

Phylogenetic position of the glassfrog “*Cochranella*” *megista* (Anura: Centrolenidae) and first records for Ecuador

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Abstract

Phylogenetic position of “*Cochranella*” *megista* (Anura: Centrolenidae) and first records for Ecuador. “*Cochranella*” *megista* is an Endangered and rarely encountered species of glass frog that, until now, had been only registered in the Colombian Andes. Here we report this species for the first time in Ecuador, expanding its known distribution ca. 530 km south of its original range. Additionally, we include *C. megista* in a molecular phylogeny for the first time and unambiguously place the species in the genus *Nymphargus*, resulting in a new combination. Habitat in both countries is fragmented and is threatened by mining concessions and agriculture.

Keywords: Amphibians, conservation, Greater Andean Glassfrog, *Nymphargus*, Río Manduriacu Reserve, threatened species.

Resumo

Posição filogenética de “*Cochranella*” *megista* (Anura: Centrolenidae) e primeiros registros para o Equador. “*Cochranella*” *megista* é uma perereca-de-vidro ameaçada e raramente encontrada, que até o momento só havia sido registrada nos Andes colombianos. Relatamos aqui a presença dessa espécie pela primeira vez no Equador, expandindo sua distribuição conhecida para cerca de 530 km ao sul de sua área de distribuição original. Adicionalmente, incluímos *C. megista* em uma filogenia molecular pela primeira vez e alocamos inequivocamente a espécie no gênero *Nymphargus*, resultando em uma nova combinação. O habitat em ambos os países é fragmentado e está ameaçado pelas concessões mineiras e pela agricultura.

Palavras-chave: anfíbios, conservação, espécie ameaçada, *Nymphargus*, perereca-de-vidro-andina-grande, Reserva do Río Manduriacu.

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Introduction

The highest diversity of glassfrogs (Centrolenidae) is concentrated in the northern Andes, with 83 species (Guayasamin *et al.* 2020). Still, new records (e.g., Culebras *et al.* 2020) and descriptions of new species (Guayasamin *et al.* 2019a, b, 2020) increase our knowledge about their diversity and biogeography nearly every year. Under this context, prolonged fieldwork programs often result in the discovery of species that are rare, have low detectability, or inhabit microhabitats that are difficult to access by herpetologists. Such efforts are particularly important in Andean forests, where glassfrog diversity is high and many species are poorly known, and generally restricted by more narrow breadths of suitable habitat than lowland species (Guayasamin *et al.* 2020). One such species is “*Cochranella*” *megista*, a relatively large glassfrog currently known only from the Pacific slopes of the Colombian Andes, from the Departments of Antioquia, Chocó, Risaralda, and Valle del Cauca at elevations of 1,400–2,100 m a.s.l. (Rivero 1985, Acosta-Galvis 2000, Rada and Guayasamin 2008, IUCN SSC Amphibian Specialist Group 2017). Little is known about this taxon with only 12 observations reported since the type specimen and Neotype (MHUA 5851; Rada and Guayasamin 2008) were collected in 1980 (Rivero 1985). A photo record of *C. megista* on iNaturalist (2012) corresponds to a field observation made during the collection effort that yielded the ICN specimens in the 1990s (M. Rada pers. comm.), from which Bernal and Lynch (2008) cite 11 museum specimens (ICN 17242-44, 27763-8, 27718, 28796). Only one additional observation was recorded after the ICN specimens were collected, which was a single observation from the type locality of Parque Nacional Orquideas, Colombia (IUCN SSC Specialist Group 2017). Moreover, in the most recent taxonomic review of glassfrog systematics (Guayasamin *et al.* 2009), “*Cochranella*” *megista* was considered of uncertain generic placement within Centrolenidae

because its unusual combination of morphological traits (i.e., presence of webbing between Fingers III and IV, absence of humeral spines) and the lack, at that moment, of complementary molecular data.

Here we report the first records of *C. megista* from Ecuador, representing a substantial range extension and only the second set of observations in over 20 years. Additionally, we include, for the first time, DNA sequences of *C. megista* into a phylogenetic analysis, thereby improving our understanding of the evolutionary relationships of the species. This new record increases the number of glassfrogs in Ecuador to 61 species (updated from Guayasamin *et al.* 2020), and is the ninth glassfrog species known to occur at the relatively small Río Manduriacu Reserve (Maynard *et al.* 2020).

Materials and Methods

Study Sites

Fieldwork was primarily carried out at the Río Manduriacu Reserve (RMR), a protected area managed by Fundación EcoMinga (<https://ecomingafoundation.wordpress.com/about/>), located on the Pacific Andean slopes in western Imbabura, Ecuador (see Lynch *et al.* 2014, Guayasamin *et al.* 2019b, Maynard *et al.* 2020; Figure 1). Opportunistic surveys were also carried out at Séptimo Paraíso (0.0293 S, 78.7652 W; 1,550 m a.s.l.), a locality 40 km from RMR, near the town of Mindo, in Pichincha Province. Habitat at RMR and Séptimo Paraíso consists of primary and mature secondary lower montane and cloud forest habitat. The habitat surveyed for *C. megista* consists of primary cloud forest and is located along a mountain crest at elevations 1,800–2,000 m a.s.l.

Sampling time frames at RMR are outlined in Guayasamin *et al.* (2019b) and Maynard *et al.* (2020); however, sampling concentrated within cloud forest habitat occurred from 01 to 06 March 2019 (RJM, SK, SJT, JC, José Maria Loaiza, Rolando Peña, Fabricio Narvaez, and



Figure 1. Geographic distribution of *Nymphargus megistus* in Colombia and Ecuador: **A**, type locality (red dot), published in the original description by Rivero (1985), Parque Nacional Natural “Las Orquídeas”, Antioquia Department; **B**, on border of the Chocó and Valle del Cauca Departments; **C**, Municipality of Pueblo Rico, Risaralda Department; **D**, Municipality of Carmen de Atrato, Chocó Department; **E**, Municipality of Sipí, Chocó Department; **F**, new record, Río Manduriacu Reserve, Imbabura Province, (yellow dot); **G**, new record, near Mindo, Pichincha Province, (yellow dot). Map constructed using Google Earth Pro (7.3.3.7699).

two assistants) and 02 to 09 December 2019 (RJM, SJT, JC, José María Loaiza, Rolando Peña, and one assistant). Visual encounter surveys in RMR were conducted along transects of various lengths within primary cloud forest, and along five narrow streams (i.e., 1–3 m wide) between 19:00 and 02:00 h. General area searches were conducted when vegetation was too dense for transects of the stream. Data collection included the following: relative humidity, ambient temperature, date, time of observation, geographic coordinates, sex, age class, behavior (if any), snout–vent length, perch height, and perch diameter (when applicable). Climate data were collected using a Kestrel 3500 Weather Meter, geographic coordinates with a

Garmin GPSmap 62s handheld unit (WGS84 datum), and SVL with dial calipers.

Collected specimens were euthanized using benzocaine and were fixed and preserved in 70% EtOH. Muscle and liver samples were preserved in 96% EtOH. Specimens were deposited at the Museo de Zoología of the Universidad San Francisco de Quito (ZSFQ) under permits No. 018-2017-IC-FAU-DNB/MAE and No. MAE-DNB-CM-2018-0105 by the Ministerio del Ambiente del Ecuador. The identification of preserved specimens as “*Cochranella*” *megista* was based on the following diagnostic traits: large body size (SVL in adults > 30 mm), green dorsum with black spots, lack of humeral spines, and moderate webbing between Fingers

III and IV (Rada and Guayasamin 2008); no other glassfrog from Pacific slopes of the Andes of Ecuador and Colombia has the aforementioned combination of traits. The study was carried out in accordance with the guidelines for use of live amphibians and reptiles in field and lab research (Beaupre *et al.* 2004), compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR).

Terminology

For taxonomic nomenclature in glassfrogs, we follow the proposal by Guayasamin *et al.* (2009); note that quotation marks around a genus name indicate that the placement of a species in that genus is uncertain. For general terminology and descriptions of morphological characters we follow the proposals by Lynch and Duellman (1973), Cisneros-Heredia and McDiarmid (2007), and Guayasamin *et al.* (2009, 2020). Webbing formulae follow the method of Savage and Heyer (1967), as modified by Guayasamin *et al.* (2006).

Molecular Sequences and Phylogenetic Analysis

Genetic sequences for a fragment of the mitochondrial gene 16S of "*Cochranella*" *megista* were generated at the Laboratorio de Biología Evolutiva, Universidad San Francisco de Quito, following Guayasamin *et al.* (2008). The phylogeny was inferred with the Maximum likelihood (ML) criterion using the taxon and gene sampling described in Guayasamin *et al.* (2019a), which includes all 12 genera recognized within the centrolenid family (Guayasamin *et al.* 2009). ML trees were estimated using GARLI 0.951 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006). GARLI uses a genetic algorithm that finds the tree topology, branch lengths, and model parameters that maximize lnL simultaneously (Zwickl 2006). Default values were used for other GARLI settings, as

per recommendations of the developer (Zwickl 2006). Bootstrap support was assessed via 1,000 pseudoreplicates under the same settings used in tree search. Genetic distances (uncorrected *p*) between the new species and its closest relatives were calculated using PAUP v.4.0a (Swofford 2002).

Results

Nymphargus megistus (Rivero, 1985)

Figures 1, 2

Centrolenella megista Rivero, 1985

Cochranella megista Ruiz-Carranza
and Lynch, 1991

"*Cochranella*" *megista* Guayasamin *et al.*, 2009

Nymphargus megistus, **new combination**

Common name.—We propose the common name of "Greater Andean glassfrog" for *Nymphargus megistus*, following the criteria defined by Coloma and Guayasamin (2020). The Greek word *megistos* means "largest" or "greatest". In Spanish the common name is "rana de cristal andina grande".

Identification.—*Nymphargus megistus* can be distinguished from all other species of *Nymphargus* by exhibiting the following combination of features: large size (SVL in adult males = 30.8–37.4 mm SVL; in adult females = 36.9–40.0 mm), green dorsum with black spots, moderate webbing between Fingers III and IV of the hand, absence of humeral spines in adult males, and lacking iridophores on the gastrointestinal peritoneum (Rada and Guayasamin 2008). *Nymphargus megistus* is an atypical species of the genus *Nymphargus*, where all species have a reduced webbing between Finger III and IV. In contrast, the hand webbing in *N. megistus* is more extensive: III 2—(1 1/2–1 3/4) IV. No additional variation or sexual dimorphism has been observed.

Evolutionary relationships and generic placement.—Our phylogenetic results (Figure 3) clearly indicate that the species is part of the genus *Nymphargus*. Note that, as explained in

the methods section, our taxon sampling included taxa from all the currently recognized genera in Centrolenidae. Thus, we formally place *Centrolenella megista* Rivero 1985 in the genus *Nymphargus* sensu Guayasamin *et al.* (2009). *Nymphargus megistus* and *N. grandisonae* are recovered as sister taxa; the genetic distance between these two species is 2.0%.

New records.—ECUADOR: one adult male, SVL: 30.8 mm; Ecuador, Imbabura Province, Río Manduriacu Reserve; 00°20.217' N, 78°51.445' W, 1,857 m a.s.l.; 21:14 h, 01 March 2019; Ross Maynard, Jaime Culebras, and Scott Trageser;

primary cloud forest, perched on broad-leaf stalk 2 cm diameter, 1 m high, 15.6°C, 95% relative humidity, light rain occurred earlier in the day; ZSFQ 4071 (field number 2019-013). ECUADOR: one adult female, SVL: 38.9 mm; Ecuador, Imbabura Province, Río Manduriacu Reserve; 00°20.206' N, 78°51.452' W, 1,850 m a.s.l.; 20:19 h, 07 December 2019; Ross Maynard, Scott Trageser, and Leslie Rochefort; primary cloud forest, perched on leaf, 60 cm high, 14.8°C, 100% relative humidity, 5 mph wind, found during heavy rain; ZSFQ 3924 (field number SCA 1600). ECUADOR: one adult

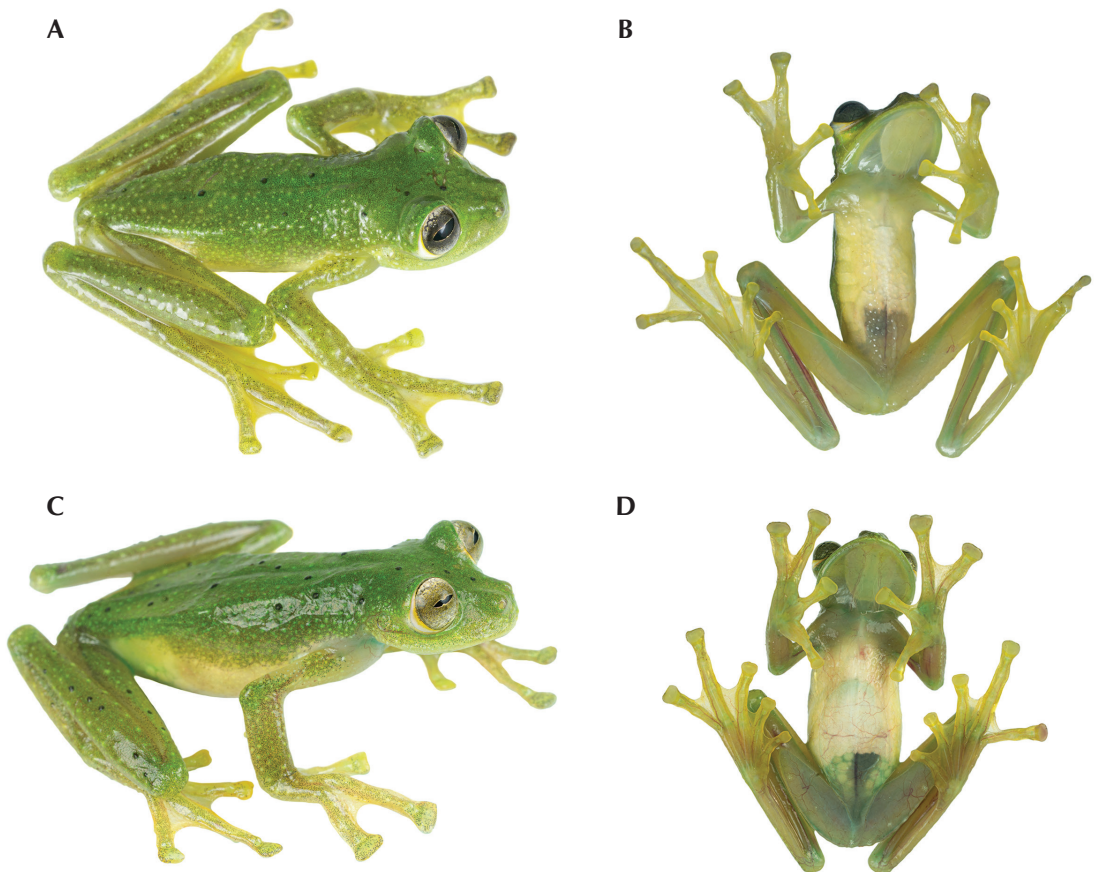


Figure 2. *Nymphargus megistus*. (A) Adult ♂, ZSFQ-4071, SVL = 30.8 mm, dorsal view. (B) Adult ♂, ZSFQ-4071, SVL = 30.8 mm, ventral view. (C) Adult ♀, ZSFQ-3924, dorsal view, SVL = 38.9 mm. (D) Adult ♀, ZSFQ-3924, ventral view, SVL = 38.9 mm.

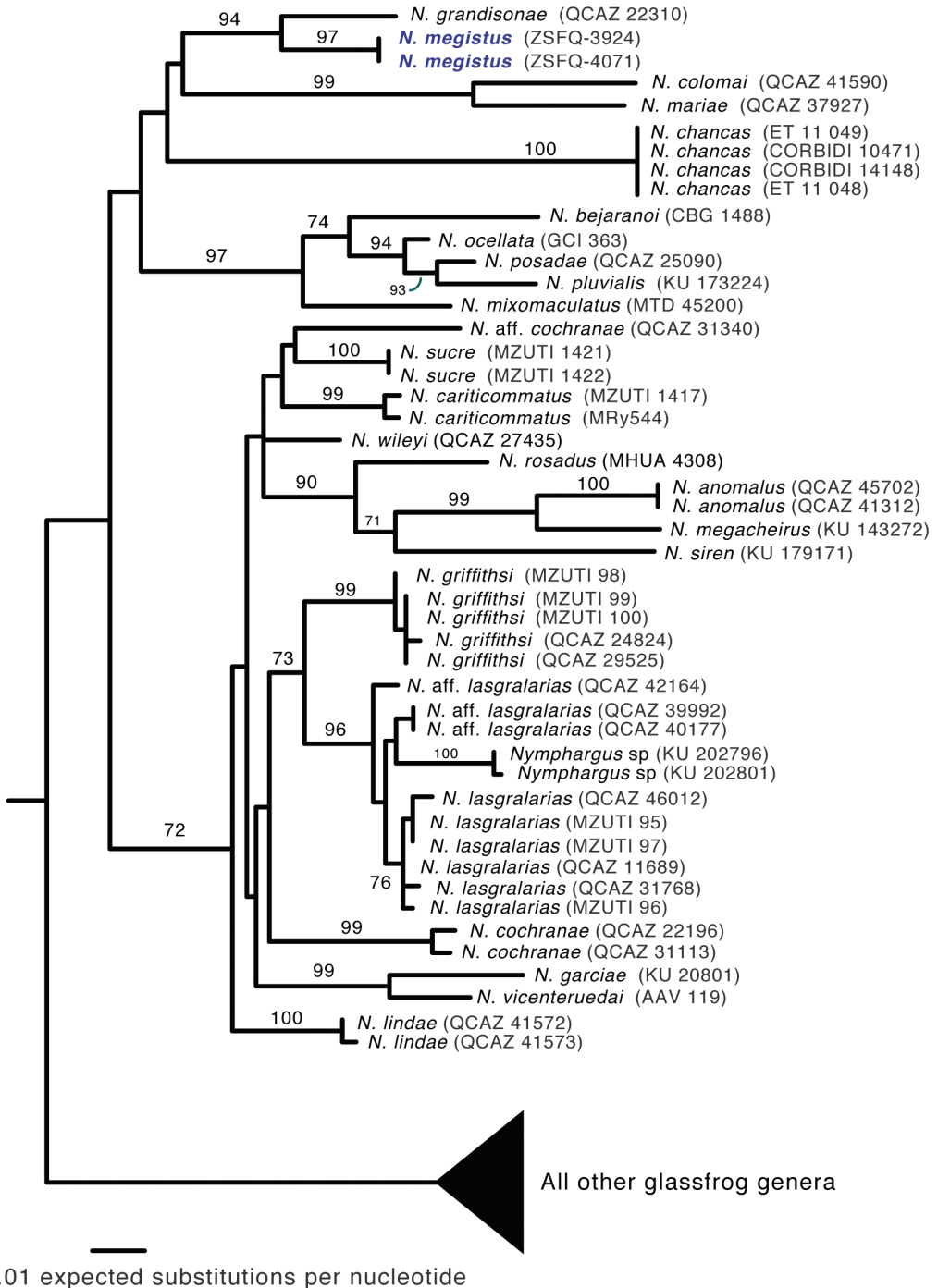


Figure 3. Phylogenetic placement of *Nymphargus megistus* inferred under Maximum Likelihood using the mitochondrial gene 16S. Genbank accession numbers for *N. megistus* are as follow: MZ314501 (ZSFQ 4071) and MZ314502 (ZSFQ 3924).

male, SVL: 35.9 mm; Ecuador, Pichincha Province, Séptimo Paraíso, nearby the town of Mindo; 00°1.758' S, 78°45.912' W, 1,550 m a.s.l.; 22:00 h, 11 February 2020; Amanda Quezada and Frank Pichardo; primary cloud forest, perched on leaf 20 cm high, found during a cloudy night, about 2 meters from creek; TH-822.

Discussion

The two records we report of *Nymphargus megistus* from the Río Manduriacu Reserve and the one individual from near Mindo represent the first observations of the species in Ecuador, extending its known distribution ca. 530 km south from the nearest record in the southern Chocó Department of Colombia (Figure 2). Although a substantial range extension, it is not unexpected; a number of anuran species are known to have similar distributions along the western slope of the Cordillera Occidental of Colombia and northern Ecuador, including other glassfrog species that have been reported from the Río Manduriacu Reserve (i.e., *Centrolene peristicta*, *Espadarana prosoblepon*, *Nymphargus balionotus*, and *N. grandisonae*; Maynard *et al.* 2020). Despite the seemingly low detectability of *N. megistus* at the localities in which it has been observed, the absence of records between the Ecuadorian localities and those from Colombia demonstrate the need for additional survey efforts within this region.

Our observations of *Nymphargus megistus* at RMR were made nine months apart (March and December 2019) in primary cloud forest near the upper reaches of RMR just below the ridgeline. Interestingly, both individuals were observed ca. 175 m from the nearest stream, and within 25 m of one another. Similar observations are recorded in the field notes of Maria Cristina Ardila-Robayo from August 1987 reporting three individuals (ICN 17242–4) found in mature forest, away from bodies of water, and 0.5–2 m above the ground. Rada and Guayasamin (2008) suggest, based off these observations, that during

non-breeding periods the adults migrate towards the interior of the forest away from streams. During early December and early March, our surveys of four streams at the upper reaches of RMR did not yield observations of *N. megistus* or their advertisement calls. Although this suggests a lack of breeding activity, the observation of the gravid female in December 2019 indicates that reproduction in this species likely does occur during this time frame, despite the fact that we did not observe this. In fact, call data for this species has yet to be recorded and described; however, males were recorded in the notes by either Maria Cristina Ardila or Pedro M. Ruiz (M. Rada pers. comm.) to have been vocalizing at an unknown date on streamside branches in Araceae plants 1.5–1.8 m above the ground (Rada and Guayasamin 2008). The incidental observation near Mindo of a male near a stream in mid-February does correspond to potential rainy season breeding activity, though it was not observed calling. Considering the proximity of our RMR observations away from a stream, and at different times of year, it is possible this area serves as an important migration corridor.

The IUCN currently recognizes *N. megistus* as Endangered (IUCN SSC Amphibian Specialist Group 2017). Although our two records from RMR extend the range of *N. megistus* considerably, it is unknown if the Ecuadorian populations are disjunct from those in Colombia. Results from the Colombia Red List Assessment Workshop (2016) indicate that the majority of the population resides in small subpopulations with no genetic exchange. Furthermore, severe fragmentation may already exist along specific corridors at elevations necessary for this species to disperse, as Andean cloud forests in Colombia are disproportionately excluded from regional and national protected area networks (Forero-Medina and Joppa 2010). Additionally, in light of increasing mining activity in Ecuador (Roy *et al.* 2018) and immediate threats facing RMR (Guayasamin *et al.* 2019b, Maynard 2020), additional surveying of the vast area between the

Colombian and Ecuadorian occurrences is necessary to better understand its distribution, natural history, and conservation status.

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