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A new species of salamander (Caudata: Plethodontidae: *Bolitoglossa*) from the subalpine rain páramo of the Cordillera de Talamanca, Costa Rica

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Abstract.—The subalpine rain páramo of Isthmian Central America is an area with a high level of endemism. The salamanders of the *Bolitoglossa subpalmata* Species Group are restricted to the highlands of Costa Rica and Panama, including the subalpine rain páramo. During explorations of the páramos in the Cordillera de Talamanca, we found populations of *Bolitoglossa* that were referred to *B. subpalmata* S.G. These new populations were compared phylogenetically with all species within the species group using the 16S and *cyt b* mitochondrial genes; and they were also compared morphometrically with *B. kamuk* and *B. pesrubra*. Herein we described a new species of the *B. subpalmata* Species Group, which inhabits the subalpine rain páramo and montane forest surrounding the páramo in the Southeastern region in Costa Rica. This new species highlights the role of the subalpine rain páramo in the speciation of salamanders in the highlands of Isthmian Central America, as well as the need to protect this unique and vulnerable habitat.

Keywords. Amphibia, caudate, Central America, páramo, phylogenetics, Plethodontidae

Resumen.—El páramo subalpino de América Central Ístmica es alto en endemismos. Las salamandras del grupo de especies *Bolitoglossa subpalmata* están restringidas a las tierras altas de Costa Rica y Panamá, incluyendo el páramo subalpino. Durante exploraciones a los páramos en la Cordillera de Talamanca encontramos varias poblaciones de *Bolitoglossa* que fueron asignadas al grupo de especies *B. subpalmata*. Estas nuevas poblaciones fueron comparadas filogenéticamente con todas las especies dentro del grupo de especies de *B. subpalmata*; además fueron comparadas morfológicamente contra *B. kamuk* y *B. pesrubra*. Aquí se describe una nueva especie del grupo de especies *B. subpalmata*, habitante del páramo subalpino y del bosque montano cerca del páramo en la región sureste de Costa Rica. Esta nueva especie resalta el rol del páramo subalpino en la especiación de las salamandras de las tierras altas de América Central Ístmica y la necesidad de proteger este hábitat único y vulnerable.

Palabras clave. América Central, Amphibia, Caudados, filogenética, Páramo, Pletodontidae

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Introduction

The subalpine rain páramo from Isthmian Central America (ICA) is an extremely reduced ecosystem that is fragmented and vulnerable. In ICA, the subalpine rain páramo is isolated in the highlands, especially in the Cordillera de Talamanca's summits, and covers less than 150 km² (Kappelle and Horn 2016). The páramo in ICA can

be considered a “sky island” located in the northwestern part of the Neotropical páramo, separated by straight-line distances of 1,100 km to Colombia's Páramo and 1,000 km to the highlands of Nuclear Central America. The subalpine páramo of ICA is characterized by low species diversity but a high level of endemism, especially among plants, although several vertebrate species are exclusive to the páramo (Kappelle and Horn 2016). Even though

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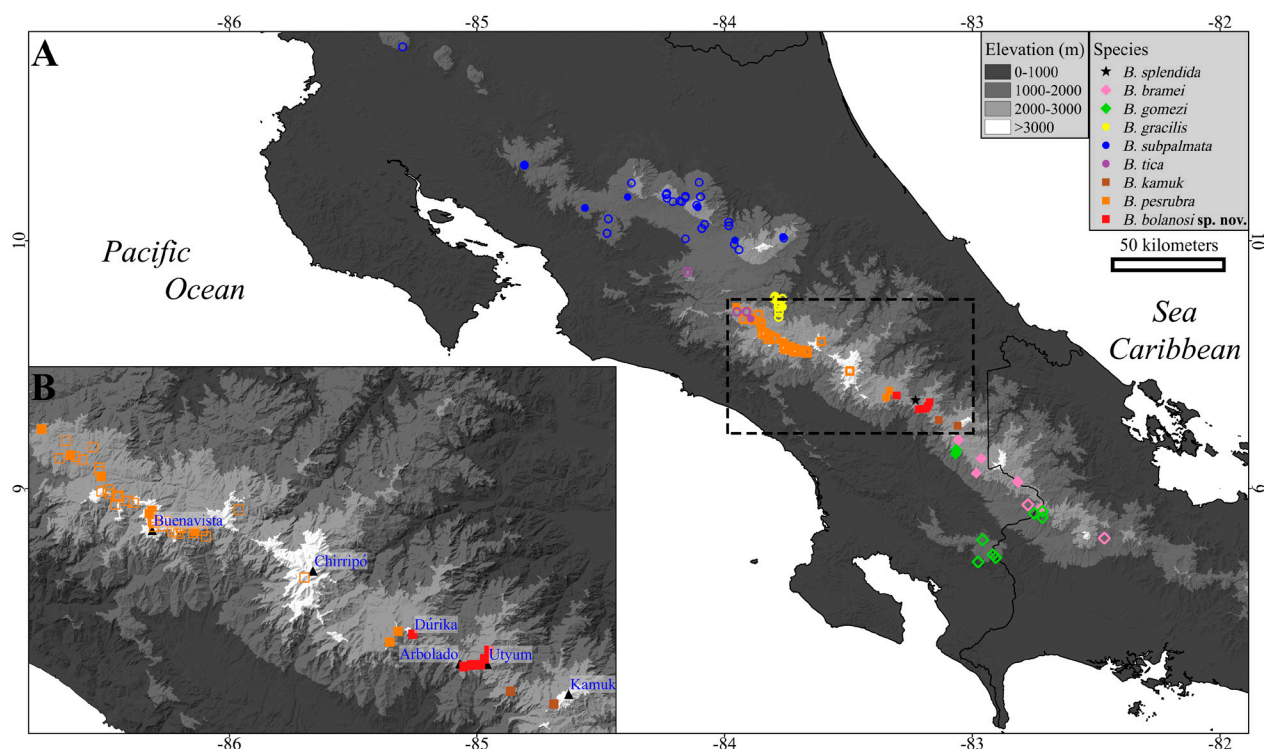


Fig. 1. (A) Map showing the known populations of the species within the *Bolitoglossa subpalmata* species group. (B) Map showing the localities for *B. bolanosi* sp. nov. and the species close to it in the páramo of the Cordillera de Talamanca. The open shapes indicate the historical data and the solid shapes indicate the localities with molecular data included herein.

the subalpine rain páramo of ICA is relatively small in area, several regions remain unexplored and several species may possibly remain unnamed. To the best of our knowledge, only three species of salamanders are exclusively found in the ICA's páramo: *Bolitoglossa kamuk*, *B. pesrubra*, and *B. pygmaea*.

Most salamanders of the ICA are associated with the highlands, especially on the Cordillera de Talamanca, where studies have documented high species turnover between sites and along elevational gradients (García-París et al. 2000; Wake 1987). The *Bolitoglossa subpalmata* Species Group (Parra-Olea et al. 2004) is an example of this pattern, containing eight species (*B. bramei*, *B. gomezi*, *B. gracilis*, *B. kamuk*, *B. pesrubra*, *B. splendida*, *B. subpalmata*, and *B. tica*) that are distributed in the highlands of Costa Rica and western Panama (Fig. 1), with high species turnover along latitudinal and elevational gradients (AmphibiaWeb 2023; Boza-Oviedo et al. 2012; García-París et al. 2008). Although the phylogenetic relationships of the *Bolitoglossa subpalmata* S.G. have been relatively well-studied (Boza-Oviedo et al. 2012), there is still little information on the distribution ranges of the species in this group, especially *B. pesrubra*, and the area between the páramo of the Cerro Chirripó and the páramo of the Cerro Kamuk has not been sampled at all.

During fieldwork in the subalpine rain páramo in the Cordillera de Talamanca, specifically the summits of the peaks Dúrika, Arbolado, Hakú, and Utyum (Fig. 1), we found some specimens of the *B. subpalmata* S.G., filling in the distribution range for the group. Based on the molecular and morphological data reported herein, we

describe these samples as a new species of *Bolitoglossa* closely related to *B. kamuk* and *B. pesrubra* of the *B. subpalmata* S.G.

Materials and Methods

Taxon sampling. In July 2013, March 2015, January 2016, and October 2016, we carried out collecting trips to Cerro Utyum (July 2013 and March 2015), Cerro Dúrika (January 2016), Cerro Arbolado (October 2016), and Cerro Hakú (October 2016) on the Talamanca Mountain range within La Amistad International Park, Costa Rica (Fig. 1). These trips involved walking a transect from Olán, Buenos Aires (9.2788° N, 83.2156° W, 1,500 m asl; all GPS coordinates in WGS84 datum) on the Pacific slope to the summits of the peaks. The salamanders were found by opening bromeliads from previously cut-off trees, and a total of 19 specimens referred to *Bolitoglossa subpalmata* S.G. were found in the sampled peak summits.

All the specimens collected for this study were humanely euthanized using a topical anesthetic. Following the euthanization process, a small tissue sample, either liver or the tip of the tail, was taken and stored in 96% ethanol. The specimens were fixed in a 10% formalin solution and transferred to 70% ethanol for long-term preservation. Individuals were deposited in the herpetological collection of Museo de Zoología at Universidad de Costa Rica (UCR) (Appendix 1). Museum collection acronyms follow Frost (2023), with the additions of EAP (Erick Arias field numbers), CRARC (Costa Rica Amphibian Research Center private

collection), and NV= No voucher.

Amplification and sequencing. Total genomic DNA was extracted from the ethanol-preserved tissues of 24 *Bolitoglossa* specimens using the phenol-chloroform standard protocol (Sambrook and Russell 2006). The large subunit ribosomal RNA (16S) and cytochrome *b* (*cyt b*) mitochondrial genes were amplified. The primers 16Sar and 16Sbr (Palumbi et al. 1991) were used for 16S, and primers MVZ15 and MVZ16 (Moritz et al. 1992) were used for *cyt b*. PCR amplifications were performed using a total volume of 15 μ L, which contained 1 μ L DNA template (at 50 ng μ L⁻¹), 0.75 U Taq polymerase (Amplificasa®, Biotecnologías Moleculares), 1X PCR buffer with 1.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3 μ M forward and reverse primers. The PCR conditions were as follows: for 16S, an initial cycle of 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C, plus a final step of 3 min at 72 °C; and for *cyt b*, an initial cycle of 2 min at 94 °C, followed by 38 cycles of 30 s at 94 °C, 1 min at 48 °C, 1 min at 72 °C, plus a final step of 8 min at 72 °C. The PCR products were cleaned with ExoSap-IT (USB Corporation) and sequenced in both directions using the original amplification primers and BigDye termination reaction chemistry (Applied Biosystems). The cycle-sequencing products were column-purified with Sephadex G-50 (GE Healthcare) and run on an ABI 3500xL Genetic Analyzer (Applied Biosystems). Consensus sequences for each individual were constructed using SEQUENCHER 5.3 (Genes Codes Corp.). The resulting sequences were deposited in GenBank (Appendix 1).

Phylogenetic analyses. The sequences obtained here were compared with the sequences of the 16S and *cyt b* mitochondrial genes for 78 specimens of the *Bolitoglossa subpalmata* species group. Sequences of *B. aurae* were used as outgroup and those from *B. compacta* were used to root all trees based on the results in Rovito et al. (2015). The list of vouchers and GenBank accession numbers used in this study are provided in Appendix 1. Sequence alignments were performed using the MUSCLE 3.7 software (Edgar 2004) with default parameters and trimmed to the point where most of the taxa had sequence data. PartitionFinder v2.1.1 software (Lanfear et al. 2017) and the Bayesian Information Criterion (BIC) were used to select the best partition scheme and the best model of sequence evolution for each partition. A single set of *branchlengths* was used across all partitions (*branchlengths*=linked), and the search for the best partition scheme used a heuristic search (*scheme*=greedy, Lanfear et al. 2012). Four subsets were defined *a priori*: one for 16S and three for *cyt b* (partitioned by codon position).

Phylogenetic analyses were performed using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The maximum likelihood analysis was performed using Garli 2.01 (Zwickl 2006). To find the best tree, ten search replicates were run with the following default setting values: *streefname* = random, *attachmentsper-taxon* = 24, *gentreshfortopoterm* =

100,000, and *significanttopochange* = 0.00001. For bootstrapping, 1,000 pseudoreplicates were run with the previous settings and with the following changes: *gentreshfortopoterm* = 10,000, *significanttopochange* = 0.01, and *treerejectionthreshold* = 20, as suggested in the Garli manual to speed up the bootstrapping. From these bootstraps, a majority rule consensus tree was obtained using Sumtrees (Sukumaran and Holder 2010a) from DendroPy package version 4.4.0 (Sukumaran and Holder 2010b). Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 (Ronquist et al. 2012) with the partition scheme and the model of sequence evolution for each partition as selected previously. Two separate analyses were run, each consisting of 50 million generations, sampled every 1,000 generations, and four chains with default heating parameters. A time-series plot of the likelihood scores of the cold chain was examined to check stationarity using Tracer 1.6 software (Rambaut et al. 2014). The first 25% of trees were discarded as burn-in and the remaining trees were used to estimate the *allcompat* consensus tree along with the posterior probabilities for each node and each parameter. The Garli and MrBayes analyses were run on the CIPRES portal (Miller et al. 2010). Genetic distances (uncorrected *p*-distances) were computed using MEGA6 (Tamura et al. 2013).

Morphometrics. The measurements used herein follow those proposed by Kubicki et al. (2022). The specimens from new localities were compared morphometrically only with the species *B. kamuk* and *B. pesrubra*, given that these are the species of the group that inhabit the subalpine páramo and occur geographically close (Fig. 1, Appendix 2). The following 29 morphological measurements were taken on each of the 31 adult specimens of *Bolitoglossa subpalmata* S.G. that were examined in this study: standard length (SL), shoulder width (ShW), head width (HeW), neck width (NeW), eye width (EW), snout length (SnL), jaw to snout length (JSL), lateral gular fold to tip of snout (LGFS), internarial distance (IND), naris to lip distance (NLP), intercanthal distance (ICD), hind limb length (HLL), front limb length (FLL), trunk width (TW), midventral gular fold to snout length (VGS), front limb to snout distance (FSL), ulna and hand length (UHL), axilla to groin length (AGL), vent length (VL), hand width (HaW), hand length (HaL), length of Finger III (LF3), width of Finger III (WF3), length of Finger II (LF2), foot width (FoW), foot length (FoL), length of Toe III (LT3), width of Toe III (WT3), and length of Toe II (LT2). The additional proportions reported here include: IND/HeW, HeW/AGL, SnL/HeW, HaL/VGS, FoL/VGS, HaW/HeW, FoW/HeW, LT2/FoL, LF2/HaL, WT3/FoW, WF3/HaW, HaL/HaW, and FoL/FoW.

The measurements were taken with a Mitutoyo Absolute 4084 digital caliper, and rounded to the nearest 0.1 mm. Given that there are very significant overlaps in the numbers of teeth found in the different *Bolitoglossa* species known to occur in Costa Rica (Boza-Oviedo et al. 2012; García-Paris et al. 2008; Savage 2002), the number of teeth in the specimens examined in this study were not counted. We believe that attempting to accurately count

the number of teeth in such small specimens (most of which were fixed with their mouths in a closed position), requires a high-level of invasive manipulation that would most likely cause significant and unnecessary damage to the mouth cavity. The limb interval is equal to the number of costal folds between the tips of the longest digits of the adpressed front and hind limbs, expressed in 0.5 increments (e.g., 4 or 4.5).

Morphometric statistics. To avoid allometric effects relative to the differences in the sizes and shapes between species and between individuals, the data were transformed using the method of Leonart et al. (2000). In this method, a logarithmic transformation of the continuous variables is performed to reduce the extreme values. All transformed variables were used in the allometric transformation by means of the equation:

$$Y_i^* = Y_i \left[\frac{X_0}{X_i} \right]^b$$

where Y^* corresponds to the value of each of the dependent variables corrected for size and shape, Y_i corresponds to the value of each dependent morphometric variable, X_0 is the average of the SL variable for all populations, X_i is the SL value for each individual, and b is the regression line intercept with the Y-axis resulting from the regression of each dependent variable with X_i . The intercept is used as an allometric transformation factor and is unique for each variable.

A discriminant analysis was performed to determine whether the morphometric variables were effective in predicting the species. Only those variables that were different among at least two of the three putative species analyzed were included in the discriminant analysis. The following variables were finally used in the discriminant analysis: HeW, JSL, ICD, HLL, VGS, HaW, HaL, HaL/VGS, FoL/VGS, and HaW/HeW. The discriminant analyses were separated by sex, and were performed using R v3.3.3 (R Development Core Team 2013).

Results

Molecular analyses. The mitochondrial dataset includes samples of all described species in the *Bolitoglossa subpalmata* S.G. The resulting data matrix had a total of 80 sequences (31 salamanders collected in this study and 49 sequences downloaded from Genbank) with a sequence length of 1,334 bp, including gaps, with 527 bp for 16S and 807 for cyt *b*. Three partitions were identified with the following substitution models: HKY+I+G for 16S + cyt *b* codon position 3, HKY+I for codon position 1 of cyt *b*, and TRN+G for cyt *b* codon position 2.

The phylogenies from Garli and MrBayes were relatively discordant in the internal topology within the *B. subpalmata* S.G. (Fig. 2). However, all phylogenetic analyses found the *B. subpalmata* S.G. to be monophyletic, with three internal clades relatively well-supported. The first clade was formed by *B. bramei* and *B. gomezi*, two species that are restricted to premontane and montane forests of the Pacific slope. The second clade was formed by *B. gracilis* sister to *B. tica* + *B.*

subpalmata, and these species are distributed in the Volcanic Central Mountain range and the northern part of the Cordillera de Talamanca. Finally, a well-supported clade was formed by *B. kamuk* sister to *B. pesrubra* + unnamed taxon, and these three species are restricted to the subalpine rain páramo of the Cordillera de Talamanca. The phylogenetic position of *B. splendida* is uncertain. In the Bayesian analysis, this species was weakly supported as sister to the clade formed by the three species of the páramo. In the ML analysis, *B. splendida* was weakly supported as sister to the clade formed by *B. bramei* + *B. gomezi*, forming the sister clade to the clade formed by *B. gracilis*, *B. subpalmata*, and *B. tica*.

The mitochondrial genetic distances are shown in Table 1. Genetic distances between the specimens of the unnamed taxon from the subalpine rain páramo and all other members of the *B. subpalmata* species group are 1.45–4.71% for 16S and 5.03–9.09% for cyt *b*.

Morphometric analyses. The specimens examined included 13 specimens (8♀ and 5♂) of the unnamed taxon from the subalpine rain páramo, 15 specimens of *B. pesrubra* (7♀ and 8♂), and three specimens of *B. kamuk* (1♀ and 2♂). The morphometric variation between the three species that inhabit the subalpine rain páramo of Costa Rica is shown in Table 2. The discriminant analysis correctly classified 100% of the specimens to the species (Fig. 3) in both sexes, showing a clear separation between the specimens of the new species described below and the specimens of *B. kamuk* and *B. pesrubra*. The ratios HaL/VGS, HaW/HeW, and IND/HeW differed among the three species of the subalpine rain páramo.

Description of New Species

Bolitoglossa bolanosi sp. nov.

Bolaños' Web-footed Salamander
(Figs. 4–6)

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Holotype. UCR 22965, an adult male from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: the summit of Cerro Arbolado, Parque Internacional La Amistad, (9.320°, -83.216°; 2,600 m asl), collected by Erick Arias and Omar Zúñiga on 19 October 2016.

Paratopotype. UCR 22964, a subadult male, same data as holotype.

Paratypes. UCR 22424, an adult male; UCR 22423, an adult female; UCR 22425, a subadult female; UCR 22422 and UCR 22426, subadult males; and UCR 22427, a juvenile from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: the summit of Cerro Utyum, Parque Internacional La Amistad, (9.323°, -83.187°; 2,870 m asl), collected by Erick Arias, Gerardo Chaves, Olmer Cordero, and Omar Zúñiga on 30 March 2015. UCR 22421, an adult female from Costa Rica: Provincia de Limón: Cantón de Talamanca: Distrito de Telire: the summit of Cerro Utyum, Parque

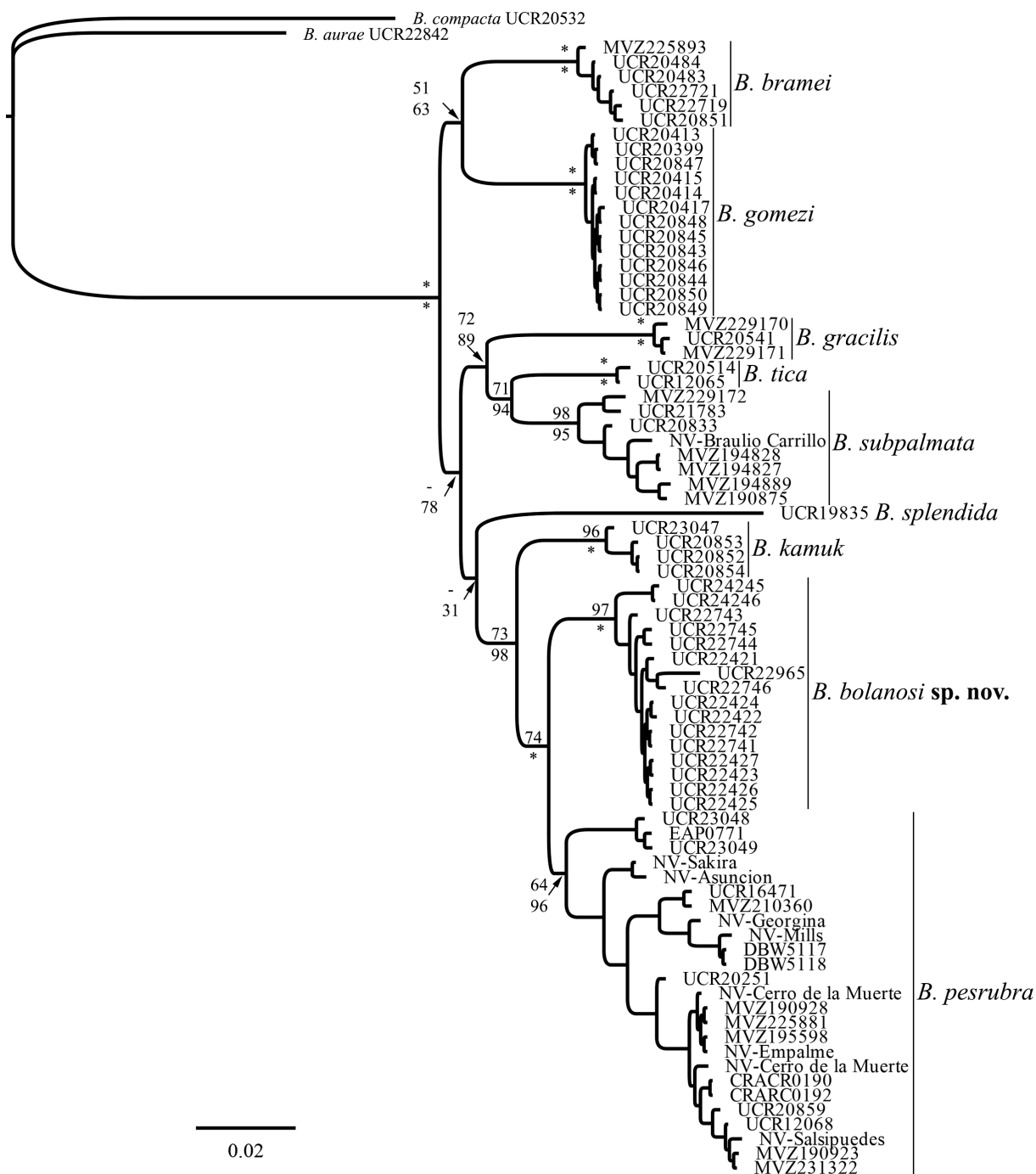


Fig. 2. Bayesian phylogenetic inference shows the relationships of the *Bolitoglossa subpalmata* species group based on the 16S and *cyt b* mitochondrial DNA gene fragments. Bootstrap proportions from maximum likelihood are shown above the branches, and numbers below the branches are posterior probabilities (multiplied by 100) from the MrBayes analysis. The scale bar refers to the estimated substitutions per site. The support values of any node within the species are not shown. The asterisks represent support >99. NV = no voucher.

Internacional La Amistad, (9.333°, -83.180°; 2,913 m asl), collected by Erick Arias, Gerardo Chaves, Olmer Cordero, and Omar Zúñiga on 30 March 2015. UCR 22745, an adult male; UCR 22741–4, adult females; and UCR 22746, a juvenile from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: the summit of Cerro Hakú, Parque Internacional La Amistad, (9.322°, -83.203°; 2,660 m asl), collected by Erick Arias and Omar Zúñiga on 28 December 2015.

UCR 24245, an adult female; UCR 24246, an adult male; UCR 24247, a subadult male; and UCR 24248, a juvenile from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: the summit of Cerro Dúrika, Parque Internacional La Amistad, (9.374°, -83.303°; 3,240 m asl), collected by Omar Zúñiga on 13 January 2016.

Generic Placement. Assigned to the genus *Bolitoglossa*

Table 1. Mean uncorrected genetic distances, as percentages, among species of the *Bolitoglossa subpalmata* species group, using the 16S (right) and *cyt b* (left) mitochondrial genes.

	<i>B. splendida</i>	<i>B. bramei</i>	<i>B. gomezi</i>	<i>B. gracilis</i>	<i>B. subpalmata</i>	<i>B. tica</i>	<i>B. kamuk</i>	<i>B. pesrubra</i>	<i>B. bolanosi sp. nov.</i>
<i>B. splendida</i>	—	3.88	3.49	4.42	2.63	4.19	2.53	2.74	3.14
<i>B. bramei</i>	10.01	—	3.36	3.20	2.66	3.76	2.32	3.36	3.44
<i>B. gomezi</i>	10.10	5.95	—	4.58	3.09	4.16	2.56	3.60	3.99
<i>B. gracilis</i>	9.30	7.84	7.06	—	2.75	3.73	3.63	4.61	4.71
<i>B. subpalmata</i>	9.53	7.71	6.96	8.12	—	1.94	1.75	2.71	2.82
<i>B. tica</i>	9.47	6.60	6.63	7.29	6.48	—	3.21	3.88	3.39
<i>B. kamuk</i>	10.29	6.56	6.71	7.75	8.46	7.43	—	1.25	1.45
<i>B. pesrubra</i>	9.11	6.51	6.72	7.35	7.78	7.29	7.06	—	1.69
<i>B. bolanosi sp. nov.</i>	9.09	6.50	6.34	7.04	7.57	6.51	5.87	5.03	—

due to having 14 costal grooves and lacking a sublingual fold, and to the subgenus *Eladinea* based on the molecular evidence presented herein.

Diagnosis. The combination of the following characteristics can be used to distinguish *Bolitoglossa bolanosi* from the other described species of the genus *Bolitoglossa*: (1) having broad hands and feet, with the distal phalanges on the fingers and toes free of palmar and plantar tissue; (2) dorsal coloration highly variable, rarely black brownish uniform and usually mottled with yellow spots, but never with red on hind limbs or forelimbs; and (3) 16S and *cyt b* mtDNA distances.

Comparisons. *Bolitoglossa bolanosi* is differentiated from members of the subgenus *Eladinea* by its 16S and *cyt b* mtDNA distances. Since *B. bolanosi* is only known to occur in Costa Rica and molecular evidence strongly supports it forming part of the *Bolitoglossa subpalmata* species group within the subgenus *Eladinea*, phenotypic comparisons are presented here only concerning the members of that clade (*B. bramei*, *B. gomezi*, *B. gracilis*, *B. kamuk*, *B. pesrubra*, *B. splendida*, *B. subpalmata*, and *B. tica*), which are endemic to mountain ranges of Costa Rica and western Panama.

Contrasting characteristics for *Bolitoglossa bolanosi* are presented in parentheses. *Bolitoglossa bramei* Wake et al., 2007 can be distinguished from *B. bolanosi* by having a rounded snout in males (snout strongly truncated in males); dorsal ground color dark brown to brownish red, usually with darker mottling or frosting of silvery-gray (dorsal ground color black to dark brown, usually with mottling or blotches of yellow to red). *Bolitoglossa gomezi* Wake et al., 2007 has fore limbs relatively shorter, FLL/SL 20% (FLL/SL 22.3–26.1%), snout rounded (snout truncate in males). *Bolitoglossa gracilis* Bolaños et al., 1987 has dorsum yellowish ground color and a distinct dark midventral stripe (dorsum variable but never with yellowish ground color and never with a distinct midventral stripe). *Bolitoglossa splendida* Boza-Oviedo et al., 2012 has a shiny black dorsum with a bright reddish-orange broad dorsal band extending from the back of the head to the base of the tail, and with bright enamel-yellow spots scattered along the lateral and ventrolateral surfaces (dorsum variable but none with a reddish dorsal band extending from the head to the tail). *Bolitoglossa subpalmata* (Boulenger, 1896) has forelimbs that are relatively shorter, FLL/SL 18.7–23.1% (FLL/SL 22.3–26.1%). *Bolitoglossa tica* García-París et al., 2008 has dorsal ground color usually a uniform reddish brown with a darker tail, rarely with mottling or blotches contrasting (dorsal ground color black to dark brown, usually with yellow to red mottling or blotchy contrasting); prominent whitish spots on the venter (venter without whitish spots).

Bolitoglossa bolanosi differs from its closest relatives as follows: *Bolitoglossa kamuk* Boza-Oviedo et al., 2012 is smaller and more slender with mean SL 34.6–38.4 mm (larger and robust, SL = 39.43–50.01 mm); internarial distance relatively shorter, IND/HeW = 0.26±0.05 (internarial distance relatively longer, IND/HeW = 0.31±0.05); shorter tail TL/SL 98–99% (tail long, TL/SL

Table 2. Morphometric data for *Bolitoglossa bolanosi* sp. nov., *B. kamuk*, and *B. pesrubra*. These data were obtained prior to morphometric correction.

Variable	<i>B. bolanosi</i> sp. nov.		<i>B. kamuk</i>		<i>B. pesrubra</i>	
	Mean±S.D. (Range)		Mean±S.D. (Range)		Mean±S.D. (Range)	
	♀ (N = 8)	♂ (N = 5)	♀ (N = 1)	♂ (N = 2)	♀ (N = 7)	♂ (N = 8)
SL	43.05±5.54 (32.71–50.01)	41.83±6.55 (34.01–49.04)	38.4	35.2±0.85 (34.6–35.8)	50.6±3 (47.6–54.3)	48.34±3.61 (47.6–54.78)
ShW	5.64±0.98 (4.25–7.49)	5.34±1.02 (4.4–7.09)	4.9	3.9±0.14 (3.8–4)	6.3±0.69 (5.2–7.3)	5.46±0.52 (5.2–6.43)
HeW	6.74±0.7 (5.45–7.7)	6.43±0.72 (5.46–7.3)	6	5.15±0.21 (5–5.3)	7.17±0.75 (6.09–8.3)	7.02±0.41 (6.09–7.76)
NeW	5.62±0.84 (3.99–6.72)	5.17±0.74 (4.26–6.17)	5	4.05±0.07 (4–4.1)	6.2±0.77 (5.3–7.4)	5.38±0.52 (5.3–6.53)
EW	2.32±0.28 (1.78–2.59)	2.5±0.48 (2.01–3.18)	2.5	2.15±0.21 (2–2.3)	2.56±0.22 (2.2–2.8)	2.51±0.22 (2.2–2.8)
SnL	2.75±0.32 (2.26–3.09)	2.88±0.43 (2.31–3.43)	2.3	1.95±0.07 (1.9–2)	3.03±0.18 (2.8–3.3)	3±0.76 (2.8–3.78)
JSL	7±0.64 (5.99–8.15)	7±0.88 (6.01–8.21)	6.8	5.95±0.49 (5.6–6.3)	8.09±0.5 (7.6–9)	8.08±0.48 (7.6–8.83)
LGFS	10.99±1.19 (8.46–12.17)	10.75±1.56 (9.06–12.9)	10.2	8.95±0.21 (8.8–9.1)	11.84±0.65 (11.2–12.9)	11.94±0.63 (11.2–12.96)
IND	1.94±0.14 (1.74–2.12)	2.25±0.56 (1.57–3.11)	1.8	1.2	2.27±0.19 (2–2.5)	2.61±0.36 (2–3.16)
NLP	0.9±0.09 (0.74–1.01)	0.87±0.15 (0.74–1.11)	1	0.75±0.07 (0.7–0.8)	1.07±0.13 (0.9–1.3)	1.05±0.16 (0.9–1.28)
ICD	3.3±0.34 (2.88–3.8)	3.27±0.38 (2.93–3.88)	2.8	2.45±0.07 (2.4–2.5)	3.14±0.57 (1.9–3.6)	3.51±0.28 (1.9–3.98)
HLL	11±1.62 (8.03–12.79)	11.07±1.54 (9.47–13.09)	10.4	8.3±0.28 (8.1–8.5)	12.26±0.85 (11.2–13.6)	12.71±1.36 (11.2–14.16)
TW	6.62±1.39 (5.16–9.2)	5.26±0.63 (4.3–6.02)	5.4	3.9±0.14 (3.8–4)	6.93±0.96 (5.8–8.4)	6.1±0.46 (5.8–6.9)
FLL	10.38±1.54 (7.75–11.91)	11.28±3.87 (8.06–17.62)	8.9	8.00	11.27±0.74 (10.1–12.4)	11.51±1.22 (10.1–13.61)
VGS	10.49±1.13 (8.18–11.68)	10.47±1.81 (8.72–13.2)	9.2	8.45±0.35 (8.2–8.7)	11.66±0.52 (11.1–12.4)	11.4±0.56 (11.1–12.2)
FSL	12.75±1.42 (10.16–14.8)	12.59±1.77 (10.91–15.17)	11.9	10.3±0.28 (10.1–10.5)	14.46±0.79 (13.5–15.6)	14.37±0.85 (13.5–15.59)
UHL	7.22±2 (2.63–8.58)	6.51±2.65 (3.2–9.01)	6.5	5.9	8.1±0.64 (7.4–9.4)	6.16±2 (7.4–9.2)
AGL	21.94±3.75 (16.14–26.4)	21.2±3.05 (16.18–24.13)	19.3	17.9±0.28 (17.7–18.1)	27.37±1.89 (25.2–30.1)	24.71±1.96 (25.2–28.35)
VL	4.34±0.7 (3.05–5.09)	4.06±0.85 (2.93–4.94)	4	2.56±0.62 (2.12–3)	4.86±0.53 (4.4–5.8)	4.36±0.64 (4.4–5.78)
HaW	3.66±0.5 (3.04–4.41)	3.79±0.84 (2.68–4.84)	3.3	2.65±0.21 (2.5–2.8)	4.24±0.44 (3.8–5.1)	4.36±0.57 (3.8–5.1)
HaL	3.85±0.64 (2.75–4.64)	3.81±1 (2.91–5.35)	3.7	2.6±0.14 (2.5–2.7)	4.04±0.43 (3.8–5)	4.2±0.49 (3.8–4.9)
WF3	0.86±0.09 (0.73–1.05)	0.79±0.15 (0.59–0.98)	0.9	0.6	0.77±0.08 (0.7–0.9)	0.82±0.11 (0.7–0.99)
LF2	1.61±0.22 (1.33–1.92)	1.81±0.29 (1.59–2.31)	1.6	1.45±0.07 (1.4–1.5)	2.01±0.22 (1.8–2.4)	2.24±0.4 (1.8–2.7)
LF3	2.02±0.25 (1.75–2.39)	2.29±0.45 (1.86–3)	2.3	1.75±0.21 (1.6–1.9)	2.56±0.24 (2.4–3.1)	2.76±0.42 (2.4–3.45)

A new species of *Bolitoglossa*

Table 2 (continued). Morphometric data for *Bolitoglossa bolanosi* sp. nov., *B. kamuk*, and *B. pesrubra*. These data were obtained prior to morphometric correction.

Variable	<i>B. bolanosi</i> sp. nov.		<i>B. kamuk</i>		<i>B. pesrubra</i>	
	Mean±S.D. (Range)		Mean±S.D. (Range)		Mean±S.D. (Range)	
	♀ (N = 8)	♂ (N = 5)	♀ (N = 1)	♂ (N = 2)	♀ (N = 7)	♂ (N = 8)
FoW	4.44±0.76 (3.44–5.42)	4.56±0.87 (3.63–5.74)	3.9	3.45±0.21 (3.3–3.6)	4.96±0.4 (4.5–5.6)	5.17±0.77 (4.5–6.02)
FoL	4.34±0.58 (3.42–5.03)	4.23±0.91 (3.27–5.53)	4.3	3.00	4.6±0.35 (4.1–5.2)	4.78±0.39 (4.1–5.3)
WT3	0.9±0.16 (0.68–1.24)	0.77±0.1 (0.67–0.92)	0.9	0.65±0.07 (0.6–0.7)	0.8±0.1 (0.7–1)	0.86±0.13 (0.7–1.1)
LT2	1.82±0.19 (1.52–2.05)	1.93±0.16 (1.77–2.15)	1.5	1.2±0.14 (1.1–1.3)	2.31±0.22 (2–2.7)	2.44±0.5 (2–3.09)
LT3	2.44±0.44 (1.66–2.94)	2.46±0.22 (2.15–2.68)	2.3	1.65±0.07 (1.6–1.7)	2.79±0.17 (2.6–3)	3.05±0.42 (2.6–3.73)
VGS/SL	0.24±0.01 (0.23–0.26)	0.25±0.01 (0.23–0.27)	0.24	0.24±0 (0.24–0.24)	0.23±0.01 (0.22–0.24)	0.24±0.01 (0.22–0.25)
IND/HEW	0.29±0.02 (0.26–0.32)	0.35±0.05 (0.29–0.43)	0.3	0.23	0.32±0.03 (0.29–0.38)	0.37±0.04 (0.29–0.42)
AGL/SL	0.51±0.03 (0.47–0.58)	0.51±0.05 (0.46–0.58)	0.503	0.51	0.54±0.01 (0.53–0.55)	0.51±0.02 (0.53–0.54)
HEW/SL	0.16±0.01 (0.15–0.17)	0.15±0.01 (0.15–0.17)	0.156	0.15±0.01 (0.14–0.15)	0.14±0.01 (0.12–0.16)	0.15±0.01 (0.12–0.16)
HEW/AGL	0.31±0.03 (0.25–0.36)	0.31±0.02 (0.28–0.34)	0.311	0.29±0.01 (0.28–0.29)	0.26±0.02 (0.23–0.29)	0.28±0.01 (0.23–0.3)
SNL/HEW	0.41±0.04 (0.36–0.45)	0.45±0.03 (0.41–0.48)	0.383	0.38±0.03 (0.36–0.4)	0.42±0.03 (0.37–0.46)	0.43±0.1 (0.37–0.49)
HLL/SL	0.26±0.01 (0.25–0.27)	0.27±0.01 (0.26–0.28)	0.271	0.24±0.01 (0.23–0.24)	0.24±0.01 (0.22–0.26)	0.26±0.02 (0.22–0.3)
FLL/SL	0.24±0.01 (0.22–0.26)	0.26±0.05 (0.23–0.26)	0.232	0.23±0.01 (0.22–0.23)	0.22±0.02 (0.19–0.24)	0.24±0.02 (0.19–0.27)
HAL/VGS	0.37±0.03 (0.33–0.4)	0.36±0.03 (0.33–0.41)	0.402	0.31±0.03 (0.29–0.33)	0.35±0.03 (0.31–0.4)	0.37±0.03 (0.31–0.43)
FOL/VGS	0.41±0.02 (0.37–0.44)	0.4±0.03 (0.37–0.43)	0.467	0.36±0.01 (0.34–0.37)	0.39±0.02 (0.37–0.42)	0.42±0.03 (0.37–0.45)
HAW/HEW	0.54±0.04 (0.48–0.59)	0.58±0.07 (0.49–0.66)	0.55	0.52±0.06 (0.47–0.56)	0.59±0.04 (0.54–0.66)	0.62±0.07 (0.54–0.76)
FOW/HEW	0.66±0.06 (0.55–0.73)	0.7±0.06 (0.64–0.79)	0.65	0.67±0.07 (0.62–0.72)	0.7±0.07 (0.63–0.84)	0.73±0.09 (0.63–0.9)
LT2/HEW	0.27±0.03 (0.24–0.3)	0.31±0.03 (0.26–0.32)	0.25	0.23±0.02 (0.22–0.25)	0.33±0.04 (0.27–0.39)	0.35±0.07 (0.27–0.45)
LT3/FOW	0.55±0.05 (0.48–0.63)	0.56±0.08 (0.47–0.64)	0.59	0.48±0.05 (0.44–0.52)	0.56±0.05 (0.51–0.65)	0.6±0.08 (0.51–0.71)
LT2/FOL	0.42±0.03 (0.37–0.48)	0.47±0.09 (0.35–0.54)	0.349	0.4±0.05 (0.37–0.43)	0.5±0.05 (0.42–0.57)	0.51±0.1 (0.42–0.68)
LF2/HAL	0.43±0.08 (0.29–0.58)	0.49±0.08 (0.42–0.6)	0.432	0.56±0.06 (0.52–0.6)	0.5±0.05 (0.45–0.58)	0.53±0.08 (0.45–0.63)
WT3/FOW	0.2±0.03 (0.16–0.24)	0.17±0.01 (0.16–0.18)	0.231	0.19±0.03 (0.17–0.21)	0.16±0.03 (0.14–0.22)	0.17±0.02 (0.14–0.21)
WF3/HAW	0.24±0.03 (0.21–0.28)	0.21±0.03 (0.16–0.26)	0.273	0.23±0.02 (0.21–0.24)	0.18±0.01 (0.17–0.21)	0.19±0.03 (0.17–0.24)

102.3–117.2%); dorsal ground color relatively uniform, orange to black (dorsal ground usually mottled or blotchy); males with rounded snout (snout strongly truncated in males). *Bolitoglossa pesrubra* (Taylor, 1952) has hands and feet more webbed (Fig. 5C–D), usually less than the ultimate phalange free (with at least the ultimate phalange free beyond the interdigital tissue margin, Fig. 5A–B); has red color on forelimbs and usually on hind limbs (dorsum variable but never with red on hind limbs or forelimbs). Hands relatively wider, HaW/HeW = 0.61 ± 0.07 (hands narrower, HaW/HeW = 0.56 ± 0.06).

Description of holotype. Adult male having a SL of 49.05 mm (Fig. 4). Head slightly wider than neck and shoulders (HeW 7.3 mm, NeW 6.2 mm, ShW 7.1 mm), with the greatest width of the head just posterior to the articulation of the jaws; truncate in dorsal outline and rounded to truncate in profile; snout moderate (SnL 3.4 mm, 6.9% of SL), with nearly terminal non-protruding small nostrils (LNH 0.6 mm, RNW 0.4 mm) directed anterolaterally; internarial area convex in dorsal outline. Snout protruding beyond the anterior margin of the lower lip in lateral view. Eyes relatively large (EW = 92% of SnL), weakly protruding beyond the dorsal and ventral outline of the head, directed anterolaterally, with a distinct suborbital groove. Top of head flat and smooth, tapering slightly toward anterior terminus, lacking contrasting interorbital or other dermal structures. Canthus rostralis weakly rounded; intercanthal area flat to slightly convex; and loreal region slightly concave. Evident cirri (nasolabial protuberances) on tip of snout; nasolabial grooves start at ventrolateral margins of nares and terminate in a cirrus. Gular fold is well-defined, starting on the dorsolateral portion of the neck, below the postorbital groove. Evident mental gland is visible under the skin of the anterior intermandibular region.

Arms relatively long and slender (FLL = 12.6 mm, 25.7% of SL), without noticeable hypertrophied forearm compared to the upper arm. Hands well-developed and slender (HaL = 5.4 mm, 40.9% of VGS; HaW = 4.8 mm, 65.8% of HeW). Fingers II, III, and IV protrude freely, with at least the ultimate phalange free beyond interdigital tissue margin (LF2 2.3 mm, LF3 3.0 mm; Fig. 5). Tips of fingers rounded; terminal pads weakly discernible on the ventral surfaces of fingers. Relative lengths of fingers on right hand: $I < IV < II < III$.

Legs moderately long and slender (HLL 13.1 mm, 26.7% of SL). Feet well-developed and slender (FoL 5.5 mm, 41.7% of VGS; FoW 5.7 mm, 78.1% of HeW). Toes II, III, IV, and V protruding freely beyond interdigital tissue margin (LT2 1.9 mm, LT3 2.7 mm), toe I with minimal indentation at interdigital spaces. Toe III is most free of interdigital tissue, with about the entire distal phalanx protruding. Tips of toes rounded; terminal pads weakly discernible on ventral distal surface of toes. Relative lengths of toes on right foot: $I < V < II < IV < III$.

Body subcylindrical (slightly wider than high) in cross-section, and relatively slender (TW = 6.0 mm; TW = 26.5% of AGL). Between the axilla and groin, 11 costal grooves are visible, 13 if counting axillary and inguinal grooves; costal grooves are most visible on ventral and

lateral portions of the body. Adpressed limbs separated by one costal fold; 12 costal folds total between axilla and groin. The tail is long, cylindrical in cross-section, with an evident constriction at the base, and some caudal grooves discernible on the anterior portion of the tail. The skin on the surfaces of the head, body, limbs, and tail is smooth.

Coloration in life. The ground color of dorsal surfaces of the head, trunk, tail, hind limbs, and forelimbs is brownish black to brownish violet with numerous fine lighter patches of chromatophores scattered throughout the dorsal surface, especially concentrated on the head. The head and hindlimbs are lighter than the trunk. A pair of irregular dorsolateral stripes, which run from the tail base to the intercanthal area crossing the superior eyelids, are formed by yellowish-bronze blotches. The iris is bright dark bronze with a dark brownish-black reticulation. The upper surfaces of the arms are lighter than the trunk, pinkish brown, with orange blotches in the proximal portion of the humerus and on the ventrolateral surface of the ulna. The upper surface of the legs is similar in color to the trunk, uniform brownish black, except in the foot which is paler. The dorsal and dorsolateral surfaces of the tail are nearly uniform brownish black.

The ventrolateral surfaces of the body, tail, hindlimbs, and forelimbs are lighter than the dorsal surface. The gular surfaces are paler than the venter, consisting of lighter brown with a paler blotch on the anterior part, the mental gland. The ventral surface of the trunk and the tail are slightly lighter than the dorsal surface. The ventral surfaces of the arms and legs are lighter than the dorsal surfaces with irregular orange blotches. The palmar and plantar surfaces are light brown with black chromatophores.

Coloration in ethanol. After more than seven years in ethanol (70%), the overall coloration of the holotype has darkened throughout and contains a principal dark brown-blackish tone.

Measurements (in mm), limb interval, and percentages of the holotype. SL 49.05; TL 57.5; ShW 7.1; HeW 7.3; NeW 6.2; EW 3.2; SnL 3.4; JSL 8.2; LGFS 12.9; LNH 0.6; RNW 0.4; IND 3.1; NLP 1.1; ICD 3.9; HLL 13.1; FLL 12.6; TW 6.0; VGS 13.2; FSL 15.2; UHL 9.0; AGL 22.8; VL 4.9; HaW 4.8; HaL 5.4; LF2 2.3; LF3 3.0; WF3 1.0; FoW 5.7; FoL 5.5; LT2 1.9; LT3 2.7; WT3 0.9. Limb interval 1. Measurements in relative percentages: VGS/SL 26.9%; IND/HeW 42.5%; AGL/SL 46.5%; HeW/SL 14.9%; HeW/AGL 32.0%; SnL/HeW 46.6%; LNH/HeW 8.2%; LNH/SL 1.2%; RNW/HeW 5.5%; RNW/SL 0.8%; HLL/SL 26.7%; FLL/SL 25.7%; HaL/VGS 40.9%; FoL/VGS 41.7%; HaW/HeW 65.7%; FoW/HeW 78.1%; LT2/FoL 34.5%; LF2/HaL 42.6%; WT3/FoW 15.8%; WF3/HaW 20.8%.

Noteworthy variation. The female paratypes (UCR 22421 and UCR 24245) have more rounded snouts, and much less defined and protruding cirri or nasolabial protuberances, which are sexually dimorphic features. In coloration, this species is extremely polymorphic, some

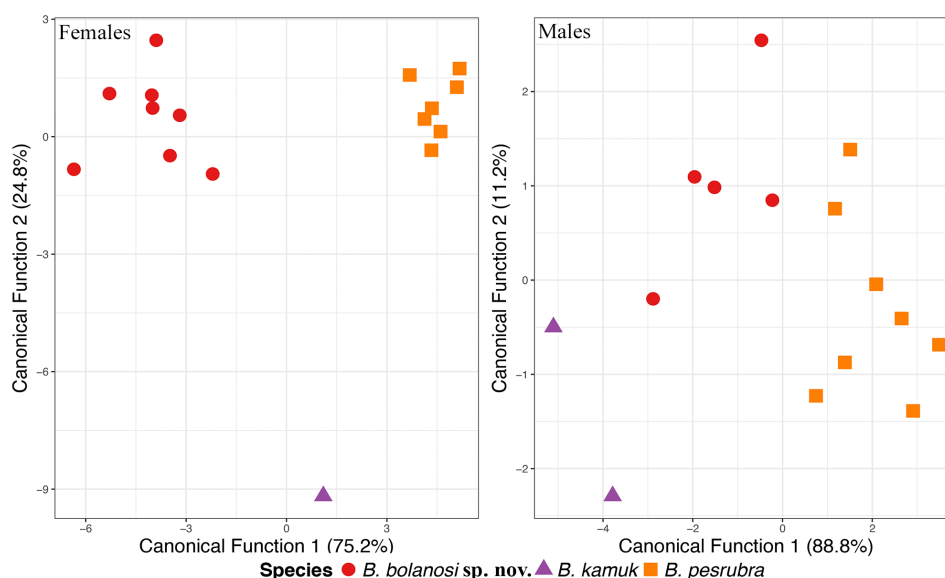


Fig. 3. Linear discriminant analysis shows the morphological separation between the new species and the two species close to it.

individuals are almost uniform black, which is common in juveniles (UCR 22247, Fig. 6B). The paratopotype (UCR 22964, Fig. 6A) has the pair of dorsolateral stripes more vivid and continuous, the blotches in the upper eyelid are iridescent green; in addition, it has a band of pale orange from posterior head to tail, where it is suffused with the red of the tail. The presence of red on the tail is common; generally the red color in the proximal portion is only on the dorsal surface, but the tail becomes completely red in the distal portion. Several specimens showed a similar coloration morpho “mottled” (Fig. 6C), with ground color brownish black to reddish brown with numerous and irregular yellow to red blotches or spots; these specimens lack the dorsolateral stripes. The female UCR 24245 (Fig. 6D) has a striking coloration pattern formed by large yellow blotches about a brownish-black ground color; although less evident this specimen has the pair of dorsolateral stripes but the blotches are larger and suffused in the back.

Measurements (in mm), limb intervals, and percentages of the paratypes. SL 39.4–50.0; ShW 4.9–7.5; HeW 6.3–7.7; NeW 5.0–6.7; EW 1.8–3.2; SnL 2.4–3.4; JSL 6.0–8.2; LGFS 10.2–12.9; IND 1.7–3.1; NLP 0.7–1.1; ICD 2.9–3.9; HLL 9.9–13.1; FLL 8.9–17.6; TW 5.2–9.2; VGS 9.6–13.2; FSL 11.4–15.2; UHL 6.8–9.0; AGL 18.6–26.4; VL 3.5–5.1; HaW 3.0–4.8; HaL 3.4–5.4; LF2 1.3–2.3; LF3 1.8–3.0; WF3 0.7–1.1; FoW 3.4–5.7; FoL 3.8–5.5; LT2 1.5–2.1; LT3 1.7–2.9; WT3 0.7–1.2. Limb intervals 3–5. Measurements in relative percentages: VGS/SL 23.4–26.9%; IND/HeW 25.8–42.6%; AGL/SL 46.4–57.6%; HeW/SL 14.5–16.9%; HeW/AGL 25.2–35.7%; SnL/HeW 35.7–47.6%; HLL/SL 24.6–26.8%; FLL/SL 22.3–26.1%; HaL/VGS 32.7–40.5%; FoL/VGS 37.1–44.4%; HaW/HeW 48.0–66.3%; FoW/HeW 54.5–78.6%; LT2/HeW 24.1–31.9%; LT3/FoW 46.7–63.0%; LT2/FoL 34.9–45.8%; LF2/HaL 29.3–46.9%; WT3/FoW 15.5–24.1%; WF3/HaW 16.2–28.3%.

Etymology. The name “*bolanosi*” is a patronym honoring

the Costa Rican herpetologist Federico Bolaños, and is used as a noun in the genitive case. We name this species after our dear friend in recognition of his scientific contributions to the knowledge of the herpetology of Costa Rica, as curator of the Herpetology section at Museo de Zoología of Universidad de Costa Rica, and as the mentor of most herpetologists present in the country.

Habitat and natural history observations. The habitat of *Bolitoglossa bolanosi* in the subalpine rain páramo is characterized by having a very short dry season (one to two months), annual precipitation ranging from 1,000 to 2,000 mm, and annual temperatures between 3 and 6 °C. The páramo vegetation consists of extensive, deep moss mats, spongy soil, ferns, and small isolated trees with arboreal bromeliads. *Bolitoglossa bolanosi* was found within both moss and bromeliads. It also occurs in Montane rain forest which is dominated by *Quercus* covered with moss, with a great abundance of bryophytes and epiphytes. The type locality occurs in the Montane rain forest (Bolaños et al. 2005; Holdridge 1967), characterized by a very short dry season (one to two months), an annual precipitation range of 2,200 to 4,500 mm, and annual temperatures from 6 to 12 °C.

Very little is known about the natural history of *B. bolanosi*, but it is important to note that females guarding a clutch of eggs were found on Cerro Hakú (December 2015) and Cerro Dúrika (January 2016), both under moss on the floor. All specimens from Cerro Utyum were found within bromeliads at heights of >3 m over the soil, despite active searches in the moss of the páramo. Unlike in the peaks Dúrika, Arbolado, and Hakú, the specimens were mainly found in the moss at <2 m over soil, despite active searches in the bromeliads at >3 m above the ground (especially on Cerro Arbolado and Cerro Hakú). In the summit of Cerro Dúrika, *B. bolanosi* is very near (~4 km) to a site with *B. pesrubra*. In the summit of Cerro Utyum, it is to only ~7 km to a site with *B. kamuk*, and in this peak *B. bolanosi* is sympatric with an unnamed miniaturized salamander related to *B. pygmaea*. In addition, the type



Fig. 4. Holotype of *Bolitoglossa bolanosi* sp. nov. (UCR 22965) in life on a white background. Photograph by Erick Arias.

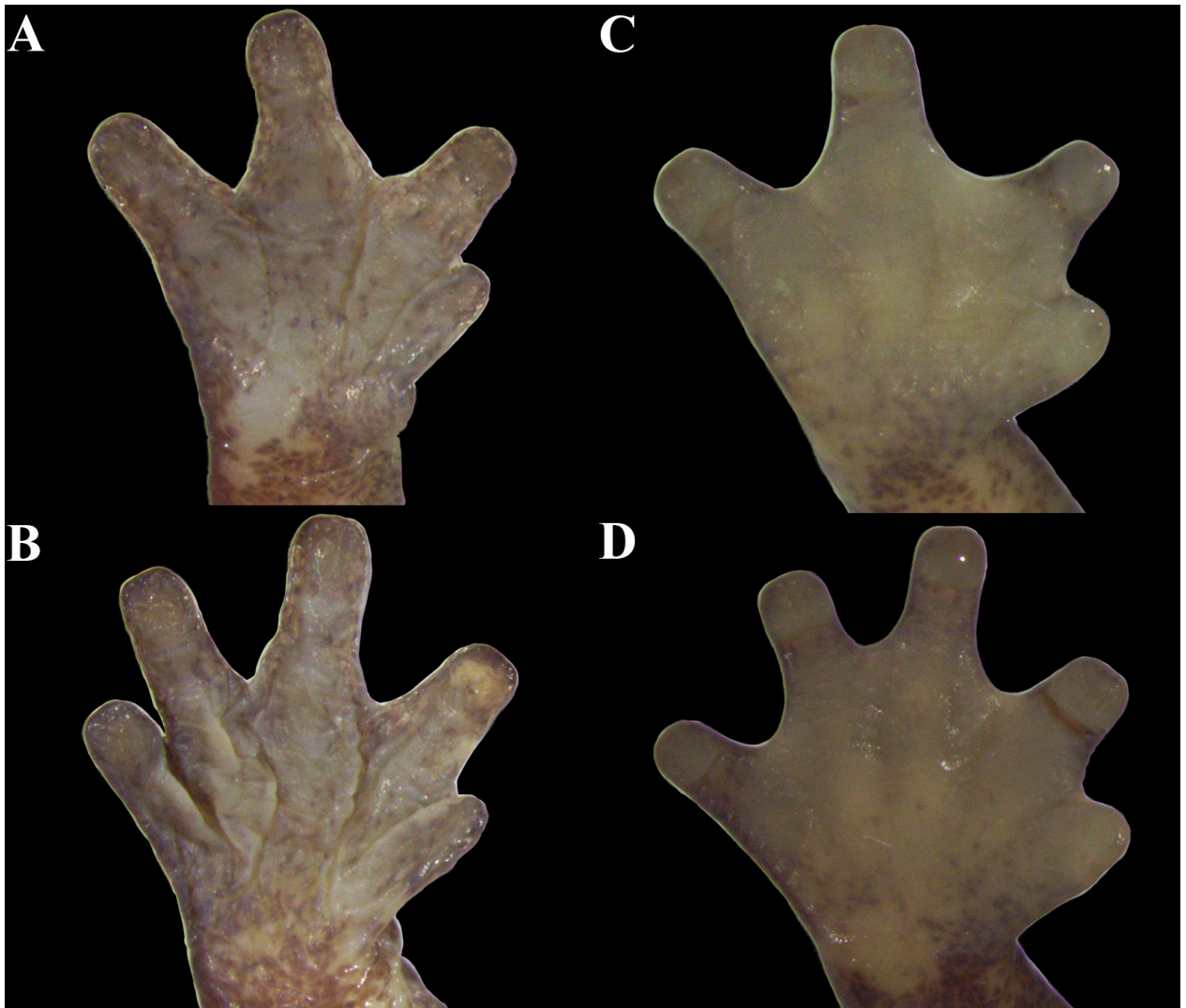


Fig. 5. Dorsal views of the hand and foot of the holotypes of (A–B) *Bolitoglossa bolanosi* sp. nov. (UCR 22965) and (C–D) *B. pesrubra* in preservation. Photograph by Erick Arias.



Fig. 6. In-life photographs of variations within *Bolitoglossa bolanosii* sp. nov. (A) Paratopotype male subadult UCR 22966, (B) paratype juvenile UCR 24247, (C) paratype adult female UCR 22421, and (D) paratype adult female UCR 24245. Photographs A and C by Erick Arias, B and D by Omar Zúñiga.

locality of *B. splendida* is only ~4 km from the site with *B. bolanosii*.

Distribution. The known distribution area of *Bolitoglossa bolanosii* is very small, restricted throughout to ~15 km on the summits of the peaks Dúrika, Arbolado, Hakú, and Utyum on the Cordillera de Talamanca (Fig. 1). The altitudinal range of the new species is 2,550–3,240 m asl. All the populations of the new species were found in primary vegetation (páramo and forest) and all are within a protected area, La Amistad International Park.

Conservation status. The conservation status of this species is uncertain; however, its known distribution range is small (<20,000 km²) and all known populations are restricted to summits of peaks, sites that are highly vulnerable to forest fires and other effects of climate change. We suggest that it should be tentatively considered as Vulnerable (VU) following the IUCN (2017) category criteria.

Discussion

Costa Rica hosts 56 species of salamanders, and it is the sixth most diverse country in the World in terms of total species richness and the most diverse relative to its total area (AmphibiaWeb 2023; Boza-Oviedo et al. 2012; Frost 2023). Several new species of salamanders have been described from ICA in this century (Arias and Kubicki

2018; Bolaños and Wake 2009; Boza-Oviedo et al. 2012; García-Paris et al. 2008; Hanken et al. 2005; Kubicki 2016; Kubicki and Arias 2016; Kubicki et al. 2022; Wake et al. 2007). Nevertheless, the taxonomic studies of salamanders in this region have been obscured due to a conservative morphology (Arias and Kubicki 2018; Boza-Oviedo et al. 2012; Kubicki et al. 2022). Recently, Kubicki et al. (2022) found low genetic distances among species of *Nototriton* from Costa Rica; however, they showed that two different species of *Nototriton* coexist (sympatry) in two sites, providing strong evidence of speciation. We also found relatively low (Table 1) genetic distances among the species of the *B. subpalmata* S.G., however, we found two sites separated by less than 7 km in a straight line from where two different species occur. In the first case, in the páramo of the Cerro Dúrika, samples of *B. bolanosii* (UCR 24245–7) were found only 4 km in a straight line from specimens of *B. pesrubra* (UCR 23049). The area separating these two sites consisted of homogeneous páramo, lacking any significant features that could be viewed as a barrier that would prevent the potential mixture of individuals or genes. However, the genetic distance among these organisms is 2.14% for 16S, providing evidence of genetic isolation. A second case was found in the páramo of the Cerro Utyum, where the *B. bolanosii* specimens (UCR 22421–7) were found only 7 km in a straight line from *B. kamuk* (UCR 23047). Similarly, these sites are separated by homogeneous páramo and we found genetic distances of 1.35–1.8% for

16S. We acknowledge the limitations of our conclusions, since our genetic analyses were based solely on the mitochondrial results. However, we think that all the evidence supports our hypothesis about the relationships within the *B. subpalmata* S.G.

Bolitoglossa bolanosi is the fourth species of salamander endemic to the páramo of ICA and the Montane rain forest surrounding it. The other three species also belong to the *B. subpalmata* S.G. Although there are relatively few species, the role of the páramos of ICA in the speciation of salamanders is interesting, especially since the páramos correspond only to 0.3% of the continental surface of Costa Rica (Kappelle and Horn 2016). The current isolated patches of páramos in ICA and its contraction-expansion dynamics in the past can explain (in part) the species formation in this habitat. In addition, it is noteworthy that the larger páramo in Costa Rica, the Cerro Chirripó, has not been sampled for salamanders. This páramo corresponds to two-thirds of all páramo in the country. There is a need for more studies in the páramos of ICA, especially due to the vulnerability of this ecosystem to climate change (Enquist 2002; Nogués-Bravo et al. 2007). In the past, *B. pesrubra* was relatively common (Bolaños and Wake 2009; Vial 1968). However, its populations have been greatly reduced (Boza-Oviedo et al. 2012; García-Paris et al. 2008), and we do not fully understand the cause of this reduction.

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Gerardo Chaves is a retired biologist, associated with the Zoology Museum of the University of Costa Rica. His undergraduate thesis focused on the arrival of Olive Ridley Sea Turtles, but most of his professional work has been oriented towards the ecology and taxonomy of the Costa Rican herpetofauna. His research activity is directed towards understanding the declines of amphibian populations in Mesoamerica and filling in information gaps in the Cordillera de Talamanca. From this research, he has published articles in several journals related to the ecology and taxonomy of Neotropical herpetofauna. Gerardo is currently the Chair of the IUCN Amphibian Specialist Group in Costa Rica.



Gabriela Parra Olea is a researcher at the Instituto de Biología, UNAM, Mexico, and curator of Mexico's national collection of amphibians and reptiles. Her research is focused on the molecular systematics and conservation of Mexican amphibians. Her laboratory is formed by students and postdocs all working on research projects in the systematics, taxonomy, conservation genetics, and impact of infectious diseases, specifically chytridiomycosis, on the conservation of amphibians.

Appendix 1. Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum collection acronyms follow Frost (2023) with the addition of EAP to refer to Erick Arias field numbers and CRARC to refer to the Costa Rica Amphibian Research Center private collection. NV= No voucher. CR = Costa Rica, HN = Honduras, MX = Mexico.

Species	Institutional voucher	Collection locality	Elevation (m)	Latitude	Longitude	16S	cyt b
<i>B. mexicana</i>	MVZ176838	Chiapas, MX	320	17.382	-91.793	GU725457	GU725470
<i>B. aurae</i>	UCR22842	Turrialba, Cartago, CR	1300	ND	ND	KX779527	KX779528
<i>B. sombra</i>	MVZ225875	Coto Brus, Puntarenas, CR	1760	8.954	-82.846	AY526136	AY526174
<i>B. compacta</i>	UCR20532	Talamanca, Limón, CR	2450	9.112	-82.962	JQ899163	JQ899193
<i>B. splendida</i>	UCR19835	Talamanca, Limón, CR	1826	9.357	-83.229	JQ899150	JQ899181
<i>B. bramei</i>	MVZ225893	Coto Brus, Puntarenas, CR	2200	8.935	-82.775	–	AF212066
<i>B. bramei</i>	UCR20483	Buenos Aires, Puntarenas, CR	2100	9.062	-82.984	JQ899159	JQ899189
<i>B. bramei</i>	UCR20484	Buenos Aires, Puntarenas, CR	2500	9.122	-82.964	JQ899160	JQ899190
<i>B. bramei</i>	UCR20851	Buenos Aires, Puntarenas, CR	2925	9.197	-83.056	JQ899142	JQ899172
<i>B. bramei</i>	UCR22719	Coto Brus, Puntarenas, CR	3160	9.031	-82.822	OR162559	OR147299
<i>B. bramei</i>	UCR22721	Coto Brus, Puntarenas, CR	3160	9.031	-82.822	OR162560	OR147300
<i>B. gomezi</i>	UCR20399	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899155	JQ899184
<i>B. gomezi</i>	UCR20413	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	–	JQ899185
<i>B. gomezi</i>	UCR20414	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899156	JQ899186
<i>B. gomezi</i>	UCR20415	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899157	JQ899187
<i>B. gomezi</i>	UCR20417	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899158	JQ899188
<i>B. gomezi</i>	UCR20843	Buenos Aires, Puntarenas, CR	2255	9.158	-83.065	JQ899140	JQ899170
<i>B. gomezi</i>	UCR20844	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899147	JQ899177
<i>B. gomezi</i>	UCR20845	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899148	JQ899178
<i>B. gomezi</i>	UCR20846	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899149	JQ899179
<i>B. gomezi</i>	UCR20847	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	–	JQ899180
<i>B. gomezi</i>	UCR20848	Buenos Aires, Puntarenas, CR	2255	9.158	-83.065	JQ899139	JQ899169
<i>B. gomezi</i>	UCR20849	Buenos Aires, Puntarenas, CR	2255	9.158	-83.065	JQ899141	JQ899171
<i>B. gomezi</i>	UCR20850	Buenos Aires, Puntarenas, CR	2100	9.139	-83.067	JQ899146	JQ899176
<i>B. gracilis</i>	MVZ229170	Paraíso, Cartago, CR	1680	9.692	-83.782	AY526121	AF212067
<i>B. gracilis</i>	MVZ229171	Paraíso, Cartago, CR	1680	9.692	-83.782	AY526122	AF212068
<i>B. gracilis</i>	UCR20541	Paraíso, Cartago, CR	1400	9.734	-83.764	OR162558	–
<i>B. tica</i>	UCR12065	Guarco, Cartago, CR	2440	9.686	-83.894	AY526137	AF212089

A new species of *Bolitoglossa*

Appendix 1 (continued). Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum collection acronyms follow Frost (2023) with the addition of EAP to refer to Erick Arias field numbers and CRARC to refer to the Costa Rica Amphibian Research Center private collection. NV= No voucher. CR = Costa Rica, HN = Honduras, MX = Mexico.

Species	Institutional voucher	Collection locality	Elevation (m)	Latitude	Longitude	16S	cyt b
<i>B. tica</i>	UCR20514	Paraíso, Cartago, CR	2300	9.712	-83.858	JQ899162	JQ899192
<i>B. subpalmata</i>	MVZ190875	Barva, Heredia, CR	1850	10.133	-84.1	–	AF212093
<i>B. subpalmata</i>	MVZ194827	San Ramón, Alajuela, CR	1500	10.033	-84.483	–	AF212090
<i>B. subpalmata</i>	MVZ194828	San Ramón, Alajuela, CR	1500	10.033	-84.483	EU448107	AF212091
<i>B. subpalmata</i>	MVZ194889	Vazquez de Coronado, San José, CR	1700	9.998	-83.964	–	AF212095
<i>B. subpalmata</i>	MVZ229172	Monte Verde, Puntarenas, CR	1500	10.3	-84.7	AF416697	AF212094
<i>B. subpalmata</i>	NV-Braulio Carrillo	Heredia, CR	ND	ND	ND	–	AF212092
<i>B. subpalmata</i>	UCR20833	Alfaro Ruiz, Alajuela, CR	1600	10.177	-84.391	OR162562	–
<i>B. subpalmata</i>	UCR21783					OR162561	OR147301
<i>B. kamuk</i>	UCR20852	Talamanca, Limón, CR	3126	9.255	-83.059	JQ899143	JQ899173
<i>B. kamuk</i>	UCR20853	Talamanca, Limón, CR	3126	9.255	-83.059	JQ899144	JQ899174
<i>B. kamuk</i>	UCR20854	Talamanca, Limón, CR	3126	9.255	-83.059	JQ899145	JQ899175
<i>B. kamuk</i>	UCR23047	Talamanca, Limón, CR	2870	9.277	-83.135	OR162582	–
<i>B. bolanosi</i> sp. nov.	UCR22421	Talamanca, Limón, CR	2913	9.333	-83.18	OR162567	OR147303
<i>B. bolanosi</i> sp. nov.	UCR22422	Buenos Aires, Puntarenas, CR	2870	9.323	-83.187	OR162571	OR147307
<i>B. bolanosi</i> sp. nov.	UCR22423	Buenos Aires, Puntarenas, CR	2870	9.323	-83.187	OR162573	OR147309
<i>B. bolanosi</i> sp. nov.	UCR22424	Buenos Aires, Puntarenas, CR	2870	9.323	-83.187	OR162570	OR147306
<i>B. bolanosi</i> sp. nov.	UCR22425	Buenos Aires, Puntarenas, CR	2870	9.323	-83.187	OR162578	OR147312
<i>B. bolanosi</i> sp. nov.	UCR22426	Buenos Aires, Puntarenas, CR	2870	9.323	-83.187	OR162574	OR147310
<i>B. bolanosi</i> sp. nov.	UCR22427	Buenos Aires, Puntarenas, CR	2870	9.323	-83.187	OR162572	OR147308
<i>B. bolanosi</i> sp. nov.	UCR22741	Buenos Aires, Puntarenas, CR	2660	9.322	-83.203	OR162580	OR147313
<i>B. bolanosi</i> sp. nov.	UCR22742	Buenos Aires, Puntarenas, CR	2660	9.322	-83.203	OR162576	OR147311
<i>B. bolanosi</i> sp. nov.	UCR22743	Buenos Aires, Puntarenas, CR	2660	9.322	-83.203	OR162579	–
<i>B. bolanosi</i> sp. nov.	UCR22744	Buenos Aires, Puntarenas, CR	2660	9.322	-83.203	OR162577	–
<i>B. bolanosi</i> sp. nov.	UCR22745	Buenos Aires, Puntarenas, CR	2660	9.322	-83.203	OR162575	–
<i>B. bolanosi</i> sp. nov.	UCR22746	Buenos Aires, Puntarenas, CR	2660	9.322	-83.203	OR162581	–
<i>B. bolanosi</i> sp. nov.	UCR22965	Buenos Aires, Puntarenas, CR	2600	9.32	-83.216	OR162566	–

Appendix 1 (continued). Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum collection acronyms follow Frost (2023) with the addition of EAP to refer to Erick Arias field numbers and CRARC to refer to the Costa Rica Amphibian Research Center private collection. NV= No voucher. CR = Costa Rica, HN = Honduras, MX = Mexico.

Species	Institutional voucher	Collection locality	Elevation (m)	Latitude	Longitude	16S	cyt b
<i>B. bolanosi</i> sp. nov.	UCR24245	Buenos Aires, Puntarenas, CR	3240	9.374	-83.303	OR162568	OR147304
<i>B. bolanosi</i> sp. nov.	UCR24246	Buenos Aires, Puntarenas, CR	3240	9.374	-83.303	OR162569	OR147305
<i>B. pesrubra</i>	CRARC0190	Dota, San José, CR	2750	9.652	-83.849	OR162585	OR147314
<i>B. pesrubra</i>	CRARC0192	Dota, San José, CR	2750	9.652	-83.849	OR162586	OR147315
<i>B. pesrubra</i>	DBW5117	ND	ND	ND	ND	–	AF212081
<i>B. pesrubra</i>	DBW5118	ND	ND	ND	ND	–	AF212082
<i>B. pesrubra</i>	EAP0771	Buenos Aires, Puntarenas, CR	2850	9.345	-83.39	OR162563	OR147302
<i>B. pesrubra</i>	MVZ190923	Dota, San José, CR	2760	9.623	-83.832	EU448104	AF212074
<i>B. pesrubra</i>	MVZ190928	Guarco, Cartago, CR	2760	9.626	-83.832	–	AF212072
<i>B. pesrubra</i>	MVZ195598	Guarco, Cartago, CR	2930	9.614	-83.804	–	AF212076
<i>B. pesrubra</i>	MVZ210360	Guarco, Cartago, CR	3230	9.567	-83.733	EU448105	–
<i>B. pesrubra</i>	MVZ225881	Guarco, Cartago, CR	2980	9.617	-83.833	–	AF212075
<i>B. pesrubra</i>	MVZ231322	Guarco, Cartago, CR	2760	9.626	-83.832	–	AF212074
<i>B. pesrubra</i>	NV-Asunción	Guarco, Cartago, CR	3230	9.567	-83.733	–	AF212080
<i>B. pesrubra</i>	NV-Cerro de la Muerte	Guarco, Cartago, CR	2980	9.617	-83.833	–	AF212077
<i>B. pesrubra</i>	NV-Cerro de la Muerte					OR162588	OR147316
<i>B. pesrubra</i>	NV-Empalme	Guarco, Cartago, CR	2700	9.626	-83.836	–	AF212078
<i>B. pesrubra</i>	NV-Georgina	Paraíso, Cartago, CR	3100	9.567	-83.717	–	AF212083
<i>B. pesrubra</i>	NV-Mills	Paraíso, Cartago, CR	3000	9.552	-83.717	–	AF212084
<i>B. pesrubra</i>	NV-Sakira	Pérez Zeledón, San José, CR	3400	9.592	-83.761	–	AF212079
<i>B. pesrubra</i>	NV-Salsipuedes	Guarco, Cartago, CR	2530	9.65	-83.85	–	AF212070
<i>B. pesrubra</i>	UCR12068	Guarco, Cartago, CR	2680	9.652	-83.849	AY526132	AF212069
<i>B. pesrubra</i>	UCR16471	Pérez Zeledón, San José, CR	3300	9.587	-83.765	OR162583	–
<i>B. pesrubra</i>	UCR20251	Paraíso, Cartago, CR	2760	9.556	-83.686	OR162587	–
<i>B. pesrubra</i>	UCR20859	Guarco, Cartago, CR	2800	9.669	-83.852	OR162584	–
<i>B. pesrubra</i>	UCR23048	Talamanca, Limón, CR	3050	9.395	-83.335	OR162564	–
<i>B. pesrubra</i>	UCR23049	Talamanca, Limón, CR	3000	9.368	-83.347	OR162565	–

Appendix 2. Specimens examined in the morphological analysis. All voucher numbers below are ‘UCR’ numbers, and the specimens were housed at the Museo de Zoología at the Universidad de Costa Rica.

Bolitoglossa bolanosi **sp. nov.**

COSTA RICA: Limón, Talamanca, Cerro Utyum: (22421♀); Puntarenas, Buenos Aires, Cerro Arbolado: (22965♂); Puntarenas, Buenos Aires, Cerro Hakú: (24245♀, 24246♂); Puntarenas, Buenos Aires, Cerro Hakú: (22741–4♀, 22745♂); Puntarenas, Buenos Aires, Cerro Utyum: (22422♂, 22423♀, 22424♂, 22425♀).

Bolitoglossa kamuk

COSTA RICA: Limón, Talamanca, Cerro Diká: (23047♀); Limón, Talamanca, Cerro Kamuk: (20852–3♂).

Bolitoglossa pesrubra

COSTA RICA: Cartago, El Guarco, Cerro de la Muerte: (23912♂, 22436♀); Cartago, Paraíso, Cerro de la Muerte: (13810♂, 19034♂, 19125♀, 19099♀, 23634♂); San José, Dota, Cerro de la Muerte: (12067♂, 20793♀, 20797-9♀); San José. Pérez Zeledón, Cuericí: (11712♂, 11757♂, 12109♂).