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Notes on the distribution and advertisement call of *Nymphargus buenaventura* (Anura: Centrolenidae), with comments on its natural history and conservation

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Abstract

Notes on the distribution and advertisement call of *Nymphargus buenaventura* (Anura: Centrolenidae), with comments on its natural history and conservation. We provide an update on the distribution of Buenaventura´s glassfrog (*Nymphargus buenaventura*), adding a new locality in Peru. We extend the known geographic range of the species 180 km southward. We report for the first time its advertisement call, which consists of a high-pitched note. Morphological and molecular analyses confirmed the identity of the specimens, with 16S rRNA sequences exhibiting minimal genetic divergence (1.08%) from those of Ecuadorian populations. Additionally, we observed egg clutches exceeding previously reported clutch sizes. Given the extended range and lack of population data, we recommend placing *N. buenaventura* in the Data Deficient category of the IUCN Red List until further studies are conducted.

Keywords: Data Deficient, Glassfrogs, High-pitched note, New locality, Peru, rRNA

Resumo

Notas sobre a distribuição e o canto de advertência de *Nymphargus buenaventura* (Anura: Centrolenidae), com comentários sobre sua história natural e conservação. Fornecemos uma atualização sobre a distribuição da rã-de-vidro-de-buenaventura (*Nymphargus buenaventura*), adicionando uma nova localidade no Peru. Ampliamos a distribuição geográfica conhecida da espécie em 180 km ao sul. Relatamos pela primeira vez seu canto de anúncio, que consiste em uma nota aguda. Análises morfológicas e moleculares confirmaram a identidade dos espécimes, com sequências de rRNA 16S exibindo divergência genética mínima (1,08%) em relação às populações equatorianas. Além disso, observamos ninhadas de ovos excedendo os tamanhos de ninhadas relatados anteriormente. Dada a extensão da distribuição e a falta de dados populacionais, recomendamos incluir *N. buenaventura* na categoria Dados Deficientes da Lista Vermelha da IUCN até que mais estudos sejam conduzidos.

Palavras-chave: Deficiente em Dados, Nova localidade, Notas de alta frequência, Pererecas-de-vidro, Peru, rRNA.

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Introduction

Glassfrogs are a group of Neotropical arboreal frogs renowned for their translucent ventral skin, which often reveals their internal organs—a trait that has fascinated biologists and inspired their common name. Currently, 167 species are recognized as belonging to this family (Frost 2025), most of them inhabiting the eastern side of the Andes. Glassfrogs from the Pacific slopes of northwestern Peru are not as diverse as those from the Andean Amazonian slopes. In contrast to the 17 species distributed in the Amazon River drainage of the northeastern Andes (Catenazzi and Venegas 2012, Twomey *et al*. 2014, Gagliardi-Urrutia *et al*. 2022), only three species have so far been recorded on the Pacific slopes of the Andes of northern Peru: *Centrolene buckleyi (*Boulenger, 1882), *Centrolene hesperia* (Cadle and McDiarmid, 1990), and *Cochranella euhystrix* (Cadle and McDiarmid, 1990) (Duellman and Wild 1993). Further north, the diversity of Glassfrogs on the Pacific drainage of southern Ecuador remains low, with four species recorded: *Espadarana prosoblepon* (Boettger, 1892), *Nymphargus buenaventura* (Cisneros-Heredia and Yañez-Muñoz, 2007) (Guayasamin *et al*. 2020), *Centrolene camposi* Cisneros-Heredia, Yánez-Muñoz, Sánchez-Nivicela, and Ron, 2023, and *C. ericsmithi* Cisneros-Heredia, Yánez-Muñoz, Sánchez-Nivicela, and Ron, 2023. Regarding frogs in the genus *Nympahrgus, N. buenaventura* marks the southernmost geographic limit of the genus in the Pacific drainage of South America (Guayasamin *et al*. 2020).

Nymphargus buenaventura is a small frog (SVL = 20.9–22.4 mm) known from four localities in southwestern Ecuador from 800 to 1925 m a.s.l. (Yáñez-Muñoz *et al*. 2014, Guayasamin *et al.* 2020, Coloma and Duellman 2025). Besides these records, little is known about this species. This lack of knowledge includes its advertisement call and natural history. Additionally, its phylogenetic relationships are controversial due to incongruences in its inferred phylogenetic position, as shown by Portik *et al*. (2023) and Montilla *et al*. (2023). So far, *N. buenaventura* has been found inhabiting vegetation alongside streams in mountain forests (Cisneros-Heredia and Yañez-Muñoz 2007). Although it was originally placed in the Data Deficient category of the IUCN Red List (Cisneros-Heredia 2008), Guayasamin *et al*. (2020) suggested moving it to the Endangered category due to its apparent endemism to southwestern Ecuador and its distribution overlapping a highly deforested area. Later, *N. buenaventura* was placed in the Endangered category after the assessment made by Cisneros-Heredia *et al*. (2022). During fieldwork in the Cordillera de Huancabamba in the northwestern Andes of Peru, we collected a small series of glassfrogs that we assigned to *Nymphargus buenaventura* based on morphological, acoustic, and DNA sequence data. The new geographic record of this glassfrog species is the first report in Peru. The results of this work are described herein.

Materials and Methods

Fieldwork

We conducted Visual Encounter Surveys (Crump and Scott 1994) during both day and night. As a result of our field survey, six adult males (CORBIDI 22316–22318, 22320–22322) and a subadult male (CORBIDI 22319) of *Nymphargus buenaventura* (Figure 1) were caught at Agua Blanca Village, Huancabamba province, Piura department, Peru (05°20'44.0" S, 79°34'1.9" W; 1800 m a.s.l.) on 6 February 2020, from 21:45 to 22:30 h by Luis A. García-Ayachi, Jesus R. Ormeño, and GC. Calling males were approximately 5–10 m apart. All specimens were deposited in the herpetological collection of Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru.

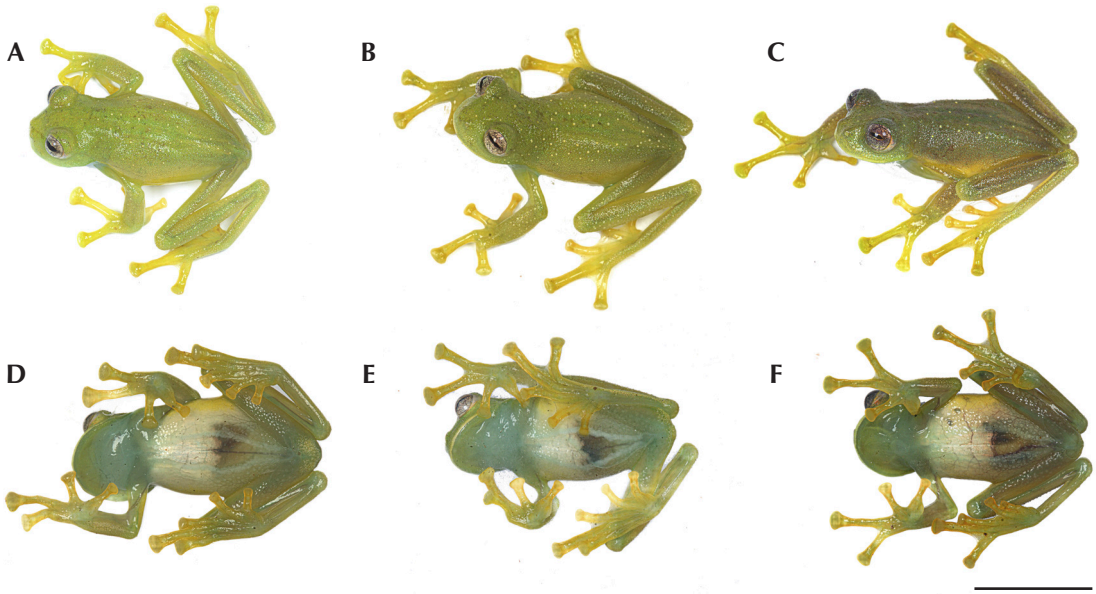

Figure 1. Coloration in life of males of *Nymphargus buenaventura* from Agua Blanca, Piura, northwestern Peru: (**A** and **D**) CORBIDI 22316 (SVL = 22.1 mm), (**B** and **E**) CORBIDI 22318 (SVL = 22.3mm), (**C** and **F**) CORBIDI 22320 (SVL = 22.3 mm). SVL = snout–vent length. Scale bar = 1.2 mm.

Morphology

We follow Guayasamin *et al*. (2020) for systematics of Centrolenidae and nomenclature of morphological characters. Specimens were sexed based on external sexual characteristics (e.g., presence of vocal sac) and by examining gonads. We measured the snout–vent length (SVL) to the nearest 0.1 mm with a digital calliper. Specimens were euthanized with an 8% benzocaine solution, fixed in 10% formalin, and stored in 70% ethanol. We also reviewed photographs of the specimen DHMECN 9471 of *Nymphargus buenaventura* from the type locality, El Oro province, Ecuador.

Bioacoustics

We recorded calls with a Marantz PMD660 digital recorder and a Marantz ME64 shotgun microphone; the resulting digital files were stored in WAV format. Recordings were performed during night surveys at 19°C, 0.5 m from a calling male (CORBIDI 22318) and about 1 m from an unvouchered male. We measured the following variables (as defined by Köhler *et al*. 2017): number of notes per call, note length (NL), and dominant frequency (DoF), which also corresponds to the fundamental frequency in this species, taken with a spectral slice over the entire note. We measured and visualised call variables on oscillograms and spectrograms (Figure 2) using Raven Lite v2.0.5 (K. Lisa Yang Center for Conservation Bioacoustics 2024). Spectrogram parameters included a 512-point window size, Hann window, and 75% overlap, providing a frequency (DFT size) resolution of ~86 Hz and a time (hop size) resolution of ~5.8 ms. Both the oscillogram and spectrogram were extracted directly from the same waveform in Raven and displayed using a logarithmic (dB) color scale.

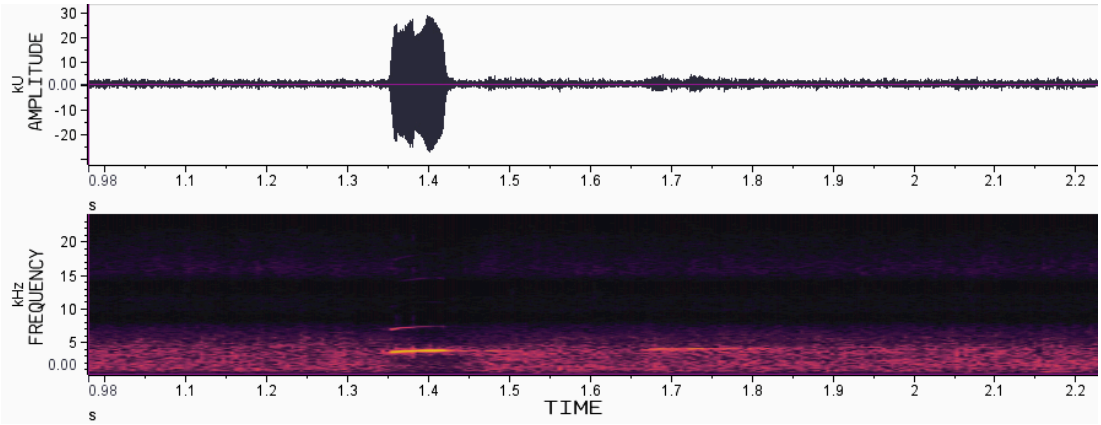

Figure 2. Advertisement call of *Nymphargus buenaventura* from Agua Blanca, Piura, northwestern Peru (CORBIDI 22318). Spectrogram is shown below oscillogram. Air temperature: 19°C.

Molecular Genetics

We sequenced a fragment of the 16S rRNA mitochondrial gene to compare its sequence with the only 16S sequence of *N. buenaventura* of a topotype available in Genbank, from QCAZ 54825 (accession code MT734665). We extracted genomic DNA from muscle tissues of CORBIDI 22316, 22320, and 22322 using the DNA extraction kit of IBI Scientific (Peosta, USA). We followed the standard protocols for amplification and sequencing of DNA (described by Chávez *et al*. 2019). We used the 16Sar forward (5'-3' sequence: CGCCTGTTTATCAAAAACAT) and 16Sbr reverse (5'-3' sequence: CCGGT CTGAACTCAGATCACGT) primers for 16S (Palumbi *et al*. 2002, von May *et al*. 2017). We used the following thermocycling conditions during the polymerase chain reaction (PCR) with a ProFlex thermal cycler (Applied Biosystems): one cycle of 96°C/3 min; 35 cycles of 95°C/30 s, 55°C/45 s, 72°C/1.5 min; one cycle 72°C/7 min. We used Exosap-IT (Affymetrix, Santa Clara, CA) to purify PCR products; MCLAB (San Francisco, CA) performed paired-end Sanger sequencing. We used Geneious R11, version 11.1.5 (Biomatters, http://www.geneious.com/) to assemble pair-end reads, to generate a consensus sequence, and to align our novel and GenBank sequences with the alignment program MAFFT v7.017 (Katoh and Standley 2013). We trimmed aligned sequences to a length of 578 bp, and estimated uncorrected p-distances, the proportion of nucleotide sites at which any two sequences are different, in MEGA X (Kumar *et al*. 2018). The newly generated sequence was deposited in GenBank (accession code for CORBIDI 22316: PV226227; fragments of the two other specimens were identical).

Results

We found all individuals on leaves 1.2–1.9 m above a stream in a small patch of secondary forest dominated by bushes and scattered small trees (3–5 m tall). We noticed some variation in the Peruvian population reported herein. Male CORBIDI 22320 (Figures 1C, 1F) has a darker green background on the dorsum with cream to pale yellow spots. The pale spots on the back are scattered in CORBIDI 22316 (Figures 1A, 1D) and CORBIDI 22320, or densely present in CORBIDI 22318 (Figures 1B, 1E) and CORBIDI 22321. The fragments of 16S of the three specimens are identical (accession code for CORBIDI 22316: PV226227) and similar to

the specimen of *N. buenaventura,* QCAZ 54825, from the type locality (uncorrected p-distance 1.08%). All adult males match the SVL previously documented (Cisneros-Heredia and Yañez-Muñoz 2007) for the species (20.9–22.4 mm).

On the same day we collected the frogs, we recorded two calls: the first one from the male CORBIDI 22318 and the second one from an unvouchered individual, both at 19:40 h. Both individuals were calling from leaves alongside a stream, 19°C air temperature. The call of *Nymphargus buenaventura* is tonal (Figure 2), composed of high-pitched calls (chirps). We analized the two calls. The first one had a duration of 0.59 s with a dominant frequency of 3.66 kHz, while the second was shorter (0.53 s) and peaked at 3.38 kHz (note duration mean = 0.56 ± 0.04 s, N = 2) and a dominant frequency of 3.37–3.65 kHz (mean = 3.51 ± 0.19 kHz, N = 2). The dominant frequency corresponds to the fundamental frequency. This is the first recording and quantitative description of an advertisement call of *N. buenaventura,* which had previously only been described onomatopoeically as a simple "tic" (Cisneros-Heredia and Yañez-Muñoz 2007). We noticed that calling activity ceased upon our approach, suggesting high sensitivity to disturbance. That is why, under these conditions, we recorded one male near us (CORBIDI 22318) and one unvouchered individual from a distant spot.

In the same stream, we also located two egg clutches with 47 and 52 eggs (Figure 3), exceeding the number of 46 eggs for a clutch known from Ecuador (Cisneros-Heredia and Yañez-Muñoz 2007, Guayasamin *et al*. 2020).

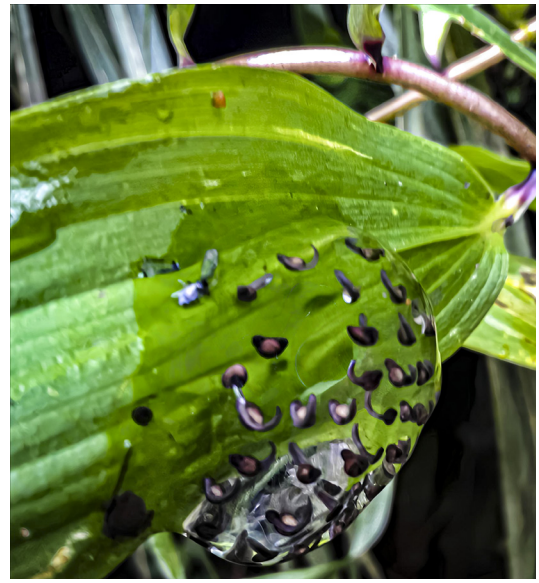

Figure 3. Egg clutch of *Nymphargus buenaventura* with 47 embryos, located at Agua Blanca, Piura, northwestern Peru, locality of the new record.

Discussion

All individuals show the diagnostic characteristics of *Nymphargus buenaventura* (Cisneros-Heredia and Yañez-Muñoz 2007), including green dorsum with small pale yellow to cream spots, reduced webbing between fingers, absence of humeral spine, and absence of iridophores on the digestive visceral peritonea, but with iridophores covering the renal capsules (see Figure 1). Additionally, our genetic analyses yielded a genetic distance of only 1.08% from the topotype QCAZ 54825, which confirms our identification. Also, uncorrected *p*-distance values between QCAZ 54825 and genetically related species (i.e. *Nymphargus cariticommatus, N. lindae,* and *N. sucre*) are more than 4% (Montilla *et al*. 2023). Our results suggest that the Peruvian population reported herein might be ± 1% genetically closer or farther from related samples reported by Montilla *et al*. (2023). Additionally, according to the phylogenetic data published by Guayasamin *et al*. (2020), *N. cariticommatus, N. sucre,* and *N. wileyi* form a clade sister to a clade formed by *N. lindae* and *N. cochranae*. This suggests that *N. buenaventura* may be genetically related to *N. cochranae* and *N. wileyi*. Further genetic research is needed to clarify its phylogenetic position.

We report the first documented occurrence of *Nymphargus buenaventura* in Peru, marking the

fourth glassfrog species recorded from the Pacific versant of the northern Peruvian Andes. This finding significantly expands the known distribution of the species, extending its range 180 km (airline) southward from the southernmost locality reported for this species, which is Marcabelí (Yánez-Muñoz *et al*. 2014), Ecuador, and 190 km (airline) from its type locality (Cisneros-Heredia and Yañez-Muñoz 2007), the Buenaventura Reserve in Guayas Province, Ecuador (see Figure 4). This range extension suggests the potential presence of *N. buenaventura* in intermediate localities, necessitating further field investigations to delineate its precise distribution. Our observations at 1800 m a.s.l. represent one of the few records of this species at altitudes exceeding 1700 m (Cisneros-Heredia and Yañez-Muñoz 2007, Yánez-Muñoz *et al.* 2014, Guayasamin *et al.* 2020, Coloma and Duellman 2025). This is not the first time this species has been recorded at this high elevation. Coloma and Duellman (2025) reported this glassfrog at an elevation of 1925 m a.s.l. The specimen QCAZ 21947 from "Luz María, 0.9 km Oeste de Luz María," Ecuador (geographic coordinates: -2.684 -79.417), reported by Yánez-Muñoz *et al*. (2014) and Guayasamin *et al*. (2020) was found at 1770 m a.s.l., not at 770 m a.s.l. as given therein. The western Andean slopes of northern Peru are characterized by a complex environmental mosaic, transitioning from equatorial dry forests at lower elevations to progressively wetter montane forests at higher altitudes (Koepcke and Koepcke 1958, Brack 1986), with rougher terrain above 300 m a.s.l. (Koepcke and Koepcke 1958, Venegas 2005). The transition to wetter conditions occurs around 1600 m a.s.l. (Koepcke and Koepcke 1958, Venegas 2005). Since the documented altitudinal range of *N. buenaventura* corresponds to the equatorial dry forest zone, updated altitudinal records between 1770–1925 m a.s.l. indicate the upper altitudinal limit of this species and its tolerance to the transitional montane forest environment.

Among *Nymphargus* from the Pacific versant between Peru and Ecuador, the advertisement call of four species have previously been described: *Nymphargus grandisonae* (Cochran and Goin, 1970), *N. griffithsi* (Goin, 1961), *N. lasgralarias* Hutter and Guayasamin, 2012, and *N. manduriacu* Guayasamin, Cisneros-Heredia, Vieira, Kohn, Gavilanes, Lynch, Hamilton, and Maynard, 2019 (all occurring in northwestern Ecuador). We add information on one more species herein. From the aforementioned species, only *N. griffithsi* and *N. manduriacu* emit single-tonal calls, as does *N. buenaventura*. Despite our small sample, we can confirm that the dominant frequency in the advertisement call of *N. buenaventura* is considerably lower: 3.37–3.65 kHz (vs 3.79–4.30 kHz in *N. griffithsi,* and 4.05–4.44 kHz in *N. manduriacu*). Additionally, only *N. manduriacu* has a combination of a single tonal call with a chirp-like sound (Guayasamin *et al*. 2019) as observed in *N. buenaventura*. Among genetically related species mentioned earlier in this section, the call is only known for *N. cochranae,* which was described as a high-pitched note by Lynch and Duellman (1973). Despite the description matching the sound we recorded from *N. buenaventura* (high-pitched note), the lack of data about spectral and temporal variables of the call of *N. cochranae* prevents us from performing additional analyses on this topic.

A negative correlation between SVL and peak frequency in glassfrogs was observed by Escalona-Sulbarán *et al*. (2018). Due to the lack of quantitative acoustic data, it is not possible to analyse genetically related species. *Nymphargus buenaventura,* which is smaller (SVL range = 20.9–22.4 mm) than *N. griffithsi* (SVL range = 20.9–22.4 mm) and *N. manduriacu* (SVL range = 20.9–22.4 mm), two species from southern Ecuador, has a lower dominant frequency range, something that does not support the hypothesis provided by Escalona-Sulbarán *et al*. (2018). This hypothesis needs to be tested using a larger dataset based mainly on genetically related species as suggested by Escalona-Sulbarán *et al*. (2018).

We noticed that embryos inside clutches are dark grey (see Figure 3), whereas embryos reported

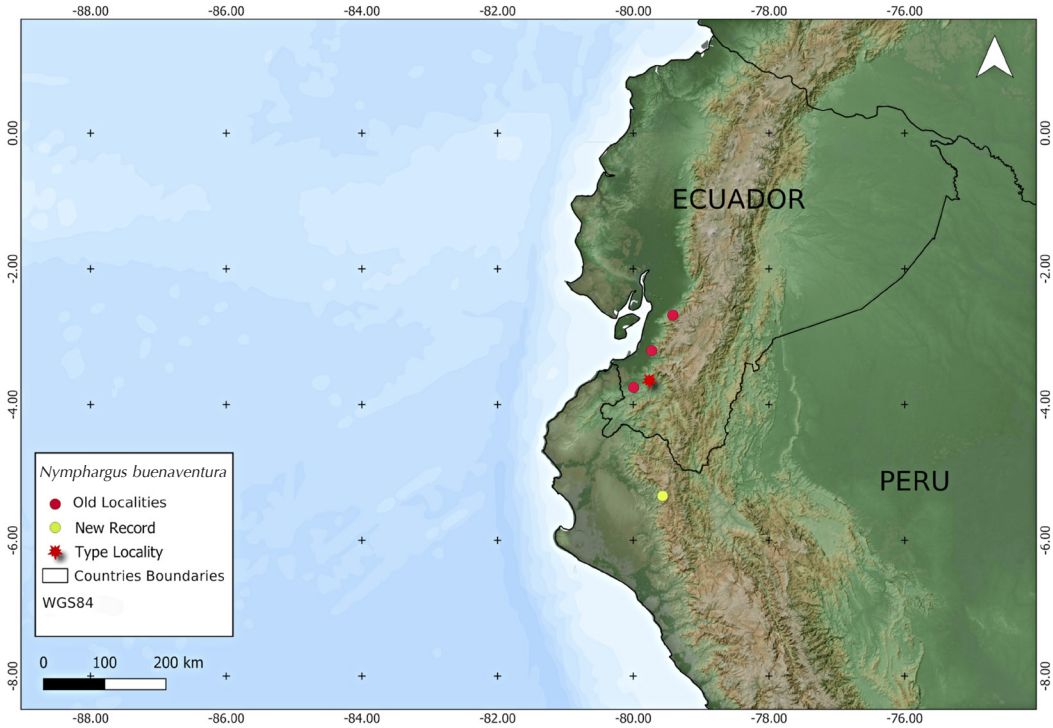

Figure 4. Map showing the distribution of *Nymphargus buenaventura*.

for Ecuadorian populations are yellow (Cisneros-Heredia and Yañez-Muñoz 2007). The ages of the clutches we located are unknown, which may explain the color differences. As reported by Cisneros-Heredia and Yañez-Muñoz (2007), both egg clutches were on the upper side of the leaf and seemed to glide toward its edge.

Despite the suggestion of Gayasamin *et al.* (2020) and Cisneros-Heredia *et al.* (2022) that *N. buenaventura* should be categorized as Endangered, we suggest keeping *N. buenaventura* in the Data Deficient category until future studies focused on its population status are conducted.

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