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Description of a new species of glassfrog from "El Quimi" Reserve in the Cordillera del Cóndor (Anura, Centrolenidae, Nymphargus)

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INDEX

LIST OF TABLES

LIST OF FIGURES

El presente trabajo se presenta en el formato de la revista ZooKeys a partir de la siguiente página.

DESCRIPTION OF A NEW SPECIES OF GLASSFROG FROM "EL QUIMI" RESERVE IN THE CORDILLERA DEL CONDOR (Anura, Centrolenidae, *Nymphargus*)

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Abstract

With 34 species, the genus *Nymphargus* is characterized by the absence of humeral spines among other characters. To differentiate between the species of this genus can be somewhat complex since you have to look at the details to identify cryptic species. In this study we present an updated phylogeny of the *Nymphargus* genus showing the new species and also the phylogenetic position of *N. buenaventura* for the first time. The new species is characterized by not having spots on the dorsum, shagreen skin and white esophagus and stomach. The new species is closely related to a couple of undescribed species.

Keywords: Taxonomy, *Nymphargus*, Ecuador, Amphibia, Sistematics.

Introduction

The genus *Nymphargus* belongs to the Centrolenidae family, which consists of 12 genera with about 159 species. This family is distributed throughout Central and South America (Guayasamin et al., 2020; AmphibiaWeb, 2021). It has been one of the families that has gradually increased its number in terms of genera and species, being that in 1973 there

were barely 2 genera (*Centrolene* and *Centrolenella*) and only 50 species approximately (Lynch et al., 1973)

Nymphargus is a relatively recent genus, its taxonomy previously placed it within the genus Cochranella, until, in 2007, Cisneros-Heredia & McDiarmid, 2007 separated it based on both molecular and morphological analyses, which elucidated the main characteristics that differentiate these two genera. Thus, it contains about 34 species (Frost, 2021; Guayasamin et al., 2009), while in Ecuador there are 21 species, of which 11 are endemic (Ron et al, 2021). This is a genus distributed throughout the Andes, from Colombia to Bolivia, given that most species inhabit elevations above 1000m, that is, it is restricted to the mountains (Guayasamin et al., 2009, 2020).

Frogs belonging to this genus are characterized by the absence of humeral spines as well as the reduction of the membranes of the outermost fingers (Cisneros-Heredia & McDiarmid, 2007; Guayasamin et al. 2009) The taxonomy of *Nymphargus* can be complex due to identification errors where details that can differentiate species are overlooked, such as the case of *Nymphargus griffithsi* (Goin, 1961) and *Nymphargus lasgralarias* (Hutter et al., 2012) which are morphologically almost identical species, so previously *N. lasgralarias* was found within *N. griffithsi* (Hutter et al., 2012).

There are several species of Centrolenidae that are still undescribed or that confused among taxa, many of them are found in local museum collections without being reported for the country and there are even some with two different names (Cisneros-Heredia et al., 2016), on the other hand the phylogenetic relationships of Centrolenidae are stable and include the majority of species. This facilitates the recognition and separation of cryptic species (Guayasamin et al., 2020).

In an expedition to the El Quimi Reserve, Cordillera del Condor, a team of field researchers from the Zoology Museum at the Pontifical Catholic University of Ecuador (QCAZ) collected a species of *Nymphargus* similar to *N. cariticommatus* that could represent a new species. The purpose of this study is to compare morphological and genetic characteristics of this population with closely related and morphologically similar species of the genus *Nymphargus* to determine its taxonomic status. The comparisons allowed determining that the discovered population represents a new species which we describe herein.

Materials and Methods

Study site

The analyzed specimens were collected in 2017 on the tepui plateau on the eastern side of the Quimi River Valley, El Quimi Biological Reserve, Morona Santiago province, Ecuador. This plateau is located in the southeast of the province, on the border between Ecuador and Peru, it is considered the geological base of the Cordillera del Cóndor and the highest plateau of the Hollin Formation (2000m – 2200m) made up of quartzite sandstone (Pérez et al., 2020; Ron et al., 2018).

Morphological analysis

The characters in the morphology of the El Quimi specimens deposited in the collection of the Zoology Museum (QCAZ) were analyzed and identified, following the taxonomic classification of Cisneros-Heredia et al. (2007)**.**

Characters that, in combination, serve to differentiate species of centrolenids were taken into account (Cisneros-Heredia & McDiarmid, 2007). These characters are the following: vomerine teeth, the shape of the snout: dorsal and lateral; the tympanum: supratympanic fold, tympanic membrane and tympanic ring; texture of the dorsal skin, cloacal ornamentations, color of the peritoneum, external structure of the liver (including hepatic condition), presence or absence of the humeral spine, nuptial excrescences, among others. The definition and terminology of the characters was based on Cisneros-Heredia & McDiarmid (2007). The specimens were compared with specimens of closely related and morphologically similar.

Morphometric analysis

In addition to qualitative characteristics, quantitative measures were also considered, such as the following: Snout–vent length (SVL), head width (HW), head length (HL), horizontal eye diameter (ED), inter–orbital distance (IOD), eye-nostril distance (EN), internarial distance between nostrils (IN), width of disc on the third finger (3DW), tibia length (TL), foot length (FL), tympanum diameter (TD), length of finger I and finger II (FLI and FLII); these variables were measured with digital calipers. Then they were analyzed and

compared with those of related species. The measurement methodology was based on Cisneros-Heredia & McDiarmid (2007).

Phylogenetic analysis

Using DNA sequences generated in the molecular laboratory of the QCAZ museum in combination with sequences deposited in GenBank, we obtained a new phylogeny for Nymphargus. For the molecular phylogenetic analyses, we obtained DNA sequences of the mitochondrial genes 12S, NADH dehydrogenase subunit 1 ND-1. We also obtained sequences for the nuclear gene C-MYC. We sequenced 3 individuals of *Nymphargus dajomesae* sp. nov. Tissues were obtained from the genome bank of the Zoology Museum, Pontificia Universidad Católica del Ecuador (QCAZ). DNA was extracted from liver or muscle tissue preserved in 95% ethanol or tissue storage buffer using guanidine thiocyanate protocol (M. Fujita, unpublished) with some modifications. Primers used for 12S amplification were obtained from Wiens *et al.* (2005), for ND-1 from Wiens *et al.* (2005), Moen and Wiens (2009) and Rivadeneira, Venegas and Ron (2018) and C-MYC from Crawford (2003) and Wiens et al., (2005) listed in Table 1.

PCR amplification was performed under standard protocols and amplicons were sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). Sequences were assembled in GeneiousPro 9.1.8 (Kearse et al., 2012). Vouchers and GenBank accession numbers for newly generated sequences are shown in Table 2.

We complemented our newly generated sequences with homologous sequences of *Nymphargus* downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank) and originally published by Guayasamin *et al.* (2008), Castroviejo-Fisher *et al.* (2014), Cisneros-Heredia *et al*., (2021), Guayasamin *et al.* (2019), Sánchez-Carvajal *et al.* (2021), Twomey, Delia and Castroviejo-Fisher (2014) and Guayasamin *et al.*, (2020). We analized 10 genes: ND1, 12S, 16S (three mitochondrial genes), BNDF, CXCR4, cmyc, POMC, RAG1, SLC8A1 and SLC8A3 (protein-coding genes). The alignment of the sequences was performed in GeneiousPro 9.1.8 (Kearse et al., 2012) with the plug-in MAFFT (Katoh et al., 2013) and a posterior manual alignment with Mesquite v 3.70 software (Maddison & Maddison, 2021). The aligned matrix is composed of 83 individuals and it's available at http://zenodo.org under doi: xxx

Phylogenetic relationships were inferred for all genes concatenated using maximum likelihood (ML) as optimal criterion. Because different evolutionary processes have influenced each gene, we partitioned the data by gene and codon position to find the best model of evolution for each partition. To accomplish this, we used the commands *-m MPF* (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017) and -spp in software IQ-TREE multicore version 1.6.8 (Nguyen et al., 2015). To find the best phylogeny we used the same software under default settings. To assess branch support, we made 1000 ultra-fast bootstrap searchers (-bb 1000 command) and 1000 replicates for the SH-like approximate likelihood ratio test (-alrt 1000 command; Guindon *et al.*, 2010). We consider that branches with ultrafast bootstrap values > 94 and SH-aLRT values > 80 had strong support.

Results

Phylogeny

The phylogeny (Fig.1) is similar to recently published phylogenies of *Nymphargus* (e.g., Guayasamin *et al.*, 2020).The new species *Nymphargus dajomesae* sp. nov. belongs to a clade, with low support (ultra-fast bootstrap = 56), that includes two subclades. One is distributed in the Pacific basin of Ecuador, in the flanks of Cordillera Occidental (*N. griffithsi* + *N. lasgralarias*). The other is distributed in the eastern flanks of the Andes of Ecuador and adjacent Cordillera del Cóndor in the Amazon basin. Remarkably, the eastern clade is composed exclusively by undescribed species: (1) *N. dajomesae* sp. nov., (2) QCAZ 31340 from Estación Cientifica San Francisco, Zamora Chinchipe province, and (3) DHMECN 2249 from San Juan Bosco, Morona Santiago province. The second and third species lack enough material to be described. Based on our genetic and morphological data we are confident to describe *Nymphargus dajomesae* sp. nov. below.

***Nymphargus dajomesae* sp. nov.**

Holotype: QCAZ 68586 – SC 59154, adult male, collected from the buffer zone of the El Quimi Biological Reserve (3,51925º S, 78,38369º O; 2067 m), Zamora Chinchipe Province, Ecuador, by Diego Almeida, Darwin Núñez, Eloy Nusirquia, Alex Achig and Ricardo Gavilanes on July 7, 2017.

Paratypes: QCAZ 68522–26, adult males collected by Diego Almeida, Darwin Núñez, Eloy Nusirquia, Alex Achig and Ricardo Gavilanes on July 7, 2017 at the same location as the holotype.

Etymology: The specific name is given in honor of Neisi Dajomes, who is the first Ecuadorian woman to win a gold medal at the Tokyo 2020 Olympic Games in the weightlifting category.

Identification: *Nymphargus dajomesae* has a uniform yellowish-green color without spots on the back, while the fingers and toes are yellow; it has iridophores that cover almost the entire parietal peritoneum (condition P4); small body size (male SVL 21.2 – 27.2 mm), absence of humeral spine. It can be confused with *Nymphargus cariticommatus* (Wild, 1994), *N. wileyi* (Guayasamin et al., 2006)*, N. griffithsi* (Hutter et al., 2012)*, N. posadae* (Ruiz-Carranza et al., 1995) and *N. buenaventura* (Cisneros-Heredia et al., 2007) . However, it differs due to the absence of spots on the back. The new species is also similar to *N. posadae* which differs by having dorsal skin with numerous small warts (dorsal skin shagreen in *N. dajomesae*), and white ventrolateral border of arm, finger IV, tarsus, and Toe V (barely perceptible enamel in *N. dajomesae*).

Nymphargus wileyi can be distinguished from the coloration of the iris, (coppery white with black reticulations in *N. wileyi*, vs. golden with fine and dark reticulations in *N. dajomesae*). *Nymphargus buenaventura* has scattered flat tubercles on the back skin corresponding to light spots and extensive micro granulations, a condition absent in *N. dajomesae. Nymphargus griffithsi* differs by the shape of the snout (truncated in both dorsal view vs. rounded in *N. dajomesae*); in addition, *N. griffithsi* has enameled ulnar and tarsal folds (ulnar folds absent and non–enameled tarsal folds in *N. dajomesae*).

Nymphargus dajomesae superficially resembles *N. cariticommatus.* However, the dorsum of *N. cariticommatus* has small yellow spots (yellow spots absent in *N. cariticommatus*). Available data suggests that both species differ in body size (the single known adult male of *N. cariticommatus* has SVL= 23.3 mm (Guayasamin et al., 2020; Wild, 1994); *Nymphargus dajomesae* adult males SVL= 21.2–27.2 mm). Also *N. cariticommatus*' iris is silvery white color with thin black reticulation and a yellow ring around pupil (Guayasamin et al., 2020)whereas that *N. dajomesae* has a golden iris with dark fine reticulations. *Nymphargus dajomesae* does not have papillae on the tip of toes and presents very dense patches of lateral glands in males (Sánchez-Carvajal et al., 2021).

Diagnosis: (1) Vomerine teeth absent; (2) snout truncated in dorsal view and profile; loreal region slightly concave; (3) tympanum evident and moderate in size (tympanum diameter 28.2% – 34.5% of eye diameter), dorsal edge of tympanic annulus covered by supratympanic fold, tympanic membrane similar in color to surrounding skin; (4) dorsal skin shagreen; (5) the ventral view of the area between the legs, shows pair of unenameled sub-cloacal warts; the postero-dorsal view shows a granular area enameled; (6) iridophores cover almost the entire parietal peritoneum (condition P4), white pericardium, iridophores restricted to the esophageal peritoneum and the stomach peritoneum, apparently there's no iridophores in the intestines (condition V2); peritoneum around the kidneys white with small translucent spots; (7) liver lobate, covered by translucent peritoneum (condition H0); (8) humeral spines absent; (9) webbing absent between inner fingers I and II, basal webbing between fingers II and III; reduced webbing between outer fingers, webbing formula III ($2^{2}/_{3}$ –$2^{1}/_{2}$) IV; (10) foot webbing formula: I (2^{-})–(2^{-}–2^{+}) II (1– $1^{1}/_{2}$)–(2^{+}–$2^{1}/_{2}$) III (1^{+}–$1^{2}/_{3}$)–(2–2^{+}) IV ($2^{1}/_{2}$–$2^{2}/_{3}$)–($1^{2}/_{3}$–2^{-}) V; (11) no dermal folds or tubercles on hands, forearms or feet; tarsal fold low, smooth, and without tubercles; (12) prepollex concealed; in males, Nuptial pad Type I; (13) finger II slightly longer than finger I; (14) width of the disc of finger III about 47.6% –72.3% of the diameter of the eye; (15) in life, the color of the dorsum varies from yellowish green to opaque or dark green, it does not have flecks or spots; green bones; (16) in preservative, in males, dorsum with lavender coloration due to melanophores in the dorsal surface from snout to low back, less concentration of melanophores as punctuations on the dorsal surface of the limbs, some can show scattered light blue spots; (17) in life, general golden coloration, dark fine reticulations, more conspicuous point on the upper side than on lower side of the iris and apparent absence of circumpupillary ring; in preservative, iris greyish with purple reticulation and some purple flecks, apparent absence of circumpupillary ring; (18) in life, yellow fingers and toes; in preservative, Pale colored hands and feet, have melanophores reaching finger III (in some cases) and finger IV and toes IV and V; (19) the spectral and temporal characteristics of the call as well as the site from which it calls are unknown; (20) Fighting behavior unknown; (21) Egg clutches and parental care unknown; (22) tadpoles unknown; (23) small body size; in males, SVL 21.2 – 27.2 mm (X= 22.6 mm; n = 6).

Holotype description: Adult male, SVL 23.3 mm. Medium-sized and slender body. Head slightly wider than long (head length 75.6% of head width). Snout rounded in dorsal view, and truncated in profile; canthus rostralis indistinct, slightly concave; slightly concave loreal region; lips slightly flared; nostril protuberant, closer to tip of snout than to eye,

directed fronto-laterally; slightly depressed inner area. Eyes large, directed antero-laterally at an angle of ~39º from the midline, transverse diameter of the disc of Finger III 55% of the diameter of the eye. Weak supratympanic fold from behind eye to insertion of arm, tympanic annulus oriented primarily vertically with slight posterolateral inclination, tympanic membrane pigmented like surrounding skin.

Dentigerous processes of vomer without teeth; tongue ovoid, not notched or indented posteriorly, vocal slits paired, extending from mid-tongue to angles of jaws. Skin shagreen on dorsal surfaces of head, body, and lateral surface of head and flanks, ventral skin granular. The cloacal area shows a granular enameled area in the posterodorsal area, two enlarged unenameled sub-cloacal warts (in preservative).

Upper arm thin, forearm moderately robust, width of upper arm about half that of forearm. Humeral spine absent; no skin folds or tubercles on hands, forearms, or feet; relative lengths of the fingers: III > IV > II > I; absent webbing between inner toes (I and II), basal webbing between fingers II and III, finger webbing formula: III ($2^{2}/_{3}$ –$2^{1}/_{2}$) IV; bulla absent, wide and slightly truncated finger discs, disc in the third finger slightly larger than those in the toes, and smaller than eye diameter, 3DW/ED = 0.55; subarticular tubercles rounded and elevated, supernumerary tubercles present; palmar tubercle large, rounded and elevated; thenar tubercle elliptic. Prepollex concealed, nuptial pad Type I, ovoid, granular, and unpigmented extending from the ventrolateral base to the dorsal surface of finger I of the hand, covering its base. Hind limbs slender, tibia length 54% of SVL, foot length 39,4% of SVL. Tarsal fold low, smooth and without tubercles; foot webbing formula: $I2^{-}$–$2^{+}II1^{+}$–$2^{+}III1^{1}/_{3}$–$2^{+}IV2^{1}/_{2}$–$1^{2}/_{3}V$; elliptical toe discs; rounded disc pads. (Fig.3)

Variation

Some individuals have sub-cloacal warts more rounded (QCAZ 68523, 68524) than others that have quite elliptical (QCAZ 685262, 68525, 68526, 68586). Variation in color of live and preserved individuals is shown in Figures 4 and 5. Variation in size is shown in Table 3.

Ecology: *Nymphargus dajomesae* sp. nov. is active during night. All individuals were found perching on leaves between 60 to 180 cm from the ground, near streams in areas with low shrubby vegetation (1.50 m in height) with widely spaced trees (height 10-15 m). Some frogs were found on bromeliads. Some males were calling.

Distribution: *Nymphargus dajomesae* sp. nov. is found between 1992 and 2090 m at a single locality in the upper part of the El Quimi Biological Reserve in Cordillera del Condor, Zamora Chinchipe province, Ecuador (Fig. 6).

Conservation status: based on IUCN criteria (IUCN, 2012), we consider *N. dajomesae* sp. nov. as Data Deficient (DD). Population status of this species is unknown. Surveys of amphibians in Cordillera del Cóndor have been scant and sporadic (Ron et al., 2018). Therefore, the possibility of the discovery of additional populations of *N. dajomesae* sp. nov. cannot be ruled out.

Discussion

White esophagus (V3 condition) was previously reported just for *N. cariticommatus* (Wild, 1994), now *N. dajomesae* apart from presenting that character, shows a white peritonea that covers the stomach. Whereas white kidney with translucent spots has been reported for *N. wileyi* (Guayasamin et al., 2006), this character is also present in *N. dajomesae*.

N. dajomesae shows papillae at the tip of toes in some individuals, this character has been seen in several centrolenid species but it is not considered a taxonomic character due to the variation of its presence even within the same species as it was reported by Sánchez-Carvajal *et al.* (2021), however, it is important to mention it, in case that future investigations manage to elucidate the papillae purpose.

Meanwhile, on previous phylogenies QCAZ31340, was reported as *N.* aff. *cochranae* (Guayasamin et al., 2008), but our phylogeny shows that is not even related to that species, it is sister to the clade of *N. dajomesae* (Fig. 1). Moreover, QCAZ31340 and DHMECN2249, sister species of *N. dajomesae*, must be reviewed to clarify the relationships within them. Our phylogeny shows, for the first time, the phylogenetic position of *N. buenaventura*.

Conclusion

Nymphargus dajomesae sp. nov. has distinctive and cryptic characters that allows us to separate from other species of *Nymphargus*. The phylogeny locates them as a separate clade

with two sister species that have not yet been described. It's geographical location, which is an almost unexplored area, makes them hard to find and also makes us guess if they are range – restricted species.

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391 **Tables**

392 **Table 1.** Genes and primers used in this study.

Gene	**FPrimer**	**RPrimer**
12S	tPhe-frog	tVal-frog
ND-1	ND1-210F	WL384
	WL379b	tMet-frog
	ND1-F1	WL384
C-MYC	cmyc1U	cmyc-ex2 R

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Table 2. GenBank accession numbers for newly generated DNA sequences used in the phylogenetic analyses.

Species	**Voucher**	**12S**	**ND-1**	**C-MYC**
Nymphargus dajomesae	QCAZ 68522	(To be added)	(To be added)	(To be added)
Nymphargus dajomesae	QCAZ 68526	(To be added)	(To be added)	(To be added)
Nymphargus dajomesae	QCAZ68586	(To be added)	–	–

436 **Table 3.** Variation of the measurements (in mm) of *Nymphargus dajomesae*. Abbreviations
437 are: SVL – Snout-vent length, HW – Head width, HL – Head length, ED – Horizontal eye
438 diameter, IOD – Inter-orbital distance, EN – Eye-nostril distance, IN – Internarial distance,
439 TL – Tibia length, FL – Foot length, 3DW - Width of disc on the third finger.

Adult males (n = 6)			
SVL	22.6 ± 2.2 (21.2 – 27.2)	EN	1.8 ± 0.3 (1.4 – 2.3)
HW	8.3 ± 0.9 (7.6 – 10.2)	IN	1.9 ± 0.2 (1.7 – 2.4)
HL	5.3 ± 0.9 (5.7 – 8.3)	TL	12.6 ± 1.6 (11.5 – 15.6)
ED	2.8 ± 0.1 (2.5 – 2.9)	FL	9.5 ± 1.7 (8.3 – 12.8)
IOD	3.6 ± 0.3 (3.3 – 4.2)	3DW	1.6 ± 0.3 (1.3 – 2.1)

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Figures

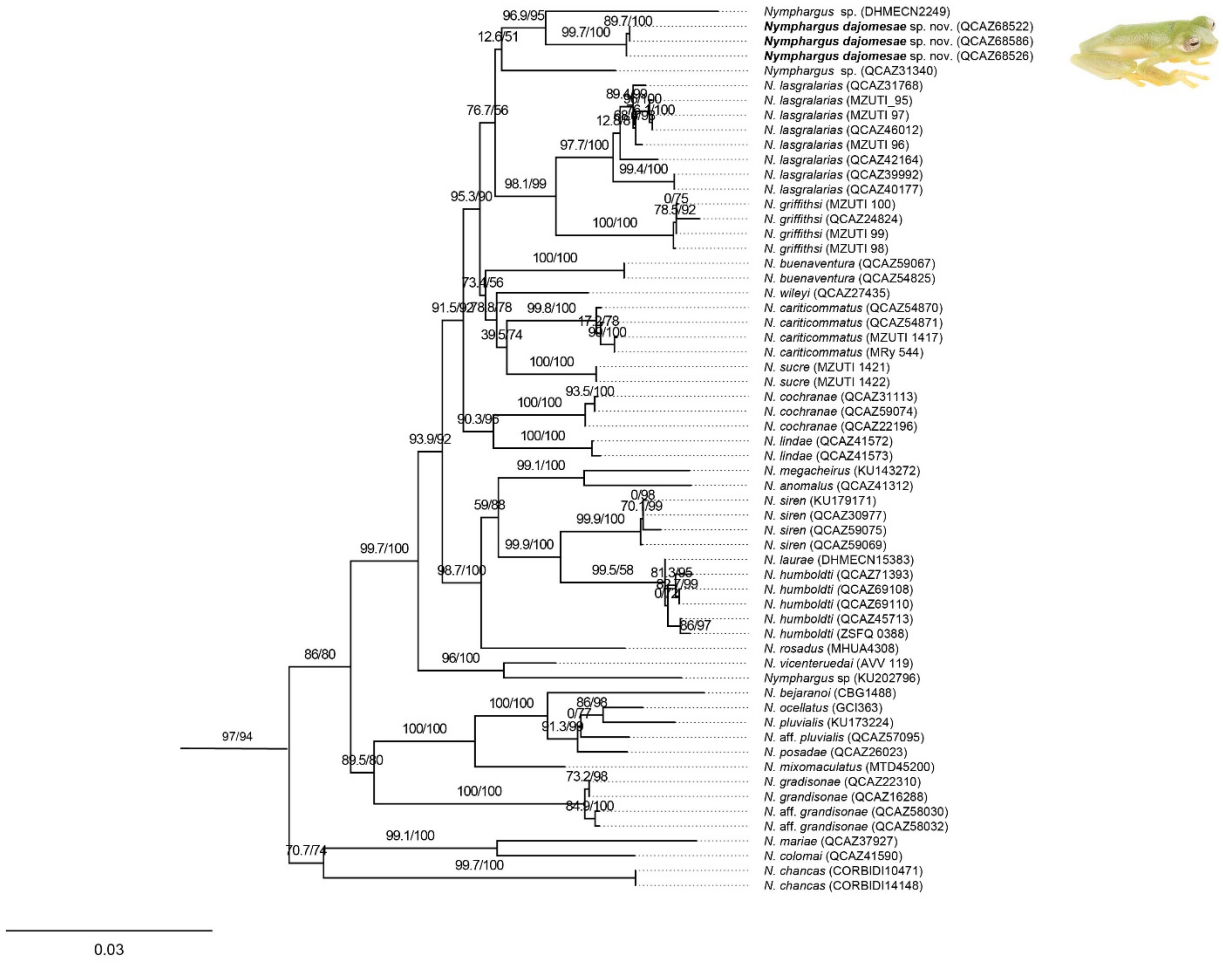


Figure 1. Maximum likelihood tree of *Nymphargus* inferred from a partitioned analysis of 6563 aligned sites of DNA sequences of 10 nuclear and mitochondrial loci. SH-aLRT support (before slash) and ultra-fast bootstrap support (after) are shown as percentages on branches. The species name is followed by the specimen voucher number in parenthesis. Outgroups are not shown. Nymphargus dajomesae sp. nov. is shown in bold.

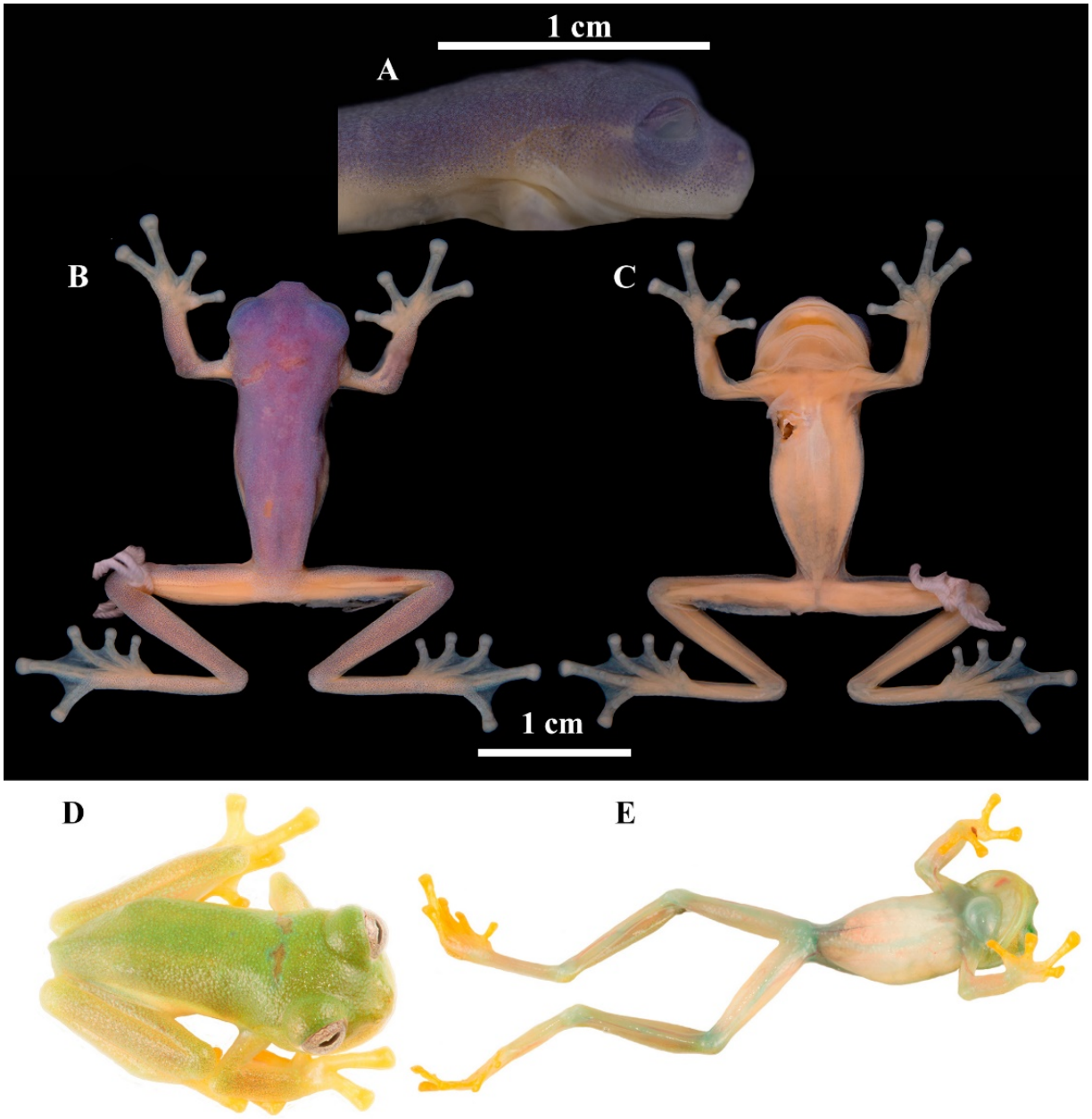


Figure 2. Holotype of *Nymphargus dajomesae* sp. nov. QCAZ68586, adult male, SVL= 23.3 mm. El Quimi Biological Reserve, Zamora Chinchipe Province, Ecuador. **A** lateral view of head of preserved individual, **B** dorsal view of preserved individual, **C** ventral view of preserved individual, **D** dorsal view of live individual, **E** ventral view of live individual Photographs **A, B, C** by Mylena Masache; **D** and **E** BIOWEB-Museo QCAZ archive.

Figure 3. Ventral views of the right hand and foot of *Nymphargus dajomesae* sp. nov. Holotype (QCAZ 68586). Photographs by Mylena Masache.

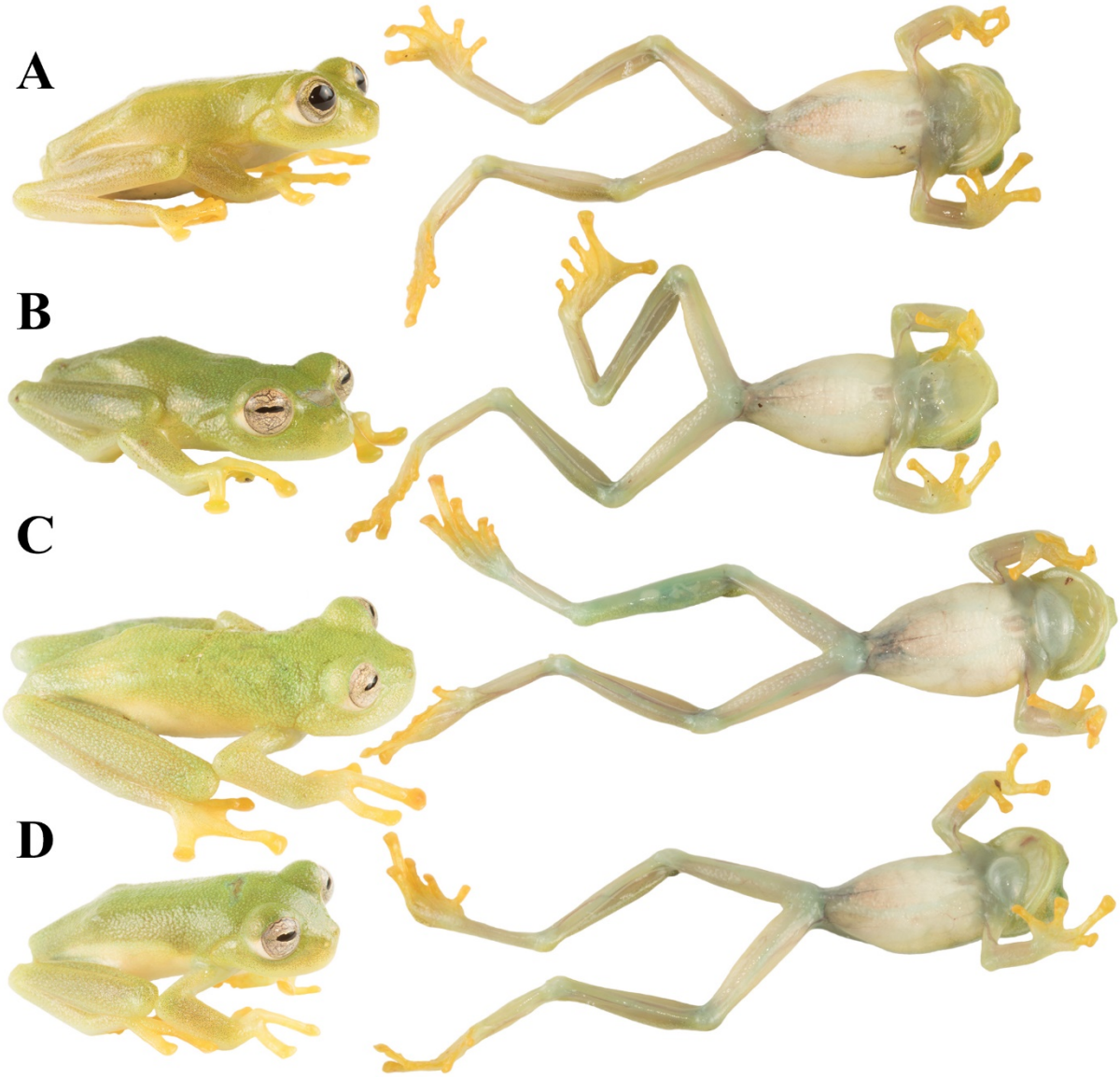
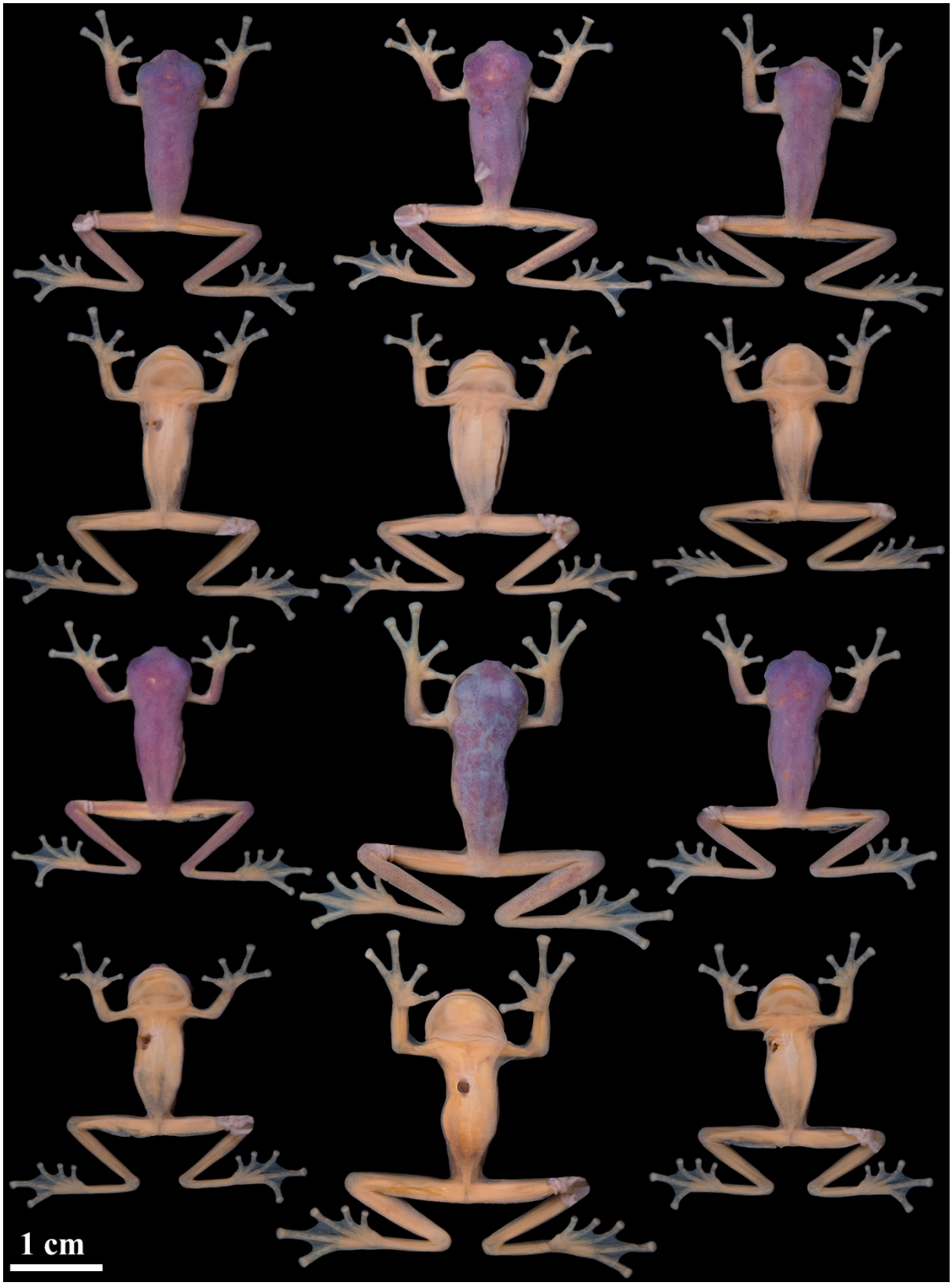


Figure 4. Variation of live specimens of *Nymphargus dajomesae* sp. nov. Dorsal and ventral view: **A, B, C, D** QCAZ 68522, 68524, 68526, 68586; all males.



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491 **Figure 5.** Variation of preserved specimens of *Nymphargus dajomesae* sp. nov. Dorsal and
492 ventral view from left to right, first and second rows: QCAZ 68522, 68523, 68524; third and
493 fourth rows: QCAZ 68525, 68526, 68586; All males. Photographs by Mylena Masache.

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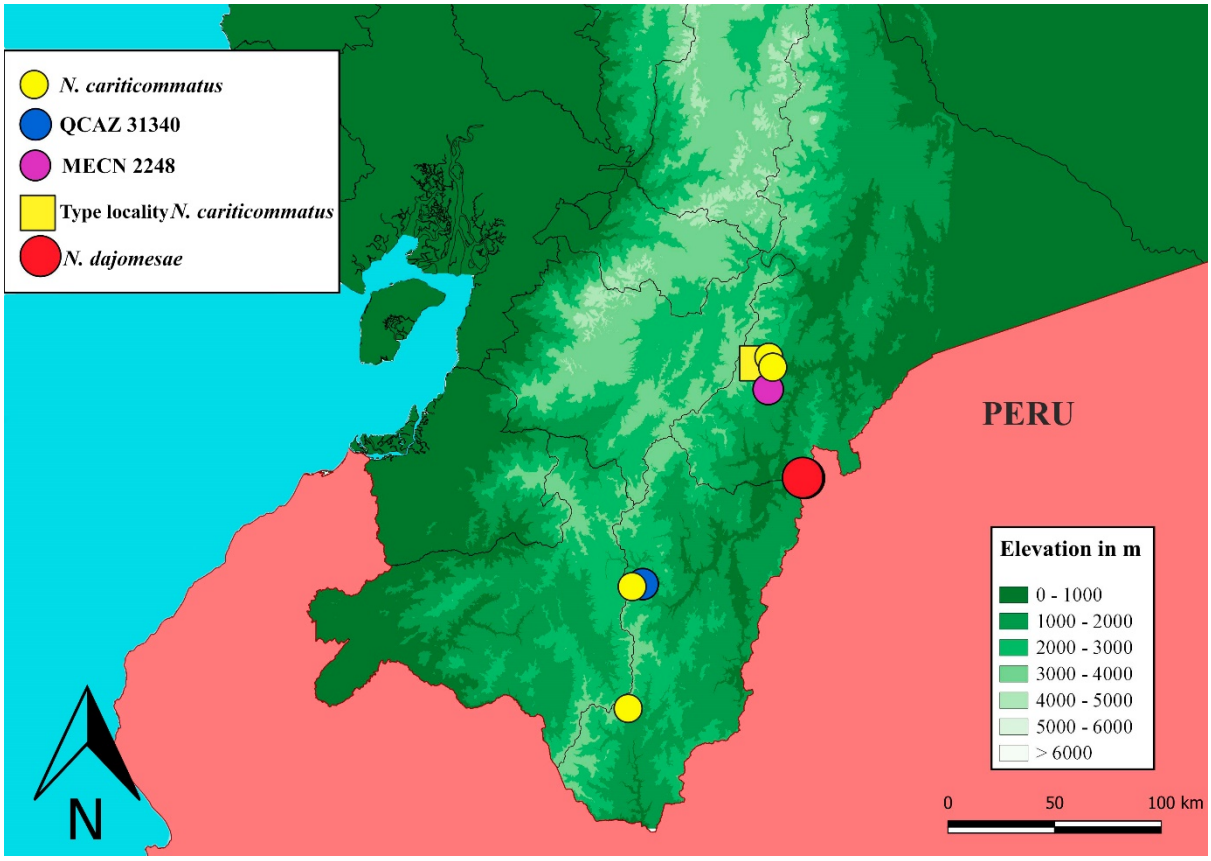

Figure 6. Distribution of *Nymphargus dajomesae* sp. nov. in Ecuador (red dot).