Abstract

- Opening sentence is awkward; please consider rephrasing for clarity and conciseness.
- The list of diagnostic features is somewhat long—streamline to highlight the most distinctive traits clearly.
- Grammar and punctuation need some polishing for smoother reading (e.g., “amphibian genus *Osornophryne* is” instead of “are”).
- The overall flow would benefit from tightening to improve readability and impact.

Introduction

- The background on *Osornophryne* is informative, but the section would benefit from clearer structure and transitions between ideas.
- Several sentences are awkwardly phrased or grammatically incorrect (e.g., subject-verb agreement, prepositions).
- Avoid first-person language (e.g., “help us to expand”) to maintain a formal scientific tone.
- Citations are sometimes redundant (e.g., Frost 2024 appears multiple times).
- Consider ending the introduction with a clearer, more focused statement of the study’s objectives.

Materials and Methods:

DNA extraction, amplification, and sequencing

- It is unclear whether the mitochondrial 16S rRNA gene is the only gene sequenced in this study. Please clarify this explicitly.
- You mention a concatenated matrix in the phylogenetic analysis, which usually implies multiple gene regions concatenated. If this is not the case, please clarify whether the concatenation refers to multiple samples or genes.
- The use of Oxford Nanopore sequencing is described, but it is not clear whether this method was applied solely to the 16S gene region or to additional regions or whole genomes. Please specify the target sequences for Nanopore sequencing.
- There is a methodological difference between the two laboratories: IKIAM performed Sanger sequencing (via Macrogen) while INABIO used Nanopore sequencing. This discrepancy might indicate different sequencing targets or approaches, but this is not explicitly addressed. Please clarify the rationale and specify whether the same gene regions were targeted in both methods.

Results:

Phylogenetic Analysis

- The type of phylogenetic inference should be explicitly stated. Although IQ-TREE is generally used for Maximum Likelihood (ML) analyses, the manuscript does not clearly mention that the approach is ML-based. Please specify this explicitly.
- The model of sequence evolution applied in the analysis (e.g., GTR+G, HKY, etc.) is not indicated. Please provide the model used and, if applicable, describe how it was selected.
- The relationship between the genetic distance calculations and the phylogenetic inference is not fully explained. Are the percent identity and genetic distances used solely for descriptive purposes, or do they inform the tree-building process? Please clarify.

Species Accounts

- Consider briefly noting whether any previously misidentified or potentially synonymous names exist, especially if specimens have been historically confused with other taxa.
- Please be consistent with the use of ";" vs. "," while listing the characters.
- Please standardize your telegraphic writing style for this whole section.

Osteological characteristics of the skull

- Consider restructuring the osteological description to emphasize diagnostic differences in direct comparison with closely related species, particularly other *Osornophryne*. While the current text provides detailed morphological information, highlighting how the new species differs from congeners (e.g., in the shape of the sphenethmoid, squamosal projections, or parasphenoid proportions) would greatly enhance the diagnostic utility of this section. A comparative format — either integrated into the description or summarized in a diagnostic table — would strengthen the argument for species delimitation based on osteological traits.

Discussion and Conclusion

- There is frequent repetition (e.g., ecotype and Toe V traits), which could be streamlined for clarity.
- Some key terms (e.g., "homoplasious condition") would benefit from clearer context or explanation.

- The final osteological comparisons are interesting but would benefit from a more cohesive synthesis rather than a list of traits.
- Grammatical issues and awkward sentence constructions appear throughout; please edit for clarity and flow.
- Consider summarizing the broader implications of the findings more clearly (e.g., evolutionary significance, conservation relevance).
- The conclusion largely restates findings already detailed in the discussion without adding new insights or a clear, impactful summary. To improve clarity and avoid redundancy, I suggest integrating the conclusion into the discussion section. Alternatively, if the authors wish to keep it separate, it should be revised to provide a concise, forward-looking summary that highlights the broader significance or future directions of the study.

New andean plump toad of the Genus *Osornophryne* (Anura: Bufonidae) from Cerro Candelaria, Ecuador (#113761)

First submission

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Please submit by **19 May 2025** for the benefit of the authors (and your token reward) .

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- Have you checked our new species policies?
- Do you agree that it is a new species?
- Is it correctly described e.g. meets ICZN standard?

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- Clear, unambiguous, professional English language used throughout.
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- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

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Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

New andean plump toad of the Genus *Osornophryne* (Anura: Bufonidae) from Cerro Candelaria, Ecuador

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The amphibian genus *Osornophryne* are endemic to the northern Andes of South America, apparent rarity has been documented over the course of the last decades, however new explorations in the humid montane forests of upper Pastaza valley are unvealing new discoveries. This paper describes a new species of Andean toad from the central Ecuadorian Andes, which has been identified through new genetic analyses, as well as morphological and cranial characters. The new species, *Osornophryne backhshalli* sp. nov., is from Cerro Candelaria in the upper Pastaza River basin and is related to *O. sumacoensis* from Sumaco Volcano. The new species is distinguished from its congeners by several unique characteristics, including a shorter Toe V than Toes I-III, presence of triangular papillae projecting from the tip of the snout, the presence of an occipital fold, the presence of large subconical and conical warts on the body, a dorsal dark brown color with small yellow reticulations, and a ventral brown pattern with bright yellow spots. The present study has updated the phylogenetic information of the genus by delimiting two high-support clades: the *Osornophryne bufoniformis* species group and the *Osornophryne guacamayo* species group. The study demonstrates that the Pastaza River does not act as a geographical barrier to the distribution of the genus *Osornophryne*, and emphasizes the need to deepen the knowledge of the genus as new explorations progress in the humid montane Ecuadorian Andes.

New Andean plump toad of the Genus *Osornophryne* (Anura: Bufonidae) from Cerro Candelaria, Ecuador.

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Abstract

The amphibian genus *Osornophryne* are endemic to the northern Andes of South America, apparent rarity has been documented over the course of the last decades, however new explorations in the humid montane forests of upper Pastaza valley are unvealing new discoveries. This paper describes a new species of Andean toad from the central Ecuadorian Andes, which has been identified through new genetic analyses, as well as morphological and cranial characters. The new species, *Osornophryne backhshalli* sp. nov., is from Cerro Candelaria in the upper Pastaza River basin and is related to *O. sumacoensis* from Sumaco Volcano. The new species is distinguished from its congeners by several unique characteristics, including a shorter Toe V than Toes I-III, presence of triangular papillae projecting from the tip of the snout, the presence of an occipital fold, the presence of large subconical and conical warts on the body, a dorsal dark brown color with small yellow reticulations, and a ventral brown pattern with bright yellow spots. The present study has updated the phylogenetic information of the genus by delimiting two high-support clades: the *Osornophryne bufoniformis* species group and the *Osornophryne guacamayo* species group. The study demonstrates that the Pastaza River does not act as a geographical barrier to the distribution of the genus *Osornophryne*, and emphasizes the

need to deepen the knowledge of the genus as new explorations progress in the humid montane Ecuadorian Andes.

Introduction

Bufonidae is a large clade of anuran amphibians, comprising over 658 species with a nearly cosmopolitan distribution (Frost, 2024). Unlike many other South American bufonids with extensive distributions (e.g., *Rhaebo*, *Rhinella*), Andean toads of the genus *Osornophryne* (Ruiz-Carranza & Hernández-Camacho, 1976) are restricted to the northern Tropical Andes (Frost, 2024; Páez-Moscoso & Guayasamin, 2012). These small toads comprises eleven species inhabiting the high Andes of Colombia and Ecuador between 2000 to 4000 meters of elevation, from the northern portion of Cordillera Central of Colombia southward to the central portion of Cordillera Real of Ecuador (Yánez-Muñoz *et al.*, 2010; Paez-Moscoso & Guayasamin, 2012; Frost, 2024).

The highest species richness of *Osornophryne* occurs in the eastern Andes of Ecuador, along the Cordillera Real, with one third of the species discovered in recent decades (Gluesenkamp & Guayasamin, 2008; Mueses-Cisneros, Yánez-Muñoz & Guayasamin, 2010; Cisneros-Heredia & Gluesenkamp, 2010; Páez-Moscoso, Guayasamin & Yánez-Muñoz, 2011).

Recent herpetological research conducted over the past 15 years by Fundación Ecominga and INABIO in the upper Pastaza River basin has led to the discovery of several new species with restricted distributions (e.g., Reyes-Puig *et al.*, 2019; INABIO *et al.*, 2023,). Cerro Candelaria, a mountain situated to the south of the Pastaza River, is of particular interest due to its high diversity and high concentration of endemic anurans (Reyes Puig *et al.*, 2010, 2014, 2023, 2024).

Expeditions to these mountain ranges have revealed new specimens of *Osornophryne* toads, together with extensive research encompassing genetic, morphological and osteological analysis. This work has led to the discovery of a new species from Cerro Candelaria, described herein. Also, these new collections help us to expand the current geographic knowledge about *O. simpsoni*.

Materials & Methods

Ethics statement. To follow the Ecuadorian regime, we use research permits to access genetic resources: NºMAE-DNB-CM-2019-0120, MAATE-ARSFC-2023-3346, MAATE-ARSFC-2024-0847 awarded by the Ministry of Environment, Water, and Ecological Transition of Ecuador. We observe the guidelines for the use of live amphibians and reptiles in field research, in agreement with Beaupre *et al.* (2004).

Nomenclature and Taxonomy. For species level recognition, we followed the species concept proposed by De Queiroz (2007) considering as new species independent lineages of metapopulations evolving separately with several supporting lines of evidence.

Species description follows the last *Osornophryne* taxonomical arrangement within the genus (Páez-Moscoso, Guayasamin & Yánez-Muñoz, 2011; Páez-Moscoso & Guayasamin, 2012).

The electronic version of this article in Portable Document Format (PDF) 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) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: [LSIDurn:lsid:zoobank.org:pub:459B3032-0A5E-4BDA-8457-9D4D53885725]. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

Taxon Sampling & Specimen Management. In the course of botanical research carried out in 2019, the first individual of *Osornophryne* was discovered serendipitously. This fortuitous finding led to the initiation of systematic monitoring of amphibians on Cerro Candelaria, which culminated in the collection of numerous significant specimens of *Osoronophryne*. Collection of individuals follows a standard and standardized techniques and methods of management for inventory and amphibian monitoring, proposed by Lips *et al.* (2001), and described in (Yánez-Muñoz et al. 2013, INABIO *et al.*, 2023, Reyes-Puig *et al.*, 2024).

During field expeditions, each captured specimen was assigned a unique number and was taken to the base camp in an individual plastic bag to confirm its sex and relative age (Angulo *et al.,* 2006). We recorded the time and date of capture, type of vegetation, substrate, activity, and climatic conditions. To facilitate the recognition of dorsal, ventral, and flanks patterns, the specimens were photographed with the unique code number and stored in a catalog of photographic references.

Collected specimens follow preservation suggestions of Rueda, Castro & Cortez (2006), were sacrificed using a local anesthetic solution, namely 2% lidocaine (which was prepared in its commercial formulation, Roxicaine), samples of tissue and liver was taken for DNA extractions, later fixed in 10% formalin for 24 hours, and then preserved in 70% ethanol. Sex and age of individuals were determined by identification of secondary sexual characters (*i.e.*, nuptial pads, males with vocal clefts, and body size) and also by direct inspection of the gonads through dorsolateral incisions.

Adult females of *Osornoprhyne sumacoensis* (MZUTI 5147) and the new species (DHMECN 18363) were dissected, removing skin and muscles from the head and mandibles, to discover the shape and structure of the squamosal and prootic, with emphasis on the crista parotica. Additionally, dermestid beetles were used for two days until the bones were free of muscle tissue and then degreased with sodium dodecyl sulfate for 24 hours, as described by Yánez-Muñoz *et al.* (2018) and Reyes-Puig *et al*. (2023).

Morphology. Examined specimens were sourced from regional and national herpetology repositories: Instituto Nacional de Biodiversidad (DHMECN), Quito, Ecuador; Instituto de Ciencias Naturales (ICN) Bogotá Colombia, and Universidad Tecnológica Equinoccial (MZUTI), Quito, Ecuador. Museum acronyms follow Frost (2024).

Examined specimens are listed in Appendix I. Characters and systematics follow those used by Paez-Moscoso & Guayasamin (2012), and previous *Osornophryne* descriptions (Yánez-Muñoz *et al.*, 2010; Páez-Moscoso, Guayasamin & Yánez-Muñoz, 2011). Study of the detailed morphology of the skull, crista parotica, and squamosal bone follows terminology used by Ruiz Carranza & Hernandez (1976) Duellman & Trueb, (1994), Vélez-Rodriguez (2005) Pramuk (2006), and Páez-Moscoso, Guayasamin & Yánez-Muñoz, (2011). Additionally, we revised differences between the structure and composition of palmar and plantar tubercles, ranging from smooth and low flat tubercles to numerous rounded and elevated tubercles with callosities. Preserved specimens in the DHMECN collection were photographed in 70% ethanol with a digital Canon camera.

Morphometric characters follow Páez-Moscoso, Guayasamin & Yánez-Muñoz, (2011). Measurements were taken with digital calipers to the nearest 0.01mm, and are as follow: (1) snout–vent length (SVL = distance from tip of snout [excluding the proboscis] to posterior margin of vent); (2) tibia length (TIB = length of flexed hind leg from knee to heel); (3) foot length (FL = distance from base of inner metatarsal tubercle to tip of Toe IV); (4) head length (HL = distance from tip of snout to articulation of jaw); (5) head width (HW = greatest width of head measured between jaw articulations); (6) interorbital distance (IOD = shortest distance between medial margins of upper eyelids); (7) upper eyelid width (EW = greatest width of eyelid measured perpendicular to medial axis of skull); (8) internarinal distance (IND = distance between internal borders of nos- trils); (9) eye–nostril distance (EN = distance from anterior corner of eye to posterior bor-der of nostril); (10) snout–eye distance (SE = distance from anterior corner of the eye to the tip of the rostrum); (11) eye diameter (ED = distance between anterior and posterior corners of eye); (12) Finger-III length (FIIIL = distance from proximal border of Finger I to distal end of Finger III); (13) Finger-IV length (distance from proximal border of Finger I to distal end of Finger IV); (14) Toe-IV length (TIVL = distance from proximal edge of Toe I to distal tip of Toe IV); (15) Toe-V length (TVL = distance from proximal border edge of Toe I to distal tip of Toe V). Sexual maturity was determined by the presence of nuptial pads in adult males and convoluted oviducts in adult females. Basic measurements of the Skull include width and lenght of the skull, width of the brain case, width of the parasphenoid alae and length of the cultriform process.

DNA extraction, amplification, and sequencing. We worked in two different laboratories using Oxford Nanopore Technologies. At the Laboratorio de Secuenciamiento de Ácidos Nucleicos INABIO, we processed 21 samples.

At IKIAM, DNA was extracted from liver tissue using the UltraClean® Tissue & Cells DNA Isolation kit (MO-BIO Laboratories, Inc., Carlsbad, CA, USA), following user instructions. The 16S rRNA (16S) ribosomal mitochondrial gene (16sSar-L-F: CGCCTGTTTATCAAAAACAT; 16sSbr-H-R: CCGGTCTGAACTCAGATCACGT) was amplified with a Polymerase chain reaction (PCR) following the specific thermocycler profile detailed in Pinto-Sánchez *et al.*, (2012). PCR products were cleaned with an Exo I/SAP digestor; samples were sequenced in Macrogen C*O.* Ltd. (South Korea).

At INABIO, DNA was extracted from liver tissue using GeneJET Genomic DNA purification Kit (K0722), following user instructions. PCR amplification, Nanopore sequencing and bioinformatics follow the same methodology detailed in Yánez-Muñoz *et al.* (2025).

A character matrix was built with the new sequences (Table S1) and aligned using MAFFT v7.017 with default settings (Katoh & Standley, 2013). To optimize the sampling of *Osornophryne*, similar sequences were searched with BLAST for 16S in the GenBank database. The concatenated matrix had 105 terminals and 2146 characters (Appendix 1). Unaligned regions were edited and suppressed.

Phylogenetic analysis. Phylogenetic analyses were realized using IQTree (Trifinopoulos *et al.*, 2016) with the aligned matrix. We run a phylogenetic inference tree with 1000 ultrafast bootstrap approximation approach (UFBoot), 1000 maximum iterations, 0.99 minimum correlation coefficient, 1000 replicates for SH-aLRT branch test and an approximate Bayes test (Guindon *et al.*, 2010). Support values mentioned herein follows this format: SH-aLRT support (%) / ultrafast bootstrap support (%).

The percentage of bases/residues that are identical between sequences (% identity) was calculated on a XX bp trimmed matrix of 16S rRNA to estimate p-genetic distances in MEGA 11 (Tamura, Stecher & Kumar, 2021).

Results

Phylogenetic relationships (Figure 1, Table 1**).**

Our phylogeny is consistent with previous hypotheses for *Osornophryne* (Páez-Moscoso & Guayasamin, 2012) in delimiting two major clades within the genus: (1) Clade A (*O. bufoniformis* species group) composed of *O. percrassa*+*O. bufoniformis*+*O.angel*+*O. puruanta*+*O. antisana*, and (2) Clade B (*O. guacamayo* species group) composed of *O. cofanorum*+*O. guacamayo*+*O. sumacoensis*+*O. occidentalis*+*O. simpsoni* (Fig. 1).

Our analysis indicates that the new species, *Osornophrye backshalli* sp. nov. belongs to clade B with high support (99.4/100 branch supports). Our inference suggests that the new species is sister to *Osornophryne sumacoensis* (92.6/100 branch supports) and closely related to the subclade *O. simpsoni*+*O. occidentalis* (73.2/87 branch supports).

Systematics.

Generic placement. We assign the new species to genus *Osornophryne* based on phylogenetic relationships and external morphology including digits of hands and feet nearly obscured by extensive webbing; and absence of paratoid glands and auditory structures; in addition *Osornophryne* species show fussed atlas and axis, reduced number of phalanges in hands and feet, sexual dimorphism, and inguinal amplexus (Ruiz-Carranza & Hernández-Camacho, 1976; Hoogmoed 1987; Gluesenkamp 1995; Gluesenkamp & Guayasamin, 2008).

Species accounts

New species

***Osornophryne backshalli* sp. nov.**

Osornophryne sp. nov. INABIO *et al.* (2023)

LSIDurn:lsid:zoobank.org:act:EE7F5D3E-66DE-47D7-B0C1-61FA7D4A7013

Proposed standard English name. Steve Backshall's Andean Toad

Proposed standard Spanish name. Osornosapo de Steve Backshall

Holotype (Figs. 2–5**).** DHMECN 15260, adult female, from Cerro Candelaria Protected Area, Rio Verde, Tungurahua province, Ecuador, (-1.444600°S, -78.302250°W; 2725 m), collected on 10 November 2019 by Eduardo Peña & Kelsey Huisman.

Paratypes (Figs. 6–9**).** A total of five (5) specimens. Adult females (3): DHMECN 18362, DHMECN 18363, from the same protected area near holotype locality (-1.436783°S, -78.301017°W; 2583 m) collected on 22 November 2022 by JPRP, Patricio Vinueza, Paulet Benavides & Eduardo Peña; DHMECN 19868, from Finca Palmonte, Rio Negro, Tungurahua Province, Ecuador (-1.438695°S, -78.265438°W; 2700 m), collected on 15 May 2024 by JPRP, José Ignacio Segovia, Evelyn Toa, Ximena Grefa, Alex Guevara and Fausto Recalde; Adult males (2): DHMECN 15821, same locality as the holotype collected on 14 March 2020 by Fausto Recalde, Juan Pablo Reyes-Puig and Martín Morales; DHMECN 18364, from the same locality and data as DHMECN 18362.

Diagnosis. *Osornophryne backshalli* sp. nov. is a member of clade B (*O. guacamayo* species group), differs from all other species of the clade by the following combination of characters: (1) small medium size toads (LRC= 22.08 – 35.4 mm.); (2) head as slightly wide as long; (3) skin on dorsal surfaces and limbs finely granular with conical and subconical warts, males more tuberculated than females; ventral surfaces with subconic tubercles; (4) snout subacuminate in dorsal view, protruding in lateral profile, with point irregular papillae on the tip; (5) crista parotica slightly arched with finely glandular skin, posterior and oblique in relation to the orbit; zygomatic ramus of the squamosal elongated with a blunt anterior border, Cultriform process with blunt anterior border (6) glandular occipital folds present discontinuous, dorsolateral folds absent instead a row of discontinuous subconical tubercles on its place; (7) pelvic folds absent (8) limbs short, heels do not touch when pressed; (9) Fingers and toes with extensive and thick webbing, fingers visible, tip of the Toes I, II, III, almost indistinguishable, Toe V short not elongated, slightly longer than Toe III, nuptial pads in males in the anterior part of Finger I; (10) dorsal surfaces light brown with yellow and grey flecks, ventral surfaces brown with yellow bright blotches; (11) short cloacal tube slightly projected medial to the thighs.

Comparisons with other species (Figs. 4–6**).** *Osornoprhyne backshalli* sp. nov. is distinguished from all other members of the genus by its dorsal and ventral skin texture with subconical and conical tubercles, and also by the bright yellow blotches on the flanks and belly, presence of occipital ridge without dorsolateral folds; other species in the eastern Andes present dorsolateral folds, at least discontinuous are *O. simpsoni*, *O.cofanorum O. bufoniformis*, *O. talipes*, *O. angel*, *O. puruanta*, *O. sumacoensis*, *O. antisana*, with the exception of *O. guacamayo* and *O. percrasa*, lacking dorsolateral ridge. Also *Osornophryne backshalli* sp. nov. differs from *O. cofanorum*, *O. guacamayo* and *O. simpsoni* by having reduced Toe V not elongated, while the latter species bears Toe V barely differentiated from the foot plate in (Figure 4, Table 2).

A systematic comparison of the key diagnostic characters of the new species with its closest congeners is presented in Table 1, and a detailed view of the lateral profile with most related congener is presented in Figures 4 and 5. In profile *Osornophryne backshalli* sp. nov., differs from its closest relative, *O. sumacoensis*, by having have a protruding snout with large triangular papillae, and smooth concave loreal region; *O. sumacoensis* bears a rounded snout with rounded papillae, and a tuberculated flat loreal region. The head is covered by elevated subconical tubercles in *O. backshalli* sp. nov., while rounded warts are present in *O. sumacoensis*. Crista parotica is slightly arched posterior and oblique in relation to the orbit, and is covered by finely glandular skin in the new species, while in *O. sumacoensis* it is posterior and more horizontal in relation to the orbit, and heavily covered by rounded warts (Fig. 5).

Osornophryne backshalli sp. nov. bears a dorsolateral row of enlarged conical tubercles, while *O. sumacoensis* bears a pustular ridge ; the forearms present large subconic tubercles in *Osornophryne backshalli* sp. nov., whereas in *O. sumacoensis* these tubercles are rounded; dorsum has subcónic elevated tubercles in the new species, contrary to the granular with rounded warts in *O. sumacoensis*. The palms and soles have smooth texture in *Osornophryne backshalli* sp. nov., while they are covered by rounded and flat supernumerary tubercles in *O. sumacoensis*, cloacal tube is more elongated and ventrally directed in *O. backshalli* sp. nov., rather than short in *O. sumacoensis* (Fig. 4).

The skull of *O. backshalli* sp.nov. presents differences from *O. simpsoni* and *O. sumacoensis* in the shape of the sphenethmoides on its dorsal view (see Fig. 6, Table 4 and Páez-Moscoso, Guayasamin & Yánez-Muñoz, 2011). In *O. backshalli* sp.nov. the anterior border is elongated and projected than the posterior border, that is short irregular and not projected posteriorly, contrary to the acuminated short anterior border in *O. sumacoensis*, instead posterior elongated border extending into interparietal suture. Additionally, the shape of the zygomatic ramus of the squamosal presents distinctive differences (Fig. 6), more projected and blunt anterior border, extending at the level of the third portion of the maxillae in *O. backshalli* sp. nov., contrary to the short broad and not projected anterior border of the zygomatic ramus in *O. sumacoensis*. Otic ramus is slightly arched at the suture with the crista parotica in *O. backshalli* sp. nov., while enlarged posteriorly and slightly oblique otic ramus in the suture of the crista parotica in *O. sumacoensis*. Cultriform process is longer in *O. backshalli* sp. nov., than in *O. sumacoensis* (Fig. 6, Table 4).

Description of the holotype (Figs. 2–3**).** DHMECN 15260, adult female, moderate in size, 35.4 mm SVL. Head length 85.75% head width; width of head greater at level of posterior margin of mouth; snout protuberant, with rostral triangular papillae in dorsal and lateral views; nostrils rounded protuberant laterally oriented; each nostril oblique and oval, frontally projected; internarial area rough and slightly concave; interorbital region tuberculated with scattered conic tubercles, skin co-ossified with underlying bones; upper eyelids strongly tuberculated bearing several subconic warts; interorbital region wider than the upper eyelid (79.36%), interorbital subconic tubercle present; outer edge of the eyelid delineated by a continuous row of warts; canthus rostralis straight and tuberculated; loreal region concave and rugouse; eyes with elliptically horizontal pupil; infraorbital and postorbital regions rugose with subconic warts posteriorly; occipital surface rough with subconic warts and two low glandular ridges forming a "Y", converging posteriorly in middle dorsal line (Fig. 2); crista parotica forming an elevated glandular ridge located posterior and oblique to the orbit. Skin of dorsum and flanks finely granular, with numerous subconic and conic warts; without dorsolateral ridge, in its place a row of elongated subconic tubercles, pelvic ridge absent; limbs tuberculated with subconic elongated warts; ventral skin with subconic tubercles in the belly and chest, becoming, more elevated in to the flanks and limbs.

Forelimbs short, slender, strongly tuberculated with rows of subconical tubercles in the fore arms; hand moderate in length, representing 21.52% of SVL; extensive webbing between fingers (Fig. 3); lengths of fingers in order of increasing length: I = II < IV < III; palms with merged flattened tubercles, subarticular tubercles not evident; large irregular palmar tubercle slightly differentiated, thenar tubercle oval.

Hind limbs short slender and covered dorsally with large subconical tubercles, tibia and foot, respectively, 27.51% and 30.64% of SVL; extensive thick skin between toes, webbing between Toes I–III,V more extensive that webbing between Toes IV (Fig. 3); lengths of Toes: I < II < III < V < IV; Toe not differentiated and longer than Toe III, soles with flattened tubercles; subarticular tubercles not evident; inner metatarsal tubercle irregular. Skin on the groin some loose joints half to the femoral region. Choanae small, rounded, widely separated; cloacal opening medial to thighs slightly projected with short cloacal tube present.

Color of holotype in life (Figs. 4, 7, 9**).** Dorsal surfaces light brown with diffuse light yellow small marks, warts on dorsum and flanks dark brown, head and snout dark brown with pale brown tones mixed, lateral surfaces of head and flanks light brown with yellow tones, limbs dark brown. Iris dark brown with yellow punctuations. Belly and ventral surfaces of limbs dark brown with bright yellow irregular blotches and marks, more concentrated on the ventral surfaces of thighs chest and shoulders, lower portion of the throat dark brown, anterior throat and chin with yellow irregular scattered flecks. Palms and soles dark brown.

Color of holotype in ethanol 70%. (Fig. 2**)**. Dorsal surfaces light brown with small light reticulations in the background, warts and tubercles dark brown; belly dark brown with light cream blotches scattered, surfaces of palms and soles, dark brown with some scattered pale brown marcs.

Osteologic diagnostic characters of the Skull. (Fig. 4**)**. The skull of the adult female paratype (DHMECN 18363), is illustrated on its dorsal, lateral and ventral views (Fig. 9), based on previous *Osornophryne* osteological descriptions (Ruiz Carranza & Hernandez 1976) ,Páez-Moscoso, Guayasamin & Yánez-Muñoz 2011), we summarized osteologic diagnostic characters for the new species (Table 4). Going forward we describe only skull bones with diagnostic differences. The cranium presents its widest portion posterior to the orbit at the level of the quadratojugal, braincase broad;t, the braincase width (measured at level of the mid orbit) is about 32.46% of the greatest width of the skull and 35.37% of the medial skull length.

Among neurocranium bones, main diagnostic characters are observed in the sphenethmoid, dorsally presents an anterior border subacuminated and projected anteriorly reaching the middle level of nasals, posterior border of sphenethmoides is short and slightly subacuminated, in ventral view sphenethmoides bears slightly acuminated anterior border that reach posterior level of the choanes, separated from premaxilla for paired nassals.

In lateral view shape and projection of squamosal components are key characters too; in lateral view otic ramus is slightly arched and broad, forming a prominent crista parotica, additionally the zygomatic ramus of squamosal present anterior elongation with a blunt border that reaches the level of the third part of the maxillae. Frontoparietal fontanelle not evident, instead the frontoparietal suture is evident. Exoccipitals are almost in contact but slightly separated medially from one another. The dorsal surface of the prootic is smooth. The epiotic eminences are not prominent.

In ventral view the skull shows the parasphenoid with its characteristic inverted T shape; the cultriform process extends anteriorly just behind the mid-level of the orbit, where it is in contact with the posterior border of the sphenethmoid. The cultriform process presents an anterior blunt margin, and reaches its maximum width at a level of the posterior border of the optic fenestra. The parasphenoid alae are robust with irregular lateral margins that are articulated with pteigoides and posteriorly with prootic and paired exoccipitals; the length of each alae is 87.8% the length of the cultriform process. Posterior margin of the parasphenoid bears a short truncated posteromedial process that terminates just anterior to the margin of the foramen magnum (Fig. 9).

Variation (Figs.7–9). *Osornophryne backshalli* sp.nov. is a sexually dimorphic species with nuptial pads on males, and differences on SVL variation, smaller males than females; measurements of the type series are presented on Table 2. There is little color variation, with general brown dorsal color and more or less irregular yellow markings on ventral surfaces (Figs. 7, 9). The male paratype DHMECN 15821 presents light brown dorsal surfaces with pale yellow tones and dark brown warts, dorsal surfaces of head dark brown with yellow tubercles, and the tip of the snout presents a yellow wart. Cantus rostralis dark brown, occipital region light brown with light yellow tones, yellow mark in the margin of the jaw. Yellow iris with dark reticulations. Flanks light brown with pale yellow marks, limbs dark brown. Belly and ventral surfaces of limbs dark brown with small yellow marks, axilae densely yellow pigmented. Palms and soles are dark brown, with small scattered yellow marks. In general the other specimens present similar color patterns, with exception of the shape and size of the bright yellow ventral pattern (Figs. 8–9).

Distribution and natural history (Figure 10**).** *Osornophryne backshalli* sp. nov., is known only from two localities in the northern and eastern slopes of Cerro Candelaria Protected area Reserve, Rio Verde Parish, Baños township, Tungurahua Province, Ecuador (Fig. 10) at an elevation range of 2568 to 2725 meters. This locations comprise montane cloud forest (Bosque siempreverde montano del norte y centro de la cordillera oriental de los Andes, Ministerio de Ambiente 2013), with a canopy of 20 meters dominated by palm trees (Ceroxylum spp.), also Clusia and Weinnmania trees covered with a dense layer of bryophytes and epiphytes, fallen trunks and deep leaf litter layer on the forest floor. The holotype was found during the day on a fallen trunk covered by moss, 50 cm over the ground, while the male paratype DHMECN 15821 was found at night in branches of bushes heavily covered by moss, 130 cm over the ground. The other paratypes were found in the leaf litter on the forest floor at night. Other amphibians sympatric with the new species include: *Osornophryne simpsoni*, *Pristimantis tungurahua*, *P. marcoreyesi*, *P. ardyae*, *P. donnelsoni* and *Niceforonia* sp.; During several amphibian surveys in Cerro Candelaria, with special emphasis on finding these species, less than 6 records were found in the last sixteen years of surveys; a relatively low abundance or rarity of the species is evident.

Etymology. The specific epithet is a patronymic in honor of the explorer and television presenter Steve Backshall, of London, UK. He has raised awareness of nature around the world and has, through his patronage of the World Land Trust, contributed directly to the conservation of the Corredor de Conectividad Ecologico Llanganates-Sangay, the habitat of this particular species and many others.

Conservation. The two localities where *Osornophryne backshalli* sp. nov. occurs are inside protected areas, Cerro Candelaria was declared as Private Protected Area by the Ecuadorian Government in 2023, and Finca Palmonte is a private reserve and Socio Bosque area managed by local family, boyh areas are contiguous on the northern and eastern slopes of Cerro Candelaria, and are part of the buffer zone of Sangay National Park and Corredor de Conectividad Llanganates Sangay, recently declared by national authorities. Based on the rarity of the species, we suggest classification under IUCN Red list criteria as Data Deficient DD Category. Future assessments must consider the small distribution range and specific habitat requirements; the species may qualify under some threat category.

Discussion

Our phylogenetic analysis provides strong support for the delineation of two major clades within the genus *Osornophryne*. Consistent with the conclusions of Páez-Moscoso and Guayasamin (2012), we identify a primary clade, which for the purposes of this study is designated as the *Osornophryne bufoniformis* species group. This clade comprises exclusively species inhabiting the high Andean ecosystems of Ecuador and Colombia (five species), characterized by their ecotype of terrestrial-semi-fossorial small stubby toads and Toe V shorter than Toes I-III. There is concurrence with the conclusions of Páez-Moscoso & Guayasamin (2012) that this group exhibits low genetic distances among its species (see Table 1), although they are distinguished by marked and distinctive morphological and morphometric characteristics.

In contrast, the second clade, which has been designated as the *Osornophryne guacamayo* species group, exhibits radiation in the humid montane forest ecosystems of the western and specially on eastern slopes of the ecuadorian Andes (six species), with high differentiation in genetics, distinctive morphologic characters and two ecotypes (Table 1, 2). The first, include terrestrial semifossorial small stubby toads, with Toe V reduced, shorter than Toes I-III (*O. sumacoensis*, *O. backshalli* sp. nov., *O. occidentalis*). The second ecotype includes small slender toads with terrestrial-arbustive habits and Toe V longer than Toes I-III (*O. guacamayo*, *O. cofanorum*, *O. simpsoni*). Although previous morphological studies suggested that Toe V may be phylogenetically informative (Hoogmoed 1987; Gluesenkamp 1995; Gluesenkamp & Guayasamin 2008; Mueses Cisneros, Yanez & Guayasamin 2010; Cisneros Heredia & Gluesenkam 2010), our data confirm that it is a homoplasious condition in response to ecological adaptations to habitat.

During several surveys in Cerro Candelaria and Sumaco, both related species with reduced Toe V (*O. backshalli* sp.nov. and *Osornophryne sumacoensis*), occurs with low relative abundance on the leaflitter and understory in a thin altitudinal band between 2500 m to 2800 m. While sympatric congeners with longer Toe V tend to have a high proportion of sightings in arbustive habitats like ferns and shrubs up to 150 centimeters (*O. guacamayo* in Sumaco and *O. simpsoni* in Cerro Candelaria).

Our last *Osornophryne* discoveries extend its distribution south of its previously known range. We recorded this genus south of the Rio Pastaza for the first time (Fig. 10). Previous *Osornophryne* southern records were limited to the mountains north of the Pastaza valley (Páez Moscoso, Guayasamin & Yanez Muñoz 2011). In addition we report new populations and distribution records of *Osornophryne simpsoni* at the northern and southern extremes of its range: DHMECN 15324 and DHMECN 15338 from Reserva Biológica Colonso Chalupas at Napo province, between 2196 m and 2539 m; and DHMECN 18365-18370, from Cerro Candelaria, and (DHMECN 18128 - DHMECN 18132) from Finca Palmonte, Tungurahua province, between 2405 m and 2583 m (see Fig.10). Discrete morphological differences related to the shape of the snout, including papilla, loreal region and crista parotica, suggest that these populations represent geographical extreme variations of the species.

The development of knowledge about the skull osteology in *Osornophryne* remains limited to the description of the genus (Ruiz Carranza & Hernandez Camacho 1976) and its latest recognized species, *O. simpsoni* (Páez-Moscoso, Guayasamin & Yánez-Muñoz, 2011). With the aim of describing and comparing the skull osteology of the new species and its near relative (*O. sumacoensis*), which was described only by morphological features and skull morphology, for first time we present diagrams of the skull of *O. sumacoensis* (MZUTI 6912) illustrating key diagnostic features, especially shape, orientation and conjunction with surrounding bones in sphenethmoides, zygomatic ramus of squamosal and parasphenoides, to discriminate with the recently discovered *O. backshalli* sp. nov.. It's evident that *O. sumacoensis* and *O. backshalli* sp. nov. , share paired nassals in contact at the middle line, frontoparietals in medial contact, without frontoparietal fontanelle, but the shape and conjunctions of sphenethmoides and squamosal differs in both species (Fig. 6, Table 4).

Detailed observation of cranium bones show correlation between shape and orientation of the crista parotica formed by the otic ramus of squamosal and covered of glandular folds (Figs. 3, 9). Another diagnostic condition is the shape and extension of the anterior border of the zygomatic ramus in the squamosal bone, finally the parasphenoides structure is very informative between species (see Páez Moscoso, Guayasamin & Yanez Muñoz 2011, Fig. 6 and Table 4).

Comprehensive and high quality computed tomography (CT-Scan) studies are fundamental to achieve a complete osteological description. Research conducted in laboratories such as those at the Alexander Koenig Museum in Bonn have demonstrated their usefulness in taxonomic studies, such as in *Hyloscirtus* (Reyes-Puig *et al.*, 2022) and *Noblella* (Reyes-Puig *et al.*, 2018). However, until more advanced technologies become available in our laboratories, traditional methods remain valuable tools, as they allow us to analyze and replicate schemes based on general anatomical patterns.

Conclusions

The integrative lines of evidence presented herein are robust in delimiting the new species and its phylogenetic relationships, thus increasing the number of species described for the genus in the northern Andes of South America to 12. It is confirmed and defined that *Osornoprhyne* is composed of two spatially separated clades between high Andean ecosystems (*O. bufoniformis* species group) and Andean slopes (*O. guacamayo* species group), sharing traits with different evolutionary origins. Examination of skull morphology identified that the state of the parotic crista, squamosal and parasphenoides structure provides key information for species differentiation, opening up a new field of research, fundamental for future taxonomic revisions.

The present study demonstrates that the Pastaza River does not act as a geographical barrier to the distribution of the genus *Osornophryne* in the southern Ecuadorian Andes. We emphasize to deepen the knowledge of the genus as new explorations progress in the Ecuadorian Andes.

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References

Angulo A; Rueda-Almohacid J, Rodríguez-Mahecha J, La Marca E. (Eds). 2006. Técnicas de Inventario y Monitoreo para los anfibios de la región tropical andina. Conservation International. Serie Manuales de Campo N° 2.

Baby P, Rivadeneira M, Christophoul F, Barragán R. 1999. Style and timing of deformation in the Oriente Basin of Ecuador. Extended Abstract, ISAG 99 (pp. 68 – 72). Göttingen.

Barragán R, Baby P. 2004. Evolución magmática actual de la zona Sub-Andina: volcanes El Reventador y Sumaco, modelos geodinámicos preliminares. In: Baby P, Rivadeneira M & Barragán R (eds), La Cuenca Oriente: Geología y Petróleo (pp. 183 – 201). Quito: Convenio IFEA-IRD-PET-ROAMAZONAS EP.

Cisneros-Heredia DF, Gluesenkamp AG. 2010. Un nuevo Sapo Andino del género *Osornophryne* (Amphibia: Anura: Bufonidae) desde el noroeste de Ecuador, con observaciones taxonómicas sobre el género. *ACI Avances En Ciencias E Ingenierías* 2(3) https://doi.org/10.18272/aci.v2i3.46

De Queiroz K. 2007.Species concepts and species delimitation. *Systematic Biology* 56:879–886 https://doi.org/10.1080/10635150701701083

Dodson CH, Gentry AH. 1991. Biological extinction in western Ecuador. *Annals of the Missouri Botanical Garde*n. 273-295.

Duellman WE, Trueb L. 1994. Biology of Amphibians. The Johns Hopkins University Press, USA.1-670.

Frost D. 2024. Amphibian Species of the World. Available at https://amphibiansoftheworld.amnh.org/ (accessed on 14 February 2024).

Gluesenkamp A. 1995. A new species of *Osornophryne* (Anura: Bufonidae) from Volcán Sumaco, Ecuador with notes on other members of the genus. *Herpetologica* 51 (3): 268–279. https://www.jstor.org/stable/3893032

Gluesenkamp A, Guayasamin JM. 2008. A new species of *Osornophryne* (Anura: Bufonidae) from the Andean highlands of northern Ecuador. *Zootaxa* 1828: 18–28. DOI https://doi.org/10.11646/zootaxa.1828.1.2

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel *O.* 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59(3):307-321

Hoogmoed MS. 1987. New *Osornophryne* (Amphibia: Anura: Bufonidae) from the Atlantic slopes of the Andes in Ecuador. *Zoologische Mededelingen* 61 (16): 209–242.

INABIO-Instituto Nacional de Biodiversidad, Fundación Ecominga, Sumak Kawsay In Situ, Waska Amazonía. 2023. Anfibios y Reptiles del Corredor Ecológico Llanganates-Sangay. Serie de Publicaciones Instituto Nacional de Biodiversidad. Publicación Miscelánea N° 17. World Wildlife Fund. Quito. Ecuador. ISBN: 978-9942-620-06-04.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30:772–780 DOI 10.1093/molbev/mst010.

Lynch JD, Duellman WE. 1997. Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in Western Ecuador: Systematics, ecology, and biogeography. The University of Kansas Natural History Museum. Special Publications 23: 1–236.

Lips KR, Reaser JK, Young BE, Ibáñez R. 2001. Amphibian Monitoring in Latin America: A Protocol Manual. Monitoreo de Anfibios en América Latina: Manual de Protocolos. Herpetological Circular N*O*. 30, Society for the Study of Amphibians and Reptiles. 1-115. https://www.amphibianark.org/wp-content/uploads/2018/07/Amphibian-monitoring-in-Latin-America.pdf

Ministerio del Ambiente del Ecuador. 2013. Sistema de clasificación de los ecosistemas del Ecuador continental. Quito, Ecuador: Subsecretaría de Patrimonio Natural Quit*O.* https://doi.org/10.13140/RG.2.1.1269.4161.

Mueses-Cisneros JJ, Yánez-Muñoz MH, Guayasamin JM. 2010. Una nueva especie de sapo del género *Osornophryne* (Anura: Bufonidae) de las estribaciones amazónicas de los Andes de Ecuador. *Papéis Avulsos de Zoologia* 50: 269–279. https://doi.org/10.1590/S0031-10492010001700001

Ortega JA, Brito J, Ron SR. 2022. Six new species of *Pristimantis* (Anura, Strabomantidae) from Llanganates National Park and Sangay National Park in Amazonian cloud forests of Ecuador. *PeerJ* 10: e13761. https://doi.org/10.7717/peerj.13761

Páez-Moscoso DJ, Guayasamin JM, Yánez-Muñoz M. 2011. A new species of Andean toad (Bufonidae, *Osornophryne*) discovered using molecular and morphological data, with a taxonomic key for the genus. *ZooKeys* 108: 73-97. https://doi.org/10.3897/zookeys.108.1129

Páez-Moscoso DJ, Guayasamin JM. 2012. Species limits in the Andean toad genus *Osornophryne* (Bufonidae). *Molecular Phylogenetics and Evolution.* https://doi.org/10.1016/j.ympev.2012.08.001

Pinto-Sánchez NR, Ibáñez R, Madriñán S, Sanjur OI, Bermingham E, Crawford AJ. 2012. The Great American Biotic Interchange in frogs: Multiple and early colonization of Central America by the South American genus *Pristimantis* (Anura: Craugastoridae). *Molecular Phylogenetics and Evolution* 62:954–972. DOI: 10.1016/J.YMPEV.2011.11.022

Pramuk JB. 2006. Phylogeny of South American *Bufo* (Anura: Bufonidae) inferred from combined evidence. *Zoological Journal of the Linnean Society* 146:407–452. https://doi.org/10.1111/j.1096-3642.2006.00212.x

Reyes-Puig JP, Yánez-Muñoz MH, Libke Z, Vinueza P, Carrión-Olmedo JC. 2024. Phylogenetic diversity of the Pristimantis anaiae species group, with a description of a new species of *Pristimantis* (Anura, Strabomantidae) from Cerro Candelaria, Tungurahua, Ecuador. *Evolutionary Systematics* 8:101–118. https://doi.org/10.3897/evolsyst.8.118855
Reyes-Puig JP, Urgilés-Merchán M, Franco-Mena D, Guayasamin JM, Batallas D, Reyes-Puig C (2023) Two new species of terrestrial frogs of the *Pristimantis gladiator* complex (Anura, Strabomantidae) from the Ecuadorian Andes, with insights on their biogeography and skull morphology. ZooKeys 1180: 257–293. https://doi.org/10.3897/zookeys.1180.107333
Reyes-Puig JP, Reyes-Puig CP, Ramírez-Jaramillo SR, Pérez-Lara MB, Yánez-Muñoz MH (2014) Tres nuevas especies de ranas terrestres Pristimantis (Anura:Craugastoridae) de la cuenca alta del Río Pastaza, Ecuador. ACI Avances en Ciencias e Ingenierías 6(2): B51–B62. https://doi.org/10.18272/aci.v6i2.179
Reyes-Puig JP, Yánez-Muñoz MH, Cisneros-Heredia DF, Ramírez-Jaramillo SR (2010) Una nueva especie de rana *Pristimantis* (Terrarana: Stabomantidae) de los bosques nublados de la cuenca alta del río Pastaza, Ecuador. ACI Avances en Ciencias e Ingenierías 2(3): B78–B82. https://doi.org/10.18272/aci.v2i3.48
Rueda J, Castro F, Cortez C. 2006. Técnicas para el Inventario y Muestreo de Anfibios: Una compilación. Pp 135- 171. En: Angulo A; Rueda-Almohacid J, Rodríguez-Mahecha J, La Marca E. (Eds). Técnicas de Inventario y Monitoreo para los anfibios de la región tropical andina. Conservation International. Serie Manuales de Campo N° 2.
Ruiz-Carranza PM, Hernández-Camacho JA. 1976. *Osornophryne* género nuevo de anfibios bufónidos de Colombia y Ecuador. *Caldasia* 11: 93–148.
Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 38(7):3022-3027
Trifinopoulos J, Nguyen LT, Von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44:W232–W235. https://doi.org/10.1093/nar/gkw256
Vélez-Rodriguez CM. 2005. Osteology of *Bufo sternosignatus* Günther, 1858 (Anura: Bufonidae) with comments on phylogenetic implications. *Journal of Herpetology* 39: 299-303. https://doi.org/10.1670/31-04W
Yánez-Muñoz MH, Altamirano-Benavides M, Cisneros-Heredia DF, Gluesenkamp AG. 2010. Nueva especie de Sapo Andino del género *Osornophryne* (Amphibia: Bufonidae) del norte de Ecuador, con notas sobre la diversidad del género en Colombia. *ACI Avances En Ciencias E Ingenierías* 2(3). https://doi.org/10.18272/aci.v2i3.44
Yánez-Muñoz MH, Reyes-Puig C, Reyes-Puig JP, Velasco JA, Ayala-Varela F, Torres-Carvajal. 2018. A new cryptic species of *Anolis* lizard from northwestern South America (Iguanidae, Dactyloinae). ZooKeys 794: 135-163. https://doi.org/10.3897/zookeys.794.26936
Yánez-Muñoz MH, Reyes-Puig JP, Reyes-Puig C, Lagla-Chimba G, Paucar-Veintimilla C, Urgilés-Merchán M, Carrión-Olmedo JC. 2025. High speciation in the cryptic *Pristimantis celator* clade (Anura: Strabomantidae) of the Mira river basin, Ecuador-Colombia. *PeerJ* 13:e18680 http://doi.org/10.7717/peerj.18680

Figure 1

Figure 1. Phylogenetic relationships of *Osornohryne backshalli* sp.nov. with its congeners.

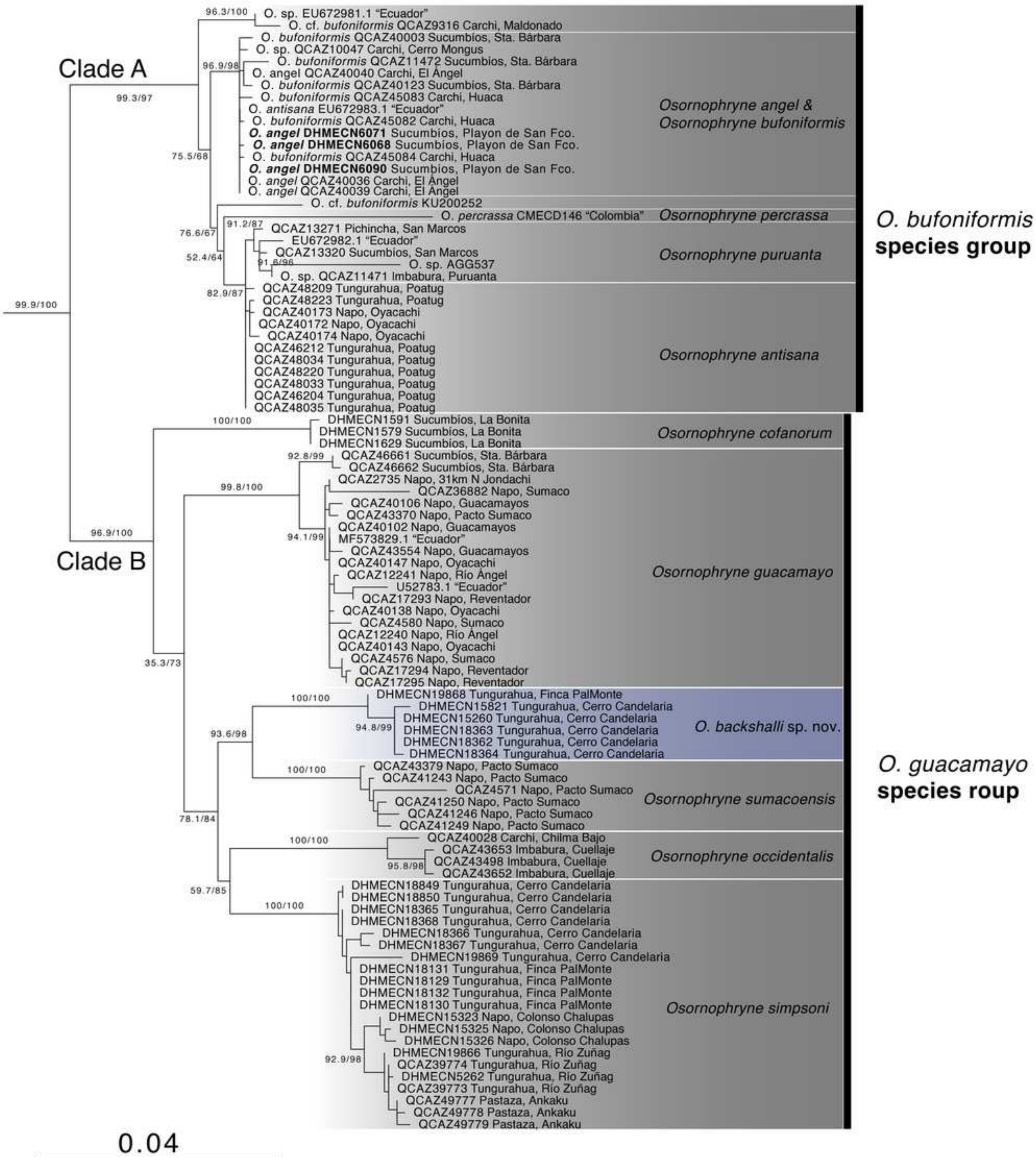
Clade A
Clade B
99.9/100
99.3/97
96.9/100
96.3/100
O. sp. EU672981.1 "Ecuador"
O. cf. bufoniformis QCAZ9316 Carchi, Maldonado
O. bufoniformis QCAZ40003 Sucumbíos, Sta. Bárbara
O. sp. QCAZ10047 Carchi, Cerro Mongus
96.9/98
O. bufoniformis QCAZ11472 Sucumbíos, Sta. Bárbara
O. angel QCAZ40040 Carchi, El Ángel
O. bufoniformis QCAZ40123 Sucumbíos, Sta. Bárbara
O. bufoniformis QCAZ45083 Carchi, Huaca
O. antisana EU672983.1 "Ecuador"
O. bufoniformis QCAZ45082 Carchi, Huaca
O. angel DHMECN6071 Sucumbíos, Playon de San Fco.
O. angel DHMECN6068 Sucumbíos, Playon de San Fco.
75.5/68
O. bufoniformis QCAZ45084 Carchi, Huaca
O. angel DHMECN6090 Sucumbíos, Playon de San Fco.
O. angel QCAZ40036 Carchi, El Ángel
O. angel QCAZ40039 Carchi, El Ángel
Osornophryne angel &
Osornophryne bufoniformis
O. cf. bufoniformis KU200252
O. percrassa CMECD146 "Colombia"
Osornophryne percrassa
76.6/67
91.2/87
QCAZ13271 Pichincha, San Marcos
EU672982.1 "Ecuador"
52.4/64
QCAZ13320 Sucumbíos, San Marcos
91.6/96
O. sp. AGG537
O. sp. QCAZ11471 Imbabura, Puruanta
Osornophryne puruanta
82.9/87
QCAZ48209 Tungurahua, Poatug
QCAZ48223 Tungurahua, Poatug
QCAZ40173 Napo, Oyacachi
QCAZ40172 Napo, Oyacachi
QCAZ40174 Napo, Oyacachi
QCAZ46212 Tungurahua, Poatug
QCAZ48034 Tungurahua, Poatug
QCAZ48220 Tungurahua, Poatug
QCAZ48033 Tungurahua, Poatug
QCAZ46204 Tungurahua, Poatug
QCAZ48035 Tungurahua, Poatug
Osornophryne antisana
O. bufoniformis
species group
100/100
DHMECN1591 Sucumbíos, La Bonita
DHMECN1579 Sucumbíos, La Bonita
DHMECN1629 Sucumbíos, La Bonita
Osornophryne cofanorum
92.8/99
QCAZ46661 Sucumbíos, Sta. Bárbara
QCAZ46662 Sucumbíos, Sta. Bárbara
99.8/100
QCAZ2735 Napo, 31km N Jondachi
QCAZ36882 Napo, Sumaco
QCAZ40106 Napo, Guacamayos
QCAZ43370 Napo, Pacto Sumaco
QCAZ40102 Napo, Guacamayos
94.1/99
MF573829.1 "Ecuador"
QCAZ43554 Napo, Guacamayos
QCAZ40147 Napo, Oyacachi
QCAZ12241 Napo, Río Ángel
U52783.1 "Ecuador"
QCAZ17293 Napo, Reventador
QCAZ40138 Napo, Oyacachi
QCAZ4580 Napo, Sumaco
QCAZ12240 Napo, Río Ángel
QCAZ40143 Napo, Oyacachi
QCAZ4576 Napo, Sumaco
QCAZ17294 Napo, Reventador
QCAZ17295 Napo, Reventador
Osornophryne guacamayo
35.3/73
100/100
DHMECN19868 Tungurahua, Finca PalMonte
DHMECN15821 Tungurahua, Cerro Candelaria
94.8/99
DHMECN15260 Tungurahua, Cerro Candelaria
DHMECN18363 Tungurahua, Cerro Candelaria
DHMECN18362 Tungurahua, Cerro Candelaria
DHMECN18364 Tungurahua, Cerro Candelaria
O. backshalli sp. nov.
93.6/98
100/100
QCAZ43379 Napo, Pacto Sumaco
QCAZ41243 Napo, Pacto Sumaco
QCAZ4571 Napo, Pacto Sumaco
QCAZ41250 Napo, Pacto Sumaco
QCAZ41246 Napo, Pacto Sumaco
QCAZ41249 Napo, Pacto Sumaco
Osornophryne sumacoensis
78.1/84
100/100
QCAZ40028 Carchi, Chilma Bajo
QCAZ43653 Imbabura, Cuellaje
QCAZ43498 Imbabura, Cuellaje
95.8/98
QCAZ43652 Imbabura, Cuellaje
Osornophryne occidentalis
59.7/85
100/100
DHMECN18849 Tungurahua, Cerro Candelaria
DHMECN18850 Tungurahua, Cerro Candelaria
DHMECN18365 Tungurahua, Cerro Candelaria
DHMECN18368 Tungurahua, Cerro Candelaria
DHMECN18366 Tungurahua, Cerro Candelaria
DHMECN18367 Tungurahua, Cerro Candelaria
DHMECN19869 Tungurahua, Cerro Candelaria
DHMECN18131 Tungurahua, Finca PalMonte
DHMECN18129 Tungurahua, Finca PalMonte
DHMECN18132 Tungurahua, Finca PalMonte
DHMECN18130 Tungurahua, Finca PalMonte
DHMECN15323 Napo, Colonso Chalupas
DHMECN15325 Napo, Colonso Chalupas
DHMECN15326 Napo, Colonso Chalupas
92.9/98
DHMECN19866 Tungurahua, Río Zuñag
QCAZ39774 Tungurahua, Río Zuñag
DHMECN5262 Tungurahua, Río Zuñag
QCAZ39773 Tungurahua, Río Zuñag
QCAZ49777 Pastaza, Ankaku
QCAZ49778 Pastaza, Ankaku
QCAZ49779 Pastaza, Ankaku
Osornophryne simpsoni
O. guacamayo
species roup
0.04

Figure 2

Figure 2. Dorsal ventral and lateral views of preserved *Osornophryne backshalli* sp.nov. holotype (DHMECN 15260), adult female.

Photographs by Mario H. Yánez-Muñoz.

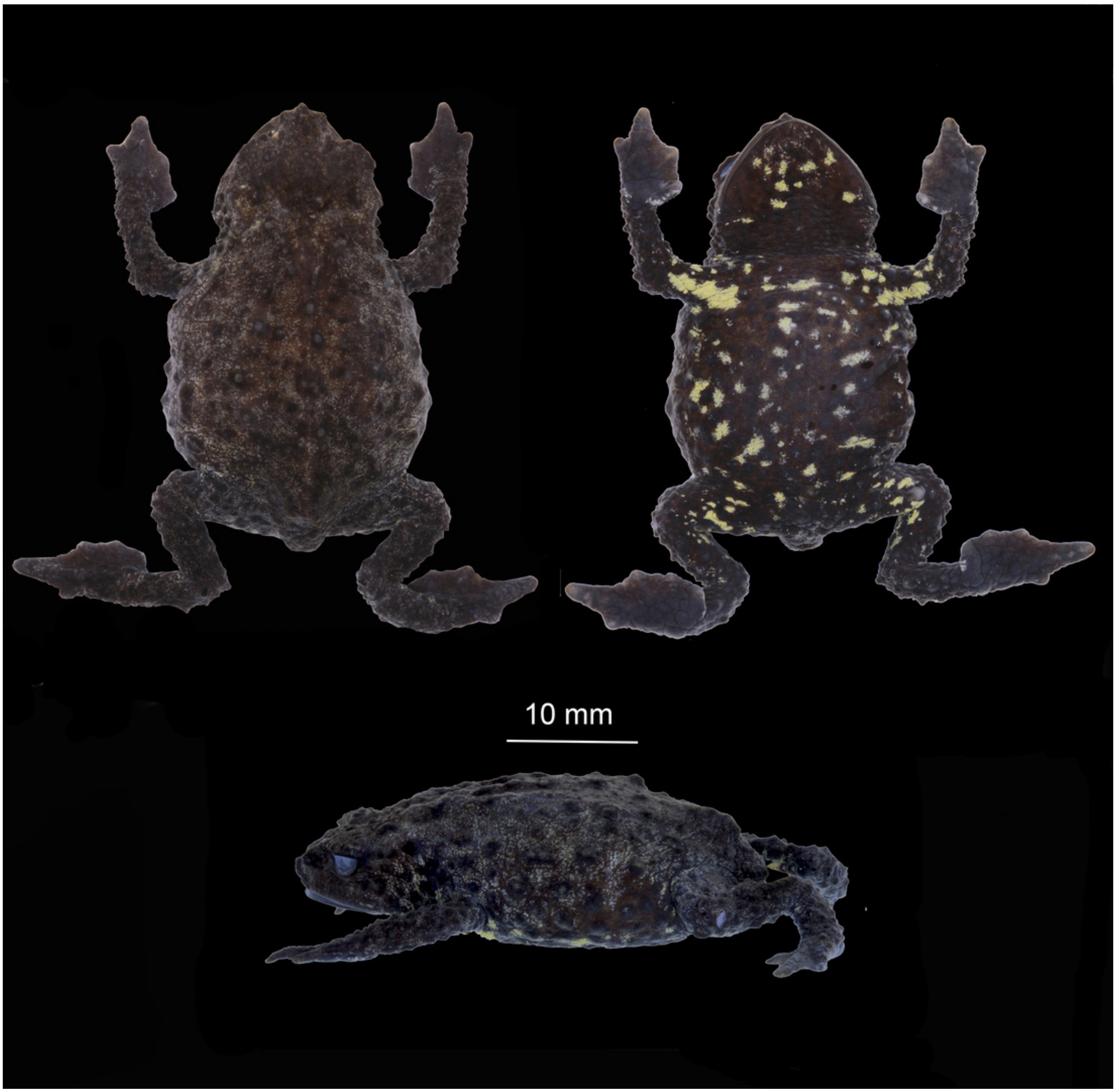

Figure 3

Figure 3. Dorsal and ventral views of hand and foot of preserved *Osornophryne backshalli* sp. nov.; holotype (DHMECN 15260), adult female.

Photographs by Mario H. Yánez-Muñoz.

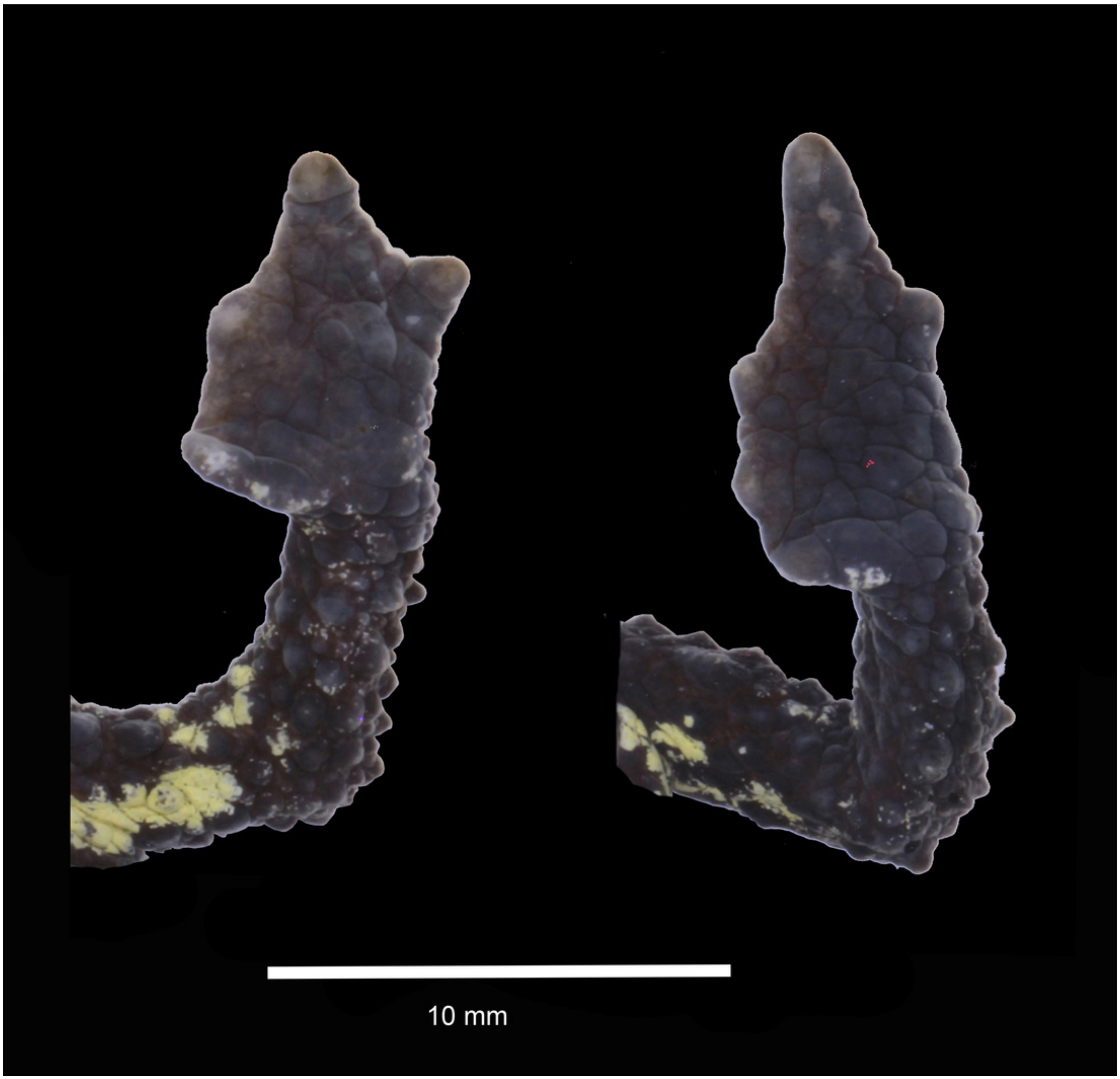

Figure 4

Figure 4. Life comparison of dosrolateral, dorsal and ventral views of female of *Osornophryne backshalli* sp. nov., with its closest relative.

A) *O. backshalli* sp. nov. DHMECN 15260, female Holotype from Cerro Candelaria ; B) *O. sumacoensis* MZUTI 6912, female topotypic from Sumaco volcano. Photographs by Mario H. Yánez-Muñoz (A) and Juan P. Reyes-Puig (B).

Figure 5

Figure 3. Preserved lateral profile view of *Osornophryne backshalli* sp. nov. in comparison with its closest relative species, showing differences among shape, orientation and skin texture of crista parotica underlined in red punctuations.

A) *Osornophryne backshalli* sp. nov., DHMECN15260, Female Holotype; B) *O. sumacoensis* QCAZ 4570, Female Holotype. Photographs by Mario H. Yánez-Muñoz (A) and Juan P. Reyes-Puig (B).

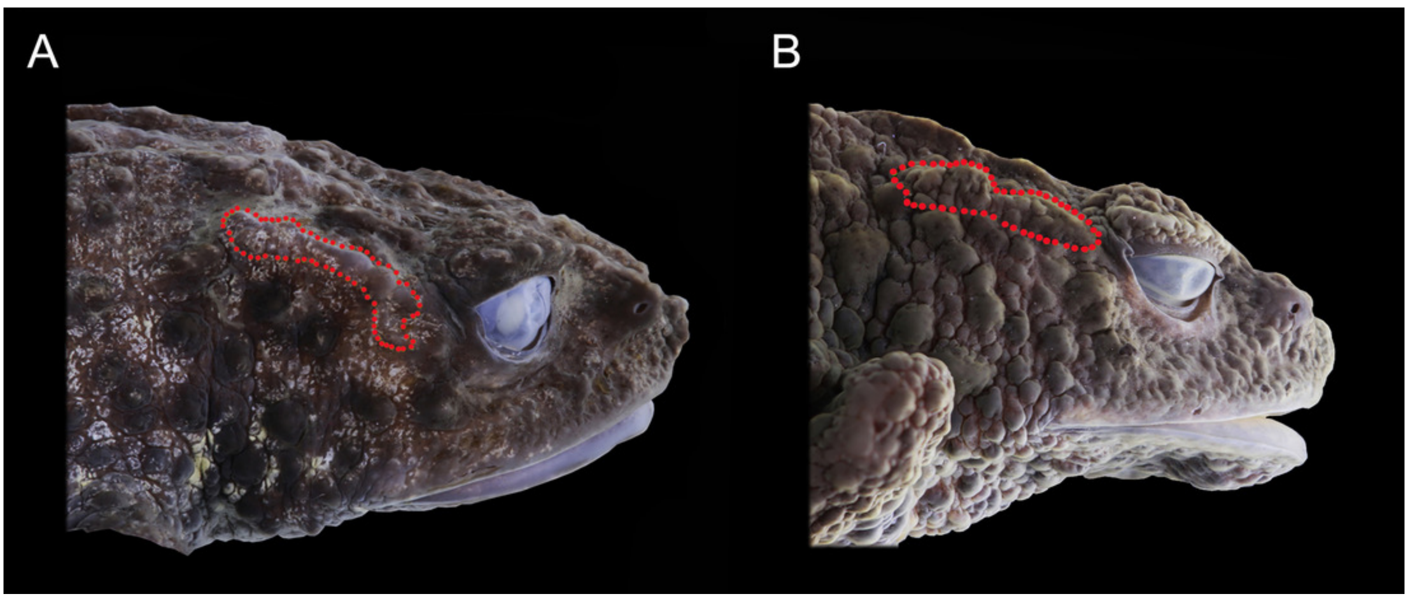

Figure 6

Figure 6. Schematic dorsal, lateral and ventral views of the skull morphology of *Osornophryne backshalli* sp. nov. and its closest relative species *O. sumacoensis*.

A) *Osornophryne backshalli* sp. nov., DHMECN 18363, female paratype; B) *O. sumacoensis,* MZUTI 5147, female . Diagrams by MAUM. Legend. Abreviations: : pmax = premaxilla; max = maxilla; spheth = sphenethmoides; fpar = frontoparietal; pter = pterigoides; pro = prootic; q = quadratojugal; sp = squamosal; exo = exoccipital; prph = parasphenoides. Diagrams Miguel A. Urgilés-Merchan.

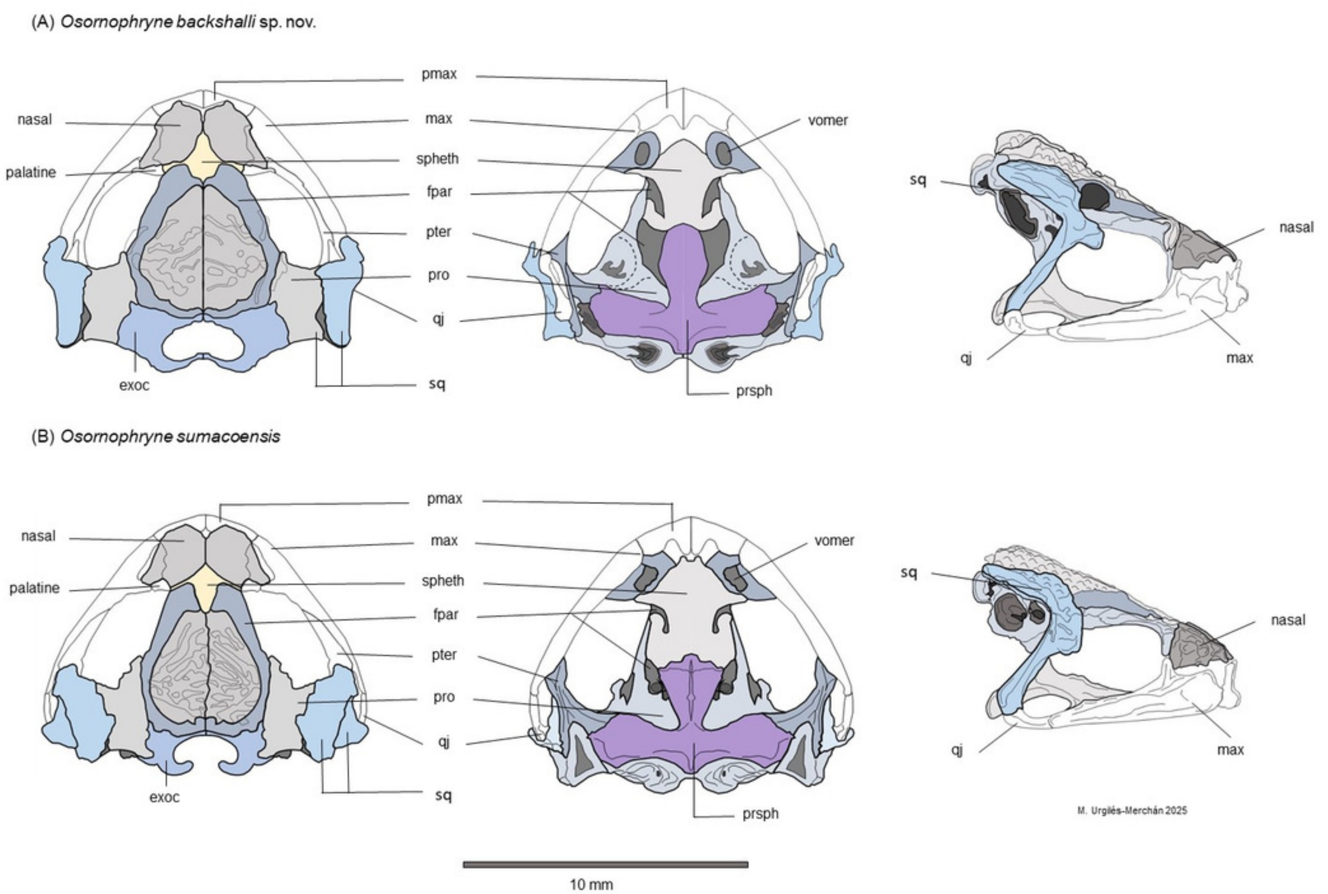

Figure 7

Figure 7. Life coloration and detailed dorsolateral view showing sexual dimorfism of *Osornophryne backshalli* sp. nov in life.

A) DHMECN 15260, Holotype, adult female B); DHMECN 18364, Paratype, adult female; C) DHMECN 18364 Paratype, adult male. Photographs by Mario H. Yánez-Muñoz (A) and Juan P. Reyes-Puig (B, C).

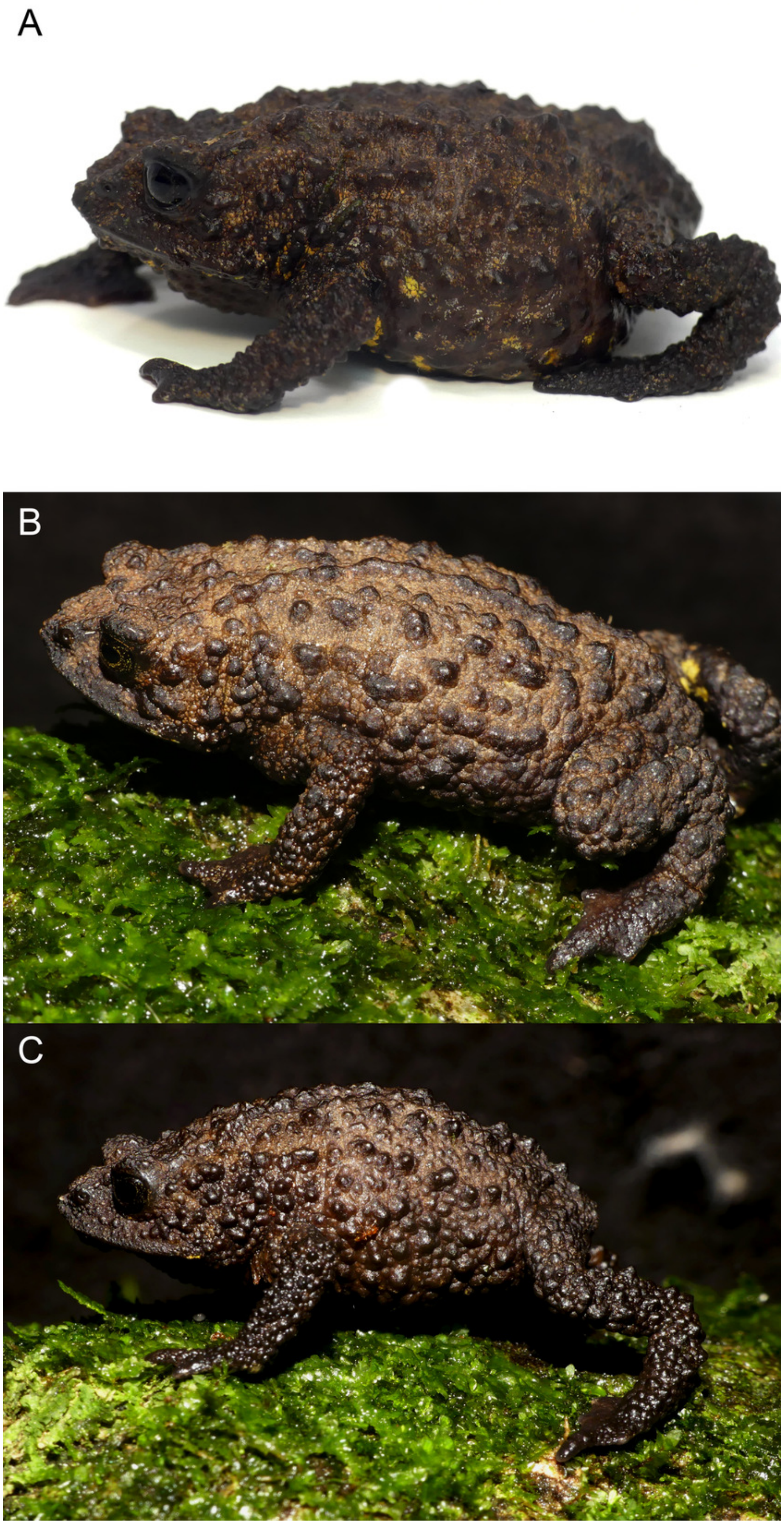
A
B
C

Figure 8

Figure 8. Preserved variation in paratypes of *Osornophryne backshalli* sp. nov.

A) DHMECN 15821, adult male; B) DHMECN 18364 adult male; C) DHMECN 18362 adult female; D) DHMECN 18363, adult female. Photographs Juan P. Reyes-Puig.

Figure 9

Figure 9. Comparison in life coloration of the type series of *Osornophryne backshalli* sp. nov.

A) DHMECN 15260, female holotype; B) DHMECN 18362, adult female paratype; C) DHMECN 18364, adult female paratype; D) DHMECN 18363, adult female paratype. Photographs by Mario H. Yánez-Muñoz (A) and Juan P. Reyes-Puig (B, C, D).


A
B
C
D

Figure 10

Figure 10. Distribution map of *Osornophryne backshalli* sp. nov., and new records of *Osornophryne simpsoni* in red.

Map by Julio C. Carríon.

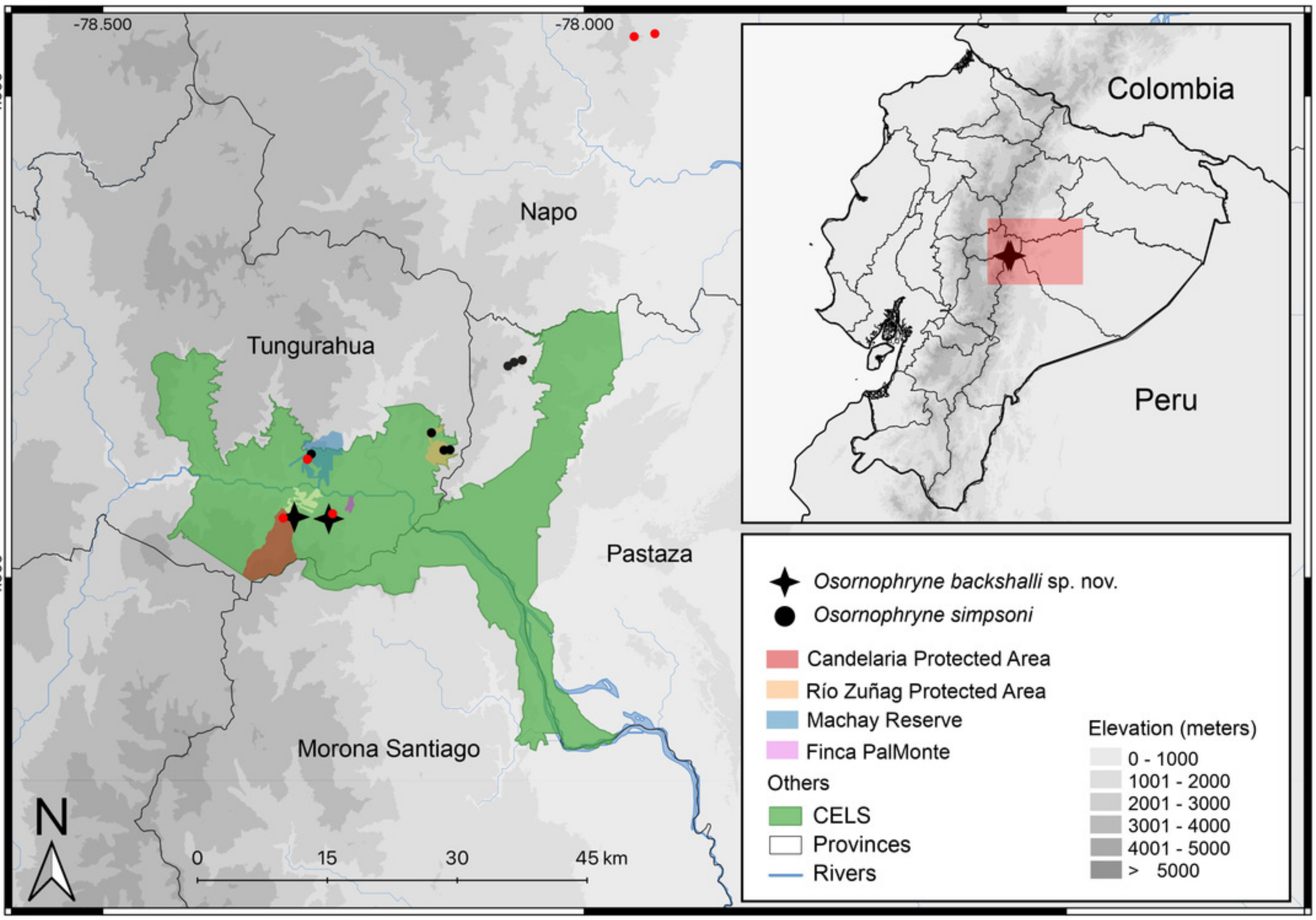

Table 1(on next page)

Estimates of Evolutionary Divergence over Sequence Pairs between species of the Osornophryne guacamayo species 2 group.The number of base differences per site from averaging over all sequence pairs between groups are shown. This analysis 3 involved 60

Characters are based on the last *Osornophryne* revision (Páez-Moscoso & Guayasamín 2012).

Table 1. Estimates of Evolutionary Divergence over Sequence Pairs between species of the *Osornophryne guacamayo* species group.The number of base differences per site from averaging over all sequence pairs between groups are shown. This analysis involved 60 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

	O cofanorum	*O guacamayo*	*O backshalli sp. nov.*	*O sumacoensis*	*O occidentalis*
O cofanorum					
O guacamayo	4.82342%				
O backshalli sp. nov.	4.57269%	3.47798%			
O sumacoensis	4.58222%	4.30845%	3.30210%		
O occidentalis	6.06343%	4.19563%	4.14410%	5.15024%	
O simpsoni	4.87768%	3.43945%	3.19880%	4.01793%	4.03872%

Table 2(on next page)

Morphological diagnostic characters of *Osonophryne backshalli* sp. nov. . in comparison with closest related species with condition of Toe V reduced.

Based on Páez & Guayasamín (2012) and this work.

Table 2. Morphological diagnostic characters of *Osonophryne backshalli* sp. nov. in comparison with closest related species with condition of Toe V reduced, characters are based on the last *Osornophryne* revision (Páez-Moscoso) & Guayasamín 2012).

SPECIES	Dorsolateral fold/ridge	Occidpital folds	Pelvic folds	Flanks texture	Dorsum	Head in dorsal view	Head in profile	Toea III-V condition	Life dorsal coloration	life ventral coloration
Osornophryne backshalli sp. nov.	Absent, row of subconical tubercles discontinuous	Glandular dermic folds forming a "V" extending into the sacrum	Absent	Subconic warts with small tubercles in males than in female	Subconic warts with small tubercles in males than in female;	Rounded with a triangular papilla on the tip of the snout/	Truncated with triangular papilla	Toes reduced with exception of Toe IV; Toe V reduced slightly longer than III/	Light brown background, with dark tones and pale-yellow flecks /	Dark Brown with irregular bright yellow blotches.
Osornophryne sumacoensis	Present pustular ridge	Present continuous or discontinuous	Present	Dominated by relatively small pustules	Dominated by relatively small pustules, with scattered large pustules	Males: Subacuminate, with papilla or very short proboscis. Females: Subacuminate, with papilla at tip	Males: Subacuminate to acuminate, with papilla or very short proboscis, Females: Round to subacuminate, with papilla at tip	Reduced, V same lenght than III	Brown to orange brown, Females dark brown	Males pale brown to orange brown; Females: Pale grayish blue, with dark gray to black blotches
Osornophryne occidentalis	Discontinuous composed of small warts	Discontinuous composed of small warts	Low or absent	Dominated by numerous warts with scattered medium size pustules	Covered of pustules of different sizes providing a wrinkled texture	Acuminated with papilla at tip	Acuminated to subacuminated with papilla at tip	Not reduced, Toe V elongated longer than toe III, and reach medial portion of toe IV	Olive green to Dark brown to black, with or without a cream to light brown dorsolateral stripes.	Males: Light brown to dark brown with few yellow pustules

Table 3 (on next page)

Table 3. Morphometric character measurements in millimeters (mm.), for the type series of *Osornophryne backshalli* sp. nov. See abbreviations on methods section.

See abbreviations on methods section.

Table 3. Morphometric character measurements in millimeters (mm.), for the type series of *Osornophryne backshalli* sp. nov. See abbreviations on methods section.

	***Osornophryne backshalli* sp. nov.**	
	Females (n=4)	Males (n=2)
SVL	30.09 - 35.4 (32.75 ± 3.75)	22.08 - 29.68 (25.88 ± 5.37)
TIB	8.67 - 9.86 (9.27 ± 0.84)	7.01 - 9.11 (8.06 ± 1.48)
FL	9.7 - 10.85 (10.28 ± 0.81)	5.96 - 9.65 (7.81 ± 2.61)
HaL	6.72 - 7.62 (7.17 ± 0.64)	4.91 - 6.9 (5.91 ± 1.41)
HL	9.18 - 10.9 (10.04 ± 1.22)	7.15 - 9.69 (8.42 ± 1.8)
HW	11.13 - 12.71 (11.92 ± 1.12)	8.23 - 10.98 (9.61 ± 1.94)
IOD	2.58 - 3.58 (3.08 ± 0.71)	2.36 - 3.31 (2.84 ± 0.67)
EW	2.52 - 2.8 (2.66 ± 0.2)	1.79 - 2.4 (2.1 ± 0.43)
IND	2.98 - 3.64 (3.31 ± 0.47)	2.72 - 3.18 (2.95 ± 0.33)
EN	1.7 - 2.17 (1.94 ± 0.33)	1.54 - 2.01 (1.78 ± 0.33)
SE	3.9 - 4.52 (4.21 ± 0.44)	3.07 - 4.19 (3.63 ± 0.79)
ED	2.54 - 3.35 (2.95 ± 0.57)	2.35 - 2.9 (2.63 ± 0.39)
FIIIL	5.78 - 6.98 (6.38 ± 0.85)	4.2 - 5.99 (5.1 ± 1.27)
FIVL	4.33 - 4.95 (4.64 ± 0.44)	3.1 - 4.53 (3.82 ± 1.01)
TIVL	8.97 - 10.05 (9.51 ± 0.76)	5.6 - 9.2 (7.4 ± 2.55)
TVL	5.9 - 6.53 (6.22 ± 0.45)	3.87 - 6.36 (5.12 ± 1.76)

Table 4(on next page)

Measurements of the skull and osteological diagnostic characters of *Osonophryne backshalli* sp. nov., in comparison with its sister species *Osornophryne sumacoensis* .

Table 4. Measurements of the skull and osteological diagnostic characters of *Osonophryne backshalli* sp. nov., in comparison with its sister species *Osornophryne sumacoensis* .

SPECIES	Skull width (mm)	Skull length (mm)	Braaincase width (mm)	Parasphenoid alae width (mm)	Cultriform proccess length (mm)	Shape of squamosal	Shape of sphenetmoides	Shape of parasphenoid
Osornophryne backshalli sp. nov.	10.35	9.5	3.36	3.24	3.69	zygomatic ramus elongated with blunt anterior border projected, otic ramus arched	anterior border subacuminated and projected anteriorly at the middle level of nasals, posterior border of sphenethmoides is short and slightly subacuminated	Cultriform process with blunt anterior border
Osornophryne sumacoensis	10.34	9.62	2.95	3.24	3.39	zygomatic ramus short and broad anterior border not projected, otic ramus oblique	anterior border short and acuminated, posterior border t	Cultriform process short and broad with irregular anterior border