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A new subspecies of glass frog, *Nymphargus grandisonae orense* ssp. n. (Anura: Centrolenidae), from southwestern Ecuador

Diego F. Cisneros-Heredia[1,2,3], Santiago R. Ron[4], Dan Cogălniceanu[5,6] & Paul Székely[7,8]

[1] Universidad San Francisco de Quito USFQ, Colegio de Ciencias Biológicas y Ambientales, Instituto de Biodiversidad Tropical IBIOTROP, Laboratorio de Zoología Terrestre, Museo de Zoología, Quito, Ecuador
[2] King's College London, Department of Geography, London, UK
[3] Instituto Nacional de Biodiversidad, Quito, Ecuador
[4] Pontificia Universidad Católica del Ecuador, Escuela de Biología, Museo de Zoología, Quito, Ecuador
[5] Ovidius University Constanța, Faculty of Natural Sciences and Agricultural Sciences, Constanța, Romania
[6] Universidad Nacional de Loja, CITIAB, Loja, Ecuador
[7] Universidad Técnica Particular de Loja, Museo de Zoología, Loja, Ecuador
[8] Universidad Técnica Particular de Loja, Departamento de Ciencias Biológicas y Agropecuarias, Laboratorio de Ecología Tropical y Servicios Ecosistémicos (EcoSs-Lab), Loja, Ecuador

Corresponding author: Dan Cogălniceanu, ORCID 0000-0003-2959-014X,
e-mail: dcogalniceanu@univ-ovidius.ro

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Abstract. We describe a new subspecies of glass frog within *Nymphargus grandisonae* from El Oro province, southwestern Ecuador, based on morphological, genetic, ecological and geographic data. *Nymphargus grandisonae* is characterized by having red dots on a green dorsum, white urinary bladder, males with humeral spines, and snout–vent length 27.5–31.5 mm in adult females and 25.1–29.4 mm in adult males. The new subspecies is distinguished from *N. g. grandisonae* and all other members of the family Centrolenidae by its distinctive colouration with red dots on a green dorsum, snout–vent length 32.1–36.2 mm in adult females and 31.5–33.6 mm in adult males, proportionally larger hands than the nominal subspecies, humeral spines in males, lobed liver, transparent hepatic peritoneum, white oesophagus and stomach fundus peritonea, and clear urinary bladder. The new subspecies was discovered at the Buenaventura Reserve in Seasonal Foothill forests at elevations between 950 and 1140 m and is probably endemic to the southern section of the Western Ecuador biogeographic province. Unfortunately, the extinction risk of the new subspecies is relatively high due to the impact of mining activities and habitat destruction. In addition, based on nuclear and mitochondrial DNA sequences, we present a new phylogeny for *Nymphargus* and comment on the phylogenetic relationships within the genus. The new subspecies is nested within *N. g. grandisonae* and is separated by low genetic distances (0.1 to 0.5% for gene 12S), suggesting a recent origin.

Key words. Amphibia, Ecuador, El Oro province, new subspecies, bioacoustics.

Resumen. Describimos una nueva subespecie de rana de cristal dentro de *Nymphargus grandisonae* de la provincia de El Oro, suroccidente del Ecuador. *Nymphargus grandisonae* tiene puntos rojos sobre el dorso verde, vejiga urinaria blanca, machos con espinas humerales y longitud hocico–cloaca 27,5–31,5 mm en hembras adultas y 25,1–29,4 mm en machos adultos. La nueva subespecie se diferencia de *N. g. grandisonae* y todos los otros miembros de la familia Centrolenidae por su coloración distintiva con puntos rojos sobre el dorso verde, longitud hocico–cloaca 32,1–36,2 mm en hembras adultas y 31,5–33,6 mm en machos adultos, manos proporcionalmente más grandes que la subespecie nominal, espinas humerales en machos, hígado lobulado, peritoneo hepático transparente, peritoneos blancos en el esófago y fundus estomacal, y vejiga urinaria transparente. La nueva subespecie fue descubierta en la Reserva Biológica Buenaventura, en bosques piemontanos estacionales a altitudes entre 740 y 1110 m y parece ser endémica de la sección sur de la provincia biogeográfica del Ecuador Occidental. Desafortunadamente, el riesgo de extinción de la nueva subespecie es relativamente alto debido al impacto de actividades mineras y a la destrucción de su hábitat. Adicionalmente, basados en secuencias de DNA nuclear y mitocondrial, presentamos una nueva filogenia de *Nymphargus* y comentamos sobre las relaciones filogenéticas dentro del género. La nueva subespecie está anidada dentro de *N. g. grandisonae* y está separada por distancias genéticas bajas (0,1 a 0,5% para el gen 12S), sugiriendo un origen reciente.

Palabras claves. Amphibia, Ecuador, provincia El Oro, subespecie nueva, bioacústica.

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Introduction

The genus *Nymphargus* Cisneros-Heredia & McDiarmid, 2007 contains 44 described species, the richest genus of the family Centrolenidae (Frost 2026). *Nymphargus* is characterised by the absence of webbing between Fingers I–IV, translucent hepatic and intestinal peritonea (without iridophores covering them), and by most species lacking humeral spines in adult males—except for *N. grandisonae* (Cochran & Goin 1970) (Cisneros-Heredia & McDiarmid 2007, Guayasamin et al. 2009, 2020, Twomey et al. 2014, Rada et al. 2017). The monophyly of *Nymphargus* is strongly supported by molecular evidence (Guayasamin et al. 2009, 2020, Twomey et al. 2014). Still, the relationships of some species within the genus remain unexplored (Cisneros-Heredia & McDiarmid, 2007, Cisneros-Heredia & Yánez-Muñoz, 2007b, Guayasamin et al., 2009, 2019, 2020, Rada et al. 2017; Sánchez-Carvajal et al. 2021).

The Pacific lowlands of Colombia and Ecuador are located below 1000 m altitude and show high geologic, geomorphological, and environmental heterogeneity. The combination of these factors has fostered the evolution of a complex and rich biodiversity, with several local hotspots concentrating high levels of endemism (Chapman 1917, 1926, Gentry 1982, 1992, Cracraft 1985, Duellman 1988, Duque-Caro 1990, Dodson & Gentry 1991, Long 1995, Morrone 2014, Veblen et al. 2015). Five species of *Nymphargus* are known from the Pacific lowlands of Colombia and Ecuador: *Nymphargus balionotus* (Duellman 1981), *N. buenaventura* (Cisneros-Heredia & Yánez-Muñoz 2007), *N. chami* (Ruiz-Carranza & Lynch 1995), *N. viglei* (Guayasamin et al. 2025) and *N. prasinus* (Duellman 1981). *Nymphargus buenaventura* is restricted to southwestern Ecuador and northwestern Peru, in areas covered by tropical seasonal forests of the Western Ecuador biogeographic province (Cisneros-Heredia & Yánez-Muñoz 2007b, Yánez-Muñoz et al. 2014, Chávez & Catenazzi 2025).

During recent surveys at the Buenaventura Reserve, we found a population similar to *Nymphargus grandisonae*. Herein, we analyse the relationships within the genus *Nymphargus* based on a phylogeny that includes new sequences of *N. grandisonae* and the *Nymphargus* population from Buenaventura. We also include in the phylogeny *Nymphargus buenaventura*, a species of unknown relationships. Our analyses combining morphological, ecological and geographic data provide evidence for presenting the new *Nymphargus* population from Buenaventura as a subspecies of *N. grandisonae*.

Material and methods

Ethics statement

We carried out this study in strict accordance with the guidelines for using live amphibians and reptiles in field research compiled by the American Society of Ichthyologists and Herpetologists, the Herpetologist's League and the Society for the Study of Amphibians and Reptiles (Beaupre et al. 2004). Research permits were issued by the Ecuadorian Ministry of Environment (005-14 IC-FAU-DNB/MA, MAE-DNB-CM-2015-0025-M-001 and MAATE-DBI-CM-2021-0181).

Nomenclatural acts

We decided to use the subspecies category after considering the multifaceted discussions about the role of subspecies (Kuzmin & Tarkhnishvili 2000, Hawlitschek et al. 2012, De Queiroz 2020, 2021, Hillis 2021, Burbrink et al. 2022, Scherz et al. 2022, Prateset al. 2023). After Hillis (2020), De Queiroz (2020) and Scherz et al. (2022), we consider that the subspecies category and nomenclatural rank are appropriate for the new population of *Nymphargus* herein described since it is an incompletely separated lineage within a more inclusive lineage that can be defined by phenotypic means but has not yet reached complete evolutionary independence. After Mayr & Ashlock (1991), "a subspecies is an aggregate of phenotypically similar populations of a species, inhabiting a geographic subdivision of the range of a species, and differing taxonomically from other subspecies". By "differing taxonomically", Mayr & Ashlock (1991) meant that subspecies are only to be named "if they differ… by sufficient diagnostic morphological characters".

The concepts for definition and diagnosis in the subspecies description are used as proposed by The International Code of Zoological Nomenclature (ICZN 1999): "definition, a statement in words that purports to give those characters which, in combination, uniquely distinguish a taxon", and "diagnosis, a statement in words that purports to give those characters which differentiate the taxon from other taxa with which it is likely to be confused."

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN). Hence, the new names in the electronic version are effectively published under that Code from the electronic edition alone. This published work and its nomenclatural acts have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved, and the associated information can be viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is urn:lsid:zoobank.org:pub:3974FDE9-4456-4956-858E-80EDFCAA24E6.

Taxonomic sampling

Morphological data used for comparisons were obtained from the direct study of specimens from all species of Centrolenidae found in the Western Ecuador biogeographic province and from 41 out of the 44 species of *Nymphargus* (see Appendix and specimens listed by Cisneros-Heredia & McDiarmid 2007). Specimens from the following collections were examined: The Natural History Museum, Department of Zoology, London (BMNH); División de Herpetología, Instituto Nacional de Biodiversidad, Quito (DHMECN); Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá (ICN); University of Kansas, Natural History Museum, Lawrence (KU); Museum of Comparative Zoology, Harvard University, Cambridge, MA (MCZ); Museo de Zoología, Universidad Técnica Particular de Loja, Loja (MUTPL); Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ); National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM); Museo de Zoología, Universidad San Francisco de Quito, Quito (ZSFQ). Data for the remaining species of *Nymphargus* were obtained from their original descriptions: *Nymphargus caucanus* Rada, Ospina-Sarria & Guayasamin, 2017, *N. pijao* Montilla, Arcila-Pérez, Toro-Gómez, Vargas-Salinas, & Rada, 2023, and *N. vicenteruedai* Velásquez-Álvarez, Rada, Sánchez-Pacheco & Acosta-Galvis, 2007.

Morphology and colouration

We follow the concepts, definitions, terminology and format for adult characters, measurements, definition, diagnosis, and description proposed by Cisneros-Heredia & McDiarmid (2007). All characteristics reported in the definition, diagnosis and description of the type series are from adult specimens. Examined specimens were photographed, euthanised using 20% benzocaine, fixed in 10% formalin, and preserved in 70% ethanol. Sex and maturity were determined by directly examining the gonads through dissections and noting the presence of secondary sexual characters (i.e., vocal slits and nuptial pads).

All morphometric data were measured under a stereomicroscope with a digital calliper (0.05 mm accuracy, rounded to nearest 0.1 mm), are reported as a range (mean ± standard deviation), and are as follows: snout–vent length (SVL); head length, measured as the straight line distance from posterior corner of mouth to tip of snout (HL); head width, measured at the corners of mouth (HW); interorbital distance, measured as the straight-line distance between anterior margins of orbits (IOD); horizontal eye diameter (ED); internarial distance between nostrils; eye–nostril distance from the anterior margin of orbit to the centre of nostril (EN); horizontal tympanum diameter (TD); hand length, from proximal edge of thenar tubercle to tip of Finger III (HAL); width of disc on Finger III (Fin3DW); tibia length (TL); foot length, from proximal edge of inner metatarsal tubercle to tip of Toe IV (FL). Life colour descriptions are based on photographs of live frogs taken in the field. The adjective "enamelled" describes the shiny white colouration produced by the accumulation of iridophores (Lynch & Duellman 1973, Cisneros-Heredia & McDiarmid 2007).

We analysed body and hand size using a Gaussian linear model with species, sex, and their interaction as fixed effects (size ~ species × sex). Because sample sizes were unbalanced among the four groups, significance of main effects was assessed using Type II sums of squares.

The terminology to describe clutches follows Altig & McDiarmid (2007). The developmental stages of embryos, hatchlings, and larvae were identified using the classification proposed by Gosner (1960). Larval characters and descriptions follow the terminology recommended by Mijares-Urrutia (1998), McDiarmid & Altig (1999) and Anstis (2013). The description of tadpoles is based on two specimens at Gosner stages 34 and 35 (MUTPL-T 24–25):

Phylogenetic analyses

We used DNA sequences to estimate the phylogenetic position of populations of *N. grandisonae* from Buenaventura. DNA was extracted from muscle or liver tissue preserved in 96% ethanol or tissue storage buffer using standard phenol-chloroform extraction protocols (Sambrook et al. 1989). We used a polymerase chain reaction (PCR) to amplify DNA fragments of mitochondrial genes 12S rRNA (12S), 16S rRNA (16S), NADH dehydrogenase subunit 1 (ND1) and subsequent tRNAs ($tRNA^{Leu}$, $tRNA^{Ile}$ and $tRNA^{Gln}$), and the nuclear genes RAG1, and C-MYC 2. PCR amplification was performed under standard protocols and sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea).

Our phylogeny is based on sequences of *Nymphargus* from GenBank (Guayasamin et al. 2009, Castroviejo-Fisher et al. 2014, Twomey et al. 2014) and new sequences of *N. cariticommatus* (Wild 1994), and *N. grandisonae*, including the new population from the Buenaventura Reserve. We analysed the mitochondrial genes 12S rRNA, 16S rRNA, ND1 and the nuclear genes BDNF, C-MYC 2, CXCR4, POMC, RAG1, SLC8A1, SLC8A3 for a total of 10 loci and up to 6338 bp. We also included GenBank sequences of *Allophryne*, *Chimerella*, *Hyalinobatrachium*, *Rulyrana*, *Sachatamia*, *Teratohyla*, and *Vitreorana*. The phylogeny was rooted with *Allophryne ruthveni* (specimen MAD1857; outgroup choice based on Twomey et al. 2014). The matrix had 49 terminals. GenBank accession numbers for newly generated sequences are available in Table 1.

Sequences were aligned with Geneious 7.1.7 software (Biomatters Ltd.) with the MAFFT 7.017 plugin and the L-INS-i algorithm (Katoh & Standley 2013). The alignment was visually inspected in Mesquite, version 3.01 (available at: http://mesquiteproject.org; downloaded 2 January 2015), and alignment errors were manually adjusted. We partitioned the matrix to allow separate evolution models for 12S, 16S-ND1, BDNF, C-MYC 2, CXCR4, POMC, RAG1, SLC8A1, and SLC8A3 for a total of 9 partitions. We used the software PartitionFinder v. 1.1.1 (Lanfear et al. 2012) to simultaneously estimate the best-fit model for each partition and the best partition strategy.

The phylogeny was estimated under maximum likelihood. The maximum likelihood analysis was done with a genetic algorithm using GARLI version 2.01 (Zwick 2006). We conducted 20 stochastic likelihood searches:

Table 1. GenBank accession numbers for newly generated DNA sequences of *Nymphargus* taxa (species and subspecies) used in the phylogenetic analyses.

Taxon	Voucher	12S	16S	ND1	c-myc	Geographic information
N. cariticommatus	QCAZ 54870	–	MT734666	MT733053	–	Ecuador: Zamora Chinchipe, Reserva Biológica Tapichalaca. Lat -4.4833, Long -79.1333
N. cariticommatus	QCAZ 54871	–	MT734667	MT733054	–	Ecuador: Zamora Chinchipe, Reserva Biológica Tapichalaca. Lat -4.4833, Long -79.1333
N. g. grandisonae	QCAZ 40388	MT735342	–	MT733060	MT711934	Ecuador: Cotopaxi, 18 km from Quillotuña, Pucayacu road. Lat -0.67843, Long -79.0156
N. g. grandisonae	QCAZ 48011	MT735341	–	MT733059	MT711935	Ecuador: Carchi, near Tres Marías waterfall, 1 km south from Chilma Bajo. Lat 0.8611, Long -78.0525
N. g. grandisonae	QCAZ 64605	MT735340	–	MT733058	MT711936	Ecuador: Carchi, Goaltal; Quebrada Gualchancito Alto. Lat 0.80187, Long -78.19835
N. g. grandisonae	QCAZ 67597	MT735343	–	MT733061	MT711937	Ecuador: Pichincha, Reserva Maquipucuna. Lat 0.0992600, Long -78.62962
N. g. orense ssp. n.	QCAZ 58030	MT735338	–	MT733056	MT711938	Ecuador: El Oro, Reserva Biológica Buenaventura. Lat -3.6530 S, Long -79.7429 W
N. g. orense ssp. n.	QCAZ 58032	MT735339	–	MT733057	MT711939	Ecuador: El Oro, Reserva Biológica Buenaventura. Lat -3.6530 S, Long -79.7429 W

10 from random starting trees and 10 from stepwise starting trees. Search parameters were set to the default values except for "genthreshfortopoterm" (10000, default 20000) and "limsprrange" (10, default 6). All 20 searches found the best trees with likelihood values within one log units of the global best tree, indicating that the search strategy lasted enough generations as it repeatedly converged in the same optimal trees. Branch support was estimated with non-parametric bootstrap from 200 pseudoreplicate searches. Search settings for each replicate were the same as those of the full search except for the number of stochastic searches (1 instead of 10).

Bioacoustics analysis

We recorded advertisement calls in the field using an Olympus LS-11 Linear PCM Recorder and a RØDE NTG2 condenser shotgun microphone at 44.1 kHz sampling frequency and 16-bit resolution in WAV file format. Air temperature and humidity were measured with a Lascar Electronics data logger (model EL-USB-2-LCD, accuracy: ± 0.5 °C; ± 5%). Call description was based on two recordings (FUTPL-A 265 from an unvouchered specimen and FUTPL-A 266 from specimen MUTPL 1288) made on 5 March 2022 near the Buenaventura Reserve (Table 2). Call recordings are deposited in full-length original form at Fonoteca UTPL (FUTPL-A 265–66, audio file names LS111222–223). Acoustic analysis was conducted using Raven Pro 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology), with temporal parameters measured from the oscillograms and the spectral parameters from the spectrograms. Spectrograms were generated with the Hanning window function of 512 samples, a 50% overlap and a grid spacing of 86.1 Hz.

We identified the calls as advertisement calls (sensu Wells 2007) and not territorial calls, mainly because no interactions were observed between the calling males (see the call types and associated behaviours of *N. g. grandisonae* in Hutter et al. 2013). The terminology and procedures for measuring call parameters follow (Cocroft & Ryan 1995, Toledo et al. 2015, Köhler et al. 2017, Guayasamin et al. 2020), with a call-centred approach to distinguish between a call and a note (sensu Köhler et al. 2017). Parameters measured were: (1) call duration, time from the beginning to the end of a call; (2) inter-call interval, the interval between two consecutive calls measured from the end of one call to the beginning of the consecutive call; (3) call rate, number of calls/minute measured as the time between the beginning of the first call and the beginning of the last call; (4) number of pulses/call; (5) pulse duration, time measured from one amplitude minimum to the next amplitude minimum of a pulse (we measured and analysed separately the regular pulses and the last, longer pulse); (6) pulse rate, number of pulses/second measured as the time between the beginning of the first pulse and the beginning of the last pulse; (7) dominant frequency, the frequency containing the highest sound energy measured along the entire call; (8) 90% bandwidth, reported as frequency 5% and frequency 95% or the minimum and maximum frequencies, excluding the 5% below and above the total energy in the selected call; and (9) frequency modulation, absolute value of the dominant frequency of the last 0.020 s of call subtracted from the dominant frequency of the first 0.020 s of call.

Table 2. Information of the call recordings of *Nymphargus grandisonae orense* ssp. n.

Code Fonoteca UTPL	FUTPL-A 265	FUTPL-A 266
Audio file name	LS111222	LS111223
Voucher	no	MUTPL 1288
Locality	Ecuador, El Oro, Reserva Buenaventura	Ecuador, El Oro, Reserva Buenaventura
Coordinates	3.6534° S, 79.7424° W	3.6534° S, 79.7424° W
Altitude (m)	951	951
Date	2022.03.05	2022.03.05
Time	23:44	23:49
Recording distance	2 m	1.5 m
Air temperature (°C)	22.5	22.5
Air humidity (%)	81.5	82.5

Geographic data, climatic information and ecological niche modelling

Coordinates of collection sites of the population of *N. grandisonae* from the Buenaventura Reserve were recorded with a global positioning system. Geographic names follow studies by Wolf (1892) and Terán (1984), validated as locally adopted by the land development and planning programmes of the municipalities of Piñas (Toledo Espejo 2015) and Atahualpa (Fernández Tinoco 2014).

Biogeographic regionalization and area taxonomy of western Ecuador follows the proposal by Morrone (2014), including the recognition of three biogeographic provinces: Choco-Darien, Western Ecuador, and Ecuadorian (Cisneros-Heredia 2006, 2007, 2019, Cisneros-Heredia & Yánez-Muñoz 2007b). Since the description of Morrone (2014) of the Western Ecuador biogeographic province was brief, we provide a more detailed geographic account. The Western Ecuador biogeographic province is centred on the Pacific lowlands and western Andean foothills of central and southern Ecuador. To the north, this biogeographic province reaches northern Ecuador and extreme southern Colombia through a narrow band along the Pacific lowlands of the Esmeraldas province (Ecuador) and the Tumaco department (Colombia). It runs south to the Pacific lowlands of Tumbes in extreme northern Peru (Brack Egg 1986, Hernández Camacho et al. 1992, Morrone, 1999, 2001, 2014, Cisneros-Heredia 2006, 2007, 2019). The Western Ecuador biogeographic province is characterised by seasonal forests, which can be evergreen or semi-deciduous and located in lowland or foothill areas. Other authors have assigned alternative names to the Western Ecuador province, including Pacific Tropical Forest (to the Peruvian portion, Brack Egg 1986), Pacific Equatorial Dominion (in part, Ab'Saber 1977), Tumaco district (to the Colombian portion, Hernández Camacho et al. 1992), Western Ecuador Moist Forest ecoregion (Dinerstein et al. 1995), or West Ecuadorian region (Cisneros-Heredia 2006, 2007). MAE et al. (2013) and Castroviejo-Fisher et al. (2014) did not recognise the Western Ecuador biogeographic province. Instead, they divided it among other biogeographic provinces. Climate information was obtained for specific localities and is based on the climate model by the "Climate data for cities worldwide" webpage (available at: https://en.climate-data.org/; accessed 2 April 2023).

Ecology provides fundamental evidence for taxa delimitation within the integrative taxonomy framework, and ecological niche modelling is a powerful tool for these analyses (Raxworthy et al. 2007, Rissler & Apodaca 2007, Minoli et al. 2014, Dagnino et al. 2017, Vázquez-López et al. 2020, Reyes-Puig et al. 2022). To evaluate ecological differences, we model the ecological niche of *N. grandisonae*. We did not model the ecological niche of the new subspecies because it is known from just three nearby localities; thus, modelling would not yield robust results. However, we evaluated if there is an overlap between the model of *N. grandisonae* and the localities of the new subspecies. We compiled a locality-points database of *N. grandisonae* using literature reporting specific localities (Duellman 1980, Cisneros-Heredia & McDiarmid 2007, Guevara-Molina & Vargas-Salinas 2014, Vanegas-Guerrero et al. 2014, Guayasamin et al. 2020, Cisneros-Heredia et al. 2022), specimens held in the following scientific collections: American Museum of Natural History, New York (AMNH); BMNH; DHMECN; ICN; KU; MCZ; MUTPL; QCAZ; USNM; ZSFQ; and data obtained from the Global Biodiversity Information Facility GBIF and SiB Colombia and the citizen science platform iNaturalist. Data search and extraction were conducted in January 2022. Each locality point was validated individually, following protocols described by Cisneros-Heredia & Peñaherrera-Romero (2020) and Cisneros-Heredia et al. (2023a). The final locality-points database was converted into decimal degree coordinates with the WGS 84 map datum and is available in Zenodo: https://doi.org/10.5281/zenodo.18371703.

We used Maxent (version 3.4.2) to obtain the ecological niche model. Recommended default values were used for convergence threshold, maximum number of iterations and maximum background points (Phillips & Dudik 2008); 25% of localities were randomly set aside as test points; regularization was set to 1. The selected format for the representation of probabilities for models was logistic. Parametrization was based on WorldClim (version 2.1; Fick & Hijmans 2017). Statistical analyses of variable contributions for data layers, including jackknife and correlation tests, were used to obtain more informative and less correlated variables. The following variables were used, ranked according to their contribution to the model: elevation, temperature seasonality (bio4), precipitation of warmest quarter (bio18), precipitation of driest quarter (bio17), precipitation seasonality (bio15), mean temperature of wettest quarter (bio8), max temperature of warmest month (bio5), min temperature of coldest month (bio6), annual precipitation (bio12), mean temperature of coldest quarter (bio11), mean temperature of warmest quarter (bio10). Models were evaluated through quantitative and qualitative tests, including threshold-independent and threshold-dependent tests, visual evaluations and evaluation of variable importance and response curves. We used binomial tests at equal sensitivity and specificity threshold and on extrinsic omission rate and predicted area at balance thresholds (Guisan et al. 2007). A geographical information system was developed based on grids from Maxent with ArcGIS Desktop to analyse spatial data and produce relevant maps (Buermann et al. 2008).

Extinction risk assessment

Extinction risk assessment was conducted following the system for classifying taxa at high risk of global extinction presented by IUCN (2012) and guidelines by the IUCN Standards and Petitions Committee (2022). The extent of occurrence (EOO) and area of occupancy (AOO, with a cell size of 2 km) were calculated using GeoCAT (Bachman et al. 2011).

Results

Our phylogeny (Fig. 1) shows relationships among *Nymphargus* that are consistent with those reported in previous studies (Guayasamin et al. 2009, 2019, 2020, Twomey et al. 2014). *Nymphargus buenaventura* is sister, with low support, to a seemingly undescribed species from Reserva San Francisco, Zamora-Chinchipe province, Ecuador (QCAZ 31340). Sequences of *N. grandisonae* from Buenaventura are nested within northern populations of *N. grandisonae*. Uncorrected p-genetic distances for gene 12S between them range between 0.1 and 0.5%. Despite the low genetic distances, the *N. grandisonae* populations from Buenaventura show morphological, chromatic and ecological differences. Body and relative hand sizes differed strongly between subspecies ($F_{1,19} = 46.76$, $p = 2.0 \times 10^{-6}$), with adult males and females from Buenaventura consistently having larger SVL and hands (relative to SVL) than all other populations of *N. grandisonae*, differences that remained large when body and relative hand size were estimated marginally across sexes. In addition, the Buenaventura individuals show a distinctive pattern of iridophore distribution on the visceral peritonea, including the lack of iridophores on the urinary bladder. Currently, *N. grandisonae* is the only *Nymphargus* and one of the few Centrolenidae with the urinary bladder covered by iridophores. Body size and chromatophore distribution on internal peritonea are key diagnostic characteristics for the taxonomy of Centrolenidae (Cisneros-Heredia & McDiarmid 2007, Guayasamin et al. 2020).

The ecological niche model of *N. grandisonae* predicts a distribution mainly restricted to montane forests in the northwestern tropical Andes. In contrast, the Buenaventura population inhabits western foothill forests, which are warmer, drier and at lower altitudes. The southernmost limit of the predicted niche of *N. grandisonae* stops about 100 km north of Buenaventura, showing no overlapping (Fig. 2a). The geographic range of *N. grandisonae* extends from western Colombia to northwestern Ecuador, between 7° N and 0.5° S. The Buenaventura populations occur south of 3.5° S. At Piñas, near Buenaventura, mean annual temperature is 22.4 °C, monthly temperature is 21.9–22.7 °C, mean annual precipitation is 1477 mm/year, and monthly precipitation is 7–286 mm. In contrast, in Mindo where *N. grandisonae* is frequent, mean annual temperature is 19.3 °C, monthly temperature is 19.0–19.9 °C, mean annual precipitation is 2525 mm/year, and monthly precipitation is 61–413 mm. Differences in their breeding biology are also apparent, with the Buenaventura populations laying more eggs per clutch than populations of *N. grandisonae* from its northern range (80–134 vs. 30–83 eggs per clutch, respectively; Rojas-Morales & Escobar-Lasso 2013, Vanegas-Guerrero et al. 2014).

Our data provide strong evidence that the populations of *Nymphargus grandisonae* from Buenaventura differ from other populations of *N. grandisonae* in morphology and ecology. However, our mitochondrial DNA phylogeny shows that the Buenaventura populations are nested within other populations, suggesting that evolutionary independence has not yet been fully achieved. Therefore, we propose that it be considered a subspecies of *N. grandisonae*.

Taxonomy

***Nymphargus grandisonae orense* ssp. n.**

Figs 3–7

ZooBank LSID: urn:lsid:zoobank.org:act:
3829FC50-0C05-4F35-9474-78F8D658E625

Holotype: QCAZ 58033 (Fig. 3), an adult male from Reserva Biológica Buenaventura (3.6530° S, 79.7429° W, 960 m), cantón Piñas, provincia de El Oro, República del Ecuador, collected by D. Cogălniceanu, D. Székely and P. Székely on 03 August 2014.

Paratypes: QCAZ 58034, adult male; QCAZ 58030–58032, adult females, collected on 7 August 2014; QCAZ 58064–58065, adult males, collected on 15 September

2014, by D. Cogălniceanu at the type locality; MUTPL 1287–1288, adult males collected on 5 March 2022 by D. Cogălniceanu, P. Székely, D. Székely and D. Armijos-Ojeda near the type locality (3.6534° S, 79.7424° W, 950 m).

Definition: The new subspecies is distinguished by the following combination of characters: (1) dentigerous process of vomer present, sometimes with vomerine teeth; (2) snout rounded in dorsal view, rounded to slightly sloping in lateral view; (3) tympanic annulus evident, lower ¾ visible, tympanic membrane coloured as dorsal skin, supratympanic fold present; (4) dorsal skin slightly granular on females, and slightly granular with microspicules on males; skin slightly elevated at sites of red (cream) spots on both sexes; (5) ventral skin granular, subcloacal area with two large subcloacal warts; (6) parietal peritoneum white, iridophores covering ⁴⁄₅ or completely the parietal peritoneum (condition P4); pericardium, oesophageal and upper stomach (fundus) peritonea white (i.e., covered by iridophores), all other visceral peritonea clear (condition V2); (7) liver lobed and hepatic peritoneum clear (lacking iridophore layer, condition H0); (8) adult males with projecting humeral spine; (9) basal webbing between fingers I, II and III, moderate webbing between fingers III

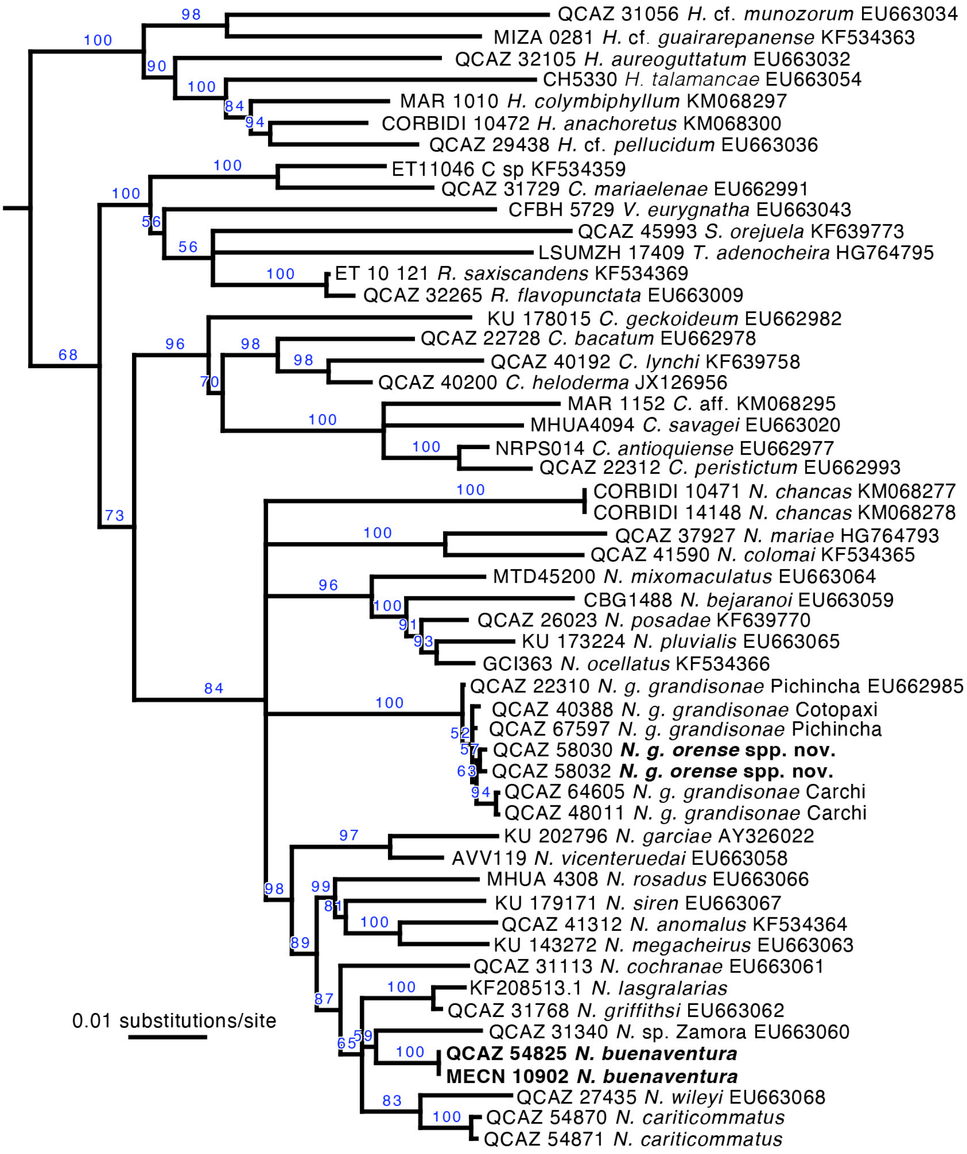

Figure 1. Maximum likelihood phylogram of *Nymphargus* showing the position of *N. grandisonae orense* ssp. n. and *N. buenaventura*. Phylogram derived from analysis of 6602 bp of mitochondrial (gene fragments 12S, 16S, ND1, tRNA Leu, tRNA Ile, tRNA Gln) and nuclear (BDNF, C-MYC 2, CXCR4, POMC, RAG1, SLC8A1, SLC8A3) DNA sequences. Voucher no. is shown before the species name, GenBank accession no., usually for 12S or 16S, is shown after. Non-parametric bootstrap (npb) support values, from 200 pseudoreplicates, are shown below branches with blue numbers. Outgroup is not shown.

and IV, III (2^+–2½)–(2^-–2^+) IV (Fig. 3); (10) toe webbing I (1^-–1⅔)–(2–2½) II 1–(2–2^+) III (1–1^+)–(2–2⅔) IV (2–2⅔)–(1–1½) V (Fig. 3); (11) lacking dermal ornamentations in the form of tubercles, folds, or fringes on hands, arms, feet, or legs; (12) nuptial excrescences type I and VI; concealed prepollex; (13) Finger I longer than Finger II; (14) diameter of eye larger than width of disc on Finger III; (15) colour in life, green dorsum with red spots, bones green; (16) colour in preservative, lavender dorsum with cream spots; (17) iris colouration in life, light grey with thin grey reticulations; (18) melanophores absent on digits of hands and present only at the base of toes; (19) males call from upper side of leaves; advertisement call formed by a whistle like, pulsed, single note, call duration 0.092–0.134 s, 15–26 pulses/call, and dominant frequency at 3445.3–3703.7 Hz; (20) fighting behaviour present, males grasp in amplexus-like position and dangle upside down while holding onto vegetation by their feet; (21) egg masses deposited on upper side of leaves, clutch size 80–134 (n = 11); males do not attend or get in contact with clutches; (22) tadpoles with elongated, oval-depressed body; sinistral spiracle; vent tube situated medially, caudal and with dextral opening; tail long, 2.2× the length of the body; oral disc medium sized, not emarginated and with M-shaped upper jaw sheath; labial tooth row formula 2(2)/3; (23) SVL in adult males 31.5–33.6 mm (32.4±0.9 mm, n = 4), and SVL in adult females 32.1–36.2 mm (34.3 ± 2.1 mm, n = 3).

Diagnosis: *Nymphargus grandisonae orense* differs from all other glassfrog taxa, except *N. g. grandisonae*, by its distinctive colouration with red dots on a green dorsum, a moderate-sized body, slightly granular dorsal skin, lobed liver uncovered by iridophores, and humeral spines in males. *Nymphargus grandisonae orense* is most similar to *N. g. grandisonae*, but the latter differs by having smaller, non-overlapping body size (27.5–31.5 mm SVL in adult females [n = 10] and 25.1–29.4 mm SVL in adult males [n = 50] of *N. g. grandisonae*, vs. 32.1–36.2 mm SVL in adult females and 31.5–33.6 mm SVL in adult males of *N. g. orense*, see Table 3); smaller hands (SVL/HdL 3.41–3.69 in adult females of *N. g. grandisonae* vs. 2.72–3.17 in *N. g. orense*), clear oesophagus and stomach fundus peritonea (white

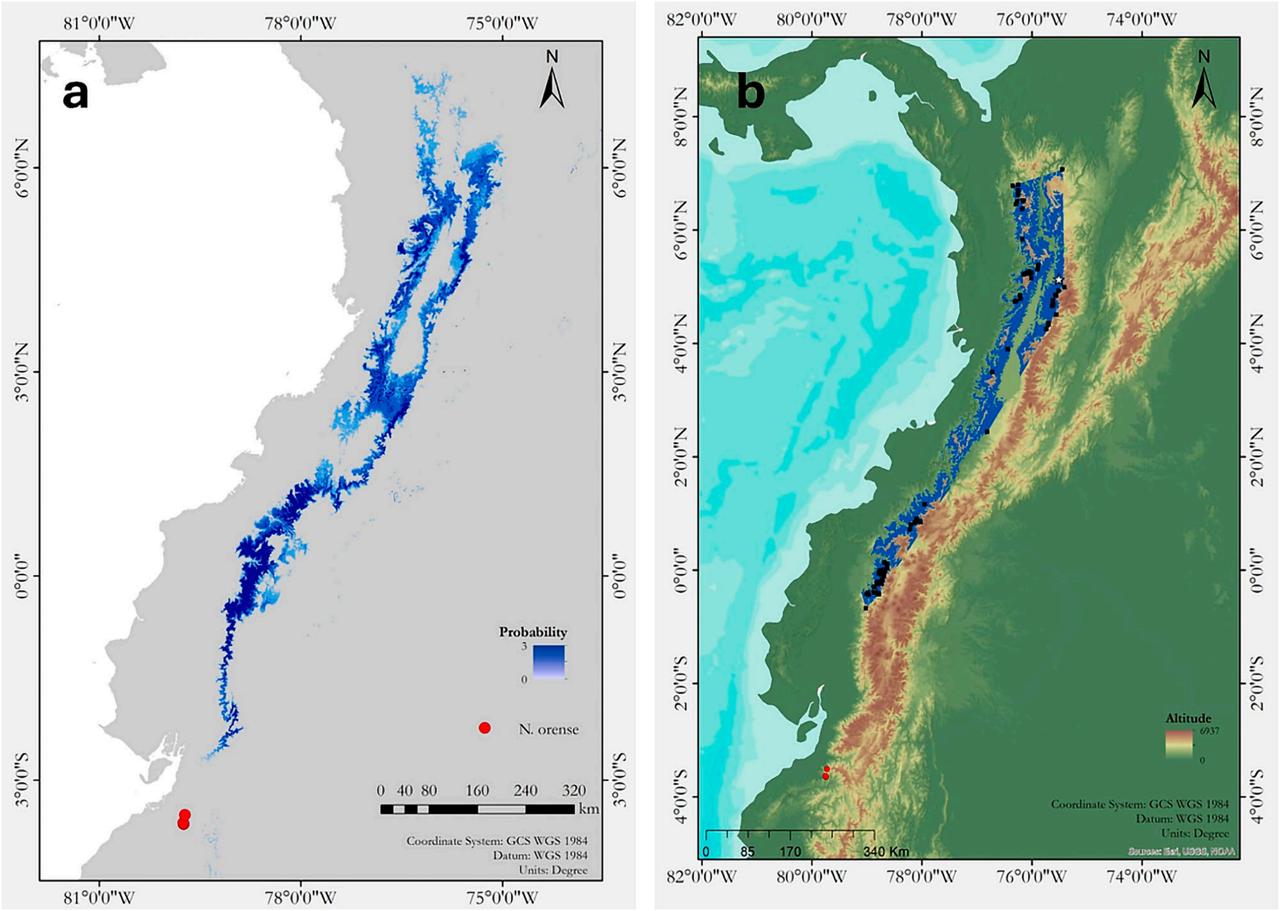

Figure 2. Map showing (a) the ecological niche model (Maxent 3.4.2) of *Nymphargus grandisonae grandisonae* (blue grading showing 0–3 probability). Notice distance (ca. 100 km) to the localities of *N. g. orense* ssp. n. (red circles); and (b) the known distributions of *N. g. grandisonae* (black squares = locality points; white star = type locality; blue = ecological niche model limited by altitude and minimum convex polygon between localities) and *N. g. orense* ssp. n. (red circles).

oesophagus and stomach fundus peritonea in *N. g. orense*), and white urinary bladder (clear in *N. g. orense*).

Nymphargus balionotus (Duellman 1981) has reddish dorsal marks, but differs from *N. g. orense* by having reddish-brown stripes and flecks and bright yellow dots on pale green dorsum (only red dots in *N. g. orense*), smaller body size (20.5–22.5 mm SVL in adult males, n = 5), humeral blade-like crest (vs. humeral spine in *N. g. orense*), truncate snout (rounded in *N. g. orense*), and smooth dorsal skin (slightly granular in *N. g. orense*).

Description of the holotype: Adult male, moderate-sized, SVL = 33.6 mm (Fig. 3). Head wider than long and wider than body (HW/HL = 1.18, HW/SVL = 0.34, HL/SVL = 0.29). Snout short (EN/HL = 0.24); rounded in dorsal view and rounded to slightly sloping in lateral view; nostrils slightly elevated, producing a shallow depression in the internarial area, loreal region concave; canthus rostralis rather indistinct (Fig. 3). Large eyes, ED/Fin3DW = 1.77, ED/HL = 0.40, directed anterolaterally at about 50° from midline, interorbital area wider than eye diameter, IOD/ED = 1.24, EN/ED = 0.59, EN/IOD = 0.48. Tympanic annulus evident and oriented dorsolaterally, lower ¾ visible, tympanic membrane coloured as dorsal skin, weak supratympanic fold above upper portion of tympanum extending down to shoulder. Dentigerous processes of vomers present, convergent, with two teeth on each side; choanae rounded, separated, closest to the distal margin of the vomerine teeth than to the margin of mouth; tongue rounded, indented posteriorly; vocal slits present, extending from anterior base of tongue to angles of jaws, subgular vocal sacs.

Skin of dorsal surfaces of body and limbs slightly granular with non-clustered microspicules (only one microspicule on each granule), skin slightly elevated at sites of red (cream) spots; head shagreen, infratympanic area with low, small warts. Skin of ventral surfaces of body granular, on throat and limbs fairly smooth. Cloacal opening directed posteriorly at upper level of thighs, no distinct cloacal sheath; subcloacal area coarsely granular with a pair of large, round, flat subcloacal warts on ventral surfaces of thighs below vent, other cloacal ornamentation absent, ventral skin granular but not enamelled.

Upper arm thinner than forearm. Humeral spine present and externally visible but not piercing the skin. Hand and ulnar folds or fringes absent. Relative lengths of fingers III > IV > I > II; basal webbing between fingers I, II and III, webbing formula between fingers III 2⅓–2^{+} IV; finger discs wider than the adjacent phalanx, nearly truncate; disc on

Figure 3. Holotype of *Nymphargus grandisonae orense* ssp. n. (QCAZ 58033), including dorsal and ventral general views, dorsal and lateral view of head, and ventral view of hand.

Table 3. Measurements (in mm) of the type series of *Nymphargus grandisonae orense* ssp. n.

	QCAZ 58033	QCAZ 58034	MUTPL 1287	MUTPL 1288	QCAZ 58031	QCAZ 58032	QCAZ 58030
Sex	Male	Male	Male	Male	Female	Female	Female
Snout–vent length	33.6	31.5	31.9	32.4	32.1	36.2	34.6
Head length	9.7	10.0	9.1	9.4	9.6	11.3	10.6
Head width	11.4	10.8	10.7	11.0	11.1	12.2	12.1
Interorbital distance	3.7	3.9	3.5	3.6	3.7	3.7	4.0
Eye diameter	3.9	3.9	3.5	3.8	3.2	4.1	3.5
Internarial distance	2.5	2.5	2.4	2.4	2.5	3.0	2.3
Eye-nostril distance	2.3	2.4	2.1	2.2	2.4	2.6	3.0
Tympanum diameter	0.9	1.0	0.9	1.0	1.0	1.0	1.1
Tibia length	18.1	18.5	19.2	18.4	19.8	19.6	20.8
Foot length	15.8	15.4	15.6	15.7	16.3	17.3	16.5
Hand length	10.7	10.7	11.6	11.5	11.8	12.2	10.9
Finger III disk width	2.2	1.9	1.9	2.4	2.6	2.8	2.5

third finger about the same size than those on toes; subarticular tubercles rounded and elevated, supernumerary tubercles present; palmar tubercle large, rounded, elevated; thenar tubercle elliptic. Concealed prepollex, unpigmented nuptial excrescences present, Type I on dorsolateral side of thumbs and Type–VI with glandular clusters extensively distributed along flanks.

Hind limbs slender; TL/SVL = 0.54, FL/SVL = 0.47. Foot or tarsal folds or fringes absent; inner metatarsal tubercle large and elliptical; outer metatarsal tubercle indistinct. Subarticular tubercles rounded and low; supernumerary tubercles small and rather indistinct. Webbing on feet 1½–2^{+} II 1–2 III 1^{+}–2⅓ IV 2½–1 V; toe discs bluntly truncate. One papilla on tip of disc of Toe I.

In life (Fig. 4), dorsum bright to lime green with 17 bright red dots; dorsal surfaces of limbs olive background with bright to lime green flecks and three bright red dots. Throat, chest, venter, flanks, hidden and ventral surfaces of limbs pale green to cream or whitish; fingers and toe pale green to whitish. Iris cream grey, yellowish tinted towards the centre (possible circumpupillary area) and fine grey reticulations towards the borders. Bones green. Ventral peritonea white.

In ethanol (Fig. 3), dorsum lavender with 17 cream dots; dorsal surfaces of limbs pale lavender with three cream dots. Throat, chest, venter, flanks, hidden and ventral surfaces of limbs cream. Iris lavender with fine dark lavender reticulations. Dorsal and ventral parietal peritonea white (i.e., entirely covered by iridophores) to the level of groins; iridophores covering pericardium, oesophagus, stomach fundus, and renal connective tissue; all other peritonea lacking iridophores, including urinary bladder. Dorsal skin under 45× magnification reveals lavender colouration produced by lavender melanophores densely packed in small aggregations, while iridophores are single and uniformly distributed. On top and around each melanophore, 4–5 bright iridophores are found. Dorsal melanophore aggregations are separated from each other, leaving spaces where only iridophores are present, although there are cream-coloured spotty areas where no chromatophore is apparent. Melanophore aggregations are more abundant on body dorsal skin than on limb dorsal skin and are absent in all ventral and flank surfaces. In aggregations or single, melanophores are present in the tympanic area and on dorsal surfaces at the base of toes but are absent from the upper lip and dorsal surfaces of arms and hands. Cream dorsal dots (red or pink until a few days after preservation) are circular, but some may have slightly irregular borders. Chromatophores forming dots are probably iridophores but less defined than iridophores across the rest of the body. Each dot on the body dorsum is surrounded by a thin line of dark melanophores (darker than other dorsal melanophores because iridophores uncovered them). This line of melanophores is absent on dots of limbs.

Variation: Based on the type series, character variation is summarized in Table 3. Snout rounded to slightly sloping in lateral view. Dentigerous process of vomer always present and noticeable, but number of teeth is variable (0–3), even between left and right sides of the same specimen (on left side, QCAZ 58034 has one tooth and QCAZ 58031 has two teeth, but both lack teeth on their right side; QCAZ 58030 has three teeth on left side and one on right side; while QCAZ 58031 lacks teeth on both sides). Dorsal skin in females is slightly granular, but it is granular with microspiculations in males. Probably, microspiculations are only present during the breeding season; thus, non-reproductive or young males may lack microspicules. Supratympanic fold may be low and look like a slightly swallowed area (QCAZ 58033). Nuptial excrescences Type I and VI are present in all males, but the latter is less noticeable and abundant in QCAZ 58034. Less noticeable nuptial excrescences are probably present in males collected at the beginning of their reproductive season. Humeral spine, vocal slits, vocal sacs and nuptial excrescences are absent in females. Subcloacal warts may be rather indistinct, although noticeable due to their size. Papilla on disc of Toe I sometimes unnoticeable (QCAZ 58032) or absent (QCAZ 58034), probably a preservation artefact. The number of dorsal red dots (cream in preservative) varies from 13–24, and those on legs between 0–8. Iridophores cover ventral parietal peritoneum down to the groins in males but cover ⅘ in females. Iridophores on renal connective tissue absent in some specimens (QCAZ 58031, 58034). Iridophores in testicular connective tissue present in male paratype QCAZ 58034.

Eggs: We found 11 egg clutches in August–September 2014, two in March 2015 at the scenic route of Buenaventura Reserve, and five in March 2022 near the type locality. Tadpoles had partially hatched in two of the clutches. The 2014 and 2015 egg clutches were found on the upper side of big Melastomataceae and Zingiberaceae leaves, between 0.5 and 0.9 m above water. Egg clutches from 2022 were found on the vegetation in the immediate vicinity of a secondary road bordered by a streamlet, 3–6 m high from the ground, with some egg clutches hanging over the road itself and not the streamlet. Recently laid clutches were deposited in a laminar array with transparent jelly. The clutches were initially placed at different sections of the leaves, and, after hydration, jelly swelled by absorbing water into a slightly globular tier and slid towards the leaf borders due to its weight, forming a drip tip (Fig. 5). While most leaves had one clutch, some had up to two clutches almost next to each other. The average number of eggs per clutch was 104 ± 15 (80–126; n = 11). Some clutches had large, empty capsules on their top and side edges. It is possible that empty capsules were present in all the clutches, but they were concealed in the swelled jelly (Fig. 5).

Tadpoles: One of the egg clutches that was encountered hanging over the road was collected on 5 March 2022 with the leaf on which it was found and transported to the laboratory. Tadpoles hatched after seven days, and two (MUTPL-DNA 122 and MUTPL-DNA 123) metamorphosed in about 200 days. The tadpoles of *N. g. orense* (Fig. 6) belong to Type IV tadpole of Orton (1953) and the exotroph, lotic, and burrower ecomorphological guild of McDiarmid & Altig (1999). For the two tadpoles, the total length was 33.3 and 33.4 mm, and the body length was 10.7 and 10.8 mm, respectively, with body length being ~ 32% of the total length.

Tadpole body elongated, oval-depressed, wider than high; snout rounded in dorsal view and sloped and round-

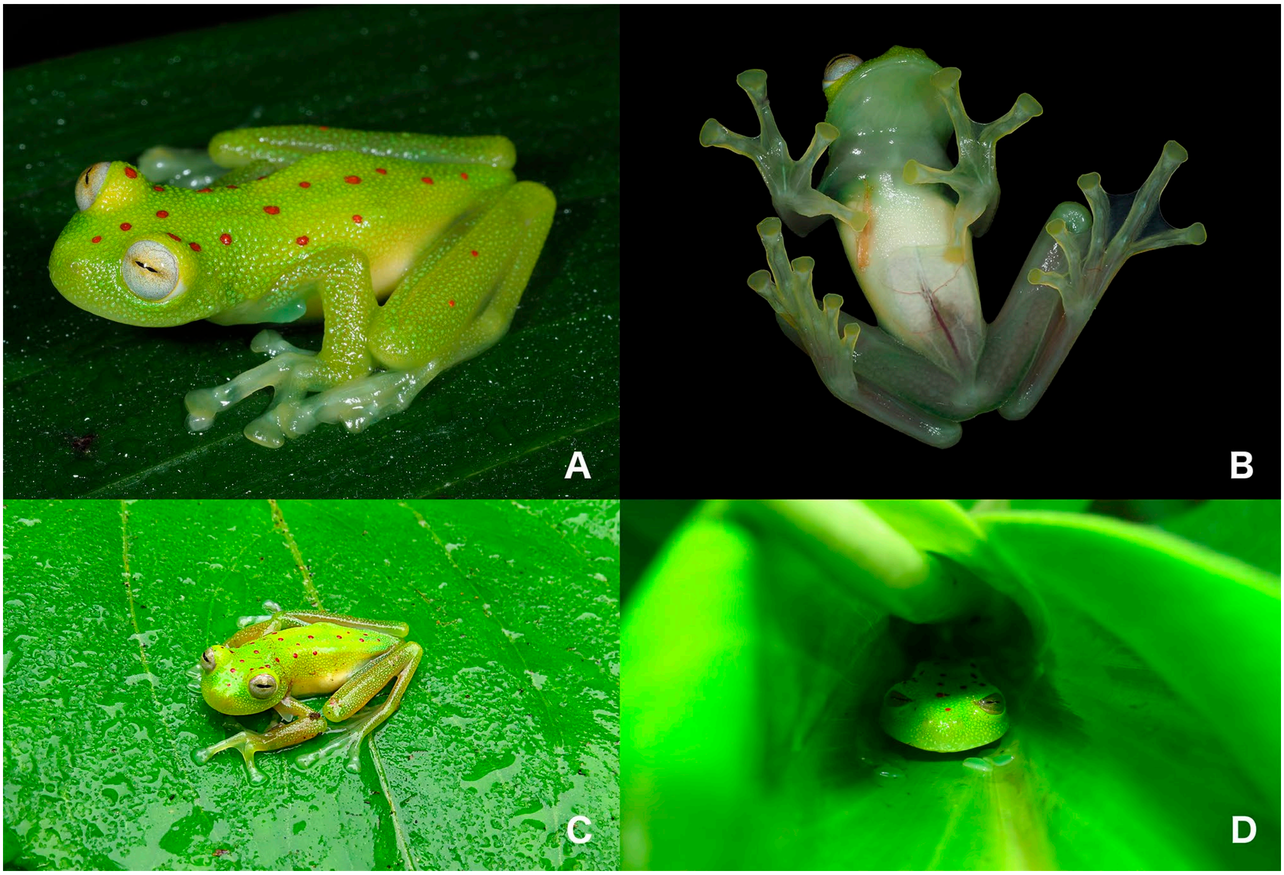

Figure 4. *Nymphargus grandisonae orense* ssp. n. in life: (A) dorsal and (B) ventral views of adult male paratype (MUTPL 1288); (C) dorsolateral view of adult male from San Juan de Cerro Azul (not collected); (D) male (QCAZ 58065) inside a leaf-cone during day.

ed in lateral view. Eyes located on the dorsal surface of the head, still a little bit C-shaped, separated by a distance 2× the eye diameter, interorbital distance smaller than internarial distance (Fig. 6). Nostrils small, positioned dorsally, protruding, with very small narial apertures oriented anteriorly. Spiracle short, single, sinistral, located at posterolateral region of body (Fig. 6A); spiracular opening slightly below body axis, oriented posteriorly and upwards (dorsoposterior orientation). Vent tube situated medially, short (about 14% of body length), attached to the ventral fin

Figure 5. Egg clutches (left and centre) and oviposition site (right, circle) of *Nymphargus grandisonae orense* ssp. n. Note the empty capsules on top and side edges (arrow).

Figure 6. Live tadpole of *Nymphargus grandisonae orense* ssp. n. (MUTPL-T 24, Gosner stage 34). (A) Left side view; (B) right side view; (C) dorsal view.

(caudal), with a dextral opening directed postero-ventrally. Tail long, ~ 2.2× body length, with rounded tip (Fig. 6). Dorsal and ventral fin originating at the junction of body and tail, dorsal fin increasing continuously at about ⅔ of tail; myotomes of tail musculature visible throughout entire tail length.

Oral disc medium sized (oral disc width ~ 55% of body width), not emarginated, located near tip of snout, directed ventrally, protruding ventrally but not laterally, beyond body. Anterior (upper) margin of labium with papillae only on lateral extremes. Marginal papillae uniserial, large, conical, distributed around the oral disc (~ 55–59 papillae), mainly on lower labium. Upper jaw sheath fully keratinized, M-shaped with finely serrated edge; lower jaw sheath fully keratinized, U-shaped, with serrated edge. Labial tooth row formula (LTRF) 2(2)/3, with tooth row A-1 complete, well developed, A-2 divided in the middle by upper jaw and rows P-1, P-2, and P-3 complete and well developed.

General colouration varied in the different developmental stages, the larvae maintaining a pinkish-red colouration until Gosner stage 40 (Figs 6–7). Greenish colouration became dominant by stages 41 and 42 (Fig. 7), when the characteristic red spots also appeared, first as yellowish orange and then completely red (Fig. 7).

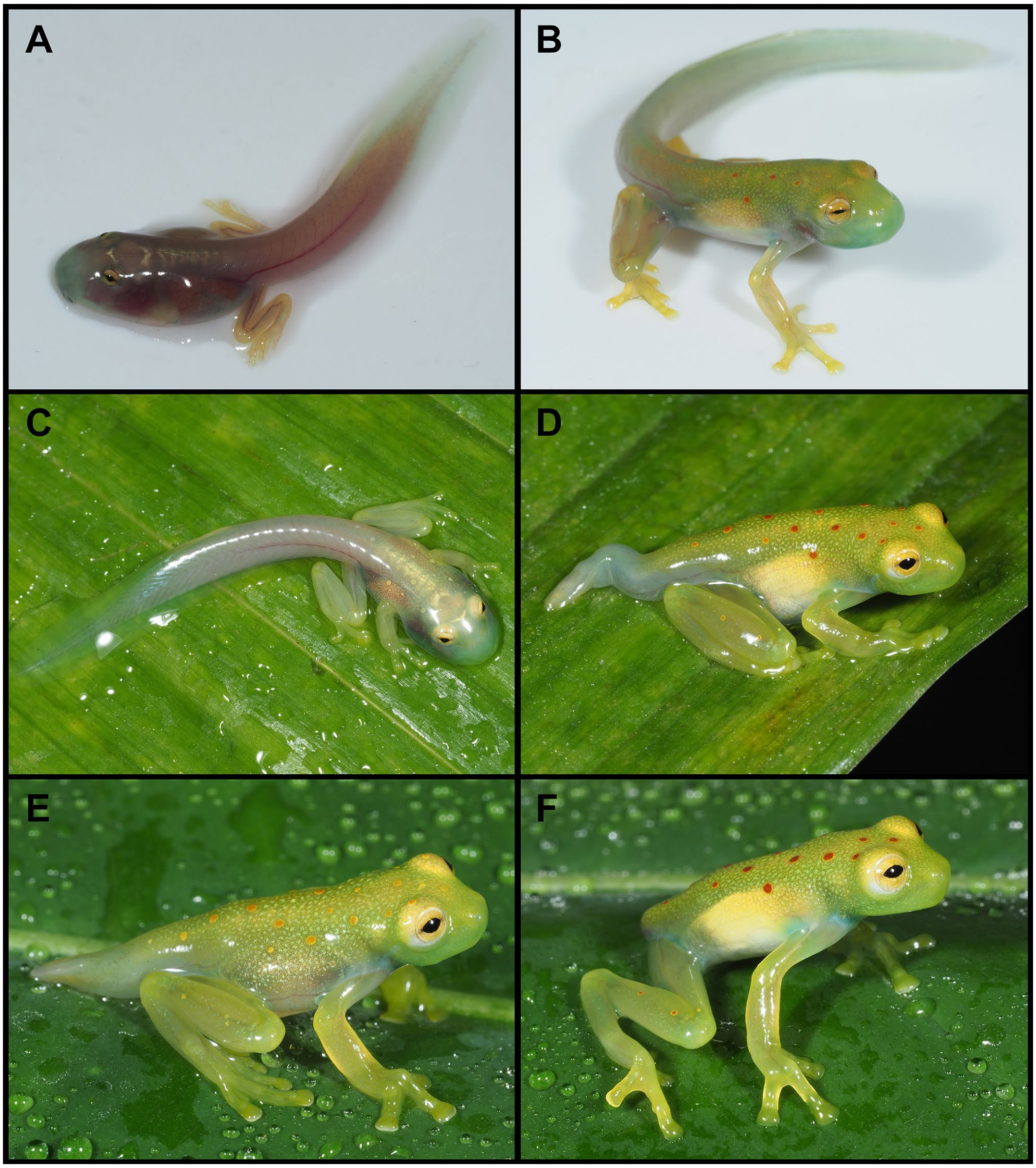

Figure 7. Ontogenetic changes in *Nymphargus grandisonae orense* ssp. n. (A) Larvae in Gosner stage 40; (B) metamorph in stage 42; (C) stage 42; (D) in stage 44; (E) stage 45; (F) stage 46; MUTPL-DNA 122 (A, C, E); MUTPL-DNA 123 (B, D, F); Photos A and B taken 30 August 2022, 172 days after hatching, C and D taken 10 September 2022, 183 days after hatching, and E and F taken 20 September 2022, 193 days after hatching.

Advertisement call: Several males were recorded calling from vegetation near a secondary road bordered by a small stream, 3–6 m high from the ground. The advertisement call of *N. g. orense* is characterized by a whistle-like, single-noted and pulsed sound (Fig. 8). The calls had a mean duration of 0.121 s, a mean inter-call interval of 37.6 s and a mean call rate of 1.62 calls/min (Tables 4–5). Each call (note) was composed on average of 21.6 pulses, with a mean pulse rate of 190.7 pulses/s; the pulses had a mean duration of 0.005 s except for the last pulse, which had a mean duration of 0.012 s (Fig. 8; Table 4). Calls had a mean dominant frequency of 3577.4 Hz, mean minimum frequency of 3410.9 Hz, mean maximum frequency of 3766.9 Hz, and a mean frequency modulation of 132.1 Hz. The fundamental frequency is not recognizable, but 2 to 3 harmonics are visible (Fig. 8). Detailed information on the recordings and the descriptive statistics of the acoustic variables are provided in Tables 4–5.

Distribution: *Nymphargus grandisonae orense* is currently known from three nearby localities in a narrow elevational band (950–1140 m) on the Chilla-Dumari Mountain ranges, southwestern Andes of Ecuador (Fig. 2b). The first two sites are in the Buenaventura Reserve (Reserva Biológica Buenaventura) on the foothills of Chilchiles, a mountain ridge branching from the Chilla-Damari Mountain ranges, part of the western slopes of the Andes. Buenaventura is a private protected reserve managed by Fundación de Conservación Jocotoco and is part of canton Piñas, province of El Oro. At Buenaventura Reserve, individuals were found at the scenic route (type locality, 3.6530° S, 79.7429° W, 960 m) and River Moromoro (3.6332° S, 79.7492° W, 1045 m). The third site is near the village of San Juan de Cerro Azul (3.5124° S, 79.7244° W, 1138 m), on the foothills of the Chilla-Damari Mountain ranges and part of canton Atahualpa, province of El Oro. Individuals from River Moromoro and San Juan de Cerro Azul were photographed but not collected (Fig. 4).

Nymphargus g. orense is apparently endemic to the southern section of the Western Ecuador biogeographic province. Intensive herpetological surveys across the province of El Oro over the last seven years have failed to detect additional populations, even in what would appear

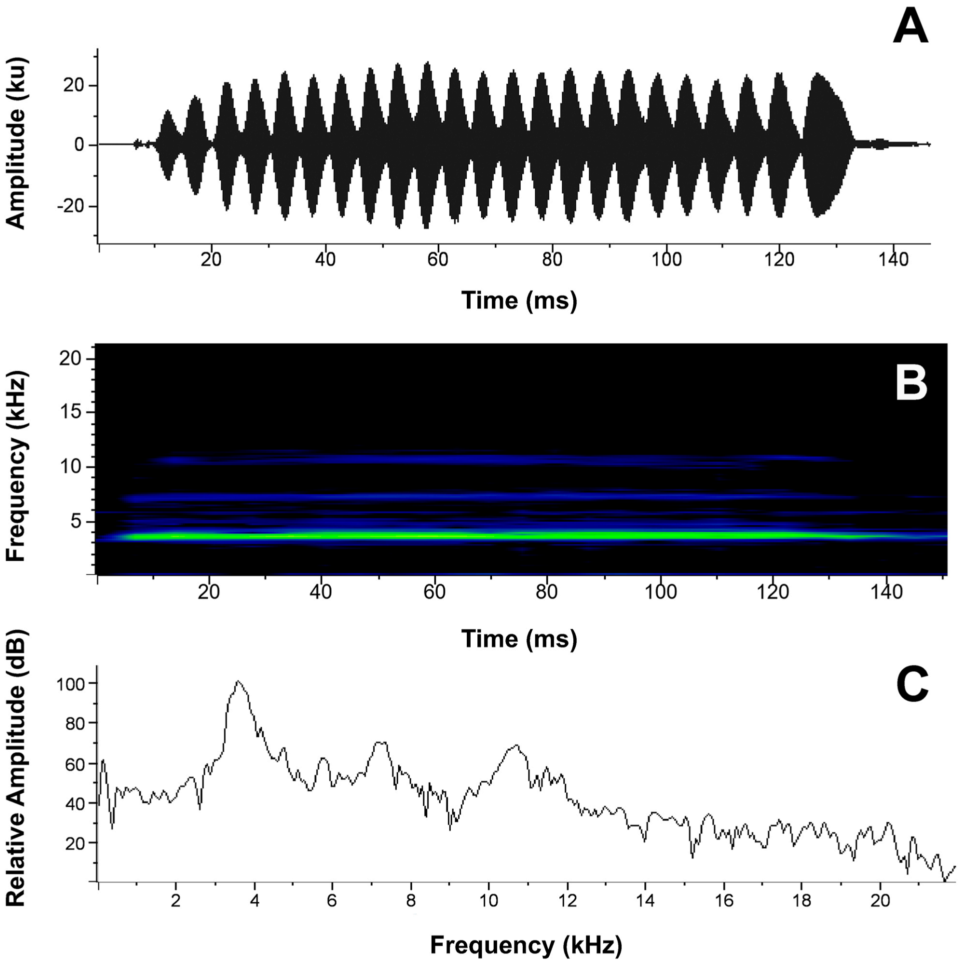

Figure 8. Advertisement call of *Nymphargus grandisonae orense* ssp. n. (FUTPL-A 265). (A) Oscilogram of a call with 23 pulses; (B) spectrogram of a call; (C) power spectrum of a call. Hanning window function at 512 bands FFT resolution.

Table 4. Quantitative description of the advertisement calls of *Nymphargus grandisonae orense* ssp. n. Values are given as average ± SD (range) and n = sample size.

Code Fonoteca UTPL	FUTPL-A 265	FUTPL-A 266
Call duration (s)	0.124 ± 0.01 (0.104–0.134) n = 9	0.117 ± 0.02 (0.092–0.134) n = 6
Inter-call interval (s)	39.5 ± 40.6 (1.5–124.5) n = 8	34.5 ± 41.8 (1.2–99.6) n = 5
Call rate (calls/min)	1.51	1.73
Number of pulses/call	22.6 ± 2.6 (18–26) n = 9	20.2 ± 3.4 (15–23) n = 6
Pulse duration (s)	0.005 ± 0.0004 (0.003–0.007) n = 194	0.005 ± 0.0007 (0.004–0.008) n = 115
Last pulse duration (s)	0.012 ± 0.003 (0.009–0.016) n = 9	0.013 ± 0.0008 (0.012–0.014) n = 6
Pulse rate (pulses/s)	194.0 ± 2.1 (189.7–196.3) n = 9	185.7 ± 3.2 (180.7–188.5) n = 6
Dominant frequency (Hz)	3541.0 ± 51.8 (3445.3–3617.6) n = 9	3632.0 ± 35.2 (3617.6–3703.7) n = 6
Freq 5% (Hz)	3368.8 ± 28.7 (3359.2–3445.3) n = 9	3474.0 ± 44.5 (3445.3–3531.4) n = 6
Freq 95% (Hz)	3722.8 ± 38.0 (3703.7–3789.8) n = 9	3832.9 ± 47.2 (3789.8–3876.0) n = 6
Frequency modulation (Hz)	134.0 ± 45.4 (86.1–172.3) n = 9	129.2 ± 72.1 (86.1–258.4) n = 6
Number of visible harmonics	2	2–3

Table 5. Comparative quantitative description of the advertisement calls (mean ± SD, and range in parentheses) of the subspecies of *Nymphargus grandisonae*. Data for *N. g. grandisonae* as reported by Guayasamin et al. (2020).

	N. g. grandisonae	*N. g. orense* ssp. n.
Number of analyzed calls (individuals)	417 (22)	15 (2)
Call duration (s)	0.115 ± 0.02 (0.056–0.158)	0.121 ± 0.01 (0.092–0.134)
Inter-call interval (s)	23.4 ± 30.1 (0.7–209.2)	37.6 ± 39.4 (1.2–124.5)
Call rate (calls/min)	3.5 ± 4.3 (0.459–25.8)	1.62 (1.51–1.73)
Number of pulses/calls	15.7 ± 2.7 (8.0–25.0)	21.6 ± 3.0 (15–26)
Pulse rate (pulses/s)	143.2 ± 18.8 (107.9–320.8)	190.7 ± 4.9 (180.7–196.3)
Pulse duration (s)	0.007 ± 0.001 (0.003–0.010)	0.005 ± 0.0006 (0.003–0.008)
Last pulse duration (s)	0.012 ± 0.006 (0.003–0.080)	0.012 ± 0.002 (0.009–0.016)
Dominant frequency (Hz)	3587.6 ± 189.6 (3100.8–4048.2)	3577.4 ± 64.0 (3445.3–3703.7)
Frequency modulation (Hz)	143.8 ± 107.9 (0–516.8)	132.1 ± 55.1 (86.1–258.4)

as suitable habitat, suggesting that *N. g. orense* is at most uncommon (MECN and GADPEO 2015; M. H. Yánez-Muñoz and J. C. Sánchez-Nivicela, pers. comm.; D. Cogălniceanu and P. Székely, pers. obs.). Nevertheless, it is possible that its geographic range could be slightly broader, and additional populations may remain undiscovered in forest remnants on foothills and slopes of the Chilla-Dumari Mountain ranges and other nearby ranges, i.e., Molleturo, Mullopungo, Tahuín, in the provinces of El Oro and Azuay.

Natural history: *Nymphargus grandisonae orense* inhabits Seasonal Foothill Evergreen forests (Catamayo-Alamor Foothill Evergreen Forest, sensu MAE 2013). Frogs were found in human-modified forest fragments on riparian vegetation close to pastures. At Buenaventura Reserve, seven frogs were found in forest borders on the limits of the reserve, along a 50 m transect. At San Juan de Cerro Azul, individuals were encountered in forest patches bordering small ravines and streams surrounded by pastures but not deforested due to their inaccessibility (Anton Sorokin, pers. comm.). Intensive sampling during August and September 2014 (total of 20 field days) at Buenaventura Reserve failed to find the subspecies in riverine areas lacking native forest remnants, including open pasturelands and croplands. It seems that *N. g. orense* can breed on riverine areas surrounded by anthropic open areas, but it probably depends on the remaining forest patches to survive.

Most individuals of the type series were active at night, on big leaves overhanging a small stream, 0.5–3 m above ground. In March 2022, during a rainy night, we encountered several calling males in the immediate vicinity of a secondary road neighbouring the type locality. Only one individual was found during the day, a male (QCAZ 58065) hiding inside a leaf cone at the type locality (Fig. 4). At River Moromoro, frogs were observed perching on vegetation next to the river at night. At San Juan de Cerro Azul, individuals were found at night on vegetation near a small rapid stream, close to a waterfall, 1–2 m above ground. No parental care, to any degree, was observed, or male interactions recorded. However, Sorokin & Steigerwald (2017) reported a male of *N. g. orense* as "*Nymphargus* aff. *grandisonae*" engaged in combat with a male of *Espadarana prosoblepon*. Sympatric frogs at the type locality included: *Pristimantis achatinus*, *P. hampatusami*, *P. subsigillatus*, *Epipedobates anthonyi*, *Hyloxalus infraguttatus*, *Espadarana prosoblepon*, *Agalychnis spurrelli*, *Hyloscirtus* sp. aff. *alytolylax*, *Scinax tsachila*, *Smilisca phaeota*, and *Rhinella bella*.

Conservation status and extinction risk: All known *N. g. orense* are from a small region in southwestern Ecuador, at Buenaventura Reserve and San Juan de Cerro Azul (airline distance between them 16 km). Habitat change and loss in the surroundings of Buenaventura and across Cerro Azul are extensive, and ca. 50% of the tree cover of Cerro Azul remains, usually in patches that have not been deforested only due to their inaccessibility. The main threats driving ongoing habitat change and loss are the expansion of the agricultural frontier due to small-holder and agroindustry grazing and farming for livestock and non-timber crops, legal and illegal mining, river pollution due to mining effluents, and to a lesser degree, domestic and urban wastewater, and legal and illicit small and large-scale logging (Yánez-Muñoz et al. 2013, Klauke et al. 2016, Carrillo-Moreno et al. 2018, Garzón-Santomaro et al. 2018, Hermes et al. 2018, Vandegrift et al. 2018). These threats are extended across both cantons (i.e., geopolitical administrative units) where the subspecies has been recorded. As of 2010, less than 55% and 66% of tree cover (canopy density > 50%) remained in Piñas and Atahualpa cantons, respectively (Fig. 9), and as of 2016, no intact or primary forests remain (Hansen et al. 2013, Global Forest Watch, available at: https://www.globalforestwatch.org; accessed 30 May 2017). Most forest remnants are small (< 100 ha) and embedded in a matrix of livestock pastures and croplands (MAE 2012, 2017, MAE et al. 2013, Sierra 2013, Fernández et al. 2014, Toledo Espejo et al. 2015, Hansen et al. 2013, Hermes et al. 2018). About 35% and 79% of the total areas of cantons of Piñas and Atahualpa have been concessioned to metallic and non-metallic mining, resulting in most rivers being heavily polluted (ARCOM 2014a, 2014b, Fernández et al. 2014, Toledo Espejo et al. 2015, Prada-Trigo 2016, INIGEMM 2017, Vandegrift et al. 2018). Over its entire range the species is considered Least Concern (Ortega-Andrade et al. 2021). The main threats for the nominal subspecies *N. g. grandisonae* are considered agricultural development, logging, human settlements, pollution and the introduction of alien predatory fish species in streams (Cisneros-Heredia et al. 2022).Based on these data, we assess the extinction risk for *N. g. orense* as follows: (1) We suspect that *N. g. orense* has suffered population reductions based on the continuous decline in habitat quality across the regions where it occurs; however, we refrain from using criterion A until more geographic and ecological data are available. (2) The subspecies has an estimated EOO of 7.74 km^2, adjusted to 12 km^2 (following IUCN Standards and Petitions Committee 2019: "If EOO is less than AOO, EOO should be changed to make it equal to AOO to ensure consistency with the definition of AOO as an area within EOO"), which is within the threshold for Critically Endangered (100 km^2) under criterion B1. If the subspecies were found in additional sites but still restricted to seasonal foothill forests of southwestern Ecuador, its extent of occurrence would be < 2500 km^2, within the threshold for Endangered (< 5000 km^2). (2) The subspecies has an estimated AOO of 12 km^2, which is within the thresholds for Endangered (10–500 km^2) under criterion B2 (Fig. 9). (3) Geographic proximity of both sites at Buenaventura Reserve and the subspecies' obligatory association with water deserves considering both as a single threat-defined location. Although threats are similar between Buenaventura Reserve and San Juan de Cerro Azul, the latter is considered a different threat-defined location due to conservation and geopolitical differences. (4) There is an ongoing decline of the subspecies terrestrial and riverine habitats, mainly due to the loss of forests to agriculture and logging and heavy and continued water pollution caused by mining. Therefore, based on the currently available information, we assess the extinction risk of *N. g. orense* as relatively high. We propose that *N. g. orense* should be classified under the IUCN category Endangered (EN) based on criteria B1ab(iii) + 2ab(iii). The IUCN category of *N. g. grandisonae* is Least Concern (Ortega-Andrade et al. 2021).

Few conservation actions in place are benefiting *N. g. orense*. Buenaventura Reserve is a private protected area preserving 28.72 km^2 between 400 and 1450 m elevation. Buenaventura mainly encompasses secondary forests and former cattle pastures managed by Jocotoco Foundation to promote habitat restoration through natural regeneration and reforestation (Spracklen & Righelato 2016). Buenaventura is the only protected area across all evergreen forests in southwestern Ecuador. Several other locations in southwestern Ecuador have been declared as "protected forests" to preserve natural resources and environmental services. However, they are not part of the National System of Protected Areas (Sistema Nacional de Áreas Protegidas SNAP), and most exist only on paper, not on the ground (Prieto-Albuja et al. 2019). Little information is available for *N. g. orense* and urgent research and monitoring actions should be established to study its life history and ecology, population size and trends, impacts by threats, and survey new sites where additional populations may exist.

National and local governments are urged to monitor and enforce compliance with conservation and environmental laws and regulations to lower deforestation and stop the negative impacts caused by mining. If new populations are found, local governments should be urged to provide official, effective protection of those sites. Recently, a proposal to declare a biological corridor in the province of El Oro, encompassing all sites of *N. g. orense* and others with seasonal evergreen forests, has been presented but remains to be officially declared and consolidated (Garzón-Santomaro et al. 2019).

Etymology: The name of this new taxon is a noun in apposition and corresponds to the Spanish demonym that identifies natives of El Oro. We dedicate this subspecies to the people and biodiversity of the El Oro province, Ecuador. El Oro is a geopolitical division in southwestern Ecuador and one of the most biodiverse areas in the country. El Oro encompasses 15 different ecosystem types and holds records of at least 45 species of amphibians, 74 reptiles, and 556 birds (MECN & GADPEO 2015). The seasonal evergreen forests of El Oro are an essential part of the Western Ecuador biogeographic province, with several endemic or quasi-endemic species of fauna. As common names, we suggest 'El Oro Red-dotted Glassfrog' in English, and 'Rana de Cristal Orense' in Spanish language.

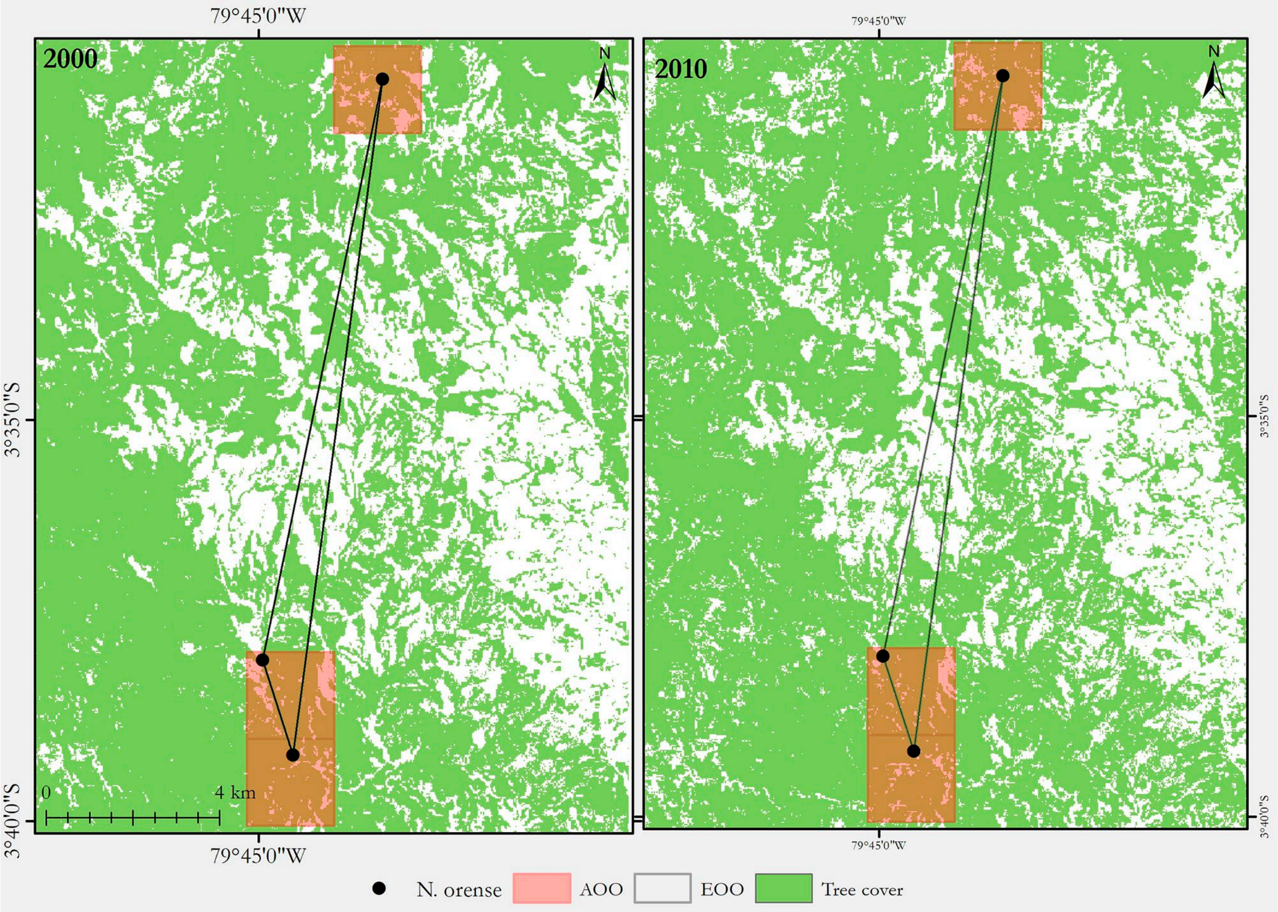

Figure 9. Map showing tree cover in years 2000 and 2010 in the distribution area of *Nymphargus grandisonae orense* ssp. n. Known localities of the species are marked with black dots, black triangle corresponds to its extent of occurrence (EOO), and 2-km pink squares to its area of occupancy (AOO). As of 2010, less than ⅔ of tree cover (canopy density > 50%) remained in the area.

Discussion

It has been stated that *Nymphargus* is a genus restricted to highland areas (over 1000 m) on the Northern and Central Andes, except for *N. mariae* (Duellman & Toft 1979, Guayasamin et al. 2009, Castroviejo-Fisher et al. 2014) and the recently described *N. vigle* (Guayasamin et al. 2025). However, speciation with niche divergence in *Nymphargus* seems more common than previously hypothesised. At least nine *Nymphargus* inhabit foothill and lowland areas: *N. balionotus*, *N. buenaventura*, *N. chami*, *N. cochranae* (Goin, 1961), *N. laurae* (Cisneros-Heredia & McDiarmid, 2007), *N. vigle* (Guayasamin et al., 2025), *N. mariae*, *N. g. orense* and *N. prasinus* (Ruiz-Carranza & Lynch 1995, Cisneros-Heredia & McDiarmid 2006, 2007, Cisneros-Heredia & Yánez-Muñoz 2007a,b, Guayasamin et al. 2020, Sánchez-Carvajal et al. 2021). The genus *Nymphargus* occurs on the western Andean slopes of Colombia and Ecuador and the eastern Andean slopes of Colombia, Ecuador, Peru, and Bolivia in the following biogeographic provinces: Cauca, Choco-Darien, Western Ecuador, Napo, and Ucayali (Duellman 1981, Ruiz-Carranza & Lynch 1995, Cisneros-Heredia & McDiarmid 2005, 2006, 2007, Cisneros-Heredia & Yánez-Muñoz 2007, Velásquez-Álvarez et al. 2007, Cisneros-Heredia & Guayasamin 2014, Yánez-Muñoz et al. 2014, Guayasamin et al. 2019, 2020, this work).

Nymphargus buenaventura had not been included in previous phylogenetic analyses. Our results support the hypothesis of Cisneros-Heredia & Yánez-Muñoz (2007) of a clade formed by *N. buenaventura*, *N. cariticommatus*, *N. griffithsi* (Goin 1961), and *N. wileyi* (Guayasamin et al. 2006), with the addition of an undescribed species previously reported as "*N.* aff. *cochranae*" (Castroviejo-Fisher et al. 2014, Guayasamin et al. 2020). Interestingly, *N. buenaventura*, a species from the western versant of the Andes, was recovered with low support as the sister of this undescribed species, which occurs on the eastern versant of the Andes, and is not paired with the most morphologically similar and geographically closer *N. griffithsi*. However, this arrangement could change as support for the clade joining *N. buenaventura* + *N. cariticommatus* + *N. griffithsi* + *N. lasgralarias* + *N. wileyi* + *N*. sp. is also low. The pattern, where species from opposite versants of the Andes of southern Ecuador and northern Peru have close phylogenetic relationships, has been reported in other clades (Musher & Cracraft 2018, Salgado-Roa et al. 2022, 2024, Cisneros-Heredia et al. 2023b).

Among centrolenids, *N. g. grandisonae* and *N. g. orense* are most peculiar by having a unique combination of characters: red-spotted dorsum, moderate hand webbing, and humeral spines (Duellman 1980, this study). Although they are separated by small genetic distances (uncorrected p-distance 0.1–0.5% for gene 12S) and not reciprocally monophyletic, their differences in adult size, proportions, and peritoneal chromatophore distribution are strong evidence to support their recognition as subspecies. Closely related species of Neotropical amphibians tend to be morphologically cryptic and genetically distinct (e.g., Elmer & Cannatella 2008, Caminer et al. 2014). However, examples of the opposite pattern, (i.e., closely related species being morphologically distinct and genetically similar) have also been documented. In a recent study, Tarvin et al. (2017) showed that the poison frogs *Epipedobates machalilla* and *E. tricolor* have distinct morphology, advertisement calls and even environmental niches, despite being separated by low genetic distances (< 1% for gene 16S). Young species, with divergent morphology and ecological niche, are frequent in rapid adaptive radiations. In the Galapagos Archipelago, for example, closely related species of Darwin Finches and Galapagos Tortoises are genetically intractable despite being adapted to separate niches and having distinct morphologies (Poulakakis et al. 2012, Lamichhaney et al. 2016).

The differences between populations are the result of rapid divergent natural selection to adapt to distinct environments. *Nymphargus grandisonae orense* inhabits southwestern Ecuador south of 3.5° S, while the range of *N. g. grandisonae* extends from western Colombia to northwestern Ecuador, between 7° N and 0.5° S (Duellman 1980, Bolívar et al. 2004, Cisneros-Heredia & McDiarmid 2007, Vanegas-Guerrero et al. 2014, Frost 2016, Guayasamin et al. 2020). *Nymphargus grandisonae orense* lives in foothill forests, which are warmer, drier and at lower altitudes than the low montane and cloud forests where *N. grandisonae* inhabits. At Piñas, near the type locality of *N. g. orense*, mean annual temperature is 22.4 °C, monthly temperature is 21.9–22.7 °C, mean annual precipitation is 1477 mm/year, and variation in monthly precipitation is 7–286 mm. In contrast, at Mindo where *N. g. grandisonae* is frequent, mean annual temperature is 19.3 °C, variation in monthly temperature is 19.0–19.9 °C, mean annual precipitation is 2525 mm/year, and variation in monthly precipitation is 61–413 mm. Differences in their breeding biology are also apparent, with *N. g. orense* laying more eggs per clutch than *N. g. grandisonae* (80–126 vs. 30–83 eggs per clutch, respectively; Rojas-Morales & Escobar-Lasso 2013, Vanegas-Guerrero et al. 2014).

Ospina-Sarria et al. (2011) described the tadpole of *N. g. grandisonae* with specimens collected from Serranía de los Paraguas, Valle del Cauca, Colombia. In Fig. 4 of their paper, the authors presented the oral disc of a tadpole (UV-CD 1912) in Gosner stage 36, this illustration being very similar to the oral disc of the *N. g. orense* tadpoles (in stages 34 and 35) observed and described by us. Most of the morphological features described by Ospina-Sarria et al. (2011) for the nominotypical subspecies are comparable with the ones described by us for *N. g. orense*. We identified several small differences, like the larger interorbital distance (distance 2× the eye diameter in *N. g. orense* vs. distance slightly larger than the eye diameter in *N. g. grandisonae*), slightly longer vent tube (about 14% of the body length vs. 12.3%), or shape of the tail tip. This last difference is the most notable one, the tail tip of *N. g. orense* being rounded (Fig. 6A, B) compared with the roundly pointed tail tip of *N. g. grandisonae* (Fig. 3A in Ospina-Sarria et al. 2011).

The advertisement call of *N. g. orense* is very similar to the call of the nominotypical subspecies (Hutter et al. 2013). Most of our measurements largely coincide with the ranges of the values reported for *N. g. grandisonae*, taking into consideration our smaller sample size. One notable difference is in the number of pulses/call, the call of *N. g. orense* being characterized by a seemingly larger number of pulses (Table 4).

The Western Ecuador biogeographic province shows high levels of endemism of flora and fauna, despite its relatively small area when compared to other tropical forests (Dodson & Gentry 1991, Lynch & Duellman 1997, Anderson & Jarrín 2002, Cisneros-Heredia 2006, 2007, Cisneros-Heredia & Yánez-Muñoz 2007, Morrone 2014). The discovery of the *N. g. orense* subspecies increases the number of endemic amphibians in the Western Ecuador biogeographic province, providing further support for its recognition as an important area of endemism for amphibians, different from the Choco biogeographic province. Within this biogeographic province, a pattern of local micro-endemisms is evident towards the southern section, in the Seasonal Lowland and Foothill forests of the provinces of Guayas, Azuay, El Oro and Loja, Ecuador, and the department of Tumbes, Peru, with several species of amphibians, reptiles, birds, and mammals circumscribed to the southern section (Peters 1973, Ridgely & Greenfield 2001, Cisneros-Heredia & Yánez-Muñoz 2007b, Torres-Carvajal et al. 2008, Passos et al. 2012, Patton et al. 2015, Yánez-Muñoz et al. 2016, Arteaga et al. 2017, 2018, Betancourt et al. 2018, Sánchez-Nivicela et al. 2020).

The biodiversity of this biogeographic province remains poorly studied and heavily threatened by unceasing habitat loss, degradation, and fragmentation due to legal and illegal logging, expansion of the agricultural frontier, industrial plantations (especially oil palm), and mining activities (Dodson & Gentry 1991, CEPF 2001, Kvist et al. 2004, Cisneros-Heredia et al. 2010, MAE 2012, 2013, 2017, Sierra 2013, Yánez-Muñoz et al. 2013). Conservation of the biodiversity and natural resources of the Western Ecuador biogeographic province depends on urgent actions to preserve the last remnants of these diverse and unique forests. We call for the urgent development of the following research and conservation actions in the near future to improve knowledge and promote conservation of *N. g. orense* and, in general, of the rich biodiversity of the southern section of the Western Ecuador biogeographic province: (i) surveying less studied areas in the provinces of Guayas, Cañar, Azuay, El Oro and Loja to determine the extent of occurrence of range-restricted taxa; (ii) studying the life history and ecology of those taxa, including impacts of habitat change on breeding and survival; (iii) monitoring population and habitat trends of range-restricted taxa; (iv) establishing or expanding public, communal and private reserves to protected remaining habitat and support the conservation by Fundación Jocotoco at the Buenaventura Reserve; (v) working with local landowners and governments to promote good practices of forest management; (vi) enhancing knowledge exchange between private and public stakeholders (including local governmental authorities) in order to promote research and conservation actions; raising local, national and international awareness about the unique and rich biodiversity of the province of El Oro; (vii) lobbying for the establishment, compliance and enforcement of national, provincial and local legislation that promotes protection and restoration of forested areas in the Western Ecuador biogeographic province.

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Appendix

Examined specimens

Cochranella litoralis: ECUADOR: Esmeraldas: Río Cachabí, DHMECN 3198; Tsejpu, Rio Zapallo; QCAZ EcoC 141; near Durango, QCAZ 27693; Pichiyacu, QCAZ 31705. COLOMBIA: Nariño: La Guayacana, ICN 13821 (holotype).

Cochranella mache: ECUADOR: Esmeraldas: Reserva Biológica Bilsa, QCAZ 22412 (holotype), 22413 (paratype); Río Balthazar, Alto Tambo, QCAZ 27747, 31327; Monte Saino, Punta Galeras, DHMECN 2611; ca. 3 km NW from Quininde, ZSFQ DFCHLQ23; Reserva Biológica Canandé, DHMECN 3560; Alto Tambo, Río Carolina, QCAZ 27764.

Espadarana prosoblepon: ECUADOR: Esmeraldas: Reserva Biológica Bilsa, USNM 541904–541915; Imbabura: 5–6 km W of Lita, QCAZ 4318, 4893; Río Verde, QCAZ 20704. Pichincha: Mashpi, ZSFQ DFCH293–295; Tandapi, KU 118041; Santo Domingo de los Tsáchilas: Santo Domingo de los Colorados, USNM 285830; Tinalandia, MCZ A-88434–35, 89877, 91189, 91191, 91460. Guayas: USNM 288438: "Guayaquil"; Los Ríos: Río Palenque Scientific Center, USNM 286718–27, MCZ A-88573, 89878; Montañas de Ila, USNM 286734–35; Bolívar: Balsapamba, QCAZ 10925; Cotopaxi: "below Sigchos", USNM 288441; El Oro: 16.8 km W of Piñas, USNM 286738–39. COSTA RICA: Alajuela, USNM 219329–33; Cartago, 219334–47; Puntarenas, USNM 219314–28, 219348–49; San José, USNM 219350–78.

Hyalinobatrachium fleischmanni: ECUADOR: Esmeraldas: Río Bogota, ZSFQ DFCH; Los Ríos: Quevedo, USNM 60520; Río Palenque, USNM 286639–40; Patricia Pilar, USNM 286645; Hacienda Cerro Chico, USNM 286646. COSTA RICA: Alajuela: USNM 219249–61; San José: USNM 219263–80; Guanacaste: USNM 219282–303. NICARAGUA: Matagalpa: USNM 220013–18; Nueva Segovia: USNM 220019–36. HONDURAS: Olancho: USNM 342162–342213. MÉXICO: Chiapas: USNM 115499.

Hyalinobatrachium valerioi: ECUADOR: Los Ríos, Río Palenque, USNM 286746–49; Cañar: Manta Real, DHMECN 0134. COSTA RICA: Puntarenas: USNM 219398–424; Alajuela: USNM 219425–38.

Nymphargus anomalus: ECUADOR: Napo: Río Azuela, KU 143299 (holotype); Volcán Sumaco, QCAZ 41312–13. Tungurahua: stream tributary of the San Jacinto River, ZSFQ 899.

Nymphargus armatus: COLOMBIA: Valle del Cauca: near Boqueron ICN 25000 (holotype), ICN 28037–49 (paratypes).

Nymphargus balionotus: ECUADOR: Carchi: Cabeceras del Río Baboso, DHMECN 0865; Pichincha: 3.5 km (by road) northeast of Mindo, KU164702 (holotype), KU 164701, KU 164703–11, ICN 23479 [formerly KU 164712] (paratypes). Imbabura: Río Manduriacu Reserve, ZSFQ 0531–533.

Nymphargus bejaranoi: BOLIVIA: Cochabamba: 58.1 km SW Villa Tunari (by road), KU 182369 (holotype), KU 182370–71 (paratypes).

Nymphargus buenaventura: ECUADOR: El Oro: Reserva Biológica Buenaventura, DHMECN 3563 (holotype), 3561–62, 2524 (paratypes); Marcabelí, DHMECN 10902; Cascadas de Manuel, DHMECN 10982.

Nymphargus cariticommatus: ECUADOR: Morona-Santiago: 11.2 km WSW Plan de Milagro, KU 202806 (holotype), KU 202805 (pararatype); El Cruzado, USNM 288435–6. Zamora-Chinchipe: Reserva Tapichalaca, DHMECN 1974, 2429.

Nymphargus chami: COLOMBIA: Risaralda: quebrada Carbones y Ventanas, ICN 32079 (holotype). Antioquia: Campamento Río Amparradó, ICN 8666, 10640 (paratypes).

Nymphargus chancas: PERU: west slope Abra Tangarana, KU 211778 (holotype).

Nymphargus cochranae: ECUADOR: Napo: Cascada de San Rafael, USNM 284304–6, 286632–36; Río Salado, USNM 286638; 14.7 km (by road) NE Río Salado, USNM 286638; 14 km (by road) SW Reventador, USNM 284304–06; Pacto Sumaco, QCAZ 31113. Orellana: "Loreto", USNM 288452; km 13 Loreto–Coca road, QCAZ 22196–97. Tungurahua: Río Topo, BMNH 1912.11.1.68. Zamora-Chinchipe: Contrafuerte de Tzunantza, DFCH D100–1; Romerillos, FHGO 2804.

Nymphargus colomai: ECUADOR: Zamora-Chinchipe: Miazi Alto, QCAZ 41590 (holotype), 41591–92 (paratypes).

Nymphargus cristinae: COLOMBIA: Antioquia: Río Encarnación, ICN 18645 (holotypes), ICN 18643–4, 18646–9 (paratypes).

Nymphargus garciae: COLOMBIA: Cauca: km 64–73 carretera Popayán a Inzá, ICN 11752 (holotype), ICN 11715–20 (paratypes). ECUADOR: Napo: 11 km ESE Papallacta, KU 164658–62; 60 km E San Miguel de Salcedo, KU 202793. Sucumbíos: 18 km E Santa Bárbara, KU 202796.

Nymphargus grandisonae: ECUADOR: Carchi: 5.9 km E of Maldonado, USNM 286647–52; Chilma Bajo, QCAZ 39964–66, 39969–71, 39980, 39982–83; Chilma Bajo, rumbo a cascada Tres Marías, QCAZ 39991, QCAZ 47983, QCAZ 48011–12; 10 km E Chilma Bajo, QCAZ 40001; QCAZ 40004, cerca a cascada Tres Marías, 1 km S de Chilma Bajo; QCAZ 40175, 40178, Chilma Bajo; Pichincha: Lloa–Mindo–San Carlos, DHMECN 5873–74; Refugio Bosque Protector Mindo–Nambillo, QCAZ 7294; Mindo, QCAZ 22310–11, 22760; Reserva Biológica Tamboquinde, DHMECN 4269; Reserva Orquideológica Pahuma, DHMECN 4162; Chiriboga, Estación Experimental "La Favorita", DHMECN 901; Bosque Protector Río Guajalito, ZSFQ DFCH (field series) 111, 117, 150, 152, 160–1, 175, QCAZ 7047–48, 7062; Quebrada Zapadores, QCAZ 16288; Quebrada La Plata, USNM 211211; 2.9 km SW of Tandayapa, USNM 211212–15; 1,4 km SW Tandayapa, QCAZ 30790; Tandapi, QCAZ 067–71, 15882; Nanegal, Curipogio, DHMECN653; Vía Toachi-Chiriboga Quebrada 1 km arriba de Río Faisanes, QCAZ 15364; 5 km from Tandapi, vía Atenas, QCAZ 14292–93, 16512, 17744–58; Santo Domingo de los Tsáchilas: without precise locality, MCZ A-106952–56; Cotopaxi: QCAZ 318–321, 327, 329, Las Pampas; Bosque Integral Otonga, QCAZ 11683, 20718, 20725, 24574, 29888–92, 32282, 36245; 18.2 km de Quillotuña, vía Pucayacu, QCAZ 40388. COLOMBIA: Caldas: Pueblo Rico, BMNH 1919.7.11.68 (holotype).

Nymphargus griffithsi: Ecuador: Pichincha: Río Saloya, 1219 m, BMNH 1940.2.20.4 (holotype), BMNH 1940.2.20.3 (paratype).

Quebrada La Plata, USNM 286659; 1.0 km SW of Tandayapa, USNM 286662–64, 286667–77.

Nymphargus humboldti: ECUADOR: Napo: Volcán Sumaco, ZSFQ 0388 (holotype), QCAZ 9402, 41071, 41073–74, 41077–78 (paratypes).

Nymphargus ignotus: COLOMBIA: Valle del Cauca: Peñas Blancas, ICN 14748 (holotype), ICN 14749–77 (paratypes).

Nymphargus lasgrallarias: ECUADOR: Cotopaxi: Otonga, QCAZ 13115, QCAZ 11689–90. Pichincha: Nanegal Grande, QCAZ 46012; 9 km SE Tandayapa, KU 164577–87.

Nymphargus laurae: ECUADOR: Orellana: Loreto USNM 288453 (holotype).

Nymphargus lindae: ECUADOR: Zamora-Chinchipe: Miazi Alto, QCAZ 41572 (holotype), QCAZ 41562–71 (paratypes).

Nymphargus luminosus: COLOMBIA: Antioquia: Corregimiento Nutibara, km 23–27 Nutibara-La Blanquita road, 1,000–1,430 m, ICN 15930 (holotype), ICN 15918–20, 15922–29, 15931–36 (paratypes).

Nymphargus luteopunctatus: COLOMBIA: Cauca: vereda La Playa, ICN 20747 (holotype).

Nymphargus manduriacu: ECUADOR: Imbabura: Reserva Río Manduriacu ZSFQ 0466 (holotype), ZSFQ 0462–65 (paratypes), ZSFQ 0464.

Nymphargus mariae: PERU: Huánuco: Serranía de Sira, KU 174713 (holotype of *Centrolenella mariae*); Pastaza: 1 km W Puyo, MCZ 91187 (holotype of *Centrolenella puyoensis*); Río Pucayacu, USNM 291298; stream tributary of Río Lliquino, QCAZ 37932; near Villano, QCAZ 39293. Napo: ca. 45 km E of Narupa, ZSFQ DFCHD285. Orellana: Río Huataracu, QCAZ 7104, 7499; Reserva Río Bigal, QCAZ 48529.

Nymphargus megacheirus: ECUADOR: Napo: 16.5 km NNE Santa Rosa, KU 143245 (holotype), 143246–72 (paratypes); Rio Azuela, USNM 286700, KU 143273–77, 166329; 14.7 km (by road) NE Río Salado, USNM 286701; Cordillera de Guacamayos, EPN; 2 km SSW Río Reventador, KU 164614. COLOMBIA: Putumayo: 10.3 km W El Pepino, KU 169664–65.

Nymphargus megista: ECUADOR: Imbabura: Río Manduriacu Reserve, ZSFQ 3924, 4071.

Nymphargus mixomaculatus: PERU: San Martín, CORBIDI.

Nymphargus nephelophila: COLOMBIA: Caquetá: 3.1 km debajo de la cresta del Alto Gabinete, ICN 24297 (holotype), ICN 24296 (paratype).

Nymphargus ocellatus: PERU: Pasco: Huancabamba, BMNH 1912.11.1.19 (holotype). Ayacucho: Huanhuachayocc on Tambo-Valle path, KU 197030.

Nymphargus oreonympha: COLOMBIA: Caquetá: 8.6 km E Alto Gabineta, ICN 20765 (holotype), ICN 20766–75 (paratypes).

Nymphargus phenax: PERU: Ayacucho: Tutumbaro, KU 162263 (holotype), 162264, 162266–7 (paratypes).

Nymphargus pluvialis: PERU: Cusco: Pistipata, KU 173224 (holotype), 173225–27 (paratypes), USNM 298950–52.

Nymphargus posadae: COLOMBIA: Cauca: ICN 11307 (H), ICN 7447–50 (P). ECUADOR: Sucumbíos: Río Chingual, ca. 20 km N of La Bonita, USNM 288464–5. Napo: Yanayacu Biological Station, QCAZ 25090, 26022–23. Zamora Chinchipe: tributary of Río Jambue, ZSFQ DFCH. (ca. 0°26' S, 77°32' W; 1890 m).

Nymphargus prasinus: COLOMBIA: Valle del Cauca: Río Calima, KU 169693 (holotype), KU 169691–92 (paratypes).

Nymphargus rosada: COLOMBIA: Caldas: km 5.8 hacia Samaná, ICN 34761 (holotype), ICN 34764–5 (paratypes).

Nymphargus ruizi: COLOMBIA: Cauca: Quebrada Sopladero, ICN 7469 (holotype), ICN 7470–71 (paratypes).

Nymphargus siren: ECUADOR: Napo: Río Azuela, USNM 286740; Río Salado, ca. 1 km upstream from Rio Coca, KU 146610 (holotype), KU 146611–23 (paratypes); 6.5 km S Baeza, MCZ A97809. Orellana: Cordillera de Galeras, ZSFQ DFCH D292–295.

Nymphargus sp.: ECUADOR: Zamora-Chinchipe: Estación Científica San Francisco, QCAZ 31340–41.

Nymphargus spilotus: COLOMBIA: Caldas: Corregimiento Florencia, sitio "Rancho Quemado", 1,940 m, ICN 35155 (holotype); sitio El Estadero, 1,850 m, ICN 35157–58 (paratypes); zona "El Estadero" (o "Rancho Quemado"), ICN 38073 (paratype).

Nymphargus sucre: ECUADOR: Morona-Santiago: Plan de Milagro, USNM.

Nymphargus truebae: PERU: Cusco: Río Cosñipata, KU 162268 (holotype), 162269–81 (paratypes); Paucartambo, USNM 298178–80, USNM 346056–59, USNM 346310–13.

Nymphargus viglei: ECUADOR: Esmeraldas: Reserva Biológica Bilsa, KU 291177 (holotype).

Nymphargus wileyi: ECUADOR: Napo: Yanayacu Biological Station, QCAZ 26028 (holotype), 26024, 29, 26057 (paratypes).

Teratohyla spinosa: ECUADOR: Pichincha: Río Blanco, USNM 288443; Los Ríos: Río Palenque, USNM 286741–44. COSTA RICA: Alajuela: USNM 219388–94.