A New Genus of Terrestrial-Breeding Frogs (Holoadeninae, Strabomantidae, Terrarana) from Southern Peru

Alessandro Catenazzi 1,*; Luis Mamani 2,3, Edgar Lehr 4 and Rudolf von May 5

1 Department of Biological Sciences, Florida International University, Miami, FL 33199, USA
2 Museo de Biodiversidad del Perú, Cusco 08000, Peru; luismamanic@gmail.com
3 Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco, Cusco 08000, Peru
4 Department of Biology, Illinois Wesleyan University, Bloomington, IL 61701, USA; elehr@iwu.edu
5 Biology Program, California State University Channel Islands, Camarillo, CA 93012, USA; rvonmay@gmail.com

* Correspondence: acatenazzi@gmail.com

http://zoobank.org/urn:lsid:zoobank.org:pub:0B8FFBEE-96AA-46E1-BA6F-541DC9FA73BF
Received: 7 April 2020; Accepted: 6 May 2020; Published: 8 May 2020

Abstract: We propose to erect a new genus of terrestrial-breeding frogs of the Terrarana clade to accommodate three species from the Province La Convención, Department of Cusco, Peru previously assigned to Bryophryne: B. flammiventris, B. gymnotis, and B. mancoincana. We examined types and specimens of most species, reviewed morphological and bioacoustic characteristics, and performed molecular analyses on the largest phylogeny of Bryophryne species to date. We performed phylogenetic analysis of a dataset of concatenated sequences from fragments of the 16S rRNA and 12S rRNA genes, the protein-coding gene cytochrome c oxidase subunit I (COI), the nuclear protein-coding gene recombination-activating protein 1 (RAG1), and the tyrosinase precursor (Tyr). The three species are immediately distinguishable from all other species of Bryophryne by the presence of a tympanic membrane and annulus, and by males having median subgular vocal sacs and emitting advertisement calls. Our molecular phylogeny confirms that the three species belong to a new, distinct clade, which we name Qosqophryne, and that they are reciprocally monophyletic with species of Microkayla. These two genera (Qosqophryne and Microkayla) are more closely related to species of Noblella and Psychrophrynella than to species of Bryophryne. Although there are no known morphological synapomorphies for either Microkayla or Qosqophryne, the high endemism of their species, and the disjoint geographic distribution of the two genera, with a gap region of ~310 km by airline where both genera are absent, provide further support for Qosqophryne having long diverged from Microkayla. The exploration of high elevation moss and leaf litter habitats in the tropical Andes will contribute to increase knowledge of the diversity and phylogenetic relationships within Terrarana.

Keywords: amphibian; Andes; Cusco; high elevation; Neotropical; Qosqophryne; tropical mountain; systematic; taxonomy

1. Introduction

Terrestrial-breeding frogs of the high Andes display an impressive degree of evolutionary convergence [1–4]. Such convergence is associated with life in the cloud forest and high-Andean grassland. Frogs in many genera of Terrarana have evolved strikingly similar body forms [4,5], typically a small, compact body with very short legs and feet, short arms and hands, loss of toe pads and discs, head wider than long, small eyes directed anterolaterally, and, in many groups, reduction or loss of tympanic structure and function [3]. The high similarity of body forms has delayed obtaining a
taxonomic arrangement that reflects the evolutionary history and phylogenetic relationships of most species of small, terrestrial-breeding frogs of the Andes [1,6,7].

Illustrating the complexity within Terrarana of identifying monophyletic groups in presence of ecological convergence, authors originally assigned frogs belonging to different evolutionary lineages to the genus Phrynopus [1,8,9]. Indeed, Phrynopus might still contain incorrectly classified species of Pristimantis that lack vocal sacs, external tympanic apparatus and toe pads [10]. Subsequent molecular analyses revealed a much greater diversity and deeper genetic structure, such that Hedges et al. [1] proposed to split Phrynopus into four genera, and to erect the new subfamily Holoadeninae to include the newly described genera Bryophryne, Niceforonia, and Psychrophrynella. Within Holoadeninae, the molecular phylogeny by Hedges et al. [1] recognized Bryophryne as a distinct clade on the basis of DNA sequences from a single species, B. cophites (formerly Phrynopus cophites Lynch, 1975). Hedges et al. [1] used morphological characters to assign to Bryophryne a second species, Phrynopus bustamantei Chaparro, De la Riva, Padial, Ochoa, and Lehr, 2007. The new genus Bryophryne, along with the other genera of Holoadeninae, was recognized using molecular data, despite the lack of morphological synapomorphies [1,2,5,11].

Since Hedges et al. [1] published their molecular phylogeny, researchers have continued discovering terrestrial-breeding frogs: the number of species of Bryophryne has increased from two to 14 species [12–17], and the number of species across all Holoadeninae genera from 36 to 151 species [8]. As far as we know, all species of Bryophryne have micro-endemic distribution, and are only known to occur at their respective type localities and immediate surroundings [2,12,14–16,18]. The most recent phylogeny included six of the 14 species of Bryophryne, and recovered Bryophryne as being the sister taxon to the clade containing Barycholos, “Eleutherodactylus bilineatus”, Euparkerella, Holoaden, and Noblella [2]. However, this phylogeny by De la Riva et al. [2] did not include sequences of the three species of Bryophryne having an external tympanum and males with subgular vocal sacs, because sequences were unavailable at the time. Additionally, De la Riva et al. [2] erected a new genus, Microkayla, to accommodate all species of Psychrophrynella from Bolivia (and one species of Psychrophrynella from Peru), as well as two new species from Peru. Because of these discoveries, the integration of molecular, acoustic and morphological approaches, and the ongoing revision of existing and new material, we have a better understanding of the diversity in this group of cryptic genera. As part of our ongoing work, we have become aware of (1) uncertainty regarding the evolutionary relationships of Noblella and Psychrophrynella [2,19,20], (2) an underestimated species richness and endemism in Noblella and Psychrophrynella [19–22], and (3) three species of Bryophryne (B. flammiventris, B. gymnitis, B. mancoinca; Figure 1) having traits not shared with any other species of Bryophryne, such as having an external tympanum and males with subgular vocal sacs and emitting advertisement calls. Here we address the latter of these findings, and propose a new genus for the only three species of Bryophryne known to produce vocalizations and possessing external tympanic membrane and annulus.
Figure 1. Holotypes of species of Qosqophryne gen. n. in dorsolateral and ventral views: (A, B) Q. flammiventris (MUSM 27613; SVL 19.8 mm); (C, D) Q. gymnotis (MUSM 25543; SVL 18.4 mm); (E, F) Q. mancoinca (MUBI 11152; SVL 26.5 mm). Photographs by E. Lehr (A, B), A. Catenazzi (C, D) and L. Mamani (E, F).

2. Materials and Methods

We are familiar with most described species of Bryophryne, which we have seen in the field or inspected in collections. We provide a complete list of examined specimens in Appendix A. We used the literature (i.e., original species descriptions) for species whose specimens we could not examine. We have described the advertisement calls of B. gymnotis and B. mancoinca [14,17], and have heard and provided a short description of the call of B. flammiventris [15]. We refer readers to the original publications for details on recording methods.

We combined DNA sequences available from GenBank with sequences from newly collected tissues to generate molecular phylogenies of Bryophryne and closely related Holoadeninae taxa (Table 1). We considered sequences for a fragment of the 16S rRNA gene (16S), a fragment of the 12S rRNA gene (12S), the protein-coding gene cytochrome c oxidase subunit I (COI), the nuclear protein-coding gene recombination-activating protein 1 (RAG1), and the tyrosinase precursor (Tyr). All taxa selected for our comparisons belong to the subfamily Holoadeninae [1,23,24].
| Taxon                          | 16S  | 12S  | COI   | RAG1  | Tyr   | Voucher Nbr | Reference            |
|-------------------------------|------|------|-------|-------|-------|-------------|----------------------|
| *Barycholos pulcher*          | EU186709 | - | - | - | EU186765 | KU 217781 | [1]                |
| *Barycholos ternestzi*        | JX267466 | - | - | JX267543 | JX267680 | CFBH 19426 | [23]               |
| *Bryophyne bakersfield*       | KT276291 | KT276283 | - | - | - | MHNC 6007 | [12]               |
| *Bryophyne bakersfield*       | MF186344 | MF186287 | - | KT276278 | - | MHNC 6009 | [12]               |
| *Bryophyne bustamantei*       | MT437052 | - | - | MT431911 | - | MUSM 24537 | This study          |
| *Bryophyne bustamantei*       | CMT437053 | - | - | MT431912 | - | MUSM 24538 | This study          |
| *Bryophyne cf. zonalis*       | MT437054 | - | MT435518 | - | - | CORBIDI 17475 | This study          |
| *Bryophyne cophites*          | EF493537 | - | - | EF493423 | EF493508 | KU173497 | [9]                |
| *Bryophyne hanssaureri*       | KY652641 | - | KY672976 | KY672961 | KY681062 | AC 270.07 | [22]               |
| *Bryophyne nubilosus*         | KY652641 | - | KY672977 | KY681084 | KY681063 | MUSM 27567 | [22]               |
| *Bryophyne phugahampetu*      | MF186349 | MF186291 | - | - | - | MUBI 5425 | [2]                |
| *Bryophyne phugahampetu*      | MF186349 | MF186291 | - | - | - | MUBI 5425 | [2]                |
| *Bryophyne zonalis*           | MT437064 | - | - | MT431917 | - | AC 41.09 | This study          |
| *Eleutherodactylus bilineatus*| JX267324 | - | - | JX267556 | JX267691 | MNRJ 46476 | [23]               |
| *Euparkerella brasiliensis*   | EF493366 | EF493378 | - | - | EF493449 | EU186779 | USNM 207945 | [9]                |
| *Holoaden bradei*             | EU186710 | EU186728 | - | EU186747 | EU186768 | MZUSP 131872 | [1]              |
| *Holoaden luederwaldti*       | JX267468 | - | - | JX267545 | JX267682 | - | [23]               |
| *Lynchius flavomaculatus*     | EU186667 | EU186667 | - | EU186745 | EU186766 | KU218210 | [1]                |
| *Lynchius nebulanastes*       | EU186704 | EU186704 | - | - | - | KU 181408 | [3]                |
| *Lynchius oblitus*            | KX470793 | KX470792 | KX470799 | KX470799 | MHNC 8614 | [25]               |
| *Lynchius parkeri*            | EU186705 | EU186705 | - | - | - | KU 181307 | [1]                |
| *Lynchius simmonsi*           | JF810004 | JF809940 | - | JF809915 | JF809894 | QZ 41639 | [26]               |
| *Microkayla adenopleura*      | MF186339 | - | - | - | - | MNCN 44809 | [2]                |
| *Microkayla adenopleura*      | MF186340 | MF186283 | - | MF186537 | MF186560 | MNCN 44810 | [2]                |
| *Microkayla ankohuma*          | MF186288 | - | - | - | - | MNKA 7280 | [2]                |
| *Microkayla cf.  iatamasi*    | MF186352 | MF186293 | MF186456 | - | - | MNCN 43787 | [2]               |
| *Microkayla boettgeri*        | MF186353 | MF186294 | - | - | MF186599 | MUBI 5363 | [2]                |
| *Microkayla boettgeri*        | MF186354 | - | - | - | - | MUBI 5364 | [2]                |
| *Microkayla chapi*            | MF186417 | MF186328 | - | MF186540 | MF186562 | MNCN 43762 | [2]                |
| *Microkayla chilina*          | MF186411 | - | - | - | - | MUBI 5350 | [2]                |
| *Microkayla chilina*          | MF186414 | MF186327 | MF186457 | MF186539 | MF186561 | MNCN 43772 | [2]               |
| *Microkayla condorirri*       | MF186358 | - | - | - | - | CBF 5988 | [2]                |
| *Microkayla guillei*          | AM039712 | AM039712 | - | AM039712 | MNCN 42052 | [2]               |
| *Microkayla kallama*          | MF186379 | - | - | - | - | CBF 5999 | [2]                |
| *Microkayla katantika*        | MF186380 | - | MF186453 | - | - | CBF 6012 | [2]                |
### Table 1.

| Taxon                     | 16S | 12S | COI    | RAG1 | Tyr | Voucher Nbr | Reference         |
|--------------------------|-----|-----|--------|------|-----|-------------|--------------------|
| Microkayla kempfi        | MF186384 | -   | -      | -    | -   | MNCN 43646 | [2]                |
| Microkayla quissocoacius | MF186407 | -   | -      | -    | -   | MNCN 42039 | [2]                |
| Microkayla saltator      | AM039642 | AM039710 | -    | -    | -   | MTD TD 1229 | [9]                |
| Microkayla sp. Coscapa   | MF186399 | -   | -      | -    | -   | CBF 6564  | [2]                |
| Microkayla sp. Khatu River | MF186409 | -   | -      | -    | -   | MNCN 42034 | [2]                |
| Microkayla tequila       | MF186400 | MF186318 | -    | MF186552 | MNCN 45702 | [2]                |
| Microkayla utararo       | MF186433 | -   | -      | -    | -   | MNCN 46987 | [2]                |
| Microkayla wettsteinii   | MF186434 | MF186338 | -    | MF186551 | CBF 6241 | [2]                |
| Niceforonia brunea       | EF493357 | -   | -      | -    | -   | KU 178258 | [9]                |
| Niceforonia dolops       | EF493394 | -   | -      | -    | -   | QCAZ 31471 | [23]               |
| Noblella heyeri          | JX267541 | JX267463 | -    | -    | -   | KU 173756 | [1]                |
| Noblella lochites        | EU186699 | EU186699 | -    | EU186777 | KU 177356 | [1]                |
| Noblella losamigos       | MN366392 | -   | MN356099 | -    | -   | MVZ 292687 | [27]               |
| Noblella losamigos       | KY652644 | -   | -      | KY672962 | KY681065 | MUSA 6973 | [22]               |
| Noblella losamigos       | MN150358 | -   | MN356098 | -    | -   | MUBI 17413 | [22]               |
| Noblella madreselva      | MN064565 | -   | MN355547 | -    | -   | CORBIDI 15769 | [22] |
| Noblella myrmecoides     | JX267542 | JX267464 | -    | -    | -   | QCAZ 40180 | [23]               |
| Noblella myrmecoides     | MN150357 | -   | -      | -    | -   | CORBIDI PV45 | [28] |
| Noblella pagmacea        | KY652645 | -   | KY672979 | KY681086 | KY681067 | MUSM 24536 | [22]               |
| Noblella sp.             | AM039646 | AM039714 | -    | -    | -   | MTD 45180 | [29]               |
| Noblella sp. R           | KY652646 | -   | KY672980 | KY681087 | KY681067 | MUSM 27582 | [22]               |
| Noblella thiani          | MK072732 | -   | -      | -    | -   | CORBIDI 18723 | [22] |
| Oreobates amarakaeri     | JF809996 | JF809924 | -    | JF809913 | JF809891 | MNCN 6975 | [26]               |
| Oreobates ayacucho       | JF809970 | JF809933 | -    | JF809912 | JF809890 | MNCN IDlR5024 | [26] |
| Oreobates cruralis       | EU186666 | EU186666 | -    | EU186743 | EU186764 | KU 215462 | [1]                |
| Oreobates gemcare        | JF809996 | JF809930 | -    | JF809909 | -    | MHCN 6687  | [26]               |
| Oreobates granulosus     | EU368907 | JF809929 | -    | JF809908 | JF809887 | MHCN 3396 | [30]               |
| Phrynopus auriculatus    | EF493708 | EF493708 | -    | -    | -   | KU 291634 | [9]                |
| Phrynopus barthlenae     | AM039653 | AM039721 | -    | -    | -   | SMF 81720 | [29]               |
| Phrynopus bracki         | EF493709 | EF493709 | -    | EF493421 | -    | USNM 286919 | [9]                |
| Phrynopus bufaloes       | AM039645 | AM039713 | -    | -    | -   | MNSM 19860 | [29]               |
| Phrynopus heimorum       | AM039635 | AM039703 | MF186462 | MF186545 | MF186580 | MTD 45621 | [29]               |
| Phrynopus horstpaulii    | AM039651 | AM039719 | -    | -    | -   | MTD 44333 | [29]               |
| Phrynopus inti           | MF651902 | MF651909 | -    | MF651917 | -    | MUSM 31968 | [3]                |
| Phrynopus kauneorum      | AM039665 | AM039723 | -    | -    | -   | MNSM 20595 | [29]               |
| Phrynopus peruanus       | MG896582 | MG896605 | MG896615 | MG896626 | MG896631 | MUSM 38316 | [3]                |
| Phrynopus pesantesi      | AM039656 | AM039724 | -    | -    | -   | MTD 45072 | [29]               |
| Phrynopus spH            | MG896589 | MG896606 | -    | MG896629 | -    | MNSM 33261 | [3]                |
| Phrynopus tautzorum      | AM039652 | AM039720 | -    | -    | -   | MNSM 20613 | [29]               |
| Phrynopus tribulosus     | EU186725 | EU186707 | -    | -    | -   | KU 291630 | [1]                |
| Pristimantis attenborough | KY954752 | -    | KY962779 | KY962759 | -    | MUSM 31186 | [10]               |
| Pristimantis plusculus   | KX155577 | -    | KY962769 | -    | -   | CORBIDI 11862 | [31] |
| Pristimantis reichlei    | EF493707 | EF493707 | -    | EF493436 | -    | MNSM 9267  | [9]                |
| Pristimantis stictogaster | EF493704 | EF493704 | -    | EF493445 | -    | KU 291659 | [9]                |
| Psychrophrynella chirihampatu | KU884559 | -   | -      | -    | -   | CORBIDI 16495 | [19] |
| Psychrophrynella chirihampatu | KU884560 | -   | -      | -    | -   | MHCN 14664 | [19]               |
| Psychrophrynella glauca  | MG837565 | -   | -      | -    | -   | CORBIDI 18729 | [20] |
| Psychrophrynella sp.     | MT437065 | -   | -      | -    | -   | MUSM 27619 | This study         |
| Psychrophrynella sp.     | MT437066 | -   | -      | -    | -   | MTD 47488 | This study         |
Table 1. Cont.

| Taxon                        | 16S    | 12S    | COI    | RAG1   | Tyr    | Voucher Nbr | Reference   |
|-----------------------------|--------|--------|--------|--------|--------|-------------|-------------|
| Psychrophrynella sp. P      | KY652660 | -      | KY672992 | KY681089 | KY681081 | AC116.09    | [22]        |
| Psychrophrynella sp. R      | KY652661 | -      | KY672993 | KY681090 | KY681082 | AC116.09    | [22]        |
| Psychrophrynella usurpator   | KY652662 | -      | KY672994 | KY672975  | KY681083 | AC186.09    | [22]        |
| Qosqophryne flammicentria   | MT437055 | -      | -      | -      | -      | MTD 46890   | This study  |
| Qosqophryne flammicentrina | MT437056 | -      | -      | MT431913 | -      | MUSM 27615  | This study  |
| Qosqophryne gymnotis        | MT437057 | -      | -      | MT431914 | -      | MUSM 24546  | This study  |
| Qosqophryne gymnota         | MT437058 | -      | -      | MT431915 | -      | MUSM 24543  | This study  |
| Qosqophryne mancoinca       | MT437059 | -      | MT435519 | -      | -      | MUBI 16068  | This study  |
| Qosqophryne mancoinca       | MT437060 | -      | MT435520 | -      | -      | MUBI 16069  | This study  |

2.1. Laboratory Work

We followed protocols of extraction, amplification, and sequencing of DNA previously used for terrestrial-breeding frogs [1,20,22]. For the focal taxa (the three species members of the new genus), we extracted DNA from tissue samples obtained from six specimens collected in the field (two specimens per species). We also obtained DNA sequences from seven specimens in five other species of Bryophrynina, and two specimens representing two species in other genera (Noblella and Psychrophrynella), and the remaining sequences are legacy data from GenBank.

We extracted DNA from liver tissue preserved in 70% ethanol by using a commercial extraction kit (IBI Scientific, Dubuque, IA, USA). We used selected primers (Table 2) to amplify DNA from each gene using the polymerase chain reaction (PCR) [22,32]. We obtained sequence data by running purified PCR products in an ABI 3730 Sequence Analyzer (Applied Biosystems), except sequences of B. mancoinca and B. phuyuhampatu, which we shipped to MCLAB (San Francisco, CA) for sequencing. We deposited all new sequences in GenBank (Table 1). We provide updated names of 86 terminals included in the analysis for 314 GenBank sequences.

Table 2. Primers used in this study.

| Locus   | Primer       | Sequence (5′-3′)                  | Reference |
|---------|--------------|----------------------------------|-----------|
| 16S     | 16SAR F      | CGGCTGTTTATCAAAAACAT             | [33]      |
|         | 16SBR R      | CCGGTCTGAAACTCAGATCAGT          | [33]      |
| 12S     | L25195 F     | AAACTGGGATTAGATACCCCACTA         | [33]      |
|         | H2916 R      | GAGGGTGACGGGCGGTGTGT            | [33]      |
| COI     | dgLCO1490 F  | GGTCAACAAATCATAAGAYATYGG         | [34]      |
|         | dgHCO2198 R  | TAAACTTCAGGTTGACGGGCGGTGT       | [34]      |
| RAG1    | R182 F       | GCCATAACTGCTGGAGCATYAT           | [9]       |
|         | R270 R       | AGYAGATGTGGCCTGGGCTT           | [9]       |
| Tyr     | Tyr1C F      | GGCAGAGGAWCRTGCCAAAGATGT        | [35]      |
|         | Tyr1G R      | TGCTGGGCRCTCTCCARTCCA          | [35]      |

2.2. Molecular Phylogenetic Analyses

We inferred the phylogenetic relationships among taxa through analysis of concatenated DNA sequences of the five gene fragments (16S, 12S, COI, RAG1, Tyr). We used Niceforonia dolops to root the tree. We aligned sequences with Geneious R6, v. 6.1.8 (Biomatters 2013), using the built-in Geneious Aligner program. We then used PartitionFinder, v. 1.1.1 [36] to select the best partitioning scheme and substitution model for each gene using the Bayesian information criterion (BIC). The best partitioning scheme included the following six subsets (best fitting substitution models are in parentheses): partition subset 1 includes 12S and 16S sequences (GTR + I + G), partition 2 is the first codon position of COI (SYM + G), partition 3 is the second codon position of COI (F81), partition 4 is the third codon position
of COI (HKY + G), partition 5 includes the first and second codon positions of RAG together with the first and second codon positions of Tyr (HKY + I + G), and partition 6 includes the third codon position of RAG together with the third codon position of Tyr (K80 + G).

We used MrBayes, v. 3.2.0 [37] to infer a molecular phylogeny for the 106 terminals and 2632 bp concatenated partitioned dataset (16S, 12S, COI, RAG1, Tyr). We performed an MCMC Bayesian analysis that included two simultaneous runs of 10 million generations, sampled once every 1000 generations. Each run had one “cold” chain and three heated chains, and the burn-in was set to discard 25% samples from the cold chain. Upon completion of the MCMC Bayesian analysis, the average standard deviation of split frequencies was 0.003916. We used Tracer version 1.5 [38] to examine the effective sample sizes (ESS), to verify convergence, and to verify that the runs reached stationarity. The observed effective sample sizes were satisfactory for all parameters (ESS > 200). Lastly, we used FigTree v. 1.4.2 [39] to visualize the majority-rule consensus tree and assess node support (based on posterior probability values).

Our research was approved by the Institutional Animal Care and Use Committee of Florida International University (18-009). The Dirección General Forestal y de Fauna Silvestre, Ministerio de Agricultura y Riego issued the permit authorizing this research (collecting permits #292-2014-MINAGRI-DGFFS-DGEFFS, SERNANP-Machu Picchu 054-2012-SERNANP-JEF, Contrato de Acceso Marco a Recursos Genéticos, No 359-2013-MINAGRI-DGFFS-DGEFFS).

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) and the associated information can be viewed through any standard web browser at http://zoobank.org/urn:lsid:zoobank.org:pub:0B8FFBEE-96AA-46E1-BA6F-541DC9FA73BF.

3. Results

We recovered a phylogenetic tree (Figure 2) that was largely congruent with previous analyses [2,24]. However, our tree recovered three species of *Bryophryne* not previously included in phylogenetic analyses (*B. gymnotis*, *B. flammiventris*, and *B. mancoincia*) as a clade that is sister to the clade containing all species of *Microkayla*. Thus, species of *Microkayla*, instead of other species of *Bryophryne*, share the most common shared ancestor with *B. gymnotis*, *B. flammiventris*, and *B. mancoincia*. The presence of large, external tympanic membrane and annulus, and males with a median subgular vocal sac and production of vocalizations, immediately distinguishes the newly recognized genus from all other species of *Bryophryne*. At least four species of *Bryophryne* were described as having small, barely visible (under the skin surface) tympanic membranes and annuli (*B. bustamantei*, *B. quellokunka*, *B. tocra*, *B. wilakunka*), but their external appearance does not look that different from the other species of *Bryophryne* known to lack a visible tympanic membrane [2,14,18]. One of these species, *B. bustamantei* was described as producing a short whistle, but there is no recording of the call nor voucher associated with a call [18]. The distribution range of *B. bustamantei* overlaps with that of *B. gymnotis* in the cloud forest near Abra Málaga [14,18,40], and thus it is possible that the call of *B. gymnotis* was erroneously associated with males of *B. bustamantei*. There also seems to be some problems identifying specimens of this species, as shown by our phylogeny where specimens identified as *B. bustamantei* by one of us do not group with sequences from one of the paratypes of *B. bustamantei* (MHNC 6019).
Figure 2. Bayesian maximum clade-credibility tree for 106 species of Holoadeninae (Terrarana) based on a 2646-bp concatenated partitioned dataset (fragments of genes 16S, 12S, COI, RAG1, and Tyr), highlighting the relationships of the three genera *Bryophryne*, *Microkayla* and *Qosqophryne* gen. n. Posterior probabilities are indicated at each node. The frog illustrated here is *Qosqophryne gymnotis*, paratype MUSM 24542 (photograph by A. Catenazzi).

We propose to erect the new genus *Qosqophryne* gen. n. to accommodate *Bryophryne gymnotis*, *B. flammiventris*, and *B. mancoinca*. Several lines of evidence support the idea that *Qosqophryne* is distinct from its sister genus *Microkayla*. The molecular phylogeny indicates there is a degree of divergence comparable to that observed between other genera of strabomantid frogs (Figure 2). Our molecular analyses show strong support for the divergence of *Microkayla* and *Qosqophryne* gen. n. The lack of geographic overlap between the two genera, with a gap region of ~320 km by airline where both genera are absent, further supports this divergence by preventing recent gene flow among species of both.
genera (Figure 3). Furthermore, several glaciated peaks, including the massive Ausangate mountains and associates peaks of the Cordillera de Vilcanota, are interspersed along this gap region of 320 km.

Figure 3. Type localities of frogs in the genera *Bryophryne* (white circles, species details not shown), *Microkayla* (squares) and *Qosqophryne* gen. n. (red asterisks) in southern Peru and northern Bolivia. The known distribution range of these frogs is limited to the type locality and immediate surroundings. For species of *Microkayla*: (1) *M. boettgeri*; (2) *M. chilina*; (3) *M. chapi*; (4) *M. katantika*; (5) *M. chaupi*; (6) *M. melanocheira*; (7) *M. colla*; (8) *M. kallawaya*; (9) *M. guillei*; (10) *M. saltator*; (11) *M. iani*; (12) *M. illampu*; (13) *M. ankohuma*; (14) *M. condoriri*; (15) *M. tegla*; (16) *M. huayna*; (17) *M. chacaltaya*; (18) *M. wettsteini*. The map does not include seven species of *Microkayla* distributed in central and southern Bolivia (type localities outside the limits of this map).

Similarly to recent phylogenies [28,41], we found that *Noblella* is not monophyletic: the species from southern Peru along with species of *Psychrophrynella* form a clade that is sister taxon to *Microkayla + Qosqophryne*, whereas the species of *Noblella* from northern Peru and Ecuador are closely related to "*Eleutherodactylus bilineatus*" and *Barycholos* (Figure 2). Because the type species *N. peruviana*
occurs in southern Peru, and the most similar species sequenced to date *N. thiuni* is part of the *Noblella*/Psychrophrynella clade [28], our findings support the hypothesis that *Noblella* occurs only in southern Peru and northern Bolivia, and that species from northern Peru and Ecuador belong to a different genus [28,41]. Furthermore, our tree suggests that species of *Noblella* and *Psychrophrynella* belong to the same lineage, as supported by the respective type genera sharing several morphological traits [2,5,20,28,42]. Therefore, the two possibilities are that some species of *Noblella* have been misidentified as *Psychrophrynella* (and vice versa), or that *Psychrophrynella* is a junior synonym of *Noblella*. We will not be able to resolve the taxonomic uncertainty associated with *Noblella* and *Psychrophrynella* until we obtain DNA sequences from the respective type species *N. peruviana* and *P. bagrecito* [2,19,20,28].

Finally, our inferred phylogeny suggests that there are at least seven additional putative new species of *Bryophryne*, *Noblella*, and *Psychrophrynella* (Figure 2), and confirms previous findings of cryptic species diversity particularly in leaf litter, cloud forest frogs in the *Noblella*/Psychrophrynella clade [22]. These putative new species, similarly to most known species of high-elevation Holoadeninae [4], are highly endemic and known from single localities (or, around those localities, from within a narrow elevational range in the same valley, [22]). Of special interest among the putative new species, *Psychrophrynella* MUSM 27619 is the first specimen of the *Noblella*/Psychrophrynella lineage known from the Vilcabamba range.

**Taxonomy**

*Qosqophryne* new genus

http://zoobank.org/urn:lsid:zoobank.org:act:7DDB98AD-CCF9-4977-B814-285D25B3D1BF

**Type species.** *Bryophryne gymnnotis* Lehr and Catenazzi, 2009

**Included species.** *Qosqophryne flammiventris* (Lehr and Catenazzi, 2010), comb. nov.; *Q. mancoinca* (Mamani, Catenazzi, Ttito, Mallqui, Chaparro, 2017), comb. nov.

**Diagnosis.** (1) Head wider than long, narrower than body, body robust, extremities short; (2) tympanic membrane and annulus present; (3) cranial crests absent; (4) prevomerine teeth and dentigerous process of vomers present (but absent in *Q. flammiventris*); (5) trips of digits narrow, rounded, circumferential grooves absent, terminal phalanges T-shaped to knobbed; (6) Finger I shorter than Finger II, nuptial pads absent; (7) Toe V shorter than Toe III; (8) fingers and toes with lateral fringes (but absent in *Q. flammiventris*); (9) subarticular tubercles small, rounded; (10) dorsolateral folds short, discontinuous or continuous; (11) discoidal fold absent (present in *Q. mancoinca*); (12) trigeminal nerve passing external to *m. adductor mandibulae externus* (‘S’ condition; Lynch, 1986); (13) snout-vent length from 16.7–19.3 mm in males and 16.0–22.2 mm in females of *Q. gymnnotis*, to 19.6–22.9 mm in males and 23.6–26.5 mm in females of *Q. mancoinca*; (14) males with median subgular vocal sac and vocal slits, nuptial pads absent; (15) advertisement call whistle-like, composed of a single, tonal note in *Q. gymnnotis*, 2–3 short notes in *Q. mancoinca*, and 3–4 short notes in *Q. flammiventris*.

There are no known morphological synapomorphies for *Qosqophryne*, but the three known species share the following traits (Table 3): (1) males with median subgular vocal sac produce whistle-like tonal calls composed of 1–4 short notes; (2) tongue ovate; (3) skin on venter smooth to weakly areolate (in *Q. flammiventris*); (4) inner tarsal fold absent. Four other genera of Holoadeninae occur south of the Apurimac canyon, a proposed biogeographic barrier for high-elevation terrestrial breeding frogs [13–15]. *Bryophryne* differs from *Qosqophryne* in lacking an externally visible tympanum, and having males without vocal sac and not emitting vocalizations [2,12,16]. *Oreobates* have head about the same width as body, smooth venter, subarticular and supernumerary tubercles large, conical or subconical, projecting, and range in snout-vent length from 20–63 mm [1,5]. *Noblella* and *Psychrophrynella* have smooth venter, elongated tongue, two prominent metatarsal tubercles, and in most species facial masks and/or a tarsal fold-like, sigmoid tubercle [2,19,20,28]. *Qosqophryne* is most similar to its sister genus *Microkayla*. Putative synapomorphies of *Microkayla* are a rounded tongue, areolate belly, and absence of prominent metatarsal tubercles [2]. It is presumed that all species of *Microkayla* vocalize, and known calls consist of a simple, short whistle-like tonal note [2,4]. *Qosqophryne* differs from...
most Microkayla in having (except for Q. flammiventris) fingers and toes with lateral fringes (absent in Microkayla), and having (except Q. flammiventris) dentigerous processes of vomers (absent in Microkayla). Future examination of osteological characters, for example through computed tomography, might help identify such characters, and resolve the condition of the tympanic apparatus in the three genera Bryophryne, Microkayla and Qosqophryne.

Table 3. Meristic traits (+ = character present, - = character absent) for the three known species of Qosqophryne gen. n.

| Characters                  | Q. gymnotis                        | Q. flammiventris                   | Q. mancoinca                      |
|-----------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Skin on dorsum              | shagreen                          | Shagreen with small scattered tubercles | Shagreen with small conical tubercles |
| Skin on venter              | smooth                            | Weakly areolate                    | smooth                            |
| Dorsolateral folds          | Discontinuous, short              | Discontinuous, short              | Continuous, short                 |
| Tympatic membrane           | +                                 | +                                 | +                                 |
| Tympatic annulus            | +                                 | +                                 | +                                 |
| Dentigerous processes of vomers | +                               | -                                 | +                                 |
| Vocal sac                   | +                                 | +                                 | +                                 |
| Vocal slits                 | +                                 | +                                 | +                                 |
| Nuptial pads                | -                                 | -                                 | -                                 |
| Fingers with lateral fringes| +                                 | -                                 | +                                 |
| Toes with lateral fringes   | +                                 | -                                 | +                                 |
| Inner tarsal fold           | -                                 | -                                 | -                                 |
| Dorsum coloration           | Reddish, grayish or purplish brown or dark gray with narrow tan middorsal stripe | Grayish brown                      | Reddish brown or grayish brown with narrow tan middorsal stripe |
| Venter coloration           | Dark brown, tan, or reddish brown with pale gray flecks | Blackish brown with yellow, orange or pink blotches | Gray or pale bluish gray with reddish-brown reticulation |

Etymology. The name refers to the city of Cusco, using the spelling Qosqo which more closely reflects the name in Quechua. Qosqo is used in apposition with phryne, from the greek for “frog”. Thus, the name for the new genus alludes to the geographic distribution of the three known species in the Peruvian Department of Cusco.

Distribution, natural history, and conservation. The three species of Qosqophryne occur within a region of ~150 km² in the upper montane forests and grasslands of the Cordilleras de Urubamba and Cordillera de Vilcabamba, Provincia La Convención, Department Cusco, Peru. These frogs inhabit cloud forests, elfin forests, montane scrub and humid grasslands (puna) from 3270 to 3800 m a.s.l. Similar to other regions in the high Andes, these habitats and their amphibian communities are threatened by pasture burning, climate change and associated expansion of agricultural activities, deforestation, and the fungal disease chytridiomycosis [43,44]. Although chytridiomycosis has caused the collapse of montane frog communities at several sites in Departamento Cusco [45,46], terrestrial-breeding frogs have generally declined the least, and several species challenged in experimental infection trials appears to resist or tolerate infection [47]. Protection of natural habitats will benefit conservation of these frogs. Two of the three species occur within naturally protected areas: Q. gymnotis within the Área de Conservación Privada Abra Málaña, and Q. mancoinca within Machu Picchu Historic Sanctuary.

Remarks. The new genus is distinguished from all species of Bryophryne by the presence of tympanum and tympanic annulus, and median subgular vocal sacs in males. Furthermore, males of all three species of Qosqophryne are known to emit advertisement calls (unknown in all species of Bryophryne, except possibly for B. bustamantei). We have described the advertisement calls of Q. gymnotis and Q. mancoinca [14,17]. One of us (LM) has recorded the advertisement call of a male Q. flammiventris (MUBI 13365) at the type locality, and this call is composed of 3–4 short notes (~15–35 ms duration) at dominant frequency ~3000 Hz. Females of Q. gymnotis attend clutches of 14–16 eggs [39], but unattended clutches of up to 19 eggs have also been found [14].
The new genus *Qosqophryne* is supported by our molecular phylogeny, the most complete to date covering three mitochondrial and two nuclear gene fragments, as well as most described species of *Bryophryne* and *Microkayla*. Despite the absence of known synapomorphies for the sister clades *Microkayla* and *Qosqophryne*, we are confident that our proposed arrangement reflects the evolutionary history of these organisms, and yet still takes into consideration taxonomic stability [48]. There is strong support (bootstrap probabilities) at the node where *Microkayla* and *Qosqophryne* diverge, and the relative branch lengths leading to their respective living species is similar, or in some cases exceed the branch lengths separating other genera within Terrarana (e.g., *Euparkerella* and *Holoaden*, or *Barycholos* and the “northern clade” of *Noblella*).

4. Discussion

Our study integrating molecular, acoustic and morphological information justifies the erection of the new genus of strabomantid frog *Qosqophryne*. The molecular phylogeny we inferred, the most complete phylogeny to date in terms of terminal sampling for genera of Holoadeninae [2,24], provides strong support for this new genus forming a sister clade to *Microkayla*. Furthermore, our phylogeny confirms taxonomic uncertainty regarding the genera *Noblella* and *Psychrophrynella* [2,19,20], suggests the presence of several undescribed species of *Noblella* and *Psychrophrynella*, and generalizes the idea of high species endemism in high elevation Andean strabomantids [2,4,19–22,49].

Morphological synapomorphies for the new genus *Qosqophryne* have not been recognized, and there does not appear to be a unique combination of meristic traits to distinguish all species of *Microkayla* from species of *Qosqophryne*. However, there are some characteristics that help distinguish the two genera. Some of the traits present in *Qosqophryne* but absent in *Microkayla* are fingers and toes with lateral fringes, venter smooth (areolate in *Microkayla*), and presence of dentigerous processes of vomers (but absent in *Q. flammiventris*). The structure of the advertisement call, when known, appears to be similar in both genera, i.e., a whistle-like call, but composed of a single note in *Microkayla* vs. 2–4 notes in *Qosqophryne* (except for *Q. gymnotis*). There is limited information on parental care, but it appears that females attend clutches in *Q. gymnotis* [39], whereas males attend clutches in *M. illimani* and *M. teqta* [50,51]. Similarly to *Qosqophryne*, females attend clutches in *B. cophites* [52], *B. hanssaueri* and *B. nubilosus* (Catenazzi, pers. obs.). However, we lack natural history information from most species of strabomantid frogs, and thus any generalization on parental care is premature.

In support of our proposed new genus, there is a wide gap, both in terms of airline distance and the highly dissected topography, in the distribution range of species of *Microkayla* and *Qosqophryne*. These are all highly endemic, terrestrial-breeding frogs most likely characterized by extreme low vagility, as suggested by their patchy distribution in cloud forests and grasslands. All species of *Microkayla* occur from extreme southern Peru (Department Puno) to the western limits of department Santa Cruz in central Bolivia (Serranía Siberia), whereas the three species of *Qosqophryne* occur in the Vilcabamba mountain range in the Peruvian Department of Cusco. The gap of 320 km by airline between the southernmost locality of *Qosqophryne* (*Q. gymnotis*; –13.07558, –72.38201) and the northernmost locality of *Microkayla* (*M. boettgeri*) overlaps with the distribution range of *Bryophryne*. At the northern limit, *B. abramalagae* and *B. bustamantei* are marginally sympatric with *Q. gymnotis*, whereas at the southern limit, *B. wilakunka* (Ayapata, Puno, –13.85294, –70.31450) occurs ~80 km NW of the type locality of *M. boettgeri* (Phara, Puno, –14.16247, –69.66250). Although many species in these genera of Holoadeninae are likely “micro-endemic”, researchers have seldom invested much effort in documenting the distribution ranges of most species, and it is possible that some of these species occur more widely than presently known. Therefore, currently five genera of Holoadeninae occur in the tropical Andes south of the Apurimac canyon in Cusco, Puno and northern Bolivia: *Bryophryne*, *Psychrophrynella* and *Qosqophryne* in the Vilcabamba mountain range; *Bryophryne*, *Noblella* and *Psychrophrynella* in the Vilcanota range; *Bryophryne*, *Microkayla*, *Noblella* and *Psychrophrynella* in the Carabaya range, and *Microkayla* south of the Apolobamba range.
Author Contributions: Conceptualization, A.C., L.M., E.L. and R.v.M.; methodology, A.C., L.M., E.L. and R.v.M.; software, A.C. and R.v.M.; validation, A.C., L.M., E.L. and R.v.M.; formal analysis, A.C. and R.v.M.; investigation, A.C., L.M., E.L. and R.v.M.; data curation, A.C., R.v.M.; writing—original draft preparation, A.C.; writing—review and editing, A.C., L.M., E.L. and R.v.M.; visualization, A.C., L.M., E.L. and R.v.M.; supervision, A.C.; project administration, A.C.; funding acquisition, A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Amazon Conservation Association, the Rufford Foundation, the Chicago Board of Trade Endangered Species Fund, and the Amphibian Specialist Group.

Acknowledgments: We thank the staff of the Museo de Biodiversidad del Perú (MUBI) for access to the herpetological collection.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Specimens Examined

**Bryophryne abramalagae**: PERU: CUSCO: Provincia La Convención: Distrito de Huayopata, Abra de Málaga (13°07'23.8'' S, 72°20'51.2'' W), 4000 m a.s.l., MUSM 27630–32, MTD 47489–91.

**Bryophryne bakersfield**: PERU: CUSCO: Provincia La Convención: Distrito de Echarate, Roquequiró de Lorohuachana, 3620 m a.s.l. (12°29'43.8'' S, 72°04'35.9'' W), MHNC 7972.

**Bryophryne bustamantei**: PERU: CUSCO: Provincia La Convención: Abra de Málaga: MUSM 24537–38.

**Bryophryne cophites**: PERU: CUSCO: Provincia de Paucartambo: Distrito Kosñipata: S slope Abra Acchicancha, 14 km NNE Paucartambo, 3400 m a.s.l.: KU 138884 (holotype); N slope Abra Acchicancha, 27 km NNE Paucartambo, 3450 m a.s.l.: KU 138885–908, 138911–5 (all paratypes); 2 km NE of Abra Acchicancha, 3280 m a.s.l.: MHNG 2698.24, 5.5 km N of Abra Acanacu [Acjanaco], 3523 m: MUSM 27895, Tres Cruces, 8.5 km N of Abra Acchicancha, 3590 m a.s.l.: MUSM 20855–56, 26283–84, 26264, 26266–67, 26313, 26315, 27896, 30414–17, Pilippo Grande, 3865 m a.s.l., near border of Manu NP: CORBIDI 11919.

**Bryophryne flaviventris**: PERU: CUSCO: Provincia La Convención: Distrito de Vilcabamba and Pampaconas, 3800 m a.s.l.: MUSM 27613 (holotype), MUSM 27612, 27614–15, MTD 46890–92 (paratypes).

**Bryophryne gymnotis**: PERU: CUSCO: Provincia de La Convención, Distrito de Huayopata: 1 km east of San Luis, 3272–3354 m a.s.l.: MUSM 24543 (holotype), MHNG 2710.28, 2710.29, MTD 46860–64, 47288, 47291–92, 47297, MUSM 24541–42, 24544–45, 24546–56, MVZ 258407–10 (paratypes).

**Bryophryne hansi**: PERU: CUSCO: Provincia de Paucartambo, Distrito de Kosñipata: Acjanaco, Manu National Park, 3266 m a.s.l.: MUSM 27567 (holotype); from near Acjanaco, Manu National Park, 3280–3430 m a.s.l.: MHNG 2698.25, MTD 46865–66, 46887–89, MUSM 24557, 27568–69, 27607–11, MVZ 258411–13 (all paratypes).

**Bryophryne mancoinca**: PERU: CUSCO: Provincia de La Convención, Hornopampa sector, near Salkantay Mountain, along the road to the Archeological Complex of Choquequirao, 3707 m a.s.l.: MUBI 11152 (holotype), MUBI 11147–11151, 11153, 11154, 11159, 16068, 16069, 16074, 16083 (paratypes).

**Bryophryne nubilosus**: PERU: CUSCO: Provincia de Paucartambo: 500 m NE of Esperanza, 2710 m a.s.l.: MUSM 26310 (holotype), MUSM 26311; near the type locality, 13°11'33.21" S, 71°12'25.17" W, 3065 m: MTD 47294; near Hito Pillahuata, 2600 m: MUSM 20970; Quebrada Toqoruyoc, 3097 m a.s.l.: MUSM 26312, MTD 47293; Esperanza, 2800 m: MHNSM 26316–17; 13°11'20.2" S, 71°35'07.3" W, 2900 m a.s.l.: MUSM 24539–40.

**Bryophryne phuyuhampatu**: PERU: CUSCO: Provincia de Paucartambo: Distrito de Paucartambo, Quispilloma valley, Área de Conservación Privada (ACP) Umkumari Llaqtata, 2795–2850 m a.s.l., 13°22'12.14" S, 71°6'49.82" W (WGS84; type locality), CORBIDI 18224–18226, MUBI 14654 and 14655.

**Bryophryne quel loyalanka**: PERU: CUSCO: Provincia de Quispicanchis: Distrito de Marcapata: Coline, 3672 m a.s.l.: MUSM 27571, 27573.
Bryophryne zonalis: PERU: CUSCO: Provincia de Quispicanchis, Distrito de Marcapata, Kusillochayoc at 3129 m a.s.l.: MUSM 27570 (holotype), MTD 46867, 46869–70, MUSM 27572, 27574–75, 27861, MVZ 258414 (paratypes); at Puente Coline, 3285 m a.s.l.: MVZ 258415 (paratype).

Microkayla boettgeri: PERU: PUNO: Provincia de Sandia, Distrito de Limbani, Phara, 3466 m a.s.l.: MHNSM 19966 (holotype), MHNSM 19967–76, MTD 46508–9, 46512–19 (paratypes).

Microkayla chapi: PERU: PUNO: Provincia de Sandia, Distrito de Limbani, 3.7 km from Sina, Hirigache River valley, 3466 m a.s.l.: MUBI 5326 (holotype), MUBI 5325, 5327, 5330, 5331, 5328, 5329 (paratypes).

Microkayla chilina: PERU: PUNO: Provincia de Sandia, Distrito de Limbani, 3.7 km from Sina, Hirigache River valley, 3466 m a.s.l.: MUBI 5355 (holotype), MUBI 5350, 5351, 5353, 5354 (paratypes).

Qosqophryne flammiventris: PERU: CUSCO: Provincia de La Convención, Distrito de Vilcabamba, road between Vilcabamba and Pampaconas, 3800 m a.s.l., MUBI 13365.

Qosqophryne gymnotis: PERU: CUSCO: Provincia de La Convención, Distrito de Huayopata: San Luis, MUBI 14315–14319.

References
1. Hedges, S.B.; Duellman, W.E.; Heinicke, M.P. New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. Zootaxa 2008, 1737, 1–182. [CrossRef]
2. De La Riva, I.; Chaparro, J.C.; Castroviejo-Fisher, S.; Padial, J.M. Underestimated anuran radiations in the high Andes: Five new species and a new genus of Holoadeninae, and their phylogenetic relationships (Anura: Craugastoridae). Zool. J. Linn. Soc. 2018, 182, 129–172. [CrossRef]
3. von May, R.; Lehr, E.; Rabosky, D.L. Evolutionary radiation of earless frogs in the Andes: Molecular phylogenetics and habitat shifts in high-elevation terrestrial breeding frogs. PeerJ 2018, 6, e4313. [CrossRef] [PubMed]
4. De La Riva, I. Unexpected Beta-Diversity Radiations in Highland Clades of Andean Terraranae. In Neotropical Diversification: Patterns and Processes; Rull, V., Carnaval, A., Eds.; Springer: Cham, Switzerland, 2020; p. 382.
5. Heinicke, M.P.; Duellman, W.E.; Trueb, L.; Means, D.B.; MacCulloch, R.D.; Hedges, S.B. A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. Zootaxa 2009, 2211, 1–35. [CrossRef]
6. Heinicke, M.P.; Lemmon, A.R.; Lemmon, E.M.; McGrath, K.; Hedges, S.B. Phylogenomic support for evolutionary relationships of New World direct-developing frogs (Anura: Terraranae). Mol. Phylogenet. Evol. 2017. [CrossRef]
7. Frost, D.R. Amphibian Species of the World: An Online Reference. Version 6.0. Available online: http://research.amnh.org/herpetology/amphibia/index.html (accessed on 23 October 2019).
8. Heinicke, M.P.; Duellman, W.E.; Hedges, S.B. Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. Proc. Natl. Acad. Sci. USA 2007, 104, 10092–10097. [CrossRef] [PubMed]
9. Lehr, E.; von May, R. A new species of terrestrial-breeding frog (Amphibia, Craugastoridae, Pristimantis) from high elevations of the Pui Pui Protected Forest in central Peru. ZooKeys 2017, 660, 17–42. [CrossRef]
10. Condori Carhuarupay, F.P. Filogenia Morfológica del Género Bryophryne Hedges, 2008 (Anura: Craugastoridae); Universidad Nacional San Antonio Abad del Cusco: Cusco, Peru, 2018.
11. Chaparro, J.C.; Padial, J.M.; Gutierrez, R.C.; De la Riva, I. A new species of Andean frog of the genus Bryophryne from southern Peru (Anura: Craugastoridae) and its phylogenetic position, with notes on the diversity of the genus. Zootaxa 2015, 3994, 94–108. [CrossRef]
12. Lehr, E.; Catenazzi, A. A new species of Bryophryne (Anura: Strabomantidae) from southern Peru. Zootaxa 2008, 1784, 1–10. [CrossRef]
13. Lehr, E.; Catenazzi, A. Three new species of Bryophryne (Anura: Strabomantidae) from the Region of Cusco, Peru. S. Am. J. Herpetol. 2009, 4, 125–138. [CrossRef]
14. Lehr, E.; Catenazzi, A. Two new species of Bryophryne (Anura: Strabomantidae) from high elevations in southern Peru (Region of Cusco). Herpetologica 2010, 66, 308–319. [CrossRef]
16. Catenazzi, A.; Titto, A.; Diaz, M.I.; Shepack, A. *Bryophrynella phygalampatu* sp. n., a new species of Cusco Andes frog from the cloud forest of the eastern slopes of the Peruvian Andes (Amphibia, Anura, Craugastoridae). *Zookeys* 2017, 685, 65–81. [CrossRef] [PubMed]

17. Mamani, L.; Catenazzi, A.; Titto, A.; Mallqui, S.; Chaparro, J.C. A new species of *Bryophrynella* (Anura: Strabomantidae) from the Cordillera de Vilcabamba, southeastern Peruvian Andes. *Phylomedusa* 2017, 16, 129–141. [CrossRef]

18. Chaparro, J.C.; De la Riva, I.; Paladí, J.M.; Ochoa, J.A.; Lehr, E. A new species of *Phrynopus* from Departamento Cusco, southern Peru (Anura: Brachycephalidae). *Zootaxa* 2007, 1618, 61–68. [CrossRef]

19. Catenazzi, A.; Titto, A. A new species of *Psychrophrynella* (Amphibia, Anura, Craugastoridae) from the humid montane forests of Cusco, eastern slopes of the Peruvian Andes. *PeerJ* 2016, 4, e1807. [CrossRef]

20. Catenazzi, A.; Titto, A. *Psychrophrynella glauca* sp. n., a new species of terrestrial-breeding frogs (Amphibia, Anura, Strabomantidae) from the montane forests of the Amazonian Andes of Puno, Peru. *PeerJ* 2018, 6, e444. [CrossRef]

21. Catenazzi, A.; Usacpi, V.; von May, R. A new species of *Noblella* from the humid montane forests of Cusco, Peru. *Zookeys* 2015, 516, 71–84. [CrossRef]

22. von May, R.; Catenazzi, A.; Corl, A.; Santa-Cruz, R.; Carnaval, A.C.; Moritz, C. Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecol. Evol.* 2017, 7, 3257–3267. [CrossRef]

23. Canedo, C.; Haddad, C.F. Phylogenetic relationships within anuran clade Terrarana, with emphasis on the placement of Brazilian Atlantic rainforest frogs genus *Ischnocnema* (Anura: Brachycephalidae). *Mol. Phylogenet. Evol.* 2012, 65, 610–620. [CrossRef]

24. Padial, J.M.; Grant, T.; Frost, D.R. Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa* 2014, 3825, 1–132. [CrossRef]

25. Motta, A.P.; Chaparro, J.C.; Pomíal, J.P.; Guayasamin, J.M.; De la Riva, I.; Paladí, J.M. Molecular phylogenetics and taxonomy of the Andean genus *Lynchihis* Hedges, Duellman, and Heinicz 2008 (Anura: Craugastoridae). *Herpetol. Monogr.* 2016, 30, 119–142. [CrossRef]

26. Padial, J.M.; Chaparro, J.C.; Castroviejo-Fisher, S.; Guayasamin, J.M.; Lehr, E.; Delgado, A.J.; Vaira, M.; Teixeira, M., Jr.; Aguay, R.; de la Riva, I. A revision of species diversity in the Neotropical genus *Oreobates* (Anura: Strabomantidae), with the description of three new species from the Amazonian slopes of the Andes. *Am. Mus. Novit.* 2012, 3752, 1–55. [CrossRef]

27. Santa-Cruz, R.; von May, R.; Catenazzi, A.; Whitcher, C.; Tejeda, E.L.; Rabosky, D.L. A new species of terrestrial-breeding frog (Amphibia, Strabomantidae, Noblella) from the upper Madre de Dios watershed, Amazonian Andes and lowlands of southern Peru. *Diversity* (Basel) 2019, 11, 145. [CrossRef]

28. Lehr, E.; Frittsch, G.; Müller, A. Analysis of Andes frogs (*Phrynopus*, Leptodactyliidae, Anura) phylogeny based on 12S and 16S mitochondrial rDNA sequences. *Zool. Scr.* 2005, 34, 593–603. [CrossRef]

29. Padial, J.M.; Chaparro, J.C.; De la Riva, I. Systematics of *Oreobates* and the *Eleutherodactylus discoidalis* species group (Amphibia, Anura), based on two mitochondrial DNA genes and external morphology. *Zool. J. Linn. Soc.* 2008, 152, 737–773. [CrossRef]

30. Shepack, A.; von May, R.; Titto, A.; Catenazzi, A. A new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from the foothills of the Andes in Manu National Park, southeastern Peru. *Zookeys* 2016, 574, 143–164.

31. von May, R.; Rabosky, D.L.; Lehr, E. Earless frogs in the Andes: Extraordinary ecological divergence and morphological diversity. *Nat. Hist.* 2018, 126, 12–15.

32. Palumbi, S.R.; Martin, A.; Romano, S.; McMillan, W.O.; Stice, L.; Grabowski, G. *The Simple Fool’s Guide to PCR (Version 2.0)*; Privately published; Palumbi, S., Ed.; University of Hawaii: Honolulu, HI, USA, 1991.

33. Meyer, C.P. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biol. J. Linn. Soc.* 2003, 79, 401–459. [CrossRef]

34. Bossuyt, F.; Milinkovitch, M.C. Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proc. Natl. Acad. Sci. USA* 2000, 97, 6585–6590. [CrossRef]

35. Lanfear, R.; Calcott, B.; Ho, S.; Guindon, S. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 2012, 29, 1695–1701. [CrossRef]

36. Ronquist, F.; Huelsenbeck, J. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003, 19, 1572–1574. [CrossRef] [PubMed]
37. Rambaut, A.; Drummond, A. Tracer. Version 1.5. 2007. Available online: http://tree.bio.ed.ac.uk/software/tracer (accessed on 30 October 2019).
38. Rambaut, A. FigTree. Version 1.4.2. 2009. Available online: http://tree.bio.ed.ac.uk/software/figtree (accessed on 30 October 2019).
39. Mamani, L.; Diaz, M.I.; Titto, J.W.; Condori, F.P.; Titto, A. Parental care and altitudinal range extension of the endemic frog Bryophryne gymnotis (Anura: Craugastoridae) in the Andes of southeastern Peru. Phyllomedusa 2017, 16, 109–112. [CrossRef]
40. Catenazzi, A.; Titto, A. Noblella thiuni sp. n. a new (singleton) species of minute terrestrial-breeding frog (Amphibia, Anura, Strabomantidae) from the montane forest of the Amazonian Andes of Puno, Peru. PeerJ 2019, 7, e6780. [CrossRef] [PubMed]
41. Reyes-Puig, J.P.; Reyes-Puig, C.; Ron, S.; Ortega, J.A.; Guayasamin, J.M.; Recalde, F.; Vieira, J.J.; Koch, C.; Yanez-Munoz, M.H. A new species of terrestrial frog of the genus Noblella Barbour, 1930 (Amphibia: Strabomantidae) from the Llanganates-Sangay Ecological Corridor, Tungurahua, Ecuador. PeerJ 2019, 7, e7405. [CrossRef]
42. De la Riva, I.; Chaparro, J.C.; Padial, J.M. The taxonomic status of Phyllonastes Heyer and Phrynopus peruvianus (Noble) (Lissamphibia, Anura): Resurrection of Noblella Barbour. Zootaxa 2008, 1685, 67–68. [CrossRef]
43. Catenazzi, A.; von May, R. Conservation status of amphibians in Peru. Herpetol. Monogr. 2014, 28, 1–23. [CrossRef]
44. De La Riva, I.; Reichle, S. Diversity and conservation of the amphibians of Bolivia. Herpetol. Monogr. 2014, 28, 46–65. [CrossRef]
45. Catenazzi, A.; Lehr, E.; Rodriguez, L.O.; Vredenburg, V.T. Batrachochytrium dendrobatidis and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. Conserv. Biol. 2011, 25, 382–391. [CrossRef]
46. Catenazzi, A.; Lehr, E.; Vredenburg, V.T. Thermal physiology, disease and amphibian declines in the eastern slopes of the Andes. Conserv. Biol. 2014, 28, 509–517. [CrossRef]
47. Catenazzi, A.; Finkle, J.; Foreyt, E.; Wyman, L.; Swei, A.; Vredenburg, V.T. Epizootic to enzootic transition of a fungal disease in tropical Andean frogs: Are surviving species still susceptible? PLoS ONE 2017, 12, e0186478. [CrossRef]
48. ICZN. International Code of Zoological Nomenclature, 4th ed.; International Trust for Zoological Nomenclature: London, UK, 1999.
49. De la Riva, I. Bolivian frogs of the genus Phrynopus, with the description of twelve new species (Anura: Brachycephalidae). Herpetol. Monogr. 2007, 21, 241–277. [CrossRef]
50. Willaert, B.; Reichle, S.; Stegen, G.; Martel, A.; Barrón, S.; Sánchez de Lozada, N.; Greenhawk, N.; Agostini, G.; Muñoz, A. Distribution, ecology, and conservation of the critically endangered frog Psychrophrynella illimani (Anura: Craugastoridae) with description of its call. Salamandra 2016, 52, 317–327.
51. De la Riva, I.; Burrowes, P.A. A new species of Psychrophrynella (Anura: Craugastoridae) from the Cordillera Real, Department La Paz, Bolivia. Zootaxa 2014, 3887, 459–470. [CrossRef] [PubMed]
52. Catenazzi, A. Phrynopus cophites. Reproduction. Herpetol. Rev. 2006, 37, 206.