ARTÍCULO CIENTÍFICO
Guayasamin et al. — New species of glassfrogs — e1148 — 185-202 https://doi.org/10.22201/fc.25942158e.2025.2.1148

# A BIOGEOGRAPHIC ODDITY IN A DISAPPEARING ECOSYSTEM: A NEW GLASSFROG (CENTROLENIDAE: *NYMPHARGUS*) FROM THE ECUADORIAN CHOCÓ

## UNA RAREZA BIOGEOGRÁFICA EN UN ECOSISTEMA QUE DESAPARECE: UNA NUEVA RANA DE CRISTAL (CENTROLENIDAE: *NYMPHARGUS*) DEL CHOCÓ ECUATORIANO

Juan M. Guayasamin[1,2]*, Daniela Franco-Mena[1,2] & Mateo A. Vega-Yánez[1,2,3]

[1]*Universidad San Francisco de Quito USFQ, Laboratorio de Biología Evolutiva, Colegio de Ciencias Biológicas y Ambientales COCIBA, Instituto BIÓSFERA-USFQ, Global Research & Solutions Center USFQ, Campus Cumbayá, Quito 170901, Ecuador.*

[2]*Universidad San Francisco de Quito USFQ, Tandayapa Cloud Forest Station, P.O. Box 17-1200-841, Quito, Ecuador.*

[3]*Universidad San Francisco de Quito USFQ, Colegio de Ciencias Biológicas y Ambientales COCIBA, Maestría en Ecología Tropical y Conservación, Quito, Ecuador.*

**Correspondence: jmguayasamin@usfq.edu.ec*

**Received:** 2024-09-22. **Accepted:** 2025-03-14. **Published:** 2025-06-06.
**Editor:** Felipe Rabanal, Chile.

**Resumen.**– *Nymphargus* es un género principalmente andino de ranas de cristal. El descubrimiento de una especie de *Nymphargus* en las tierras bajas del Chocó representa una rareza biogeográfica. Aquí describimos este nuevo taxón basándonos en los siguientes rasgos: (i) ausencia de membranas entre los dedos de las manos, (ii) dorso verde con numerosas manchas negras y algunos falsos ocelos color amarillo, (iii) ausencia de dientes vomerinos y (iv) tamaño corporal relativamente grande (SVL > 30 mm). A pesar de los extensos esfuerzos de muestreo en la zona, no se han encontrado más especímenes, lo que indica que la nueva especie es rara y vulnerable. Dada su limitada área de distribución en la ecorregión del Chocó y las amenazas actuales de destrucción del hábitat (i.e., tala, expansión agrícola, minería), clasificamos este nuevo taxón como En Peligro Crítico, siguiendo los criterios de la UICN. Por último, analizamos la cobertura forestal del Chocó ecuatoriano durante 1985–2022; en este periodo se han deforestado 194.007 hectáreas, lo que supone una destrucción del 20 % del ecosistema original. Estimamos que cada año se pierde una media de 5.243 hectáreas de bosque; estos datos ponen de manifiesto la urgencia de emprender acciones de conservación en el Chocó.

**Palabras clave**.– Ecorregión Chocó, Amphibia, nueva especie, solitario, taxonomía.

**Abstract.**– *Nymphargus* is a genus of glassfrogs primarily known from the Andes. The discovery of a *Nymphargus* species in the lowland Chocó region represents a biogeographic oddity. Herein we describe this new taxon based on the following main traits: (i) absence of hand webbing, (ii) green dorsum with numerous black flecks and scarce yellow false ocelli, (iii) absence of vomerine teeth, and (iv) relatively large body size (SVL > 30 mm). Despite extensive subsequent herpetological efforts in the area, no additional specimens have been found, underscoring its rarity and vulnerability. Given its limited range in the Chocó ecoregion and the ongoing threats of habitat destruction (i.e., logging, agricultural expansion, mining), we classify this new taxon as Critically Endangered, according to IUCN criteria. Lastly, we analyzed the forest cover of the Ecuadorian Chocó during 1985–2022; during this time period 194,007 hectares have been deforested, representing a 20 % destruction of the original ecosystem. We estimate that an average of 5,243 hectares of forest are lost annually; this data highlights the urgency for conservation actions in the Chocó.

**Keywords**.– Chocó ecoregion, Amphibia, new species, singleton, taxonomy.

## INTRODUCTION

*Nymphargus* is a glassfrog genus originally described by Cisneros-Heredia & McDiarmid (2007); its diagnosis and species content were later modified by Guayasamin et al. (2009) to render the taxon monophyletic. *Nymphargus* represents one of the few glassfrog genera where morphological diagnosis is straightforward. All species in *Nymphargus* are easily recognized by having a reduced (or absence) webbing between fingers (Guayasamin et al., 2009). Moreover, all species in this

genus exhibit a partially transparent venter, and a transparent peritoneal covering the heart, liver, and digestive system (Guayasamin et al., 2009). Biogeographically, *Nymphargus* originated and is mostly restricted to the Andes (Castroviejo-Fisher et al., 2014; Guayasamin et al., 2009, 2020), with only four species found below 1,000 m on the Pacific slopes of the Andean Cordillera (Fig. 1): *N. balionotus* (Duellman 1981), *N. buenaventura* (Cisneros-Heredia & Yánez-Muñoz 2007), *N. chami* (Ruiz-Carranza & Lynch 1995), and *N. prasinus* (Duellman 1981).

The endemicity of *Nymphargus* to the Andes has been explained by a combination of niche conservatism and allopatric speciation (Hutter et al., 2013; Castroviejo-Fisher et al., 2014; Guayasamin et al., 2020). Thus, it came as a biogeographic surprise finding a *Nymphargus* in collections made by Gregory Vigle at Reserva Biológica Bilsa, in the Ecuadorian Chocó Ecoregion, more than 20 years ago (2001). The specimen exhibits all the diagnostic traits of *Nymphargus* and is described herein as a new species. After this initial finding, several batrachologists have worked in the area (Guayasamin & Bonaccorso, 2004; Ortega-Andrade et al., 2010, 2013; Vigle et al., 2020; Jones et al., 2024), but no additional specimens have been found. Given the morphological and biogeographical uniqueness of the species, we describe this new species of *Nymphargus*, and list it as Critically Endangered.

## METHODS

### To taxonomy and species description

We follow the generic classification of Centrolenidae proposed by Guayasamin et al. (2009), which includes a monophyletic *Nymphargus*. Species are considered separately evolving lineages, following the conceptual framework developed by Simpson (1951, 1961), Wiley (1978), and De Queiroz (2007). As an operational criterion for lineage identification, we rely mainly on morphological data.

### Study site

The Reserva Biológica Bilsa (RBB) is a private reserve owned by Fundación Jatun Sacha, with an area of approximately 3,300 hectares at elevations of 300–800 m a.s.l. (Ortega-Andrade et al., 2010), in the Mache-Chindul mountains at the border between the Esmeraldas and Manabí provinces, Ecuador (Fig. 2). It contains Foothill Evergreen Forest (~500–800 m a.s.l.) and Lowland Evergreen Forest (~350–500 m a.s.l.). The rainy season occurs from January to May, followed by a drier season from June to December (annual average rainfall of 1,500–2,000 mm; Ortega-Andrade et al., 2013); during the drier period, the reserve is often covered by fog at ground level (and dense cloud cover above) and subject to an undetermined but substantial amount of fog drip. Most of the RBB is marked by steeply rolling terrain with alternating ridges and streams in narrow valleys. Surveys at RBB have been carried out by numerous researchers in the last 20 years both in primary and old secondary forest, along several streams, but mainly in the Duchas and Aguacatal-Duchas streams (Guayasamin & Bonaccorso, 2004; Ortega-Andrade et al., 2010, 2013; Vigle et al., 2020; Jones et al., 2024). A map illustrating the location of all trails and streams at RBB is published in Ortega-Andrade et al. (2010:fig.1).

### Loss of vegetation cover in the Ecuadorian Chocó

For our analyses, we delimited the Ecuadorian Chocó region using the biogeographic classification map of continental Ecuador provided by the Ministerio del Ambiente, Agua y Transición Ecológica. This classification is based on vegetation physiognomy, landscape criteria, climatic conditions and floristic components (MAE, 2012). We have also complemented this classification with literature references (Fagua et al., 2019; Sierra, 1999). Based on the defined study area, we obtained geospatial data on the annual dynamics of land cover and land use through the Google Earth Engine platform. For this purpose, we accessed the MapBiomas collection (available at http://ecuador.mapbiomas.org), which provides information at a spatial resolution of 30 meters from the supervised classification of satellite images. For the analysis in the Ecuadorian Chocó we downloaded data for the years 1985, 1990, 1995, 2000, 2005, 2010, 2015, 2020 and 2022. With the downloaded information on land use and land cover in different years for the study area, a spatial analysis was carried out with the ArcGIS Pro software. First, all data layers were converted to the WGS 1984 UTM Zone 17S spatial reference system to ensure a correct overlay and comparison of the geographic information. A temporal trend analysis was performed, estimating the area of vegetation cover for each year in order to quantify the loss of vegetation in the Ecuadorian Chocó in space and time. With the vegetation area data obtained for each year, the rate of vegetation change and average deforestation were calculated for each five-year interval using the equations proposed by Puyravaud (2003). For the year 2022, only the vegetation area was calculated in order to maintain consistency in comparisons between five-year intervals. Due to the absence of vegetation cover data in MapBiomas for the year 2024, we have estimated the area using a second-degree polynomial model that adjusts to the available data. The analyses were performed in R software.

### Species description

The diagnosis and description of the new species follow the terminology described by Lynch & Duellman (1973), Cisneros-Heredia & Mcdiarmid (2007), and Guayasamin et al. (2009, 2020). Webbing formula follows Savage & Heyer (1967), as modified by Guayasamin et al. (2006). We obtained morphological data with

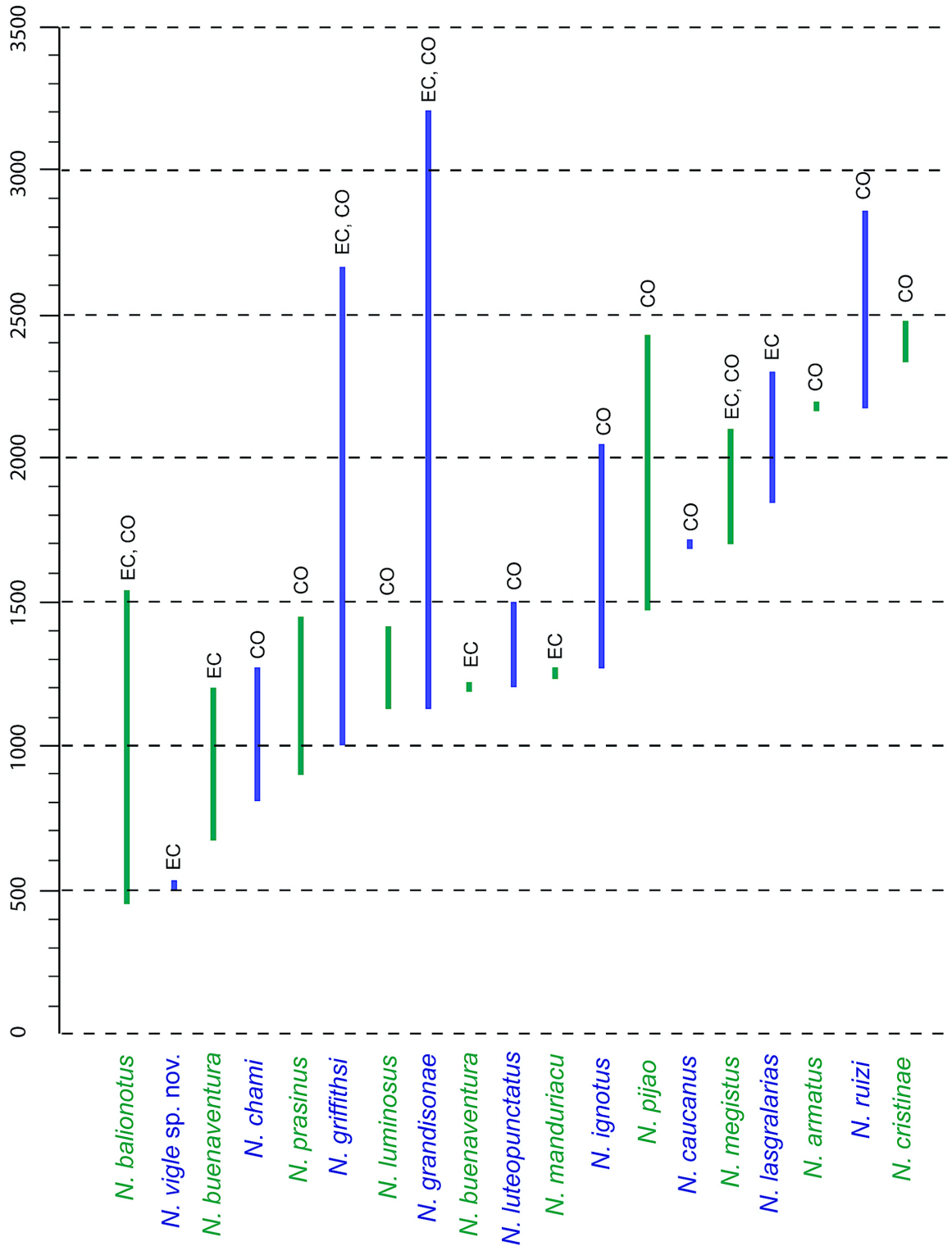


**Figura 1.** Distribución altitudinal (en metros) del género *Nymphargus* presente en las vertientes del Chocó y el Pacífico de los Andes de Ecuador y Colombia. La distribución por país se indica en la parte superior de la barra para cada especie: Colombia (CO), Ecuador (EC).

**Figure 1.** Elevational distribution (in meters) of the genus *Nymphargus* found in the Chocó and Pacific slopes of the Andes of Ecuador and Colombia. The country distribution is noted at the top of the bar for each species: Colombia (CO), Ecuador (EC).

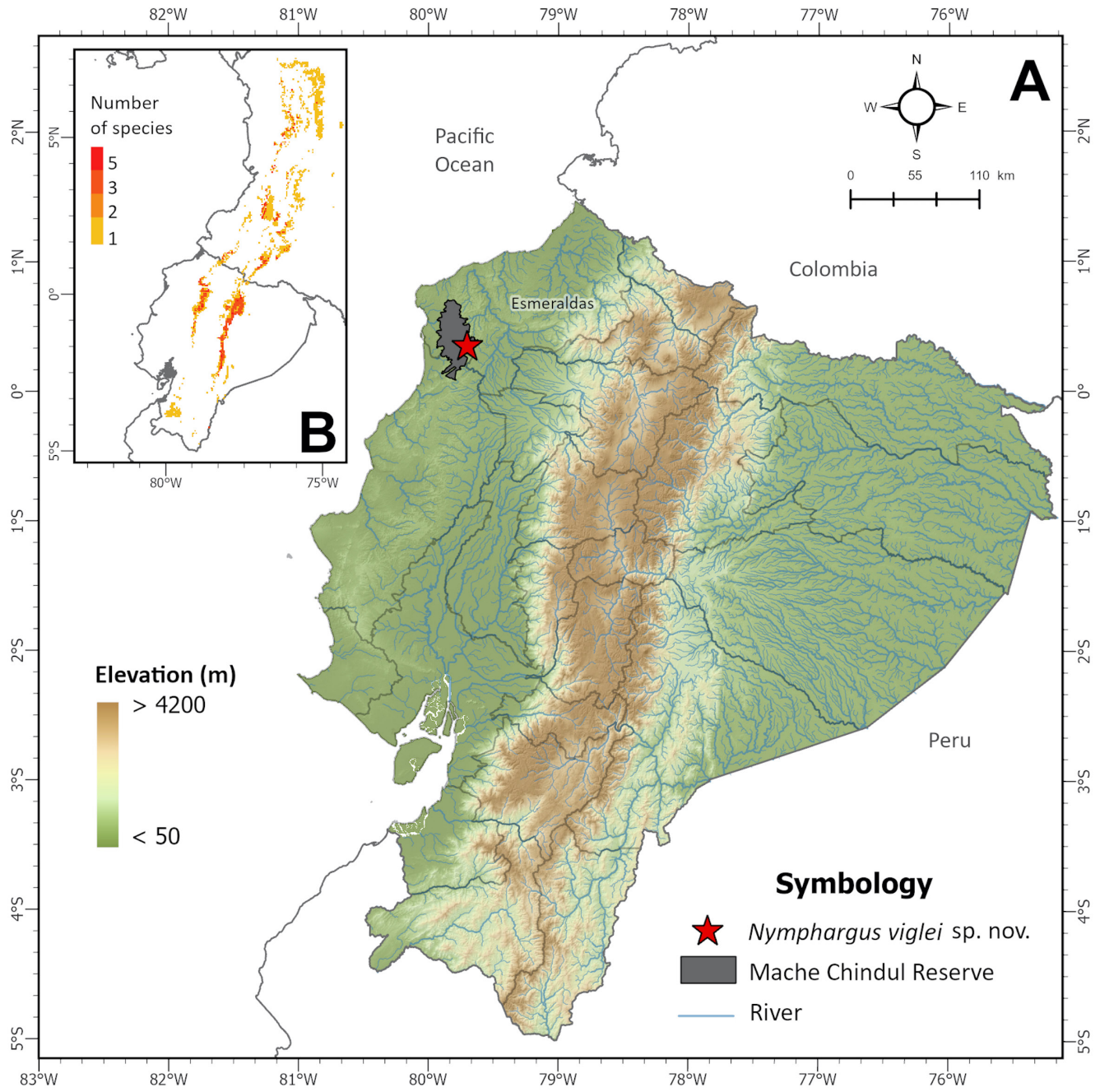


**Figura 2.** (A) Distribución de *Nymphargus viglei* sp. nov. en Ecuador. (B) Distribución de especies amenazadas (UICN) del género *Nymphargus* en el norte de los Andes.

**Figure 2.** (A) Distribution of *Nymphargus viglei* sp. nov. in Ecuador. (B) Distribution of IUCN threatened species of the genus *Nymphargus* in the northern Andes.

a Mitutoyo digital caliper to the nearest 0.1 mm, as described in Guayasamin et al. (2022): (1) snout–vent length (SVL) = distance from tip of snout to posterior margin of vent; (2) femur = distance from cloaca to knee; (3) tibia = length of flexed leg from knee to heel; (4) foot = distance from proximal edge of Toe I to tip of Toe IV ; (5) head length = distance from tip of snout to posterior angle of jaw articulation; (6) head width (HW) = width of head measured at level of jaw articulation; (7) interorbital distance (IOD) = shortest distance between upper eyelids, a measurement that equals to the subjacent frontoparietal bones; (8) eye = distance between anterior and posterior borders of the eye; (9) tympanum = distance between anterior and posterior borders

of tympanic annulus; (10) arm = length of flexed forearm from elbow to proximal edge of Finger I at the level of articulation with arm; (11) hand = distance from proximal edge of Finger I to tip of Finger III; (12) Finger I = distance from outer margin of hand to tip of Finger I; (13) Finger II = distance from outer margin of hand to tip of Finger II; and (14) width of Finger.

We compared the new taxon to specimens housed at the following collections (Appendix I): Centro Jambatu de Investigación y Conservación de Anfibios, Quito, Ecuador (CJ); NHMUK Natural History Museum, United Kingdom (formerly BMNH: British Museum Natural History); División de Herpetología, Museo Ecuatoriano de Ciencias Naturales (DHMECN); Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN); University of Kansas, Museum of Natural History, Division of Herpetology, Lawrence, Kansas, USA (KU); Museo de Zoología, Universidad Tecnológica Indoamérica, Quito, Ecuador (MZUTI); and Museo de Zoología, Universidad San Francisco de Quito, Quito, Ecuador (ZSFQ). We performed a one-sample t-test to determine if there are differences in body size between similar species. The conservation assessment of the new taxon follows the criteria detailed by the IUCN (2012).

## RESULTS

### *Nymphargus viglei* new species

Figures 3, 4, 5

LSID: urn:lsid:zoobank.org:pub:8C86252B-AE45-4223-A4B0-612791CF8605

Nomenclatural Act: urn:lsid:zoobank.org:act:6B9D0D8B-E5E3-4CC4-9B8C-B6FD366E59D2

Common name in English: Vigle's Chocó Glassfrog.

Common name in Spanish: Rana de Cristal Chocoana de Vigle.

**Holotype:** KU 291177 (field number GV-2000-0051), adult female, collected in tributary of Duchas/Aguacatal stream at Reserva Biológica Bilsa (0.3447° N, 79.7100° W; 510 m a.s.l.), within the Reserva Ecológica Mache-Chindul, Esmeraldas Province, Ecuador, by Gregory O. Vigle on 03 July 2001.

**Etymology:** The specific epithet is a noun in the genitive case, and a patronym for Gregory Owen Vigle (who collected the holotype), in recognition of his contributions to the study and conservation of the herpetofauna of Ecuador (e.g., Vigle & Miyata, 1980; Vigle, 2008; Vigle et al., 2020). By association, this name also commemorates the decades of support and encouragement of his father, John B. Vigle, Jr., mother Marjorie B. Vigle, brother Sean C. Vigle, and son John S.T.B Vigle, as well as his daughter Silvana V.M. Goberdhan-Vigle, and spouse, Z. Neesha Batcha Vigle.

**Generic placement:** The new species is placed in the clade *Nymphargus* (sensu Guayasamin et al., 2009) based on morphological traits. All species in *Nymphargus* share as a synapomorphy the absence or reduced webbing between Fingers III and IV. Other diagnostic traits in the genus (also present in the new species) are: partially transparent venter; transparent peritonea covering the heart, liver, and digestive system; adults lack humeral spines, although males in some populations (i.e., *N. armatus* [Ruiz-Carranza & Lynch 1996], *N. balionotus* [Duellman 1981], *N. grandisonae* [Cochran & Goin 1970], *N. griffithsi* [Goin 1961]) can present an expanded crista ventralis of the humerus, closely resembling a humeral spine. Biogeographically, most *Nymphargus* species are restricted to the Andes (Guayasamin et al., 2020).

**Identification and similar species:** Among glassfrogs found in the Chocó and the Pacific slope of the Andes, *Nymphargus viglei* sp. nov. is unique by having the following combination of traits: (i) absence of webbing between fingers, (ii) dorsum green, with numerous black flecks and few yellow false ocelli, and (iii) relatively large female SVL (31.1 mm; n = 1). Only four species of *Nymphargus* have been documented within the Chocó biogeographic region; these species are: *N. balionotus*, *N. buenaventura*, *N. chami*, and *N. prasinus* (Fig. 6). Differences between the new species and other Chocoan *Nymphargus* are summarized in Table 1. Other species that could be confused with *N. viglei* sp. nov. are *N. griffithsi* (Goin 1961) and *Espadarana prosoblepon* (Boettger 1892); both species may present black flecks on the dorsum, and some populations of *E. prosoblepon* also exhibit false yellow ocelli. The new species is easily differentiated from *N. griffithsi* by having false ocelli (absent in *N. griffithsi*), and being considerably larger (SVL in *N. griffithsi* < 25.8 mm; SVL in *N. viglei* sp. nov. = 31.1 mm). Additionally, *N. griffithsi* is restricted to the Andes at elevation of 1,220–2,430 m (Hutter & Guayasamin, 2012; Guayasamin et al., 2020), whereas the new taxon is known only from the Chocoan lowlands (ca. 500 m). *Espadarana prosoblepon* has been reported at the same locality as *N. viglei* sp. nov. (Ortega-Andrade et al., 2010); these species are readily distinguished by the presence of webbing between Fingers III and IV in *E. prosoblepon* (absent in the new species; Figs. 5, 7), vomerine teeth (present in *E. prosoblepon*, absent in the new species), and body size (SVL in *E. prosoblepon* < 28.6 mm, n = 25; SVL in *N. viglei* sp. nov. = 31.1 mm). Statistically, body

**Figura 3.** *Nymphargus viglei* sp. nov. en su entorno natural. Ilustración: Valentina Nieto Fernández.

**Figure 3.** *Nymphargus viglei* sp. nov. in its natural environment. Illustration: Valentina Nieto Fernández.

size in *N. viglei* sp. nov. is significantly larger ($p < 0.001$) than *E. prosoblepon*. Also, all examined specimens of *E. prosoblepon* (25 adult females) exhibit a conspicuous membrane between outer fingers; webbing formulae: III (12/3 –2+) — (1+ –2) IV. In contrast, hand webbing in *N. viglei* sp. nov. is very much reduced: III 3 — 23/4 IV. Finally, in the dorsal view, the head of *E. prosoblepon* is round, whereas *N. viglei* sp. nov. has a truncate head (Figs. 5, 7).

**Diagnosis:** (1) Dentigerous process of the vomer lacking teeth o; (2) snout truncate in dorsal and lateral view; (3) tympanum oriented almost vertically, with slight lateral and posterior inclinations, its diameter about 26% of eye diameter; upper border of tympanic annulus obscured by supratympanic fold; tympanic membrane pigmented as surrounding skin; (4) dorsal skin shagreen, lacking spicules; males unknown; (5) venter areolate; pair of enlarged subcloacal warts; (6) white parietal peritoneum covering about anterior two-thirds of venter; white pericardium; translucent peritonea on kidneys, intestines, stomach, gall and urinary bladders; (7) liver lobate, covered by translucent peritoneum; (8) humeral spines absent; (9) webbing absent between fingers; (10) feet about two-thirds webbed; webbing formula: I 2– —21/3 II 11/4 —22/3 III 11/2 — 3– IV 3—2– V; (11) ulnar and tarsal folds present, low; (12) concealed prepollex; condition of nuptial pads unknown; (13) Finger II longer than Finger I; (14) disc of Finger III width about 55% of eye diameter; (15) in life, dorsum green with numerous black flecks and yellow false ocelli; bones green; (16) in preservative, dorsum cream with numerous dark lavender flecks and few unpigmented spots; (17) in life, iris silvery white, with numerous minute grey to dark grey spots; (18) melanophores on dorsal surfaces of Fingers III and IV and Toes IV and V; (19) large body size in females (SVL = 31.1 mm; n = 1); males unknown.

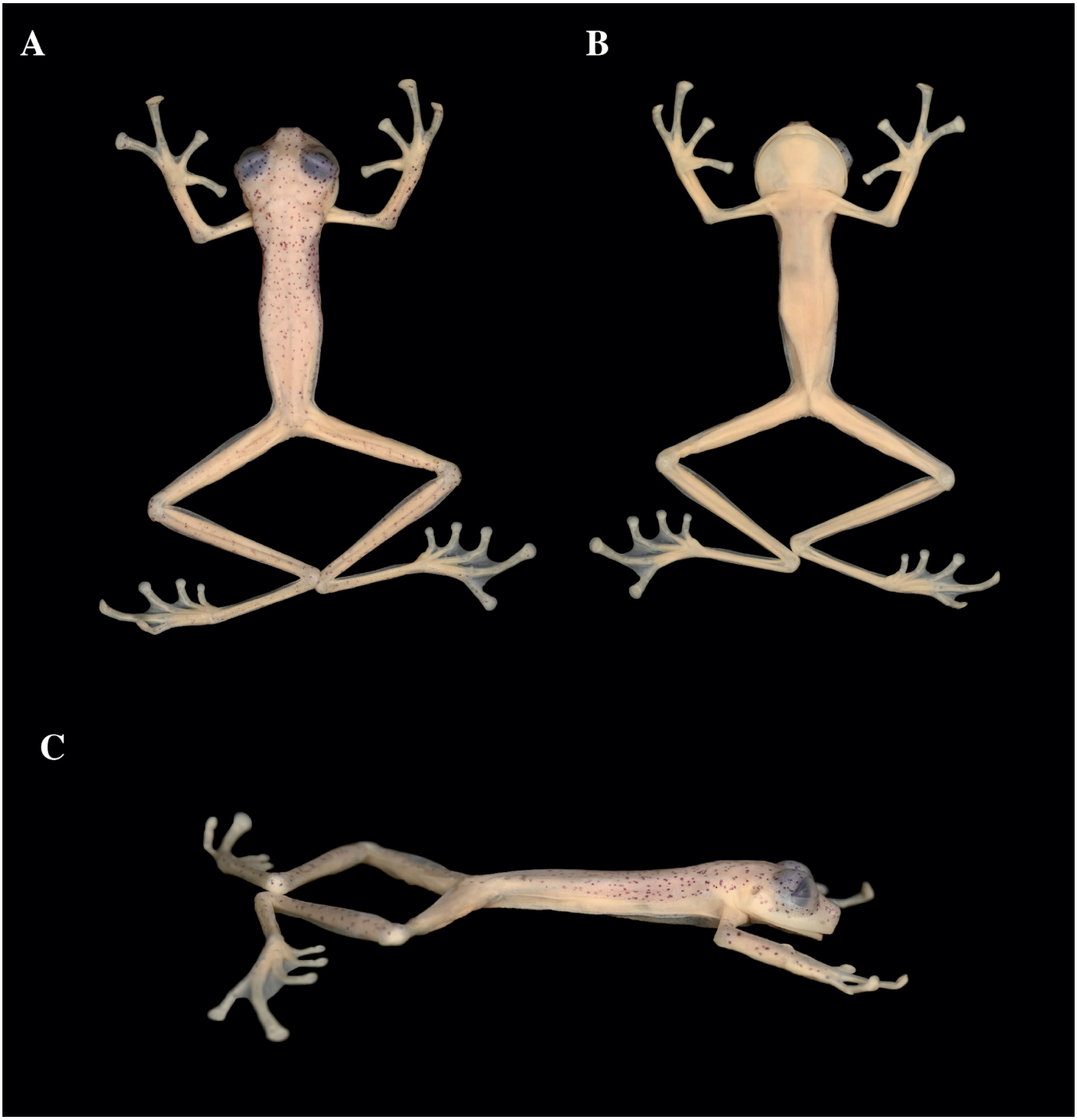


**Figura 4.** Holotipo de *Nymphargus viglei* sp. nov. en alcohol. (A) Vista dorsal. (B) Vista ventral. (C) Vista lateral.
**Figure 4.** Holotype of *Nymphargus viglei* sp. nov. in alcohol. (A) Dorsal view. (B) Ventral view. (C) Lateral view.

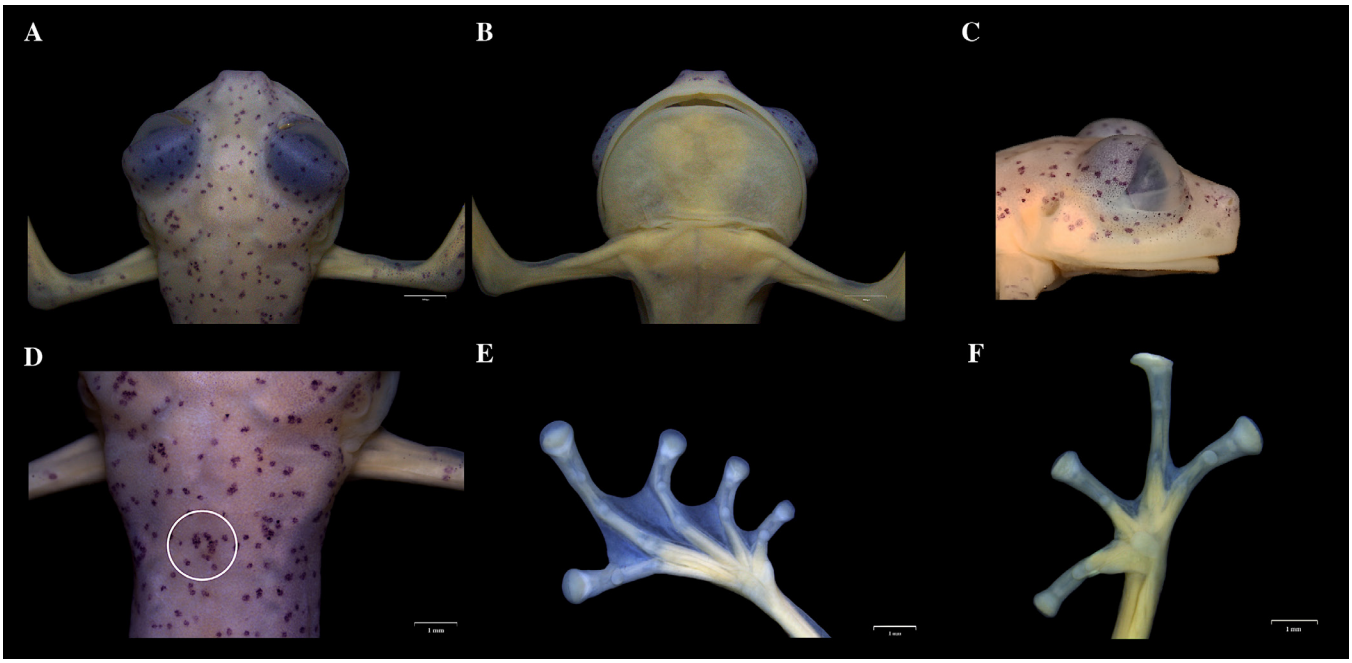


**Figura 5.** Detalles morfologicos del holotipo preservado de *Nymphargus viglei* sp. nov. (A, B, C) Cabeza en vistas dorsal, ventral y lateral. (D) Vista parcial del cuerpo en vista dorsal; marcado con un circulo blanco un falso ocelo. (E) Pie en vista ventral. (F) Mano en vista ventral.

**Figure 5.** Morphological details of preserved *Nymphargus viglei* sp. nov., holotype. (A, B, C) Head in dorsal, ventral, and lateral views. (D) Partial view of body in dorsal view; note white circle around a false ocellus. (E) Foot in ventral view. (F) Hand in ventral view.

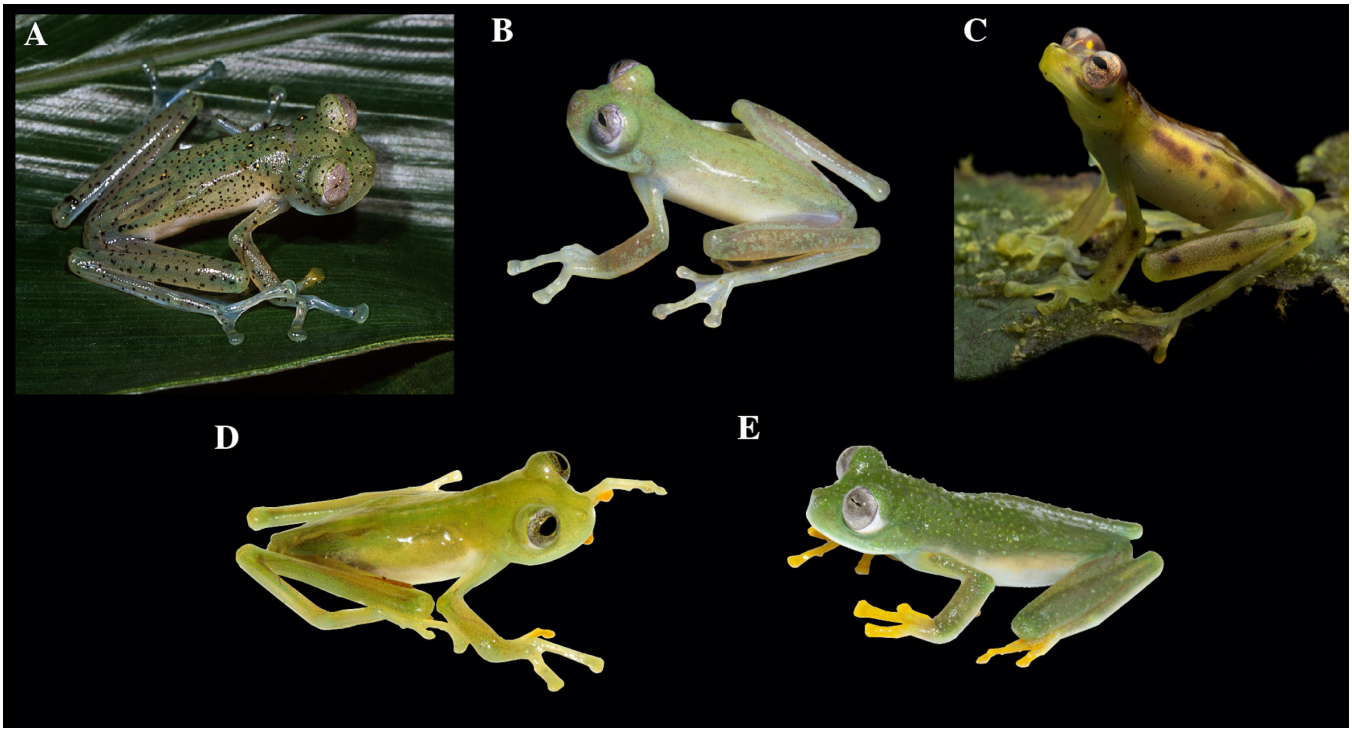


**Figura 6.** *Nymphargus viglei* sp. nov. y especies similares en vida. (A) *Nymphargus viglei* sp. nov., holotipo. Foto: Greg Vigle. (B) *N. prasinus*, ICN 19645, Foto: John D. Lynch. (C) *N. balionotus*, ZSFQ 533. Foto: José Vieira. (D) *N. buenaventura*, DHMECN 10982, Foto: Juan Carlos Sánchez Nivicela. (E) *N. chami*, MAR-2869. Foto: Marco Rada.

**Figure 6.** *Nymphargus viglei* sp. nov. and similar species in life. A. *Nymphargus viglei* sp. nov., holotype. Photo: Greg Vigle. (B) *N. prasinus*, ICN 19645. Photo: John D. Lynch. (C) *N. balionotus*, ZSFQ 533. Photo: José Vieira. (D) *N. buenaventura*, DHMECN 10982. Photo: Juan Carlos Sánchez Nivicela. (E) *N. chami*, MAR-2869. Photo: Marco Rada.

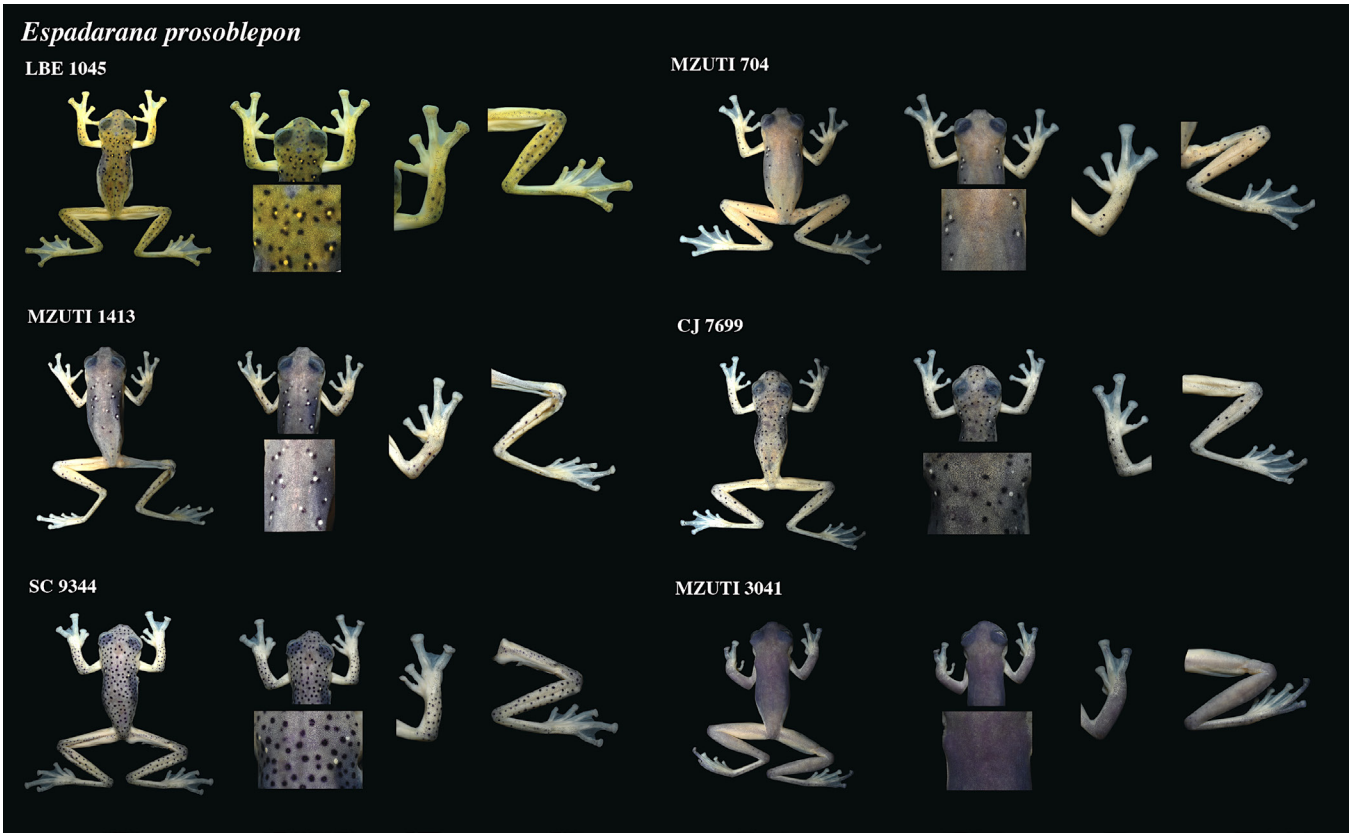


**Figura 7.** Variación morfológica de *Espadarana prosoblepon* (hembras adultas). Para cada especimen mostrado, las vistas de de izquierda a derecha son: tamaño corporal en vista dorsal, cabeza y acercamiento del patron de color dorsal, membranas interdigitales en manos y pies. Contraste las membranas interdigitales en manos y forma de la cabeza de *E. prosoblepon* y la nueva especie (mostrada en la Figura 5).

**Figure 7.** Morphological variation of *Espadarana prosoblepon* (adult females). For each specimen we show, from left to right, the following views: body size in dorsal view, head and close-up of dorsal color pattern, hand webbing, and foot webbing. Contrast hand webbing and head shape of *E. prosoblepon* and the new species (depicted in Figure 5).

**Tabla 1.** Diferencias entre las especies chocoanas de ranas cristal del género *Nymphargus*. Tamaño corporal (SVL) dado en mm.

**Table 1.** Differences among Chocoan species of glassfrogs in the genus *Nymphargus*. Body size (SVL) is given in mm.

| | Male SVL | Female SVL | Dorsal coloration in life | Skin texture | Source |
|---|---|---|---|---|---|
| ***N. balionotus*** | 19.0–21.0 (n = 6) | 21.7–22.0 (n = 2) | Pale green with reddish-brown dorsolateral stripes, small reddish-brown spots, and larger yellow spots | Smooth to shagreen | Duellman (1981), Guayasamin et al. (2020), Maynard et al. (2020) |
| ***N. buenaventura*** | 20.9–22.4 (n = 4) | 23.5 (n = 1) | Green with minute, scattered pale yellow spots. | Shagreen | Cisneros-Heredia & Yánez-Muñoz (2007), Guayasamin et al. (2020) |
| ***N. chami*** | 30.5–34.6 (n = 6) | 37.6 (n = 1) | Green with numerous small white warts | With abundant subconical tubercles | Ruiz-Carranza & Lynch (1995) |
| ***N. viglei sp. nov.*** | Unknown | 31.1 (n =1) | Green with numerous small black flecks and few yellow false ocelli | Shagreen | This study |
| ***N. prasinus*** | 31.6–31.8 (n = 3) | Unknown | Uniform dark green | Smooth to shagreen | Duellman (1981) |

**Description of holotype:** Adult female, SVL 31.1 mm. Head slightly wider than long (head length 97% of head width); snout truncate in dorsal and lateral profiles; canthus rostralis indistinct, slightly concave; loreal region slightly concave; lips slightly flared; nostril protuberant, closer to tip of snout than to eye, directed frontolaterally; internarial area barely depressed. Eyes large, directed anterolaterally at an angle ~ 50°; transverse diameter of disc of Finger III 55% eye diameter. Supratympanic fold low, obscuring the upper portion of tympanic annulus; tympanum oriented mostly vertically, but with slight posterolateral inclination; tympanic membrane translucent, pigmented as surrounding skin. Dentigerous process of vomer low, situated transversely between choanae, lacking teeth; choanae large, longitudinally rectangular; tongue ovoid, with ventral posterior fifth not attached to floor of mouth and posterior margin slightly notched. Humeral spine absent. Low ulnar folds evident on external and internal ventrolateral margins of arm; relative lengths of fingers: III > IV > II > I; hand webbing absent between inner finger and greatly reduced between outer finger: III 3 — 23/4 IV; discs expanded, nearly elliptical; disc pads nearly triangular shaped; subarticular tubercles of medium size, round, simple; few low supernumerary tubercles present; palmar tubercle elliptical, simple. Length of tibia 63% SVL; low inner and outer tarsal folds evident; foot two-thirds webbed; webbing formula of foot: I 2– —21/3 II 11/4 —22/3 III 11/2 — 3– IV 3—2– V; discs on toes round to elliptical, lacking papillae; disc on Toe IV slightly narrower that disc on Finger III; disc pads almost triangular-shaped; inner metatarsal tubercle large, ovoid; outer metatarsal tubercle absent; supernumerary tubercles absent. Skin on dorsal surfaces of head, body, and lateral surface of head and flanks shagreen, with very few warts, and lacking spicules; throat smooth; belly and lower flanks areolate; cloacal opening directed posteriorly at upper level of thighs; numerous cloacal warts present, unpigmented. Ventral surface of thighs with a pair of enlarged tubercles.

**Coloration in life (Figs. 3, 6):** Dorsal surfaces of head, body, and limbs are green with numerous black flecks and few false ocelli, which consist of a black outline —formed by the accumulation of flecks—, circling a yellow spot. Dorsally, the distal halves of inner fingers and toes have mustard coloration. The anterior two-thirds of the venter is white, whereas the posterior third is translucent. The iris is silvery white, with numerous minute grey to dark grey spots, especially around the pupil. Bones are green.

**Coloration of holotype in ethanol (Fig. 4):** Dorsal surfaces of head, body, and limbs cream with numerous dark lavender flecks, some of which cluster to form false ocelli. Anterior two-thirds of venter cream-white, posterior third cream. White pericardium; translucent peritoneal covering digestive tract, liver, kidneys, gall and urinary bladders. Iris silvery white with minute dark spots.

**Measurements of holotype (mm):** *Nymphargus viglei* sp. nov., adult female: SVL = 31.1, head length = 9.5, head width = 9.8, snout length = 4.3, interorbital distance = 2.8, upper eyelid width = 2.7, eye diameter = 3.8, tympanum diameter = 1.0, tympanum-eye distance = 2.3, femur = 18.3, tibia = 19.5, foot length = 13.4, hand length = 10.8, disc of Finger III width = 2.1, disc of Toe IV width = 1.8.

**Ecology:** The single specimen was found resting on a large palm leaf (probably *Heliconia sp.*) approximately 1.2 meters over a very small unnamed tributary stream that feeds a larger stream (in a steep valley) historically known locally as the Río Aguacatal/Duchas. The holotype was collected on the first night of a sampling period that included a total of ten nights in the Aguacatal/Duchas stream valley between July 3 and July 14, 2000. On that first night, two other centrolenid species were observed, including ten individuals of *Espadarana prosoblepon*, and a single specimen of a species that was initially misidentified by Greg O. Vigle as *Cochranella euknemos* (Savage & Starrett 1967), but later described as a paratype of a new species (*Cochranella mache*; Guayasamin & Bonaccorso, 2004).

Over the entire 15-day span of sampling in 2000 (July 1–15), a total of 42 individuals of *E. prosoblepon* were recorded from the Aguacatal/Duchas stream, with an additional 11 records from other areas of the RBB, but no other centrolenid species were found after a total of 85 hours of sampling effort. Later and more extensive survey efforts at the RBB over a span of six years (2004 to 2009) recorded four additional species of centrolenids including *Hyalinobatrachium tatayoi* Castroviejo-Fisher et al. 2007, *H. valerioi* (Dunn 1931), *Sachatamia albomaculata* (Taylor 1949), and *Teratohyla pulverata* (Peters 1873) (Ortega-Andrade et. al., 2010), and searches in 2008–2009 alone yielded 129 records of *C. mache* from all localities sampled at the RBB (Ortega-Andrade et. al., 2013). Despite those efforts, no additional records of the new species described herein are currently known from the RBB, or from any other locality. A complete list of the amphibians documented at RBB is included in Appendix II.

**Distribution (Fig. 2):** *Nymphargus viglei* sp. nov. is known from a single locality at Reserva Biológica Bilsa (0.3447°N, 79.7100°W; 510 m), a private reserve within the Reserva Ecológica Mache-Chindul, Esmeraldas Province, Ecuador. Given that glassfrog species tend to occupy narrow elevational gradients (Guayasamin et al., 2020), it is likely that the new species is restricted to the remnant forest of the Chocó.

**Conservation status:** Following IUCN (2012) criteria, we place *Nymphargus viglei* sp. nov. in the category of Critically Endangered, which means that the species is facing an extremely high risk of extinction. The new species meets the criterion B1a, B2a (known from a single locality) and B1biii, B2biii (continuing decline inferred from habitat loss and fragmentation). The main threats for this species are habitat destruction linked to timbering, cattle farming, cacao, and African oil palm plantations. Also, evidence suggests that *N. viglei* sp. nov. is a rare species; several herpetological studies have conducted extensive fieldwork at Reserva Biológica Bilsa and nearby localities (Guayasamin & Bonaccorso, 2004; Ortega-Andrade et al., 2010, 2013; Vigle et al., 2020; Jones et al., 2024), but no additional individuals have been found. The rarity of *N. vigle* sp. nov., especially at well-preserved areas such as Bilsa Reserve is difficult to explain; the species could be naturally rare and/or inhabit the forest canopy, making it challenging to observe. Surveys at canopy level would certainly give us insights about the ecology of the Chocoan amphibian community. We also note that this apparent scarcity of some glassfrog species in nature is reflected in the high number of singleton descriptions (see Discussion).

A recent worrisome event is that the Jatun Sacha foundation, which used to manage the more than 3,000 ha of Reserva Biológica Bilsa, has abandoned the location. As a consequence, there have been several invasions into the Reserva, which have required the intervention of the police and military force. The current situation requires strong support and funding of the Reserva Ecológica Mache-Chindul by the Ecuadorian government and NGOs. On the positive side, FCAT (Fundación para la Conservación de los Andes Tropicales) is working in an area nearby the type locality of the new species, protecting and restoring critical habitats.

## DISCUSSION

### Singleton descriptions are, oftentimes, justified

Tropical biodiversity contains large proportions of rare species (Magurran & Henderson, 2003; Cooper et al., 2024). Thus, as a logical consequence, it is expected that collections will house numerous specimens representing such rare taxa. Taxonomists have the responsibility to describe them and, at the same time, minimize the possibility of generating invalid species.

Species descriptions based on one specimen —known as 'singletons'— are relatively common. Lim et al. (2012) in their review of descriptions published between 2000 and 2010 found that 30 % of invertebrates and 19 % of vertebrates are singletons. Within *Nymphargus*, 8 out of the 43 described species are singletons (19 %); in other words, almost 1 in every 5 species was originally described with a single specimen, which means that singletons, in this glassfrog genus, are relatively common. Although these descriptions are problematic in many ways (e.g., lack of information regarding intraspecific variation such as polymorphisms, ontogenetic changes, and sexual dimorphism), we concur with Köhler & Padial (2016) in that the advantages of recognizing well-supported singleton species as targets for further research and conservation greatly surpass the inconveniences of naming them. We argue that simple guidelines should reduce taxonomic errors when describing singletons (modified from Köhler & Padial, 2016; Guayasamin et al., 2018): (i) morphological diagnosis should be based on traits that present low intraspecific variation in the group of study; (ii) whenever is possible, different datasets should be included the description (e.g., morphological, acoustic, molecular, ecological, biogeographic); (iii) use of specimens that do not show evidence of poor preservation (e.g., dry, twisted, rotten specimens); (iv) precise locality data for accurate comparison with similar species. *Nymphargus viglei* sp. nov. falls within the argumentation explained above, with morphological and biogeographical data supporting its validity. Its recognition also adds to the list of species endangered by the destruction of the Chocó (see below).

### The Chocó - An Endangered Ecosystem

The species that inhabit the Chocó face the challenge to survive in one the most endangered ecosystems of South America (Orme et al., 2005). Deforestation and forest fragmentation within the Ecuadorian Chocó has been rapid and ongoing since the 1960s, mainly because of agriculture and logging (Sierra & Stallings 1998; Fagua et al., 2019; Finer & Mamani 2020) and, more recently, mining (Mestanza-Ramón et al., 2021). Our results show that between 1985–2022 a total of 194,007 hectares of the Ecuadorian Chocó forest have been deforested, which represents an average loss of 5,243 ha/year. The highest forest losses are located in the provinces of Esmeraldas (including the type locality of *Nymphargus viglei* sp. nov.) and Manabí (Fig. 8). Although some time periods have shown a slight increase in vegetation cover (1985–1995, 2000–2005), during the last 15 years (2005–2020), the Ecuadorian Chocó has shown a negative rate of change and an average annual deforestation of 5.4%. In particular, the period 2010–2015 shows the highest reduction in vegetation, with an average annual deforestation of 7.9 % (Table 2). Based on the most recent vegetation cover data (year 2022), the Ecuadorian Chocó has a total forest cover of only 779,581 hectares, which we estimate will be reduced to 742,649 hectares during 2024. This means that in less than 40 years, the Chocó has lost 20 % of its extension, this has a relationship with high population densities and human activities (Kleemann et al., 2022).

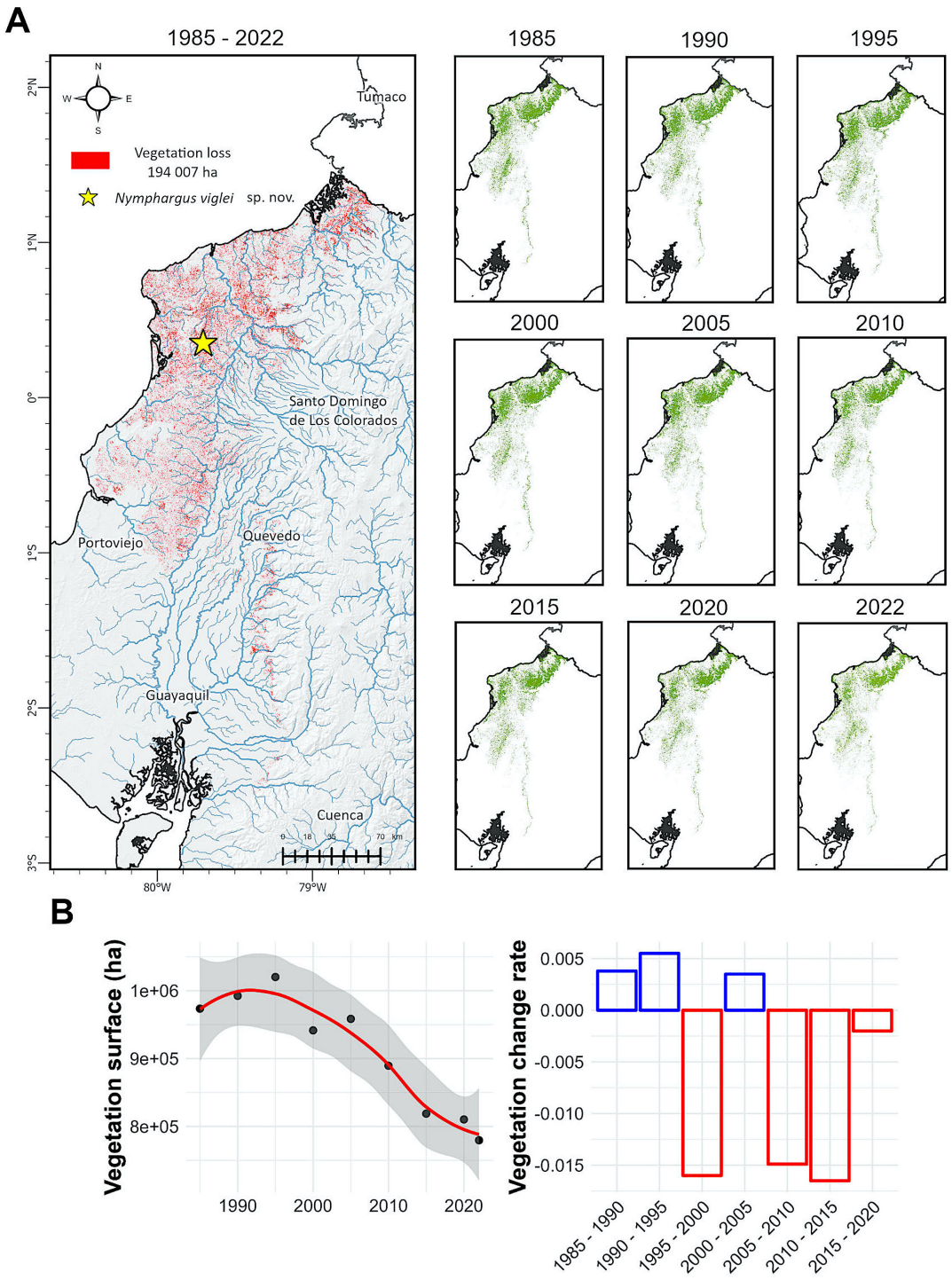


**Figura 8.** (A) Cambios en la cobertura vegetal en el Chocó ecuatoriano de 1985 to 2022. (B, izquierda) Dinámica temporal de la superficie de vegetación en la bioregión del Chocó ecuadoriano. (B, derecha) Tasa de cambio en la superficie de vegetación a través del tiempo.

**Figure 8.** (A) Changes in vegetation cover in the Ecuadorian Chocó from 1985 to 2022. (B, left) Temporal dynamics of the vegetation surface in the Ecuadorian Chocó bioregion. (B, right) Rate of change in vegetation surface over time.

**Tabla 2.** Tasa de cambio de vegetación y promedio de deforestación en el Chocó ecuatoriano. Valores negativos (-) están relacionados con deforestación, mientras que los positivos (+) representan ganancias en la cobertura vegetal.

**Tabla 2.** Rate of vegetation change and average deforestation in the Ecuadorian Chocó. Negative (-) values are related to deforestation, whereas positive (+) values represent gain in forest cover.

| Years | Rate of change | Annual vegetation change (hectares) | Percentage of deforestation (%) |
|---|---|---|---|
| **1985–1990** | 0.0038 | 3760 | - |
| **1990–1995** | 0.0055 | 5559 | - |
| **1995–2000** | -0.016 | -15727 | 7.7 |
| **2000–2005** | 0.0035 | 3384 | - |
| **2005–2010** | -0.0149 | -13814 | 7.2 |
| **2010–2015** | -0.0165 | -14125 | 7.9 |
| **2015–2020** | -0.002 | -1702 | 1 |

The accelerated rate of deforestation in the Chocó is an imminent threat to biodiversity and ecosystem services in the region. This habitat loss is leading to the extinction of Chocó flora lineages that have diversified since the Pliocene (~ 5 million years ago), as well as numerous endemic fauna species (Pérez-Escobar et al., 2019). Current deforestation is driven by illegal mining, monocultures, and timber extraction, which are combined with increasing levels of violence. Thus, strengthening the Reserva Ecológica Mache-Chindul is imperative. Additionally, recent analyzes (Lessmann et al., 2014; Cuesta et al., 2017) shows that many important areas of high biodiversity in the Chocó remain without any protection. We highlight that eradicating illegal activities and violence in the regions should be a priority. This includes restricting those activities in areas of high ecological sensitivity. The persistence of Chocoan endemics, such as *Nymphargus viglei* sp. nov., depends on the urgent conservation of this ecosystem.

**Acknowledgements.–** We thank Adrián Rodríguez-Meza for support on MapBiomas. MVY thanks the Research Grants (Grant ID 26379) from the Masters in Tropical Ecology and Conservation at Universidad San Francisco de Quito. JMG thanks Universidad San Francisco de Quito for constant support to his research (ID 5467, 16871, 16808). We thank curators and collections managers of the following institutions for grant access to the herpetological collections: CJ (Andrea Terán, Luis A. Coloma), DHMECN (Mario Yánez-Muñoz), ICN (John D. Lynch), MZUTI (Mónica Páez, David Salazar), ZSFQ (Carolina Reyes-Puig, Diego F. Cisneros), and KU (Linda Trueb, Rafe Brown). For photos included in this work, we thank Jose Vieira, Juan Carlos Sánchez Nivicela, and Marco Rada. For the illustration of the new species, we thank Valentina Nieto Fernández.

## LITERATURE CITED

Barbour, T. 1905. The vertebrata of Gorgona Island, Colombia: Reptilia; Amphibia. Bulletin of the Museum of Comparative Zoology 46:98-102.

Boettger, O. 1892. Katalog der Batrachier-Sammlung im Museum der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt am Main. Gebrüder Knauer, Frankfurt, Deutschland.

Boulenger, G.A. 1898. An account of the reptiles and batrachians collected by Mr. W.F.H. Rosenberg in Western Ecuador. Proceedings of the Zoological Society of London 1898:107-126.

Boulenger, G.A. 1899. Descriptions of new batrachians in the collection of the British Museum (Natural History). Annals and Magazine of Natural History 3:273-277.

Boulenger, G.A. 1902. Descriptions of new batrachians and reptiles from north western Ecuador. Annals and Magazine of Natural History 9:51-57.

Boulenger, G.A. 1913. On a collection of batrachians and reptiles made by Dr. H.G.F. Spurrell, F.Z.S., in the Choco, Colombia. Proceedings of the Zoological Society of London 1913:1019-1038.

Castroviejo-Fisher, S., J. Ayarzagüena & C. Vilà. 2007. A new species of *Hyalinobatrachium* (Centrolenidae: Anura) from Serranía de Perijá, Venezuela. Zootaxa 1441:51-62.

Castroviejo-Fisher, S., J.M. Guayasamin, A. Gonzalez-Voyer & C. Vilà. 2014. Neotropical diversification seen through glassfrogs. Journal of Biogeography 41:66-80.

Cisneros-Heredia, D.F. & R.W. McDiarmid. 2007. Revision of the characters of Centrolenidae (Amphibia: Anura: Athesphatanura), with comments on its taxonomy and the description of new taxa of glassfrogs. Zootaxa 1572:1-82.

Cisneros-Heredia, D.F. & M.H. Yánez-Muñoz. 2007. A new species of glassfrog (Centrolenidae) from the southern Andean foothills on the West Ecuadorian region. South American Journal of Herpetology 2:1-10.

Cochran, D.M. & C.J. Goin. 1970. Frogs of Colombia. Bulletin of the United States National Museum 288:1-655.

Coloma, L.A. 1995. Ecuadorian frogs of the genus Colostethus (Anura: Dendobatidae). University of Kansas, Natural History Museum. Miscellaneous Publications 87:1-75.

Cope, E.D. 1862. On some new and little known American Anura. Proceedings of the Academy of Natural Sciences of Philadelphia 14:151-159.

Cuesta, F., M. Peralvo, A. Merino-Viteri, M. Bustamante, F. Baquero, J.F. Freile & O. Torres-Carvajal. 2017. Priority areas for biodiversity conservation in mainland Ecuador. Neotropical Biodiversity 3:93-106.

De Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology 56:879-886.

Duellman, W.E. 1981. Three new species of centrolenid frogs from the Pacific versant of Ecuador and Colombia. Occasional Papers of the Museum of Natural History, University of Kansas 88:1-9.

Dunn, E.R. 1931. New frogs from Panama and Costa Rica. Occasional Papers of the Boston Society of Natural History 5:385-401.

Dunn, E.R. 1943. An extraordinary new *Hyla* from Colombia. Caldasia 2:309-311.

Fagua, J.C., J.A. Baggio & R.D. Ramsey. 2019. Drivers of forest cover change in the Chocó-Darien Global Ecoregion of South America. Ecosphere 10:e02648.

Flores, G. 1988. Two new species of Ecuadorian *Eleutherodactylus* (Leptodactylidae) of the *E. crucifer* assembly. Journal of Herpetology 22:34-41.

Finer, M. & N. Mamani. 2020. Amazon Deforestation Hotspots 2020. Monitoring of the Andes Amazon Program, MAAP:132.

Frost, D.R. 2024. Amphibian Species of the World: an Online Reference. Version 6.2. Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. [Accessed on 01 September 2024].

Funkhouser, J.W. 1956. New frogs from Ecuador and southwestern Colombia. Zoologica 41:73-80.

Fowler, H.W. 1913. Amphibians and reptiles from Ecuador, Venezuela, and Yucatan. Proceedings of the Academy of Natural Sciences of Philadelphia 65:153-176.

Goin, C.J. 1961. Three new centrolenid frogs from Ecuador. Zoologischer Anzeiger 166:95-104.

Guayasamin, J. M. & E. Bonaccorso. 2004. A new species of glass frog (Centrolenidae: *Cochranella*) from the lowlands of northwestern Ecuador, with comments on the *Cochranella granulosa* group. Herpetologica 60:85-94.

Guayasamin, J.M., M.R. Bustamante, D. Almeida-Reinoso & W.C. Funk. 2006a. Glass frogs (Centrolenidae) of Yanayacu Biological Station, Ecuador, with the description of a new species and comments on centrolenid systematics. Zoological Journal of the Linnean Society 147:489-513.

Guayasamin, J.M., S. Castroviejo-Fisher, L. Trueb, J. Ayarzagüena, M. Rada & C. Vilà. 2009. Phylogenetic systematics of Glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. Zootaxa 2100:1-97.

Guayasamin, J.M., A. Arteaga & C.R. Hutter. 2018. A new (singleton) rainfrog of the *Pristimantis myersi* group (Amphibia: Craugastoridae) from the northern Andes of Ecuador. Zootaxa 4527:323-334.

Guayasamin, J.M., D.F. Cisneros-Heredia, R. W. McDiarmid, P. Peña & C. R. Hutter. 2020. Glassfrogs of Ecuador: Diversity, evolution, and conservation. Diversity 12:1-285.

Guayasamin, J.M., R.M. Brunner, A. Valencia-Aguilar, D. Franco-Mena, E. Ringler, A.M. Armijos & J. Culebras. 2022. Two new glassfrogs (Centrolenidae: *Hyalinobatrachium*) from Ecuador, with comments on the endangered biodiversity of the Andes. PeerJ 10:e13109.

Heyer, W.R. 2005. Variation and taxonomic clarification of the large species of the *Leptodactylus pentadactylusspecies* group (Amphibia:

Leptodactylidae) from Middle America, northern South America, and Amazonia. Arquivos de Zoologia 37:269-348.

Hutter, C.R. & J.M. Guayasamin. 2012. A new cryptic species of glassfrog (Centrolenidae: *Nymphargus*) from Reserva Las Gralarias, Ecuador. Zootaxa 3257:1-21.

Hutter, C.R., J.M. Guayasamin & J.J. Wiens. 2013. Explaining Andean megadiversity: the evolutionary and ecological causes of glassfrog elevational richness patterns. Ecology Letters 16:1135-1144.

Jiménez de la Espada, M. 1875. Vertebrados del Viaje al Pacífico Verificado de 1862 a 1865 por una Comisión de Naturalistas Enviada por el Gobierno Español. Batracios. A. Miguel Ginesta. Madrid, España.

Jones, S.H., D. Franco-Mena, D. Zambrano Cantos, J.C. Loor Vélez, C. Aulestia, R. A. Zahawi & J.M. Guayasamin. 2024. Range expansion for the Critically Endangered poison-dart frog *Leucostethus bilsa* Vigle et al., 2020 (Amphibia, Dendrobatidae), demonstrating the importance of small forest reserves in the Ecuadorian Chocó. Check List 20:853-858.

Jongsma, G.F.M., R.W. Hedley, R. Duraes & J. Karubian. 2014. Amphibian diversity and species composition in relation to habitat type and alteration in the Mache-Chindul Reserve, northwest Ecuador. Herpetologica 70:34-46.

Köhler, J. & J.M. Padial. 2016. Description and phylogenetic position of a new (singleton) species of Oreobates Jiménez de la Espada, 1872 (Anura: Craugastoridae) from the Yungas of Cochabamba, Bolivia. Annals of Carnegie Museum 84:23-38.

Lim, G.S., M. Balke & R. Meier. 2012. Determining species boundaries in a world full of rarity: singletons, species delimitation methods. Systematic Biology 61:165-169.

Lynch, J.D. & W.E. Duellman. 1973. A review of the Centrolenid frogs of Ecuador, with descriptions of new species. Occasional Papers of the Museum of Natural History, The University of Kansas 16:1-66.

Lynch, J.D. 1974. A new species of *Eleutherodactylus* (Amphibia: Leptodactylidae) from the Pacific lowlands of Ecuador. Proceedings of the Biological Society of Washington 87:381-388.

Lynch, J.D. & P.M. Ruiz-Carranza. 1996. A remarkable new centrolenid frog from Colombia with a review of nuptial excrescences in the family. Herpetologica 52:525-535.

Magurran, A. & P. Henderson. 2003. Explaining the excess of rare species in natural species abundance distributions. Nature 422:714-716.

Maynard, R.J., S.J. Trageser, S. Kohn, P.S. Hamilton, J. Culebras & J.M. Guayasamin. 2020. Discovery of a reproducing population of the Mindo Glassfrog, *Nymphargus balionotus* (Duellman, 1981), at the Río Manduriacu Reserve, Ecuador, with a literature review and comments on its natural history, distribution, and conservation status. Amphibian & Reptile Conservation 14:172-184.

MapBiomas Ecuador Project - Collection 1.0 of the Annual Land Cover and Land Use Series for Ecuador. Available at: http://ecuador.mapbiomas.org. [Accesed on 01 August 2024].

Menéndez-Guerrero, P.A., S.P.L. dos Santos, M.-J. Salazar-Nicholls, D.M. Green & S.R. Ron. 2024. Cryptic diversity in toads of the *Rhinella marina* species group (Anura, Bufonidae) with a subjectively beautiful new species from Western Ecuador. Zoological Journal of the Linnean Society 197:1-26.

Mestanza-Ramón, C., S. Paz-Mena, C. López-Paredes, M. Jimenez-Gutierrez, G. Herrera-Morales, G. D'Orio & S. Straface. 2021. History, current situation and challenges of gold mining in Ecuador's litoral region. Land 10:1220.

Ministerio del Ambiente del Ecuador (MAE). 2012. Sistema de clasificación de los ecosistemas del Ecuador continental. Subsecretaría de Patrimonio Natural. Quito 5:65-74.

Orme, C. D. L., R. G. Davies, M. Burgess, F. Eigenbrod, N. Pickup, V. A. Olson, A. J. Webster, T. Ding, P. C. Rasmussen, R. S. Ridgely, A. J. Stattersfield, P. M. Bennett, T. M. Blackburn, K. J. Gaston & I. P. F. Owens. 2005. Global hotspots of species richness are not congruent with endemism or threat. Nature 486:1016-1019.

Ortega-Andrade, M.H., J. Bermingham, C. Aulestia & C. Paucar. 2010. Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. Check List 6:119-154.

Ortega-Andrade, H.M., O. Rojas-Soto & C. Paucar. 2013. Novel data on the ecology of *Cochranella mache* (Anura: Centrolenidae) and the importance of protected areas for this critically endangered glassfrog in the Neotropics. PLoS ONE 8:1-13.

Pérez-Escobar, O.A., E. Lucas, C. Jaramillo, A. Monro, S. K. Morris, D. Bogarín & A. Antonelli. 2019. The origin and diversification of the hyperdiverse flora in the Chocó biogeographic region. Frontiers in Plant Science 10:1328.

Peters, W.C.H. 1873. Über eine neue Schildrötenart, Cinosternon Effeldtii und einige andere neue oder weniger bekannte Amphibien. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin 1873:603-618.

Puyravaud, J.P. 2003. Standardizing the calculation of the annual rate of deforestation. Forest Ecology and Management 177:593-596.

Ruiz-Carranza, P.M. & J.D. Lynch. 1995. Ranas Centrolenidae de Colombia VI. Cuatro nuevas especies de *Cochranella* de la Cordillera Occidental. Lozania 63:1-15.

Savage, J.M. & W.R. Heyer. 1967. Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, central America: With 6 figures. Studies on Neotropical Fauna and Environment 5:111-131.

Savage, J.M. & P.H. Starrett. 1967. A new fringe-limbed tree-frog (family Centrolenidae) from lower Central America. Copeia 1967:604-609.

Sierra, R. & J. Stallings. 1998. The dynamics and human organization of tropical deforestation in Northwest Ecuador, 1983-1995. Human Ecology 26:135-161.

Sierra, R. 1999. Propuesta Preliminar de un Sistema de Clasificación de Vegetación para el Ecuador Continental. Proyecto INEFAN/GEF-BIRF y EcoCiencia, Quito, Ecuador.

Simpson, G.G. 1951. The species concept. Evolution 5:285-298.

Simpson, G.G. 1961. Principles of Animal Taxonomy. Columbia University Press, New York, USA.

Taylor, E.H. 1949. Costa Rican frogs of the genera *Centrolene* and *Centrolenella*. University of Kansas Science Bulletin 33:257-270.

Tanner, W.W. 1962. A new *Bolitoglossa* (salamander) from southern Panama. Herpetologica 18:18-20.

Thominot, A. 1884. Note sur un batracien d'espèce nouvelle provenant de Panama. Bulletin de la Société Philomathique de Paris. Series 7, 8:151-152.

Vaillant, L. 1911. Cheloniens et batraciens urodèle, recuillis par M. le Dr. Rivet. Mission du Service Géographique de l'Armée pour la Mesure d'un Arc de Méridien Equatorial en Amérique du Sud. Volume 9 (Zoologique). Part 2 (Reptiles, Poisson, Batraciens):45-60.

Vigle, G.O. 2008. The amphibians and reptiles of the Estación Biológica Jatun Sacha in the lowland raiforest of Amazonian Ecuador: A 20-year record. Breviora 515:1-30.

Vigle, G.O. & K. Miyata. 1980. A new species of *Dendrobates* (Anura: Dendrobatidae) from the lowland rain forests of western Ecuador. Breviora. Museum of Comparative Zoology, Cambridge, Massachusetts 459:1-7.

Vigle, G.O., L.A. Coloma, J.C. Santos, S. Hernandez-Nieto, H.M. Ortega-Andrade, D.J. Paluh & M. Read. 2020. A new species of *Leucostethus* (Anura: Dendrobatidae) from the Cordillera Mache-Chindul in northwestern Ecuador, with comments on similar Colostethus and Hyloxalus. Zootaxa 4896:342-372.

Werner, F. 1901. Ueber Reptilien und Batrachier aus Ecuador und Neu Guinea. Verhandlungen des Zoologisch-Botanischen Vereins in Wien 51:593-614.

Wiley, E.O. 1978. The evolutionary species concept reconsidered. Systematic Zoology 27:88-92.

**Apéndice 1.** Specimens examined.

**Appendix 1.** Specimens examined.

*Espadarana prosoblepon*: Ecuador: Provincia de Azuay: 12.9 km W Luz María (2.6889°S, 79.474°W, 740 m), KU 217502, QCAZ 12603–04; Provincia de Bolívar: Balzapamba (1.7667°S, 79.1833°W, 800 m), KU 132555–56, 132462–65; Provincia de Carchi: near Maldonado (0.9 °N, 78.1°W, 1410 m), KU 178156–57; Río Chinambí (0.828°N, 78.297°W; 1045 m), CJ 8543. Provincia de Cotopaxi: km 8 of the Pucayacu-Sigchos road (1.0097°S, 79.23769 W), QCAZ 40674; Reserva Natural Pristirana (0.425°S, 78.959°W; 1488 m), CJ 10976. Provincia de Esmeraldas: Reserva Itapoa (0.3447°N, 79.1348°W, 411 m), MZUTI 3041; Reserva Biológica Bilsa (0.3447°S, 79.71°W, 500 m), KU 291165–75, QCAZ 22414–17; Viruela, QCAZ 10267; Reserva Biológica Bilsa (0.3447°S, 79.71°W,

500 m), KU 291165–75; Reserva Ecológica Cotacachi-Cayapas, Charco Vicente (0.792 °N, 79.1978°W, 60 m), QCAZ 11364–65; 5 km W of Durango (1.0858 °N, 78.74 W), QCAZ 13206, 13212, 13242. Provincia de El Oro: near Valle Hermoso (3.50194°S, 79.81722°W, 379 m), QCAZ 37249; Río Chillayacu (3.32834°S, 79.58102°W, 395 m), 16.8 km W of Piñas (3.667°S, 79.667°W, 600 m), USNM 286738–39. Provincia del Guayas: Chongon-Colonche hills near Guayaquil, (ca. -2.1°S, -80.15 W), USNM 288438; Provincia de Imbabura: near Lita (0.833 °N, 78.4667°W, 520 m), KU 133482–83; 6 km E of Lita (0.79472 °N, 78.4286 W), QCAZ 4318–19; Zona de amortiguamiento de Reserva Cotacachi Cayapas, near Rio Aguas Verdes (0.331010°N, 78.93152°W; 670 m), QCAZ 46009; km 5 in the Lita-Ibarra road (0.84773 °N, 78.42175 W), QCAZ 39919; Bosque Protector Los Cedros (0.303°N, 78.787°W; 1332 m), CJ 7699; Intag, sector Junín (0.2783°N, 78.6675°W; 1380 m), CJ 5230; Cabañas EcoJunín (0.2795°N, 78.6682°W; 1302 m), CJ 10999, 11006; Reserva Junín (0.3116°N, 78.659°W; 1707 m), SC 11556; Manduriacu (0.2984°N, 78.86°W; 1133 m), CJ 14134, 14136. Provincia de Los Ríos: Estación Biológica Río Palenque, 56 km N of Quevedo (0.55°S, 79.3667°W, 220 m), KU 164616–22. Provincia de Santo Domingo de los Tsáchilas: Río Orito, on the Toachi-Chiriboga road (0.30561°S, 78.882°W, 1315 m), QCAZ 15356; Río Faisanes, on the Toachi-Chiriboga road (0.2608°S, 78.845°W, 1400 m), QCAZ 15357, 15360, 15362; La Florida (0.28361°S, 79.0189 W), QCAZ 20726–28, 20730–32; Otongachi, near La Unión del Toachi (0.3167°S, 78.95°W, 900 m), QCAZ 25094; 5 km NE of La Florida (0.25694°S, 79.0539 W), QCAZ 7184–893; 4 km NE of the Dos Ríos–Chiriboga road (0.305139°S, 78.884333°W, 1270 m), QCAZ 31982; Santo Domingo de los Colorados (0.25°S, 79.15°W, 660 m), KU 121054–55; 4 km NE of Dos Ríos (0.30278°S, 78.8678°W, 1140 m), KU 164623–34; 2 km E and 1 km S of Santo Domingo de los Colorados (0.24512°S, 79.15509°W, 600 m), KU 178158–66; La Palma (0.3167°S, 78.9167°W, 920 m), KU 178167. Provincia de Manabí: Jama-Coaque (0.1221°S, 80.1179°W; 569 m), MZUTI 4113. Provincia de Pichincha: Mindo, Santuario de las Cascadas (0.0854°S, 78.7640°W; 1418 m), MZUTI 2270; Mindo, Sachatamia Lodge (0.0237°S, 78.7584°W; 1683 m), MZUTI 1413; Cascadas de Mindo (0.07917°S, 78.7634°W; 1392 m), MZUTI 704; Reserva Maquipucuna (0.12429 °N, 78.62936°W, 1343 m), QCAZ 42179; 6 km NW (by air) of Pedro Vicente Maldonado (0.10421 °N, 79.10279°W, 544 m), QCAZ 35430; La Concordia, Bosque Protector La Perla (0.01 °N, 79.4°W, 190 m), QCAZ 12602; Reserva Mashpi (0.1667°N, 78.879°W; 900 m), CJ 2699; Comunidad Mashpi (0.1826°N, 78.9087°W; 555 m), CJ 974.

*Nymphargus balionotus*: Ecuador: Provincia de Carchi: Cabeceras del Río Baboso (00°53” °N, 78°27”°W, 1400 m), DHMECN 0865. Provincia de Pichincha: 3.5 km NE Mindo (0.0322°S, 78.761°W, 1540 m), KU 164702 (holotype), 164701, 164703–11. Provincia de Imbabura: Reserva Río Manduriacu, 1240–1254 m, ZSFQ 0531–33.

*Nymphargus buenaventura*: Ecuador: Provincia de El Oro: Reserva Buenaventura (03°38´°S, 79°45´°W, 1200 m), DHMECN 3563 (holotype), 2524, 3561, 3562 (paratypes).

*Nymphargus chami*: Colombia: Departamento de Risaralda: Municipio de Mistrató, Corregimiento Puerto de Oro, quebradas Carbones y Ventanas, vertiente occidental de la Cordillera Occidental (5°25´°N, 76°0´W), 1060–1140 m, ICN 32079 (holotype).

*Nymphargus griffithsi*: Ecuador: Provincia de Cotopaxi: 11.5 km W of Pilaló (0.94815°S, 78.989633°W; 1500 m), QCAZ 34113; Provincia de Pichincha: Río Saloya, 1219 m, BMNH 1940.2.20.4 (holotype), BMNH 1940.2.20.3 (paratype); km 14 on the San Juan de Chillogallo–Chiriboga road (0.275895°S, 78.721647°W; 2120 m), QCAZ 29531; km 16 on the San Juan de Chillogallo– Chiriboga road (0.278161°S, 78.706067°W; 2430 m), QCAZ 29524–30; La Victoria (0.16285°S, 77.909667°W; 2104 m), QCAZ 24801; Tandapi (0.416388°S, 78.7988°W), QCAZ 351, KU 118009–20; Reserva Las Gralarias “Hercules Giant Tree Frog Creek” (0°01.529´°S, 78°42.243´°W, 2175 m), MZUTI 100, 102, 099; Reserva Las Gralarias “Five Frog Creek” (0°01.870´°S, 78°42.358´°W, 2150 m), MZUTI 101; Reserva Las Gralarias “Heloderma Creek” (0°01.245´°S, 78°42.370´°W, 2200 m), MZUTI 098; 1 km SW San Ignacio (0.44861°S, 78.74777°W; 1920 m), KU 178108–21; 3.5 NE Mindo (0.03222°S, 78.76138°W; 1340 m), KU 164564–76; 5 km ESE Chiriboga (0.245277°S, 78.7261°W; 2010 m), KU 164519–37; 5.6 km SE Tandayapa (0.0333°S, 78.7166°W; 1910 m), KU 202792. Provincia de Santo Domingo de los Tsáchilas: 14 km W of Chiriboga (0.26527778°S, 78.847778°W; 1960 m), KU 164544–63; 4 km W Chiriboga (0.24277°S, 78.7855°; 2120 m), KU 142649.

*Nymphargus lasgralarias*: Ecuador: Provincia de Cotopaxi: Bosque Integral Otonga (0.55°S, 79.46667°W, 2000 m), QCAZ 13115; Reserva Otonga (0.676°S, 76.397°W, 1950 m), QCAZ 11689–90. Provincia Imbabura: Provincia de Pichincha: Five Frog Creek (0°01.870´°S, 78°42.358´°W, 2150 m) at Reserva Las Gralarias, MZUTI 096 (holotype), MZUTI 091–095, 097; Kathy’s Creek (0°01.398´°S, 78°43.772´°W, 2000 m), Reserva Las Gralarias, MZUTI 091–095; Hercules Giant Tree Frog Creek (0°01.529´°S, 78°42.243´°W, 2175 m), Reserva Las Gralarias, MZUTI 097; Nanegal Grande (0.1167 °N, 78.6667°W, 2300 m), QCAZ 46012; 9 km SE Tandayapa (0.01667°S, 78.6833°W, 2160 m), KU 164577–87.

*Nymphargus prasinus*: Colombia: Departamento de Valle: Río Calima, 1.5 km (by road) west of Lago Calima (4° 00´°N, 76° 35´°W, 1230 m), KU 169691–93 (type series).

**Apéndice 2.** Anfibios documentados en la Reserva Biológica Bilsa. Los registros provienen de las siguientes fuentes: Ortega-Andrade et al. (2010), Guayasamin & Bonaccorso (2004), Jongsma et al. (2014), Guayasamin et al. (2020), Vigle et al. (2020) and Frost, 2024.

**Appendix 2.** Amphibians documented in Reserva Biológica Bilsa. Records come from: Ortega-Andrade et al. (2010), Guayasamin & Bonaccorso (2004), Jongsma et al. (2014), Guayasamin et al. (2020), Vigle et al. (2020) and Frost, 2024.

Bufonidae: *Rhaebo haematiticus* (Cope 1862), *Rhinella alata* (Thominot 1884), *Rhinella bella* Menéndez-Guerrero, Santos, Salazar-Nicholls, Green & Ron 2024.

Centrolenidae: *Cochranella mache* Guayasamin & Bonaccorso 2004, *Espadarana prosoblepon* (Boettger 1892), *Hyalinobatrachium tatayoi* Castroviejo-Fisher, Ayarzagüena & Vilà 2007, *H. valerioi* (Dunn 1931), *Sachatamia albomaculata* (Taylor 1949), and *Teratohyla pulverata* (Peters 1873).

Dendrobatidae: *Epipedobates boulengeri* (Barbour 1905), *Hyloxalus awa* (Coloma 1995), *Leucostethus bilsa* Vigle et al. 2020, *Oophaga sylvatica* (Funkhouser 1956), *Paruwrobates erythromos* (Vigle & Miyata 1980).

Hylidae: *Agalychnis spurrelli* Boulenger 1913, *Boana pellucens* (Werner 1901), *Boana picturata* (Boulenger 1899), *Boana rosenbergi* (Boulenger 1898), *Ecnomiohyla phantasmagoria* (Dunn 1943), *Scinax quinquefasciatus* (Fowler 1913), *Smilisca phaeota* (Cope 1862).

Leptodactylidae: *Leptodactylus labrosus* Jiménez de la Espada 1875, *Leptodactylus rhodomerus* Heyer 2005.

Strabomantidae: *Pristimantis achatinus* (Boulenger 1898), *P. latidiscus* (Boulenger 1898), *P. muricatus* (Lynch & Miyata 1980), *P. rosadoi* (Flores 1988), *P. subsigillatus* (Boulenger 1902), *P. walkeri* (Lynch 1974).

Plethodontidae: *Bolitoglossa biseriata* Tanner 1962, *Bolitoglossa sima* (Vaillant 1911).