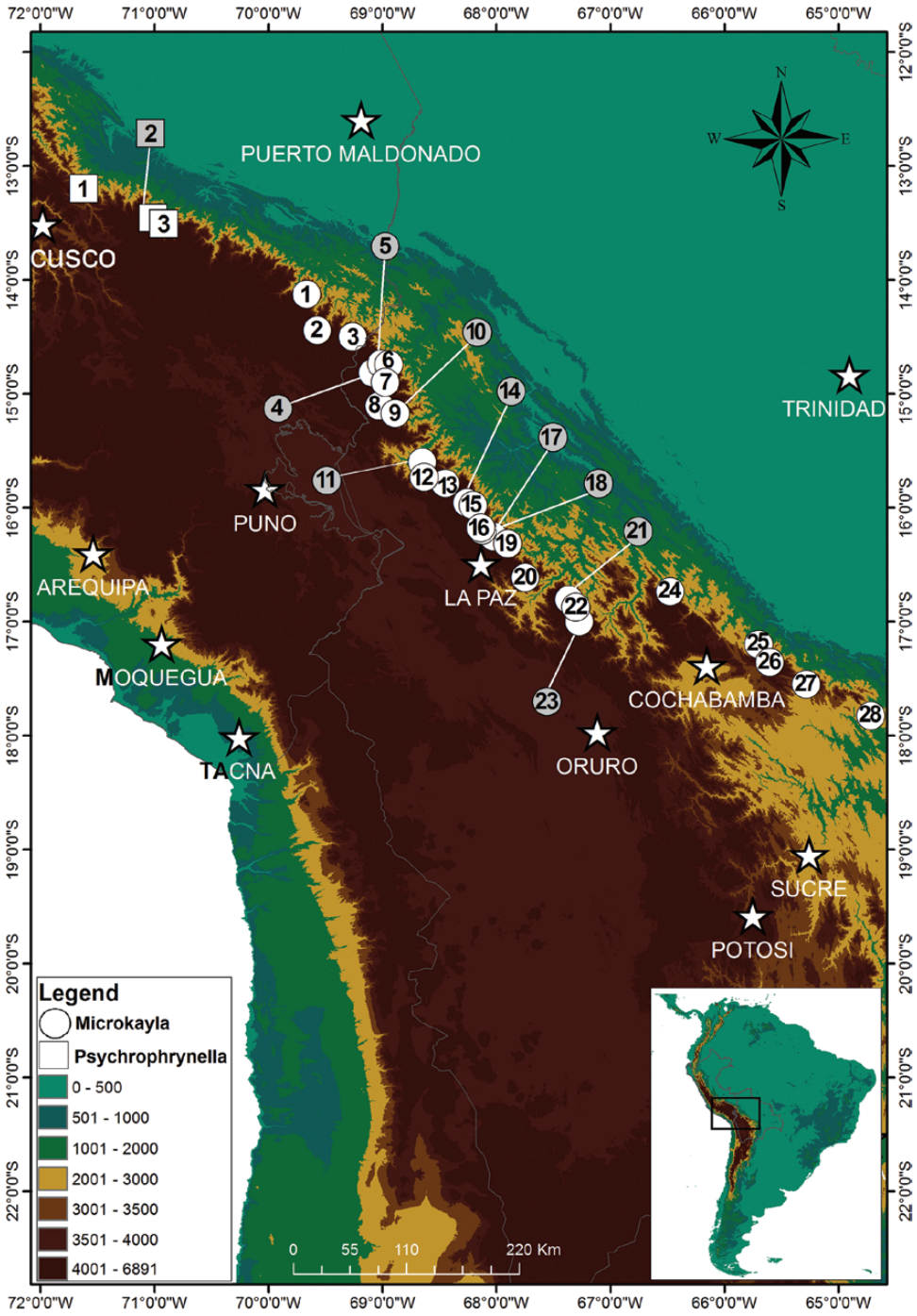

Figure 10. Map of south-eastern Peru and central Bolivia showing the distribution (type localities only) of the three nominal species of *Psychrophrynella* (squares), and 24 nominal and four unnamed species of ***Microkayla* gen. nov.** (circles). *Psychrophrynella*: (1) *P. usurpator*; (2) *P. chirihampatu*; (3) *P. bagrecito*. *Microkayla*: (1) *M. boettgeri*; (2) ***M. chilina* sp. nov.**; (3) ***M. chapi* sp. nov.**; (4) *M. katantika*; (5) *M. chaupi*; (6) *M. colla*; (7) *M. melanocheira*; (8) *M. kallawaya*; (9) *M. guillei*; (10) *M. saltator*; (11) *M. iani*; (12) *M. illampu*; (13) *M. ankohuma*; (14) *M. condoriri*; (15) *M. teqta*; (16) *M.* sp. 'Coscapa'; (17) *M. chacaltaya*; (18) *M.* aff. *chacaltaya*; (19) *M. wettsteini*; (20) *M. illimani*; (21) *M. pinguis*; (22) *M. quimsacruzis*; (23) *M.* sp. 'Khatu River'; (24) *M. harveyi*; (25) *M. iatamasi*; (26) *M.* sp. 'Ututuro'; (27) *M. adenopleura*; (28) *M. kempffi*.

prominent; tongue large, oval; choanae small, broadly separated; dentigerous processes of vomers absent; limbs short; fingers short, lacking fringes, tips of digits round, lacking circumferential grooves and ungual flap; ulnar tubercle and fold absent, but a row of low warts forming a ridge; inner palmar tubercle oval, smaller than round outer; fingers moderately short, not fringed; subarticular tubercles of the base of fingers large, round, swollen; supernumerary tubercles round, barely visible; relative length of fingers 1 < 2 < 4 < 3; tibia length 30.1% of SVL; tarsal fold absent; two metatarsal tubercles, oval inner slightly smaller than round outer; supernumerary tubercles round, poorly marked; subarticular tubercles round; toes lacking basal webbing or lateral fringes, toe tips round, lacking circumferential groves and ungual flap; relative length of toes 1 < 2 < 3 = 5 < 4; foot length 36.2% of SVL.

In preservative, dorsum brown with a pale middorsal thin line; venter and throat mostly cream with irregular brown areas; a pair of large and conspicuous bold black lumbar spots surrounded by a thin white line; a white thin line along posterior surface of thigh, from cloaca to level of shanks; axillae, groins and inner surface of forearms and shanks cream. In life, the dorsum was mostly uniformly brown above, with some pale areas; it had small reddish-orange irregular areas on axillae and groins; the venter was pale brown with irregular darker areas; the digits were reddish-orange; the iris was dark brown below and greenish-yellow in the upper third, with fine black reticulation.

Measurements (in mm) of the holotype: SVL, 19.9; HL, 6.2; HW, 6.9; IND, 1.6; END, 1.4; ED, 2.4; TL, 6.0; FL, 7.2.

Variation: The holotype has less warty dorsal skin, but other specimens have larger and sharper warts and short folds, sometimes forming short discontinuous dorsolateral or middorsal ridges (MUBI 5327, 5328). The overall coloration is more or less similar in all specimens examined, while venter varies from almost uniformly cream (MUBI 5331) to almost uniformly dark greenish-brown (MUBI 5325, 5330) and all intermediate patterns; the throat varies from cream (MUBI 5329) to brown (MUBI 5330); the pale lines of the posterior surface of thighs can be absent (MUBI 5330); some specimens have an inguinal dark spot (MNCN 43765, 43766); the tympanic annulus can be appreciable beneath the skin (MUBI 5330) or not (MUBI 5327). For morphometric variation, see Table 3.

Distribution and natural history: Known only from the type locality. Individuals were found by day under stones, in highly humid wet puna/elfin forest, and were common in only a very small area (*c.* 3 has), but no individuals were found beyond that point, despite the same kind of habitat being found over a larger area (Fig. 11). At night, with mist and full moon and an air temperature of 10 °C, males called with low intensity from inside moss on the ground and on stones. The call consisted of a single non-pulsed note, modulated

Figure 11. Habitat at the type locality of ***Microkayla chapi* sp. nov.** near Sina, Hirigache River valley, province Sandia, department Puno, Peru, 3504 m a.s.l.

in amplitude, with most intensity distributed between 3000 and 3300 Hz, a duration of 72–91 ms, emitted at a rate of 2.6–10.4 notes/minute (Fig. 12, Table 4) (call record number 8217, www.fonozoo.com).

Etymology: The species epithet is used as a name in apposition, and derives from the word 'chapi', meaning tin in Quechua, or 'Ch´api', meaning thorn in Aymara.

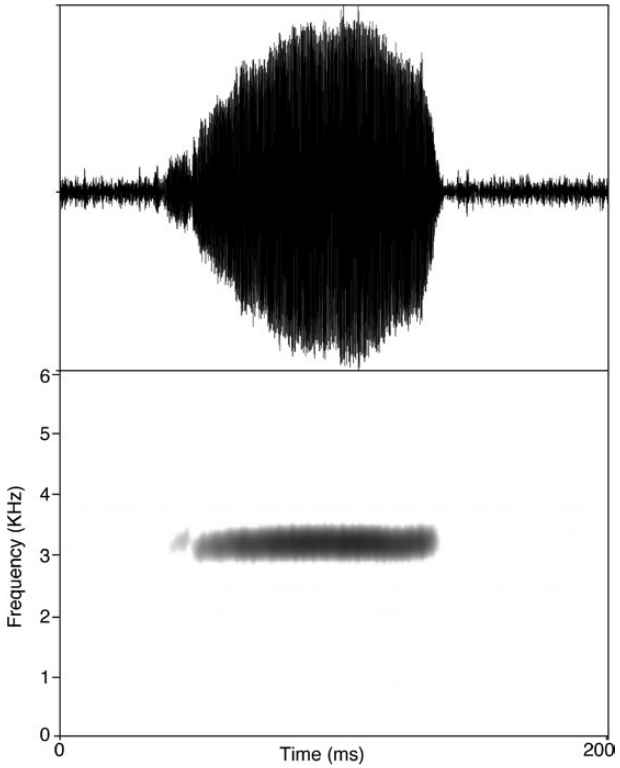

Figure 12. Oscillogram and sound spectrogram of the advertisement call of ***Microkayla chapi* sp. nov.** (MNCN 43764), recorded on 10 February 2006 at Hirigache river valley, Sina, Puno, Peru. Air temperature, 10 °C.

We use the two meanings of chapi to refer to the 'thorns' in the skin of the new species and to the tin roofs of the miner's shacks in 'La Rinconada' (5100 m), a gold mine and the highest village in the world, close to the type locality of this species. Chapi is also the name of a mountain (5400 m) near La Rinconada, on the border of the districts of Ananea and Sina, in the cordillera of Apolobamba.

MICROKAYLA CHILINA SP. NOV.

(FIG. 13)

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Holotype: MUBI 5355 (field number 4580), adult male from the joint of rivers Sayaco and Huacuyo, province Sandia, department Puno, Peru, 14°26′42.2″S, 69°34′11.5″W, 3792 m (Fig. 10), collected on 14 February 2006 by I. De la Riva, J. M. Padial, S. Castroviejo-Fisher, J. C. Chaparro and J. Bosch.

Paratopotypes: MUBI 5352 (field number 4573) (male); MUBI 5350-51, 5353 (field numbers 4569, 4572, 4574) and MNCN 43770–75 (field numbers 4570, 4571, 4575, 4577, 4578, 4579) (females); MUBI 5354 (field number 4576) (juvenile), same data as the holotype.

Diagnosis: *Microkayla chilina* is characterized by: (1) skin on dorsum warty to coarsely warty (warts round, low, subconical to conical), with slightly larger warts on flanks; conspicuous and incomplete dorsolateral ridges; belly, throat, groin and chest coarsely areolate; (2) tympanic membrane and annulus not discernible beneath the skin, tympanic fold prominent; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid lacking tubercles, bearing conical warts, cranial crests absent; (5) dentigerous processes of vomers absent; (6) vocal slits present, vocal sac subgular,

Table 4. Numerical parameters of the advertisement calls of two Peruvian species of *Microkayla*

	Sample size (specimens, calls)	Call/min	Note duration (ms)	Dominant frequency (Hz)	Change in intensity (Hz)	°C Air (substrate)	SVL	Vouchers
M. boettgeri	5, 27	8.3–21.6 (13.4)	102–145 (120.4)	2659–3043 (2793)	0–231 (44.7)	8 (–)	17.9–19.2	One from the series MNCN 43776-8
M. chapi	5, 14	2.6–10.4 (6.8)	72–91 (83.4)	3086–3171 (3146)	–	10 (12)	16.3–19.1	One from the series MNCN 43763-9

Figure 13. Live specimens of ***Microkayla chilina* sp. nov.** from the confluence of rivers Sayaco and Huacuyo, Sandia, Peru. (A) Adult female (MUBI 5351; SVL 24.3 mm); (B) Adult male holotype (MUBI 5355, SVL 24.3 mm). (C–D) Adult male (MNCN 43775, SVL 23.6 mm) from the type locality.

nuptial pads absent; (7) Finger I slightly shorter than Finger II, tips of digits rounded, lacking circumferential grooves and ungual flap; (8) fingers lacking lateral fringes; (9) ulnar region bearing warts, sometimes coalescing in an irregular ridge; (10) heel lacking tubercles; tarsus warty, lacking tubercles and folds; (11) two metatarsal tubercles, inner slightly larger than outer; supernumerary plantar tubercles low, numerous; (12) toes markedly short, lacking lateral fringes; webbing absent; Toe III longer than V, tips of digits rounded, lacking circumferential grooves and ungual flap; (13) dorsal coloration from reddish-brown to dark brown or black, sometimes with scattered yellow irregular blotches; ventral coloration dark grey to black with greyish-white and orange irregular blotches; groin, axillae, shanks and distal portions of hands and feet with orange flash marks; (14) females larger than males, SVL 25.5 in an adult female, 23.2–24.3 mm in adult males (n = 4) (Table 3).

The sister and geographically closest species to *M. chilina* is *M. boettgeri* (Lehr, 2006) (type localities separated by 36.6 km straight line distance). *Microkayla boettgeri* possesses a protruding snout, a sharp ulnar ridge formed by small conical granules, and sharp and protruding eyelids. *Microkayla chilina* has a more slender body than *M. boettgeri*, which has globular body shape (Fig. 14). In *M. chilina* the tympanic membrane and annulus are not discernible, while they are in *M. boettgeri*, in which the tympanic membrane reaches *c.* 50% of eye length in diameter. Some differences are also evident in coloration. *Microkayla chilina* often has irregular yellowish-cream blotches on dorsum, which are not present in *M. boettgeri*; this species usually has some reddish-orange coloration on venter, digits, axillae, and groins, while in *M. chilina* these areas are yellowish-orange. To the east, *M. chapi* sp. nov. is found at 33.7 km (straight line) from the type locality of *M. chilina* and is sister to the clade formed by *M. boettgeri* and *M. chilina*. *Microkayla chilina* is readily distinguished from *M. chapi* by having incomplete dorsolateral ridges (sharp and well-developed dorsolateral folds in *M. chapi*), tympanic membrane and annulus not discernible beneath the skin (a large and conspicuous tympanic membrane), and shorter toes, more areolate belly, and warty skin (smooth to granular).

Description of the holotype: An adult male, 24.3 mm SVL. Body robust; dorsal skin warty, with small irregular warts scattered all over; ventral skin areolate; dorsolateral folds present, incomplete, running from above ocular region to level of midbody, from where they continue as interrupted ridges of warts; two oblique and inconspicuous middorsal folds on central part of dorsum; pectoral fold absent; head wider than long, HW 33.7% of SVL, HL 32.9% of SVL; snout moderately short, rounded in dorsal view and in profile; nostrils not prominent, closer to snout than to eyes; canthus rostralis straight in dorsal view, concave in

Figure 14. Live specimens of *Microkayla boettgeri* from the type locality, Phara, Puno, Peru. (A–B) Adult male (MNCN 43778, SVL 18.6 mm); (C–D) Adult male (MNCN 43776, SVL 19.0 mm).

profile; eye–nostril distance 74.2% of eye length; loreal region concave; cranial crests absent; tympanic membrane and tympanic annulus not visible externally; supratympanic fold barely visible; tongue large, oval; choanae small, rounded, broadly separated; dentigerous process of vomers absent; vocal slits present; a subgular vocal sac; ulnar tubercle and fold absent (a ridge formed by connected warts); inner palmar tubercle nearly oval, slightly smaller than round outer; no nuptial pads; fingers moderately short, not fringed, lacking circumferential grooves and ungual flap; subarticular tubercles round, bulky; supernumerary tubercles round, of variable sizes; first finger approximately equal or slightly shorter than second, relative length of fingers $1 \leq 2 = 4 < 3$; limbs short; tibia length 30.0% of SVL; tarsal fold absent; two metatarsal tubercles, oval inner slightly larger than round outer; supernumerary and subarticular tubercles low, irregular; toes lacking basal webbing or lateral fringes, toe tips round, lacking circumferential grooves and ungual flap; relative length of toes $1 < 2 < 3 = 5 < 4$; foot length 37.4% of SVL.

In preservative, dorsal surfaces uniformly grey, venter and throat brownish-grey with an irregular beige area in central part of venter; palmar and plantar surfaces and inner surface of forelimbs mostly brown, digits pale cream. In life, the dorsum was mostly uniformly brown above; there were small orange irregular blotches on axillae and groins; the venter was greyish-brown with an irregular dirty-yellow pattern; the digits were yellowish-orange; the iris was dark brown.

Measurements (in mm) of the holotype: SVL, 24.3; HL, 8.0; HW, 8.2; IND, 2.4; END, 2.3; ED, 3.1; TL, 7.3; FL, 9.1.

Variation: All specimens are nearly identical in skin texture and overall colour pattern. MNCN 43773, 43774, and, especially, 43775, have some small, irregular pale grey blotches on dorsum (dirty-yellowish in life); a dark brown spot can be present on the anterior surface of the forearm (e.g. MUBI 5351, MNCN 43771) and/or the inner surface of the shank (MNCN 43772). Males are small and lack vocal slits, external vocal sac and nuptial excrescences.

Distribution and natural history: Known only from the type locality. Individuals were found during the day under stones in open wet puna. They were not common; almost two hours of collecting by five persons yielded only 12 specimens.

Etymology: The species epithet is used as a name in apposition, and derives from the Quechua word 'chilina', meaning the colour of a ripe orange (reddish-yellow), and refers to the spots of this colour present in this new species.

MICROKAYLA BOETTGERI (LEHR, 2006)

Remarks: On 16 February 2006 we sampled the wet puna around the village of Phara (district of Limbani, province Sandia, department Puno), and found a

population of *Microkayla* that corresponds to what was originally named as *Phrynopus boettgeri* by Lehr (2006) based on specimens collected in 2004 by J. Boettger at the very same locality. Specimens were found under rocks during the day and calling at night from within moss on the ground or on stones. Among the specimens we collected (MUBI 5363-5, MNCN 43776-78; 14), some characters are observed that complement those described by Lehr (2006), and we provide a brief description of those as well as of the undescribed advertisement call of this species.

Lehr (2006) mentioned the lack of vocal sac and vocal slits, but male specimens collected by us do have a vocal sac and vocal slits. Also, several specimens possess a protruding, translucent callosity on the tip of the snout, that covers the anterior area of the snout and part of the upper lip. So far, in Holoadeninae, this structure has been only described in males of the Bolivian species *M. teqta* (De la Riva & Burrowes, 2014). Those males were guarding egg clutches in subterranean chambers under stones; thus, the mentioned peculiar rostral morphology is probably a structure for digging (De la Riva & Burrowes, 2014). Also, we found colour variants lacking in those described by Lehr (2006). One specimen (MNCN 43778; Fig. 14A–B) has a bright orange to bright red belly reticulated with black and metallic blue. The underside of thighs and shanks also posses metallic blue blotches. Orange and red flash marks also extend to the groin, axillae, and hands and feet. Another specimen (MNCN 43776; Fig. 14C, D), is mostly white ventrally, with bold black reticulations and spots, shades of bright orange to red in the posterior part of the belly, and a few blue blotches on the ventral sides of shanks and thighs.

We recorded the call of *M. boettgeri* at its type locality on 16 February 2006, at 19:40 h, at an air temperature of 8 °C. The call consists of a single non-pulsed note with duration of 102–145 ms, emitted at a rate of 8.3–21.6 notes/minute (Table 4; Fig. 15) (call record numbers 8227–28, www.fonozoo.com). It is modulated in amplitude, with most intensity distributed between 2500 and 3000 Hz. There was a weak modulation in intensity (increasing to the end) in one of the specimens recorded. The difference in intensity reached 231 Hz from the beginning to the end of the call. The call of *M. boettgeri* differs from that of *M. chapi* by having a longer note with higher repetition rate and lower dominant frequency.

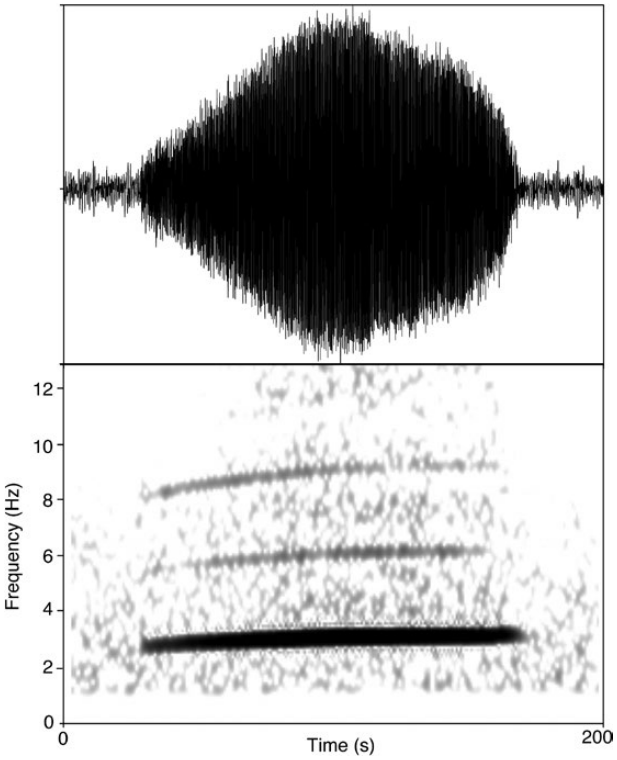

Figure 15. Oscillogram and sound spectrogram of the advertisement call of *Microkayla boettgeri* (specimen nor collected), recorded on 16 February 2006 at Phara, Puno, Peru. Air temperature, 8 °C.

Genus *Psychrophrynella* Hedges, Duellman, & Heinicke, 2008, emended

Included species: *Psychrophrynella bagrecito* (Lynch, 1986) (type species), *P. chirihampatu* Catenazzi & Ttito, 2016, and *P. usurpator* De la Riva, Chaparro & Padial, 2008.

Diagnosis: (1) head narrow, not as wide as body, extremities relatively long; (2) tympanic membrane and annulus differentiated (annulus and membrane visible beneath skin); (3) cranial crests absent; (4) prevomerine teeth, dentigerous process of vomers, and dentigerous ramus absent; pterygoid not in contact with parasphenoid; anterior parasphenoid ramus short, not reaching palatines; ear fully developed; (5) pectoral girdle anatomically arciferal but functionally firmisternal (halves of the epicoracoid cartilages fused); (6) nasal bones widely separated medially; (7) tongue long and narrow, much longer than wide; (8) tips of digits narrow and rounded, not expanded, lacking circumferential groves and pads; (9) terminal phalanges T-shaped to knobbed; phalangeal formulae of hands and feet 2-2-3-3 and 2-2-3-4-3, respectively; (10) Finger I equal to or slightly shorter than Finger II; (11) two subarticular tubercles on Finger IV; (12) Toe V slightly longer than Toe III; (13) lateral fringes and webbing absent on digits; (14) two metatarsal tubercles both prominent and subconical; inner edge of tarsus bearing a prominent, elongate, sigmoid-shaped or fold-like tubercle not contiguous with inner metatarsal tubercle (Fig. 8); (15) dorsum finely shagreen; belly smooth; (16) trigeminal nerve passing external to *m.*

adductor mandibulae externus ('S' condition; Lynch, 1986); (17) eggs large, not pigmented; (18) males with median subgular vocal sac and lacking nuptial asperities; (19) mating call composed of a series of notes.

DISCUSSION

With the recognition of *Microkayla* we highlight a well-supported and diagnosable clade of 24 species of terraranas distributed through the paramos and cloud forests of the eastern side of Andes from central Bolivia to southern Peru. This proposal will benefit future research by facilitating the analysis of the numerous populations that might constitute unnamed species and by calling attention to the origin and evolution of this particular clade.

With the recognition of *Microkayla* we also set a well-defined clade aside from the taxonomic uncertainty affecting its sister taxon, *Psychrophrynella*, from which it is separated by a long branch and conspicuous phenotypic differences. Morphological evidence further indicates that *P. bagrecito,* type species of *Psychrophrynella,* as well as *P. usurpator* and *P. chirihampatu* share a considerable number of traits with *Noblella*, especially with *N. peruviana*, the type species of this genus (Lynch, 1975; Lehr, Aguilar & Lundberg 2004; Lehr 2006; De la Riva, Chaparro & Padial, 2008a, b; Catenazzi & Ttito, 2016) and not with *Microkayla*. *Psychrophrynella bagrecito* and *P. usurpator* are outwardly similar to *Noblella peruviana* – *P. usurpator* was once considered conspecific with *N. peruviana* (De la Riva *et al.*, 2008a, 2008b), as they share a pectoral girdle anatomically arciferal but functionally firmisternal, an extensive reduction of the prevomers, a conspicuous tarsal tubercle, a pair of conical metatarsal tubercles, a smooth belly, a long and slender tongue, and *P. bagrecito* has swollen and pointed finger tips, a trait characteristic of *Noblella*. This evidence prompted Lynch (1986) to suggest that *P. bagrecito* and *P. usurpator* (as *Phrynopus peruvianus*) could be closely related to *Noblella* (as *Phyllonastes* Heyer, 1977), an opinion later shared by others (De la Riva & Köhler, 1998; Lehr *et al.*, 2004; Lehr, 2006; De la Riva *et al.*, 2008b). But neither the position of *N. peruviana* nor *P. bagrecito* have been investigated phylogenetically, which leave several potential scenarios open for further scrutiny. On the one hand, *N. peruviana* might form a clade with *P. bagrecito, P. chirihampatu* and *P. usurpator*, all of them occurring in the high Andes of southern Peru. In this case *Psychrophrynella* Hedges, Duellman, & Heinicke, 2008 would become a junior synonym of *Noblella* Barbour, 1930. On the other hand, *N. peruviana* and *P. bagrecito* might not be closely related, with at least one of them nested within the clade of species currently representing *Noblella* in phylogenetic analyses. This is the alternative assumed by the taxonomy in place that considers *N. peruviana* related to Ecuadorian and lowland Amazonian species of *Noblella* that are distantly related to *Psychrophrynella* (Hedges *et al.*, 2008). A third, and more complex scenario, would place both *N. peruviana* and *P. bagrecito* in a clade distant to *P. usurpator*, which would also require a new name for the sister group of *Microkayla*. In summary, the affinities of both *N. peruviana* and *P. bagrecito,* type species of their respective genera, remain unclear, and inferring their position is key to gaining a proper understanding of the relationships of a large portion of Holoadeninae and to attain a more stable taxonomy.

The diversity of terraranas in the wet puna

Systematic research during the last decades has greatly improved our knowledge of species diversity for many amphibian taxa (Frost *et al.*, 2006; Frost, 2017). One of the most significant outcomes has been the progressive discovery of a profusion of terraranas in the cold and humid grasslands of the Amazonian versant of the Andes in Peru and Bolivia – a kind of habitat known as subparamos or wet puna (Olson *et al.*, 2001). These discoveries are driving a dramatic shift in our perception of the diversity in these ecosystems and of the diversification of terraranas. As explained below, the diversity of terraranas in the grasslands of the high Andes is the result of several independent radiations that are not only largely underestimated and poorly studied, but could constitute some of the most diverse and interesting evolutionary radiations in the Neotropics.

Among the large diversity of species of New World direct-developing frogs or terraranas (*c.* 1000 species), the vast majority of species inhabits tropical forests of Central and South America at moderate altitudes. Only a few clades have species in the cold grasslands of the high Andes above the tree line. In the northern Andes these grasslands are known as paramos and have a broad distribution across Venezuela, Colombia, Ecuador and northern Peru (Olson *et al.*, 2001). These paramos are inhabited by a diverse fauna of terraranas (*Geobatrachus* Ruthven, 1915, *Hypodactylus*, *Lynchius, Niceforonia,* multiple clades of *Pristimantis* Jiménez de la Espada, 1870 [Hedges *et al.*, 2008]), and several other groups of amphibians, (Duellman, 1999). South of the Huancabamba depression and especially in central Peru, the geography of the landscape changes dramatically. Here the humid grasslands are confined mostly to the Amazonian versant of the Andes and form a narrow and discontinuous belt segmented by steep ridges and glacial valleys. These grasslands, known as wet puna, subparamos, or wet

montane grasslands (Ribera-Arismendi, 1992), run intermittently from northern Peru to Central Bolivia and constitute a distinctive ecosystem with a unique biodiversity (Olson *et al.*, 2001).

Up to the late nineties the anuran fauna of the wet puna was poorly known in comparison to that of the northern paramos (e.g. Duellman, 1999). A few species of *Hypsiboas* Wagler, 1830, *Gastrotheca* Fitzinger, 1843, *Pleurodema* Tschudi, 1838, *Rhinella* Fitzinger, 1826 and *Telmatobius* Wiegmann, 1834 and 11 species of terraranas (grouped in the genus *Phrynopus*; Lynch, 1975) formed most of the anuran diversity of this vast area that stretches along more than 2000 km. Surveys in the early 2000 started to reveal a much larger diversity of terraranas both in Bolivia (De la Riva, 2007) and Peru (e.g. Lehr *et al.*, 2002a; Lehr, 2007) and a new perspective on their distribution and diversity patterns emerged. As De la Riva (2007) first pointed out, highland terraranas do not have large distributions in the region but are instead confined to small patches of habitat often encircled by snow-covered peaks above and by dense elfin and cloud forests beneath them. Most species are indeed allopatric and occur at similar altitudes but in different valleys or river basins separated by sharp ridges or rocky massifs of unsuitable habitat (see Fig. 16 for some examples). Even species inhabiting the elfin forests have small distributions, never encompassing more than a few adjacent drainages (e.g. *M. iatamasi*; *M. kempffi*). In a few cases, more than one species has been found on the same drainage, either segregated by altitude and habitat (e.g. *M. saltator* and *M. guillei*), and sympatry seems to be restricted to species in distant lineages (*B. cophites* and *P. usurpator*). As a result, these frogs present an unexpected and remarkably high beta-diversity within the vertebrate fauna of the wet puna, an ecoregion traditionally seen as biologically poor in contrast to the lush neighbouring cloud and elfin forests (Duellman, 1999).

Ten years ago De la Riva (2007: p. 274) concluded that 'each valley on the Amazonian slopes of the Andes with a suitable patch of habitat along the stretch of the Andean Cordillera Occidental between central Peru and central Bolivia is likely to contain an endemic species of *Phrynopus*' [at that time the split of *Phrynopus* sensu Lynch (1975) had not yet taken place]. As the present study clearly shows, De la Riva's (2007) prediction holds for the genera *Bryophryne*, *Microkayla*, *Phrynopus* and perhaps *Psychrophrynella*. With the exploration of valleys and isolated ridges on the

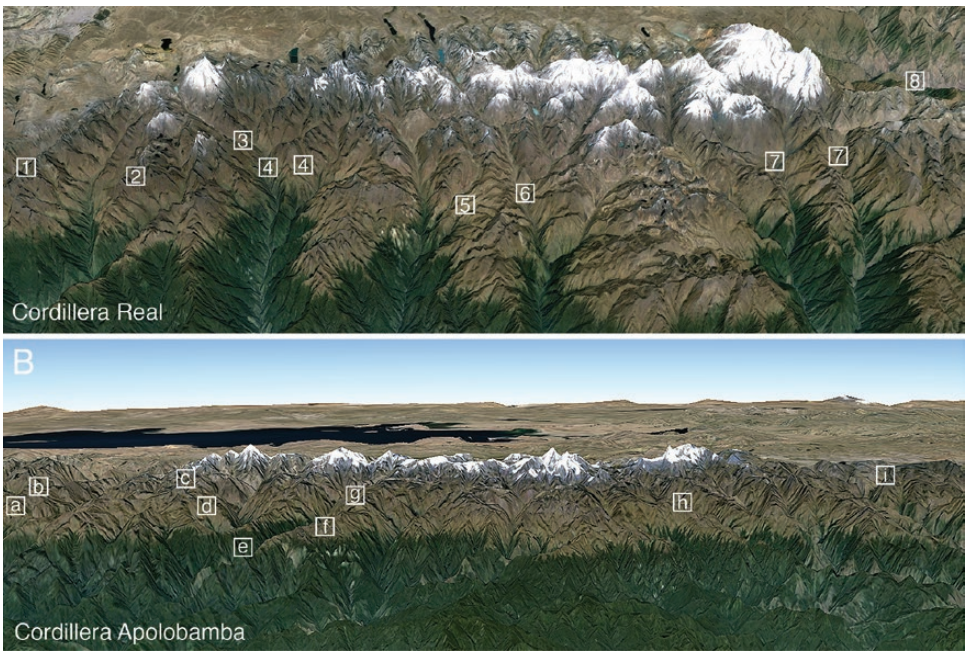

Figure 16. Partial views, as seen from the north (Google Earth's vertical proportions × 3), of the Cordillera Real (Bolivia) and Cordillera de Apolobamba (Bolivia and Peru; the dark extension in the background is the Lake Titicaca), showing the allopatric distribution of *Microkayla* species along the wet puna of the Amazonian versant of the Andes. Cordillera Real: (1) *M. wettsteini*, (2) *M. chacaltaya*, (3) *M.* aff. *chacaltaya*, (4) *M.* sp. 'Coscapa', (5) *M. teqta*, (6) *M. condoriri*, (7) *M. ankohuma*, (8) *M. illampu*. Cordillera Apolobamba: (a) *M. saltator*, (b) *M. guillei*, (c) *M. kallawaya*, (d) *M. melanocheira*, (e) *M. colla*, (f) *M. chaupi*, (g) *M. katantika*, (h) ***M. chapi* sp. nov.**, (i), ***M. chilina* sp. nov.** Straight line distance between (1) and (8) is c. 100 km, and that between (a) and (i), c. 110 km. *M. boettgeri* (not shown) is 36.6 km west of (i).

Amazonian versant of the Andes of Peru and Bolivia the number of species in these genera has increased from 9 to 59 only in this century. However, many suitable areas remain unexplored and these could harbour new species or be populated by already known species. There are few roads crossing the cordillera as they have to go through passes at about 5000 m and all new species reported in this study resulted from the exploration of such accessible areas. As the vast majority of valleys and ridges potentially inhabited by unknown species are difficult to access and require logistically complex and time-consuming expeditions, we expect a large portion of species diversity and distribution patterns to remain incompletely described for a long time.

THE DIVERSITY AND ORIGIN OF THE WET PUNA RADIATIONS

Despite our still limited knowledge of species diversity, there are several hypotheses about the origin and diversification of terraranas in the wet puna and the paramos that can be deduced from our current understanding of distribution patterns and phylogenetic relationships.

As outlined above, high Andean terraranas occupy humid grasslands from northern Colombia to central Bolivia. Most genera of terraranas with representatives in the high paramos and wet puna belong to the clade of Holoadeninae (*Bryophryne*, *Microkayla, Niceforonia*, *Oreobates*, *Phrynopus* and *Psychrophrynella*) (Padial *et al.*, 2014). These genera, however, do not form a single monophyletic group but are, instead, intermingled with other geographically distant and ecologically diverse clades of holoadenines that include Amazonian, Atlantic forest and Pacific forest species. For example, *Bryophryne* is sister to a clade that includes species from low montane forests of the Andes and the Amazon lowlands (*Noblella*), as well as species from eastern Brazil [*Barycholos ternetzi* (de Miranda Ribeiro, 1937) and '*E. bilineatus*'] and the Pacific versant of the Andes [*Barycholos pulcher* (Boulenger, 1898)]. The clade of *Bryophryne* and others is in turn the sister group to the clade of *Microkayla* and *Psychrophrynella*, which includes mostly wet puna specialists and elfin forest species. *Phrynopus* is another clade that only occurs in the wet puna and elfin forests of the Andes of central Peru (Fig. 17), and is sister to the clade including *Lynchius* and *Oreobates*. While species of *Lynchius* occur in the transition from the wet puna to the paramos (northern Peru and southern Ecuador) and in the cloud forests of Cordillera del Cóndor (one species, sister to the other ones), *Oreobates* has two species in the wet puna, nested within a large radiation of cloud forest and montane forest species that also includes species from the Amazon lowlands, the savannahs of the cerrado and even the distant Atlantic Forest.

Terraranas from the northern paramos belong instead to two different clades, Holoadeninae (*Hypodactylus, Lynchius* and *Niceforonia*) and Ceuthomantinae Heinicke, Duellman, Trueb, Means, MacCulloch & Hedges, 2009 (several clades of *Pristimantis* and *Tachyramantis* Heinicke, Barrio-Amorós & Hedges, 2015). *Hypodactylus* is distributed from the Amazon lowlands to the paramos of northern Peru and Ecuador, and has been inferred by our analyses as either the sister group of Holoadeninae or as the sister group of the clade including *Lynchius*, *Oreobates* and *Phrynopus*. The several high Andean clades of *Pristimantis* replace each other along the paramos that extend from northern Peru to northern Venezuela (Hedges *et al.*, 2008; Pinto-Sánchez *et al.*, 2012). *Niceforonia*, in turn, is restricted to the paramos above 3000 m in the Cordillera Central and Cordillera Oriental in Colombia and its phylogenetic position remains unknown.

The pattern just described suggests four to five colonizations of the Andean humid grasslands within Holoadeninae: one for *Bryophryne* (wet puna in southern Peru), one for *Hypodactylus* (paramos of Ecuador and Peru), one for the common ancestor of *Microkayla* and *Psychrophrynella* (wet puna of Bolivia and southern Peru) – or for the common ancestor of *Bryophryne*, *Microkayla* and *Psychrophrynella* (according to maximum likelihood analyses) –, one for the common ancestor of *Lynchius* and *Phrynopus* (paramos of Ecuador and Peru) and one for *Oreobates* (two species in the wet puna in central Peru). Furthermore, inferred relationships support connections among distant parts of South America (i.e. broader ancestral distributions), such as the Atlantic Forest and the Andes, the Amazon and the Cerrado. For example, *Barycholos ternetzi* is sister to *B. pulcher*, and while the former occurs in the Pacific forests of Ecuador, the later inhabits the Atlantic forest of Brazil, and these two species form the sister group of an Andean-Amazonian clade (*Noblella*). The clade of *Oreobates* also entails connections between eastern Brazil and the Andes, as *O. remotus* Teixeira, Amaro, Recoder, Sena & Rodrigues, 2012 (from the dry Atlantic Forest) and *O. heterodactylus* (de Miranda Ribeiro, 1937) (from the cerrado of Bolivia and Brazil) form a clade that is sister to an Andean-Amazonian clade. Historical connections among the Atlantic Forest and the Andes-Amazonia are also supported by phylogenetic relationships of species in the genera *Adelophryne* Hoogmoed & Lescure, 1984 (Eleutherodactylidae) (Fouquet *et al.*, 2012), *Chiasmocleis* Méhely, 1904 (Microhylidae) (Peloso *et al.*, 2014), *Gastrotheca* (Hemiphractidae) (e.g. Castroviejo-Fisher *et al.*, 2015; Duellman, 2015), *Pristimantis* (Craugastoridae) (e.g. Canedo & Haddad, 2012), and several others, which points at broad distributions of ancestral radiations across the Amazonian Craton.

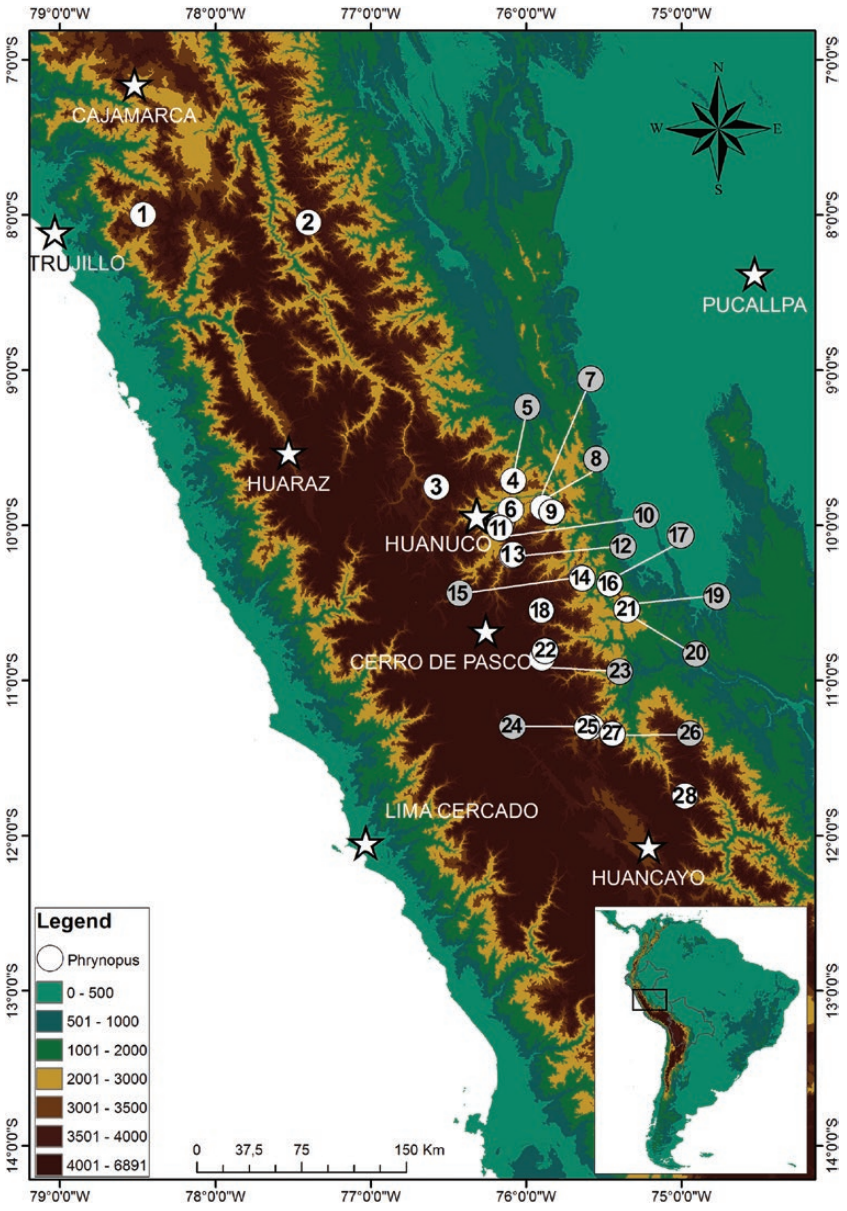

Figure 17. Map of central Peru showing the distribution (type localities only) of the 28 species of *Phrynopus* described hitherto. (1) *P. thompsoni*; (2) *P. valquii*; (3) *P. daemon*; (4) *P. vestigiatus*; (5) *P. lechriorhynchus*; (6) *P. interstinctus*; (7) *P. dagmarae*; (8) *P. kauneorum*; (9) *P. kotosh*; (10) *P. heimorum*; (11) *P. horstpauli*; (12) *P. barthlenae*; (13) *P. tautzorum*; (14) *P. nicoleae*; (15) *P. miroslawae*; (16) *P. curator*; (17) *P. badius*; (18) *P. pesantesi*; (19) *P. auriculatus*; (20) *P. bracki*; (21) *P. tribulosus*; (22) *P. paucari*; (23) *P. bufoides*; (24) *P. juninensis*; (25) *P. montium*; (26) *P. oblivius*; (27) *P. peruanus*; (28) *P. chaparroi.*

Within the overall morphological similarity that characterizes wet puna terraranas, there is some external variation and, indeed, another important deduction from phylogenetic patterns bears on the origin of such morphological diversity. In high Andean terraranas, geographically distant species often resemble each other in overall body shape and structures of fingers and toes (e.g. species in *Hypodactylus*, *Microkayla*, *Niceforonia*, *Noblella, Phrynopus* and *Psychrophrynella*), while sister species found in adjacent valleys are often markedly distinct, showing conspicuous differences in skin structures and coloration (e.g. *B. tocra* vs. *B. wilakunka*; Figs 5 and 7). Meanwhile, sympatric and parapatric species seem to rarely be sister taxa. This scenario supports the idea that morphological and species diversity in these frogs is not driven by competition with closely related species, as seems to be the case in other important

radiations (e.g. cichlid fishes or sticklebacks; Schluter, 2000) but, rather, it could likely be driven by a rather neutral divergence in isolation coupled with adaptation to local conditions. Similarity in distantly related species inhabiting the wet puna and paramos further suggests that the evolution of traits associated with living in these environments occurred independently several times and always in a similar fashion. For example, Holoadeninae species from both the paramos and the wet puna lack expanded finger discs and t-shaped terminal phalanges, and have short extremities and robust rounded bodies. In all cases, selective pressures seem to have shaped the extremely convergent morphology of different groups. These pressures must be so strong that other completely unrelated frogs also converged to the same morphology in these habitats, as exemplified by two species of the Microhylidae genus *Ctenophryne* Mocquard, 1904 (Lehr *et al.*, 2002b; Lehr & Trueb, 2007).

In contrast, arboreal or partially arboreal species in Holoadeninae, such as *Oreobates* from the Cerrado have expanded discs, fully t-shaped terminal phalanges, long extremities and slender bodies, while wet puna species such as *O. ayacucho* have the typical wet puna adaptive morphology. Similarly, the expanded discs of arboreal species of *Pristimantis* contrast with the reduced discs of terrestrial species in the same genus inhabiting the paramos (e.g. frogs of the *P. devillei* and *P. myersi* groups). It seems thus that similar variation in anatomical structures that led taxonomists to place most wet puna and paramo species under a single taxon (the genus *Phrynopus*) is the result of multiple events of diversification and adaptation to the high Andean environments.

One more interesting recurrent pattern observed in many high Andean species of Holoadeninae is the bright coloration composed of flash marks in the underside of extremities, belly and throat, which stand in strong contrasts with the mimetic coloration of the dorsum. These strong yet hidden colours suggest a certain role in defensive and/or reproductive behaviour although their function remains unknown.

CONCLUSIONS

Despite recent efforts to accelerate exploration and species description, the diversity of high Andean frogs remains highly underestimated. As a result, we are still unable to properly understand processes that led to the colonization and diversification of species in these habitats of the high Andes. In our study, we have contributed to fill this gap and have outlined evolutionary scenarios supported by the necessarily incomplete patterns inferred so far. Our analyses support that the high humid grasslands of the Amazonian versant of the Andes in Peru and Bolivia harbour a large diversity of allopatric species with small altitudinal and horizontal distributions, and which replace each other along the north to south axis of the Andes. These species belong to different lineages whose closest relatives are forest species, often from distant parts of the continent. These patterns suggest that high Andean environments were most likely colonized by species derived from forest ancestors, and that colonization took place several times independently and resulted in species with remarkably similar ecomorphologies. Diversification was thus probably driven by intense vicariance during Andean orogeny while selective pressures decisively directed morphological variation. These hypotheses deduced from patterns revealed by our analyses should constitute the subject of future research. Morphological, physiological, ecological, behavioural and molecular changes involved in adaptation, as well as the tempo and degree of determinism and randomness in the processes of transformation also constitute interesting subjects for future research, and may provide a new and general hindsight into the origin of biological diversity.

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APPENDIX 1

SPECIMENS EXAMINED

Bryophryne

Bryophryne bakersfield: PERU: Department Cusco: Roquerío de Lorohuachana, 3620 m a.s.l. (12°29′ 43.8″S, 72°04′ 35.9″W), MUBI 7972 (holotype), MUBI 7973, MUSA 2362, 2360–1, 2363–2365 (all paratypes); 2.7 km NW of Roquerío de Lorohuachana, 3560 m a.s.l. (12°29′ 11.55″S, 72°06′ 03.45″W), MUBI 5993,

5996–5997, 6000–1 (all paratypes); Tambo Inca, 3651 m a.s.l. (12°29′ 09.74″S, 72°04′ 04.66″W), MUBI 6006–8, 6010, 6012–14, 6022–6023, MNCN 43707; Tres Claveles, 8.7 km SW, Roquerío de Lorohuachana, 3393 m a.s.l. (12°32′ 43.9″S, 72°08′ 22.7″W), MUBI 7974–5, MUSA 2357 (all paratypes); surroundings of Yanacocha Lakes, 4.3 km SW of Roquerío de Lorohuachana, 3506 m a.s.l. (12°31′36.8″S, 72°05′59.5″W), MUSA 2358, 2359 (paratypes); Cajoniyoc Pass surroundings, 3.0 km SW of Roquerío de Lorohuachana, 3604 m a.s.l. (12°28′8.10″S, 72°04′12.77″W), MUSA 2367–2368 (paratypes); Cabecera Timpia, 3579 m a.s.l. (12°29′ 11.55″S, 72°06′ 03.45″W), MUBI 5999, 6002.

Bryophryne bustamantei: PERU: Department Cusco: Carrizales, MUBI 800, 811–14 (referred specimens); Canchayoc (13°07′16.2″S, 72°19′53.0″W, 3663 m a.s.l.), near Abra de Málaga, MUBI 6018 (holotype); Canchayoc (13°06'49.7″S, 72°21'17.8″W, 3555 m a.s.l.), MUBI 6015 (paratype), MUBI 796–8, 837, 931–36, 938 (referred specimens); near Canchayoc (13°06′49.7″S, 72°21′17.8″W, 3555 m a.s.l.), MUBI 6016 (paratype); near Canchayoc (13°07′20.9″S, 72°19′28.0″W, 3741 m a.s.l.), MUBI 6017 (paratype); near Canchayoc (13°06′56.4″S, 72°20′29.0″W, 3621 m a.s.l.) MUBI 6019 (paratype); Cochayoc, MUBI 861, 921–26 (referred specimens).

Bryophryne cophites: PERU: Department Cusco: Southern slope of Abra Acjanacu, 14 km NNE Paucartambo, 3400 m, KU 138884, (holotype).

Microkayla

Microkayla adenopleura: BOLIVIA: Department Cochabamba: 10 km al N de la población de Monte Punko (Parque Nacional Carrasco), department de Cochabamba, Bolivia (17°33′S, 65°17′W), 3350 m a.s.l., MHNC-B 1-ABC (holotype).

Microkayla ankohuma: BOLIVIA: Department La Paz: Cooco (15°47′14.4″S, 68°26′55.9″W, 3540 m a.s.l.), CBF 5982–83, MNCN 43229–30, 43232–36, MNK A7279, MNK A7280 (holotype), A7281–87; Ankho Uma (Ancoma) (15°44′33.0″S, 68°29′22.8″W, 3690 m a.s.l.), CBF 5984, MNCN 43228, MNK A7277–78 (paratypes).

Microkayla boettgeri: PERU: Department Puno: Quebrada Cullochoci, 1.6 km from Phara and 9.5 km from Limbani, along the road (14°9′45″S, 69°39′45″W 3592 m a.s.l.), MUBI 5363–5, MNCN 43776, 43778 (type locality).

Microkayla chacaltaya: BOLIVIA: La Paz: Sanja Pampa (16°15′13.8″S, 68°01′41.8″W, 3900 m a.s.l.), CBF 4161 (holotype), CBF 4160, CBF 4162–72 (paratypes); Zongo valley, 1.7 km above Central Botijlaca (16°11′47″S, 68°07′35″W, 3600 m a.s.l.), CBF 5985–87, MNCN 42050–52, 42053, MNK A7265–67 (paratypes).

Microkayla condoriri: BOLIVIA: Department La Paz: Amaguaya (15°57′24.7″S, 68°15′32.0″W, 3760 m a.s.l.), CBF 5988 (holotype), 5989–90, MNCN 43237–38 (paratypes).

Microkayla guillei: BOLIVIA: Department de La Paz: *c.* 4 km E of Chullina (15°10′17 ″S, 68°53′10″W, 3590 m a.s.l.), AMNH 165108 (holotype), AMNH 165109, CBF 5861 (paratypes).

Microkayla harveyi: BOLIVIA: Department de Cochabamba: 2.5 km N of Jatum Incacasani Bolivia (16°44′11″S, 66°28′39″W, 3600 m a.s.l.), MHNC-B A989 (holotype), MNCN 42029, MHNC-B A990–91 (paratypes).

Microkayla iani: BOLIVIA: Department La Paz: Tucurmani Molino and Achapampa (15°36′S, 68°39′W), CBF 2145 (holotype), CBF 2129–44 (paratypes).

Microkayla iatamasi: BOLIVIA: Department Cochabamba: 7 km de la carretera antigua al Chapare, Zona de Aguirre, límite NO del Parque Nacional Carrasco (17°12′S, 65°42′W), MHNC-B 1-ACX (holotype).

Microkayla illampu: BOLIVIA: Department La Paz: 18.5 km from Sorata on Sorata-Mapiri road (15°44′07″S, 68°38′11″W, 3840 m a.s.l.), CBF 5991 (holotype), CBF 5992–99, MNCN 42021–28, MNK A7274–76 (paratypes).

Microkayla illimani: BOLIVIA: Department La Paz: Río Caballuni, 5 km from Totoral on the road to Cooperativa 15 de Agosto (16°36′48″S, 67°44′50″W, 3594 m a.s.l.), CBF 6000 (holotype), CBF 6001, MNCN 42070–71 (paratypes).

Microkayla kallawaya: BOLIVIA: Department La Paz: Caalaya (15°06′31″S, 69°01′47″W, *c.* 3600 m a.s.l.), CBF 6005 (holotype), CBF 6002–04, CBF 6006–07, MNCN 42056–61 (paratypes).

Microkayla katantika: BOLIVIA: Department La Paz: Pelechuco (14°49′S, 69°05′W, 3600 m a.s.l.), CBF 6008 (holotype), CBF 6009–15, MNCN 42 062–69 (paratypes).

Microkayla kempffi: BOLIVIA: Department Santa Cruz: 30 km by road from Comarapa towards

Cochabamba, Serranía La Siberia (17° 50′S, 64° 45′W, 2600 m a.s.l.), EBD 2884 (holotype).

Microkayla pinguis: BOLIVIA: Department La Paz: Choquetanga Chico, CBF 1906 (holotype).

Microkayla quimsacruzis: BOLIVIA: Department La Paz: between Choquetanga and Mina Caracoles (16°52′39″S, 67°18′23″, 3660 m a.s.l.), CBF 6016 (holotype), CBF 6021–23, MNCN 42 034–36 (paratyes).

Microkayla teqta: BOLIVIA: Department La Paz: Pablo Amaya (15°58'52.55″S, 68°12′19.6″W, 3700 m a.s.l.), CBF 6725 (holotype), CBF 6726–9, MNCN 45 702–6 (paratypes).

Microkayla saltator: BOLIVIA: Department La Paz: *c.* 15 km (by road) from Charazani on the road to Apolo (15°10′45″S, 68°53′29″W, 2550 m a.s.l.), CBF 6031 (holotype), CBF 6032–38, MNCN 42044–49, 43231, 44208 (paratypes).

Psychrophrynella

Psychrophrynella bagrecito: PERU: Department Cusco: Río Marcapata below Marcapata, *c.* 2740 m a.s.l., KU 196512 (holotype), KU 196513–18, 196520–21, 196523–25 (paratypes); Quebrada de Iskaybamba, Marcapata (13°30′15.22″S, 70°54′58.84″W, 2244 m a.s.l.), MUBI 5255–56.

Psychrophrynella usurpator: PERU: Department Cusco: northern slope of Abra Acjanacu, 29 km NNE Paucartambo (13°12′S, 71°37′W, 3400 m a.s.l.), KU 138939 (holotype), KU 138937–8, 138940–9, 138951–62 (paratypes); Pantillacocha, Kosñipata valley, Province Paucartambo (13°12′S, 71°33′W, 3539 m a.s.l.), MUBI 4642–3 (paratypes).

Noblella

Noblella carrascoicola: BOLIVIA: Department Cochabamba: Sehuencas (*c.* 65°16′W, 17°30′S, 2150–2230 m a.s.l.), ZFMK 59573 (holotype).

Noblella peruviana: PERU: Department Puno: Inca Mine, near Santo Domingo de Carabaya, AMNH 14526 (holotype).

Noblella heyeri: PERU: Department Piura: 33 km SW Huancabamba, 3100 m a.s.l., KU 196529 (holotype).

Noblella lochites: ECUADOR: Province Morona-Santiago: Río Piuntza, on the northern end of the cordillera del Cóndor (approximately 3°15′S, 78°20′W, 1550 m a.s.l.), KU 147070 (holotype).

Noblella lynchi: PERU: Department Amazonas: east slope of Abra Chanchillo, 42 km (by road) ENE of Balsas (06°49′S, 77°54′W, 2870 m a.s.l.), KU 212318 (holotype).

APPENDIX 2. Sequence primers used in this study

Locus	Primer name and priming region (5′-3′)
16S	16Sar: CGCCTGTTTATCAAAAACAT
	16Sbr: CCGGTCTGAACTCAGATCACGT
16S	16L19: AATACCTAACGAACTTAGCGATAGCTGGTT
	16H24: TACCTTCGCACGGTTAGKRTACCGCGGCCGTT
16S	16S-JMP-F: CATGGTAAGTRTACCGGAAGGTG
	16S-JMP-R: ACCAGCTATDACTAAGTTCG
12S-tRNA$_{phe}$	12S-t-Phe-frog: ATAGCRCTGAARAYGCTRAGATG
	12S-frogRa: TCRATTRYAGGACAGGCTCCTCTAG
12S-tRNA$_{val}$	12S-t-Val-frog: TGTAAGCGARAGGCTTTKGTTAAGCT
	12S-frogFa: CAAACTRGGATTAGATACCCYACTATG
c-myc	cmyc1U: GAGGACATCTGGAARAARTT
	cmyc3L: GTCTTCCTCTTGTCRTTCTCYTC
COI	AnF1: ACHAAYCAYAAAGAYATYGG
	AnR1: CCRAARAATCARAADARRTGTTG
POMC	POMC-1: AATGTATYAAAGMMTGCAAGATGGWCCT
	POMC-2: TAYTGRCCCTTYTTGTGGGCRTT
RAG1	R182: GCCATAACTGCTGGAGCATYAT
	R270: AGYAGATGTTGCCTGGGTCTTC
TYR	Tyr1C: GGCAGAGGAWCRTGCCAAGATGT
	Tyr1G: TGCTGGGCRTCTCTCCARTCCCA

APPENDIX 3. Voucher numbers, locality data and GenBank accession numbers for specimens sequenced in this study

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
Bryophryne							
B. bakersfield	Peru: Cusco: La Convención: Tambo Inca	12.48604	72.06796	3651	20992	*MUBI 6022*	MF186284, MF186341, MF186445, MF186452, MF186525, MF186528
B. bakersfield	Peru: Cusco: La Convención: Tambo Inca	12.48604	72.06796	3651	20993	*MUBI 6023*	MF186285, MF186342, MF186529
B. bakersfield	Peru: Cusco: La Convención: Tambo Inca	12.48604	72.06796	3651	21319	*MUBI 6007*	MF186286, MF186343
B. bakersfield	Peru: Cusco: La Convención: Cabecera Timpia	12.48654	72.10096	3579	21320	*MUBI 6009*	MF186287, MF186344, MF186527
B. bakersfield	Peru: Cusco: La Convención: Cabecera Timpia	12.48654	72.10096	3579	21322	*MUBI 5999*	MF186295, MF186355, MF186543, MF186581
B. bustamantei	Peru: Cusco: La Convención: Valle de Umasbamba, Abra de Málaga	13.11381	72.35494	3555	21338	*MUBI 6016*	MF186295, MF186355, MF186543, MF186581
B. bustamantei	Peru: Cusco: La Convención: near Canchayoc, Valle de Umasbamba, Abra de Málaga	13.11567	72.34139	3621	21340	*MUBI 6019*	MF186296, MF186356, MF186524, MF186544, MF186548
B. tocra sp. nov.	Peru: Puno: Carabaya: Entre Ollachea y desvío a Corani	13.88856	70.51028	3859	20646	*MNCN 44214*	MF186395
B. quellokunka sp. nov.	Peru: Cusco: Ouispicanchis: Qorpinte, a 2 km de Tambopampa hacia Marcapata, Valle del río Palquilla	13.60522	71.13494	3964	5489	*MNCN 43780*	MF186309, MF186387, MF186526
B. quellokunka sp. nov.	Peru: Cusco: Ouispicanchis: Qorpinte, a 2 km de Tambopampa hacia Marcapata, Valle del río Palquilla	13.60522	71.13494	3964	5490	*MUBI 5374*	MF186310, MF186388, MF186447
B. quellokunka sp. nov.	Peru: Cusco: Ouispicanchis: Qorpinte, a 2 km de Tambopampa hacia Marcapata, Valle del río Palquilla	13.60522	71.13494	3964	5491	*MUBI 5375*	MF186311, MF186389, MF186479
B. quellokunka sp. nov.	Peru: Cusco: Ouispicanchis: Qorpinte, a 2 km de Tambopampa hacia Marcapata, Valle del río Palquilla	13.60522	71.13494	3964	5495	*MNCN 43782*	MF186390, MF186446, MF186523

APPENDIX 3. *Continued*

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
B. quellokunka sp. nov.	Peru: Cusco: Ouispicanchis: Qorpinte, a 2 km de Tambopampa hacia Marcapata, Valle del río Palquilla	13.60522	71.13494	3964	5496	*MNCN 43783*	MF186391
B. quellokunka sp. nov.	Peru: Cusco: Ouispicanchis: Qorpinte, a 2 km de Tambopampa hacia Marcapata, Valle del río Palquilla	13.60522	71.13494	3964	5498	*MNCN-DNA 5498*	MF186392
B. tocra sp. nov.	Peru: Puno: Carabaya: Entre Ollachea y desvío a Corani	13.88856	70.51028	3839	5564	MNCN 43786	MF186314, MF186396, MF186443, MF186522
B. tocra sp. nov.	Peru: Puno: Carabaya: Entre Ollachea y desvío a Corani	13.88856	70.51028	3839	5565	MUBI 5418	MF186397, MF186444, MF186521
B. tocra sp. nov.	Peru: Puno: Carabaya: Entre Ollachea y desvío a Corani	13.88856	70.51028	3839	5566	MNCN 43786	MF186315, MF186541, MF186583
B. tocra sp. nov.	Peru: Puno: Carabaya: Entre Ollachea y desvío a Corani	13.88856	70.51028	3839	5567	MUBI 5419	MF186316, MF186398
B. wilakunka sp. nov.	Peru: Puno: Carabaya: Valle de Ayapata	13.85294	70.31450	3947	5575	MUBI 5425	MF186291, MF186349, MF186435, MF186520
Microkayla							
M. adenopleura	Bolivia: Cochabamba: Carrasco: Aproximadamente 1.500 m al este de Jatun Pino, entre Sehuencas y Monte Punco	17.56917	65.28000	3330	34799	MNCN 44809	MF186339, MF186460, MF186487
M. adenopleura	Bolivia: Cochabamba: Carrasco: Aproximadamente 1.500 m al este de Jatun Pino, entre Sehuencas y Monte Punco	17.56917	65.28000	3331	34800	MNCN 44810	MF186283, MF186340, MF186488, MF186537, MF186565
M. ankohuma	Bolivia: La Paz: Larecaja: Cooco	15.78733	68.44886	3558	6217	MNKA 7280	MF186288, MF186346, MF186509, MF186560
M. ankohuma	Bolivia: La Paz: Larecaja: Cooco	15.78733	68.44886	3558	6223	CBF 5982	MF186289, MF186347
M. boettgeri	Peru: Puno: Sandia: A 1.6 km de Pahra y 9.5 km de Limbani por carretera, quebrada Cullocochi	14.16247	69.66250	3592	5470	*MNCN 43776*	MF186351, MF186483

APPENDIX 3. *Continued*

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
M. boettgeri	Peru: Puno: Sandia: A 1.6 km de Pahra y 9.5 km de Limbani por carretera, quebrada Cullocochi	14.16247	69.66250	3592	5472	*MNCN 43778*	MF186293, MF186352, MF186456, MF186470
M. boettgeri	Peru: Puno: Sandia: A 1.6 km de Pahra y 9.5 km de Limbani por carretera, quebrada Cullocochi	14.16247	69.66250	3592	5473	*MUBI 5363*	MF186294, MF186353, MF186559
M. boettgeri	Peru: Puno: Sandia: A 1.6 km de Pahra y 9.5 km de Limbani por carretera, Quebrada Cullocochi	14.16247	69.66250	3592	5474	*MUBI 5364*	MF186354, MF186484
M. cf. *iatamasi*	Bolivia: Cochabamba: Chapare: Old road from Cochabamba to Villa Tunari	17.18162	65.66604	3206	20927	*MNCN-DNA 20927*	MF186365
M. cf. *iatamasi*	Bolivia: Cochabamba: Chapare: Old road from Cochabamba to Villa Tunari	17.18162	65.66604	3206	20929	*MNCN-DNA 20929*	MF186367
M. chacaltaya	Bolivia: La Paz: Murillo: Valle de Zongo	16.19639	68.12639	3900	6254	*MNCN 42052*	MF186357, MF186532
M. chapi sp. nov.	Peru: Puno: Sandia: A 3.7 km de Sina, Valle del Río Hirigache	14.50269	69.26231	3504	5183	MNCN 43762	MF186328, MF186417, MF186481, MF186540, MF186562
M. chapi sp. nov.	Peru: Puno: Sandia: A 3.7 km de Sina, Valle del Río Hirigache	14.50269	69.26231	3504	5185	MNCN 43763	MF186418, MF186490
M. chapi sp. nov.	Peru: Puno: Sandia: A 3.7 km de Sina, Valle del Río Hirigache	14.50269	69.26231	3504	5187	MNCN 43764	MF186419
M. chapi sp. nov.	Peru: Puno: Sandia: A 3.7 km de Sina, Valle del Río Hirigache	14.50269	69.26231	3504	5188	*MNCN 43765*	MF186420
M. chapi sp. nov.	Peru: Puno: Sandia: A 3.7 km de Sina, Valle del Río Hirigache	14.50269	69.26231	3504	5191	*MNCN 43766*	MF186421
M. chapi sp. nov.	Peru: Puno: Sandia: A 3.7 km de Sina, Valle del Río Hirigache	14.50269	69.26231	3504	5192	*MNCN 43767*	MF186422
M. chilina sp. nov.	Peru: Puno: Sandia: Confluencia de quebradas Sayaco y Huacuyo	14.44506	69.56986	3792	5441	*MUBI 5350*	MF186411, MF186493
M. chilina sp. nov.	Peru: Puno: Sandia: Confluencia de quebradas Sayaco y Huacuyo	14.44506	69.56986	3792	5442	*MNCN 43770*	MF186412, MF186496
M. chilina sp. nov.	Peru: Puno: Sandia: Confluencia de quebradas Sayaco y Huacuyo	14.44506	69.56986	3792	5443	*MNCN 43771*	MF186413

APPENDIX 3. *Continued*

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
M. chilina sp. nov.	Peru: Puno: Sandia: Confluencia de quebradas Sayaco y Huacuyo	14.44506	69.56986	3792	5447	*MNCN 43772*	MF186327, MF186414, MF186457, MF186494, MF186539, MF186561
M. chilina sp. nov.	Peru: Puno: Sandia: Confluencia de quebradas Sayaco y Huacuyo	14.44506	69.56986	3792	5449	*MNCN 43773*	MF186415, MF186458, MF186495
M. chilina sp. nov.	Peru: Puno: Sandia: Confluencia de quebradas Sayaco y Huacuyo	14.44506	69.56986	3792	5450	*MNCN 43774*	MF186416, MF186459
M. condoriri	Bolivia: La Paz: Larecaja: Amaguaya	15.95686	68.25889	3760	5591	*MNCN 43237*	MF186297
M. condoriri	Bolivia: La Paz: Larecaja: Amaguaya	15.95686	68.25889	3760	5592	*CBF 5988*	MF186298, MF186358, MF186439, MF186480
M. condoriri	Bolivia: La Paz: Larecaja: Amaguaya	15.95686	68.25889	3760	5593	*MNCN 43238*	MF186299, MF186359
M. condoriri	Bolivia: La Paz: Larecaja: Amaguaya	15.95686	68.25889	3760	5594	*CBF 5989*	MF186300, MF186360, MF186530, MF186550
M. condoriri	Bolivia: La Paz: Larecaja: Amaguaya	15.95686	68.25889	3760	5595	*CBF 5990*	MF186361
M. iatamasi	Bolivia: Cochabamba: Chapare: Old road from Cochabamba to Villa Tunari	17.29691	65.74333	4192	567	*MNCN 42054*	MF186304, MF186316, MF186368, MF186398, MF186461, MF186478, MF186536, MF186558
M. iatamasi	Bolivia: Cochabamba: Chapare: Represa Corani	17.26667	65.90000	3257	6319	*MNCN-DNA 6319*	MF186301, MF186362, MF186511
M. iatamasi	Bolivia: Cochabamba: Chapare: Old road from Cochabamba to Villa Tunari, represa T7	17.25163	65.78755	3060	20928	*MNCN-DNA 20928*	MF186366
M. illampu	Bolivia: La Paz: Larecaja: A 18,5 km de Sorata por carretera en dirección a Mapiri	15.73528	68.63639	3787	558	*CBF 5998*	MF186305, MF186369, MF186534, MF186549
M. illampu	Bolivia: La Paz: Larecaja: A 20 km de Sorata en dirección a Mapiri	15.73528	68.63639	3787	6230	*MNKA 7275*	MF186370, MF186510
M. illampu	Bolivia: La Paz: Larecaja: A 18 km de Sorata hacia Mapiri	15.73528	68.63639	3787	8993	*MNCN 42028*	MF186371

APPENDIX 3. *Continued*

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
M. illampu	Bolivia: La Paz: Larecaja: A 18 km de Sorata hacia Mapiri	15.73528	68.63639	3787	8994	*MNCN 42027*	MF186372
M. illampu	Bolivia: La Paz: Larecaja: A 18 km de Sorata hacia Mapiri	15.73528	68.63639	3787	8995	*CBF 5999*	MF186373, MF186512
M. illimani	Bolivia: La Paz: Sud Yungas: Río Caballuni, a 5 km de Totoral hacia la Cooperativa 15 de Agosto	16.61333	67.74722	3600	2428	*MNCN 42071*	MF186374, MF186482
M. illimani	Bolivia: La Paz: Sud Yungas: Río Caballuni, a 5 km de Totoral hacia la Cooperativa 15 de Agosto	16.61333	67.74722	3600	2429	*CBF 6000*	MF186375
M. kallawaya	Bolivia: La Paz: Saavedra: Arroyo a 900 m. de Caalaya	15.10861	69.02972	3600	575	*MNCN 42061*	MF186291, MF186306, MF186349, MF186376, MF186575, MF186575
M. kallawaya	Bolivia: La Paz: Saavedra: Arroyo a 900 m. de Caalaya	15.10861	69.02972	3600	576	*MNCN 42057*	MF186377, MF186437, MF186576
M. kallawaya	Bolivia: La Paz: Saavedra: Arroyo a 900 m. de Caalaya	15.10861	69.02972	3600	577	*MNCN 42058*	MF186378, MF186438, MF186577
M. kallawaya	Bolivia: La Paz: Saavedra: Arroyo a 900 m. de Caalaya	15.10861	69.02972	3600	578	*MNCN 42509*	MF186379, MF186507
M. katantika	Bolivia: La Paz: Franz Tamayo: Pelechuco	14.81667	69.08333	3600	583	*CBF 6012*	MF186380, MF186440, MF186453, MF186492
M. katantika	Bolivia: La Paz: Franz Tamayo: Pelechuco	14.81667	69.08333	3600	584	*CBF 6013*	MF186307, MF186381, MF186442, MF186455, MF186533, MF186576
M. katantika	Bolivia: La Paz: Franz Tamayo: Pelechuco	14.81667	69.08333	3600	585	*CBF 6014*	MF186382
M. katantika	Bolivia: La Paz: Franz Tamayo: Pelechuco	14.81667	69.08333	3600	586	*CBF 6015*	MF186383, MF186441, MF186454, MF186491
M. kempffi	Bolivia: Santa Cruz: Serranía de la Siberia	17.83689	64.71244	3800	6323	*MNCN 43646*	MF186308, MF186384, MF186504, MF186538, MF186566

APPENDIX 3. *Continued*

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
M. kempffi	Bolivia: Santa Cruz: Serranía de la Siberia	17.83689	64.71244	3800	6324	*MNKA 7727*	MF186385
M. kempffi	Bolivia: Santa Cruz: Serranía de la Siberia	17.83689	64.71244	3800	6325	MNCN 43645	MF186386, MF186486
M. quimsacruzis	Bolivia: La Paz: Inquisivi: Entre Choquetanga y Caracoles	16.87317	67.30383	3660	568	MNCN 42063	MF186323, MF186505
M. quimsacruzis	Bolivia: La Paz: Inquisivi: Entre Choquetanga y Caracoles	16.87317	67.30383	3660	2328	*MNCN 42038*	MF186405
M. quimsacruzis	Bolivia: La Paz: Inquisivi: Entre Choquetanga y Caracoles	16.87317	67.30383	3660	2379	*MNCN 42037*	MF186406
M. quimsacruzis	Bolivia: La Paz: Inquisivi: Entre Choquetanga y Caracoles	16.87317	67.30383	3660	2381	*MNCN 42039*	MF186407, MF186485
M. saltator	Bolivia: La Paz: Saavedra: Carretera 15 km antes de Charazani desde Apolo	15.17417	68.88817	2550	550	*CBF 6033*	MF186326, MF186410, MF186508
M. sp. Khatu River	Bolivia: La Paz: Inquisivi: Cruce de carreteras a Quime y Choquetanga	17.00033	67.27483	3730	570	*MNCN 42036*	MF186324, MF186408, MF186489
M. sp. Khatu River	Bolivia: La Paz: Inquisivi: Cruce de carreteras a Quime y Choquetanga	17.00033	67.27483	3730	571	*MNCN 42034*	MF186325, MF186409, MF186506, MF186535, MF186563
M. sp. Coscapa	Bolivia: La Paz: Murillo: Carretera a Coscapa	16.11939	68.13333	3550	59247	*CBF 6564*	MF186317, MF186399, MF186476
M. teqta	Bolivia: La Paz: Larecaja: Pablo Amaya	15.98125	68.20544	3700	59248	*MNCN 45702*	MF186318, MF186400, MF186471, MF186552
M. teqta	Bolivia: La Paz: Larecaja: Pablo Amaya	15.98125	68.20544	3700	59249	*MNCN 45703*	MF186319, MF186401, MF186472, MF186557
M. teqta	Bolivia: La Paz: Larecaja: Pablo Amaya	15.98125	68.20544	3700	59250	*CBF 6726*	MF186320, MF186402, MF186473, MF186553
M. teqta	Bolivia: La Paz: Larecaja: Pablo Amaya	15.98125	68.20544	3700	59251	*MNCN 45704*	MF186321, MF186403, MF186475, MF186555
M. teqta	Bolivia: La Paz: Larecaja: Pablo Amaya	15.98125	68.20544	3700	59252	*CBF 6727*	MF186322, MF186404, MF186474, MF186554

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65254	MNCN 46979	MF186331, MF186425, MF186498, MF186567
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65255	MNCN 46980	MF186332, MF186426, MF186499, MF186568
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65256	MNCN 46981	MF186427, MF186564
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65257	MNCN 46982	MF186333, MF186428, MF186500, MF186569
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65258	MNCN 46983	MF186334, MF186429, MF186503, MF186570
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65259	MNCN 46984	MF186335, MF186430, MF186573
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65260	MNCN 46985	MF186336, MF186431, MF186497, MF186571
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65261	MNCN 46986	MF186432, MF186502, MF186574
M. utururo	Bolivia: Cochabamba: Carrasco: San José, Utururo Bajo	17.35833	65.60506	3800	65262	MNCN 46987	MF186337, MF186433, MF186501, MF186572
M. wettsteini	Bolivia: La Paz: Nor Yungas: A 1 km de Unduavi por carretera hacia Coroico	16.30589	67.89757	3380	559	*CBF 6241*	MF186338, MF186434, MF1864369, MF186477, MF186531, MF186551
Phrynopus							
P. auriculatus	Peru: Pasco: Oxapampa: Abra Esperanza	10.53186	75.34981	2790	20998	*MUBI 6471*	MF186290, MF186348, MF186466, MF186582
P. barthlenae	Peru: Huánuco: Ichoacan, near Laguna Gwengway	10.18467	76.09333	3680	4156	*MHNSM 20609*	MF186292, MF186350, MF186449, MF186464, MF186519

	Locality	Latitude (°S)	Longitude (°W)	m a.s.l.	MNCN DNA	Vouchers	GB
P. heimorum	Peru: Huánuco: *Polylepis* forest	9.99556	76.16111	3420	4158	*MTD* 45621*	MF186302, MF186363, MF186462, MF186515, MF186545, MF186580
P. horstpauli	Peru: Huánuco: Ichoacan, Juntaloma-Forest	10.16933	76.12000	3100	4154	*MTD 44335*	MF186303, MF186364, MF186450, MF186518, MF186584
P. horstpauli	Peru: Huánuco: Ichoacan, Juntaloma-Forest	10.16933	76.12000	3276	4155	*MHNSM 20600*	MF186451, MF186465, MF186517
P. miroslawae	Peru: Pasco: Oxapampa: Santa Bárbara	10.33717	75.64647	3363	20996	*MUBI 6469*	MF186312, MF186393, MF186463, MF186516, MF186542, MF186585
P. nicoleae	Peru: Pasco: Oxapampa: Santa Bárbara	10.34342	75.63831	3589	20994	*MUBI 6441*	MF186313, MF186394, MF186468, MF186513, MF186546, MF186577
P. tribulosus	Peru: Pasco: Oxapampa: Santa Bárbara	10.33997	75.63939	3466	20995	*MUBI 6451*	MF186329, MF186423, MF186448, MF186469, MF186514, MF186578
P. tribulosus	Peru: Pasco: Oxapampa: Refugio Cedro	10.54500	75.35800	2600	20997	*MUBI 7166*	MF186330, MF186424, MF186467, MF186547, MF186579

* Senckenberg Naturhistorische Sammlungen Dresden

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Accession numbers for legacy GenBank sequences used in this study.