



A new species of *Bolitoglossa* (Caudata: Plethodontidae) of the *Bolitoglossa franklini* group from an isolated cloud forest in northern Guatemala

HELLEN DAHINTEN-BAILEY^{1,6}, MARCELO J. SERRANO^{2,11}, MARGARITA ALONSO-ASCENCIO^{1,7}, JOSE J. CRUZ-FONT^{2,8}, ISABELLA ROSITO-PRADO^{2,9}, KENNEDY J. A. RUIZ-VILLANUEVA^{2,10}, CARLOS VÁSQUEZ-ALMAZAN^{3,4} & DANIEL ARIANO-SÁNCHEZ^{1,5*}

¹Centro de Estudios Ambientales y Biodiversidad, Universidad del Valle de Guatemala, 11 calle 15-79 z.15 V.H. III, Ciudad de Guatemala, Guatemala.

²Departamento de Biología, Universidad del Valle de Guatemala, 11 calle 15-79 z.15 V.H. III, Ciudad de Guatemala, Guatemala.

³Museo de Historia Natural, Escuela de Biología, Universidad de San Carlos de Guatemala. Calle Mariscal Cruz 1-56 zona 10 Guatemala, Guatemala.

⁴Fundación para el Ecodesarrollo y la Conservación, Guatemala.

⁵crvasal@yahoo.com.mx; <https://orcid.org/0000-0002-9574-5536>

⁶Department of Natural Sciences and Environmental Health, Faculty of Technology, Natural Sciences and Maritime Sciences, University of Southeast Norway, 3800 Bø, Telemark, Norway

⁷hellenush1@gmail.com; <https://orcid.org/0000-0003-0518-3398>

⁸Mageealonso@gmail.com; <https://orcid.org/0000-0002-6307-2074>

⁹jjcruz96@gmail.com; <https://orcid.org/0000-0002-1493-8126>

¹⁰isarossito94@gmail.com; <https://orcid.org/0000-0003-0112-3160>

¹¹ruizk.0993@gmail.com; <https://orcid.org/0000-0002-1447-4253>

*Corresponding author. serranomarclo.d@gmail.com; <https://orcid.org/0000-0001-5919-029X>

dariano@uvg.edu.gt; <https://orcid.org/0000-0003-4955-5018>

Abstract

We describe *Bolitoglossa qeqom* **sp. nov.** from an isolated cloud forest in Cerro Guachmalén, Alta Verapaz, Guatemala, based on multiple lines of evidence (morphological, molecular, and biogeographic data). This region comprises a mountain ridge without previous herpetological surveys. The new species is a large salamander with uniform purplish-black coloration and is distinguished by having relatively long legs with only one costal groove between adpressed limbs, numerous maxillary teeth, few vomerine teeth, only one phalange free of webbing in digit III of feet, and a relatively short tail. It is geographically closest to its sister clade of *B. lincolni* + *B. franklini* and the xeric Chixoy river canyon appears to be the major biogeographic barrier that isolated the new taxon. The cloud forest inhabited by this species has undergone severe habitat destruction in the region and land conservation actions are urgent.

Key words: Amphibia, *Bolitoglossa franklini* species group, *16S*, *cytb*, restrict endemism, Chixoy River

Resumen

Describimos a *Bolitoglossa qeqom* **sp. nov.** de un bosque nuboso aislado en Cerro Guachmalén, Alta Verapaz, Guatemala, basándonos en múltiples líneas de evidencia (datos morfológicos, moleculares y biogeográficos). Esta región comprende una cresta montañosa en la cual no se han realizado estudios herpetológicos previos. La nueva especie es una salamandra grande con coloración uniforme negro púrpura y es distinguida por poseer patas relativamente largas con solo un surco costal entre las extremidades presionadas al cuerpo, numerosos dientes maxilares, pocos dientes vomerinos, poseer únicamente una falange libre de membrana en el dígito III de los pies, y una cola relativamente corta. Esta geográficamente cercana a su clado hermano *B. lincolni* + *B. franklini* y el cañón xérico del río Chixoy parece ser la barrera biogeográfica que aisló al nuevo taxón. El bosque nuboso donde habita esta especie ha sufrido una severa destrucción de hábitat en la región por lo que las acciones de conservación son urgentes.

Palabras clave: Amphibia, Especies pertenecientes al grupo *Bolitoglossa franklini*, *16S*, *cytb*, endemismo restricto, río Chixoy

Introduction

Bolitoglossa Duméril, Bibron & Duméril is a highly diverse genus of salamanders belonging to Plethodontidae in the Neotropics. The genus is distributed from Tamaulipas, Mexico through Central America to Bolivia and Brazil (Parra-Olea *et al.* 2004). The center of diversity of this group is found in Central America and the Mesoamerican Highlands of Mexico, with a second center of diversity in the southern Appalachian Highlands of eastern North America (Jaramillo *et al.* 2020). Costa Rica and Panamá have a high diversity of salamanders due to radiation in the Talamancan mountain range but it is poorly known because the area is in general difficult to access (Bolaños & Wake 2009). Species of this genus are found in habitats ranging from alpine grasslands on the tops of volcanoes and mountains to lowland forests at sea level, cloud forests, humid forests, and mesic formations (Parra-Olea *et al.* 2004).

Bolitoglossa differs from the rest of tropical salamanders by the absence of a sublingual fold and moderately to highly webbed feet. The monophyly of the genus is well supported on both a molecular and morphological basis (Wake & Elias 1983; Parra-Olea *et al.* 2004; Jaramillo *et al.* 2020). Within *Bolitoglossa*, the *franklini-lincolni* species group is composed of a complex of closely related salamanders located within the sub-genus *Magnadigita* (Stuart 1943; Wake & Lynch 1976; Wake & Lynch 1982). This group is characterized by having a large size, elongated fingers with an interdigital membrane that covers up the last phalanx, and an overall black background color marked with red, yellow, or silver-green patches in many populations (Wake and Lynch, 1982).

In nuclear Central America, of which Guatemala is a part, the high elevations of cloud forests separated by xeric lowlands created biogeographical barriers that promoted allopatric speciation (Castoe *et al.* 2009; Ornelas *et al.* 2013; Cano *et al.* 2018). These resulted in high endemism of multiple taxa (Schuster *et al.* 2003; Campbell *et al.* 2010; Woodman *et al.* 2012; Gutiérrez-Rodríguez *et al.* 2021). Guatemala is a region with a high richness of salamander species and genera, and new species continue to be described (Vásquez-Almazán *et al.* 2009; Campbell *et al.* 2010; Rovito *et al.* 2010; Brodie *et al.* 2012; Vásquez-Almazán & Rovito 2014; Rovito *et al.* 2015). Different geographical barriers, such as the Motagua-Polochic fault system in northeastern Guatemala, have led to divergence and speciation of salamanders (Rovito *et al.* 2015). Herein, we describe a new species of *Bolitoglossa* from an isolated cloud forest from a previously unexplored mountain ridge in the southernmost part of the Maya Highlands province in Guatemala.

Material and methods

Fieldwork. We conducted herpetological sampling within Pamac II natural reserve in Cerro Guachmalén, San Cristobal Verapaz, Alta Verapaz, Guatemala (15.40987 N, 90.60520W; 1,946 m elevation), from June 2018 to August 2020. We collected and preserved two individuals seen, a male (holotype) and a female (paratype). We found other specimens found at the type locality. However, we did not preserve these other specimens because a mutual agreement with the landowners and local Poqomchi communities was a requirement to gain access to the study site. We euthanized the collected specimens with Benzocaine hydrochloride following AVMA (2018) ethical guidelines. We fixed them with 10% formalin solution and processed over 70% ethanol for long-term preservation. The holotype and the paratype were deposited at the Biological Collections of Universidad del Valle de Guatemala. Additionally, in a trip to the type locality in August 2020, eight other specimens were collected alive, photographed, measured, and then released back at the collection site, after following biosecurity procedures to avoid contamination with pathogens. These topotypes are presented in the species description section.

Morphology. Measurements and counts of salamander morphology were made using a digital caliper and a stereoscope as follows: SVL, snout-vent length (distance from snout to posterior part of the cloaca); AG, axilla-groin length (distance between limbs at the right side); HW, head width (behind ocular orbits); FLL, forelimb and HLL, hind limb length (from the axilla to the bone joint and to the end of webbing); SGF, snout-gular fold (from terminal end of snout to the gular fold); TL, tail length (from the posterior end of the vent to the end of tail); PECW, chest width (from axilla to axilla); DN, internarial (distance between inner margins of narial opening); EN, orbitonarial (distance between the tip of eye opening to narina); DBE, interorbital (distance between eyes); HWD, distance between exterior lateral margin of digit I to exterior lateral margin of digit V; FW, distance between exterior margin of toe I to exterior margin of toe V; LiiiF, length of third digit of hand; LiiiT, length of third toe; LvT, length of fifth

toe; number of costal grooves; limb interval (costal grooves between adpressed limbs); VT, vomerine teeth; MT, maxillary teeth; PT, premaxillary teeth. Morphological measurements of *B. franklini*, *B. lincolni*, *B. meliana*, *B. qeqom*, and *B. tenebrosa* were analyzed through a Non-Metric Multi-Dimensional Scaling analysis (NMDS) using the packages *vegan* (Oksanen *et al.* 2019) and *ggplot2* (Wickham 2016). All analyses were conducted in R 3.6.2 using the Rstudio 1.3.959 interface (R Core Team 2020).

Genetic analyses. Liver and heart tissue from the collected specimens were preserved in 95% ethanol. DNA extraction followed Jain *et al.* (2007), using the Kit DNeasy (QIAGEN). Sequencing of DNA was done for large subunit ribosomal RNA (*16S*) and cytochrome b (*cytb*) mitochondrial genes using the same primers and polymerase chain reaction conditions as in Rovito *et al.* (2010) using primer MVZ15Bol and Pleth16SiR1 (Rovito *et al.* 2013), MVZ 117-98 (Palumbi 1996) in Macrogen Korea using NovaSeq 6000 (Illumina). We obtained sequences of other species of *Bolitoglossa* (*Magnadigita*) from GenBank using previous data from Campbell *et al.* (2010) and Vásquez & Rovito (2014); GenBank accession numbers and voucher information are given in Table 1.

TABLE 1. Genbank accession numbers and corresponding voucher information of *Bolitoglossa* used in the phylogenetic analyses.

Species	Voucher	Country	<i>16S</i>	<i>Cytb</i>
			GenBank accession	GenBank accession
<i>B. carri</i>	USNM 523276	Honduras	AY526138	AY526175
<i>B. cataguana</i>	UF 51786	Honduras	KJ628089	KJ628090
<i>B. celaque</i>	SMF 78087	Honduras	AY526140	AY526177
<i>B. conanti</i>	MVZ 257800	Guatemala	GU725445	GU725458
<i>B. cuchumatana</i>	MVZ 252541	Guatemala	GU725454	GU725467
<i>B. decora</i>	USNM 497533	Honduras	AY526143	AY526180
<i>B. diaphora</i>	MVZ 263440	Honduras	GU725447	GU725460
<i>B. dunni</i>	MVZ 263454	Honduras	GU725446	GU725459
<i>B. engelhardti</i>	MVZ 251495	Guatemala	GU725448	GU725461
<i>B. eremia</i>	UTA A-58429	Guatemala	-	HQ009992
<i>B. flavimembris</i>	MVZ 177786	México	GU725449	GU725462
<i>B. franklini</i>	MVZ 185991	México	AY526147	AY526184
<i>B. heriroreias</i>	MVZ 233028	El Salvador	AY526154	AY526191
<i>B. helmrichi</i>	MVZ 257804	Guatemala	GU725450	GU725463
<i>B. lincolni</i>	MVZ 263475	Guatemala	GU725451	GU725464
<i>B. longissima</i>	USNM 523285	Honduras	AY526149	AY526186
<i>B. meliana</i>	MVZ 265621	Guatemala	KJ175100	KJ175105
<i>B. mexicana</i>	MVZ 176838	México	GU725457	-
<i>B. morio</i>	MVZ 257825	Guatemala	KJ175098	KJ175106
<i>B. oresbia</i>	USNM 579667	Honduras	KJ175101	-
<i>B. porrasorum</i>	MVZ 225852	Honduras	AY526151	AY526193
<i>B. rostrata</i>	MVZ 251521	Guatemala	KJ175099	KJ175107
<i>B. suchitanensis</i>	UTA A-58422	Guatemala	-	HQ010001
<i>B. synoria</i>	SMF 78084	Honduras	AY526156	AY526193
<i>B. tenebrosa</i>	MVZ 264289	Guatemala	KJ175103	KJ175110
<i>B. zacapensis</i>	MVZ 257803	Guatemala	GU725455	-

Phylogenetic analyses. We edited sequences using Sequencher DNA 5.4.6. We then aligned sequences using Mega 7 (Kumar *et al.* 2018). This tool grouped the salamanders with fewer allelic variations within their sequences, resulting in the possible clades. We obtained sequences of other species of *Bolitoglossa* (subgenus *Magnadigita*) and one outgroup (*Bolitoglossa mexicana*, subgenus *Bolitoglossa*) from GenBank (Table 1). We concatenated both alignments (1,078bp; 527bp for *16s* and 551bp for *cytb*) and estimated the mitochondrial gene tree using both

maximum likelihood and Bayesian inference. Our phylogeny reconstruction analysis was produced with the raxml-GUI2.0 (Edler *et al.* 2021) with 1,000 replications using a nucleotide substitution model with Gamma patterns distributed in five discrete categories (GTRGAMMA). The Bayesian analysis was conducted using MrBayes v3.2.7a (Huelsenbeck & Ronquist 2001). It consisted of two runs of four chains each with a Markov chain Monte Carlo (MCMC) of 2×10^7 generations. The chain was sampled every 1,000 generations and the first 25% of the samples were discarded as burn-in. PSRF (Potential scale reduction factor) values were 1.00, and ESS (Estimated Sample size) values were higher than 100, indicating that the number of generations sampled was adequate.

Specimens examined for morphological analyses. *Bolitoglossa franklini* ($n = 10$), UTA A- 18233–35, UTA A- 42569–71, UTA A-48365, UTA A-48520–21, UTA A-48669; *Bolitoglossa lincolni* ($n = 28$), UTA A-18237–39, UTA A-51388, UTA A-51447–50, UTA A-51469–86, UTA A-51499, UTA A-58148; *Bolitoglossa meliana* ($n = 38$), MVZ 150809, MVZ 160384, MVZ 160736–39, MVZ 160741, MVZ 160744, MVZ 160746, MVZ 160748, MVZ 160750, MVZ 265621, UTA A-32877, UTA A-38591–613, UTA A-48587, UTA A-58565; *Bolitoglossa qeqom* ($n = 10$), UVG A7015–16, UTADC 9633–40; *Bolitoglossa tenebrosa* ($n=11$) MVZ 108854, MVZ 113160, MVZ 150813, MVZ169038–39, MVZ 150789, MVZ 150805–6, MVZ 150812, MVZ 264289, USAC 2114; *Bolitoglossa tzultacaj* ($n=1$) UTA A-38590.

Species description

Bolitoglossa qeqom sp. nov.

Fig. 1 A–F, Tables 2 & 3

Holotype. Adult male, UVG-A7015, collected on 4 June 2018 by Kennedy Ruiz-Villanueva in Pamac II natural reserve, Cerro Guachmalén (15.411282 N, -90.602701 W; 1,862 m elevation), San Cristobal Verapaz, Alta Verapaz, Guatemala. The individual was found active at night crawling above the leave of an understory palm (*Chamaedorea* sp.).

Paratype. Adult female, UVG-A7016, collected on 2 January 2019 by Marcelo Serrano and José Cruz-Font within Pamac II natural reserve (15.409986 N, -90.605156W; 1,947 m elevation), Cerro Guachmalén, San Cristobal Verapaz, Alta Verapaz, Guatemala. The individual was found active at night climbing on a tree fern (*Cyathea* sp.).

Additional topotypes. A series of eight individuals (UTADC 9633–9640), collected on 15 August 2020 by Isabella Rosito-Prado, Hellen Dahinten-Bailey, José Cruz-Font, and Marcelo Serrano (Figs. 2 & 3, Table 3). All individuals were found active at night, on understory fern leaves and some were coming out from the debris and rotten trunks at the type locality.

Diagnosis. This is a large uniformly purplish-black colored *Bolitoglossa* of the *franklini* species group (Stuart 1943), subgenus *Magnadigita* (Taylor 1944). We have assigned it to *Bolitoglossa* because it lacks a sublingual fold and to *Magnadigita* based on its reduced interdigital webbing, well-defined digits with broad tips, and its phylogenetic relationships according to molecular data. Molecular data shows that *B. qeqom* is the sister taxon of *B. lincolni* + *B. franklini* with high support (bootstrap proportion [BS] = 91, posterior probability [PP] = 1) with relatively large genetic distances from other members of this clade (Fig. 4). *Bolitoglossa qeqom* differs from all other species of the sub-genus by its large adult size except from *B. franklini* Wake & Lynch 1982, *B. lincolni* Elias 1984, *B. meliana* Wake & Lynch 1982, *B. omniusanctorum* Campbell *et al.* 2010 and *B. tenebrosa* Vásquez-Almazán & Rovito 2014. The new species differs from *B. franklini*, *B. lincolni*, *B. meliana*, *B. tenebrosa*, and *B. tzultacaj* Campbell *et al.* 2010 by having only one phalange free of webbing in digit III of feet and relatively longer legs with only one costal groove between adpressed limbs (Table 2). Differs from *B. franklini*, *B. lincolni*, *B. meliana*, and *B. tzultacaj* (sub-adult) by having more maxillary + premaxillary teeth, and two phalanges free of webbing in digit III of hand. The new species can be distinguished from *B. franklini*, *B. meliana*, and *B. tenebrosa* by having fewer vomerine teeth, relatively shorter tail, and by having only one phalange free of webbing in digit III of feet. The new taxon can be distinguished from other related species through the combination of several morphometric characteristics as is shown in the NMDS analysis (Fig. 5).

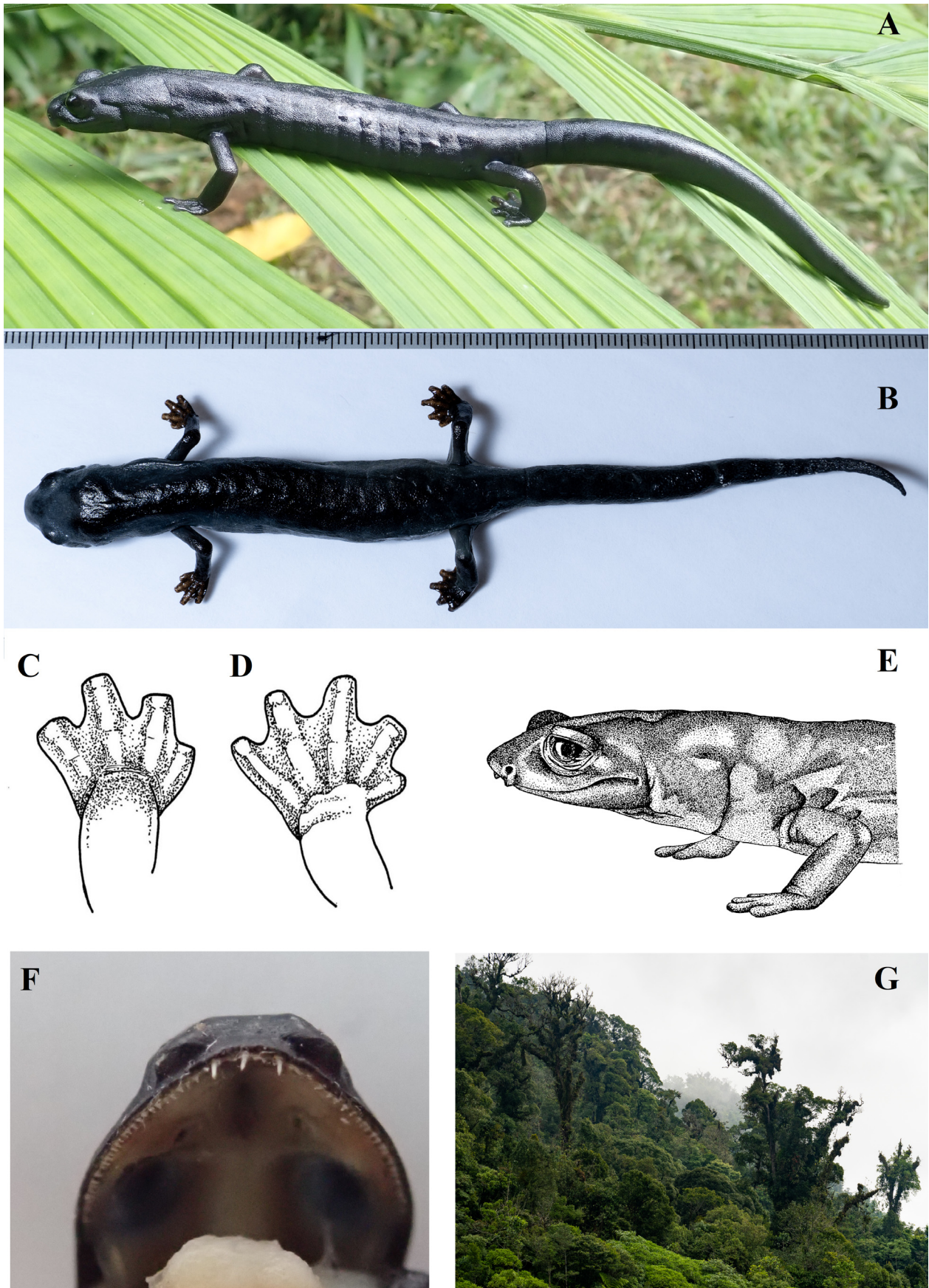


FIGURE 1. General view in life (A), after preservation (B), left hand (C), left feet (D), lateral view of head (E), maxillary and premaxillary teeth (F), and landscape view of the type locality (G) of the holotype of *Bolitoglossa qeqom* (UVG-A7015), SVL 49.9 mm. Drawings by M. Alonso-Ascencio.

TABLE 2. Comparison of average selected features of large black *Bolitoglossa* spp. from the mountain ridges of northern Guatemala. The new species is marked in bold.

	<i>B. franklini</i> (n=10)	<i>B. lincolni</i> (n = 28)	<i>B. meliana</i> (n = 38)	<i>B. qeqom</i> (n = 10)	<i>B. tenebrosa</i> (n = 11)	<i>B. tzultacaj</i> (n=1)
Vomerine teeth	25 ± 7	25 ± 6	22 ± 7	19 ± 1	25 ± 3	14
Maxillary + premaxillary teeth	48 ± 15	48 ± 16	52 ± 19	67 ± 15	70 ± 2	36
Limb interval	2 ± 0.4	3 ± 0.5	3 ± 0.6	1 ± 0.0	3 ± 0.2	4
HW/SVL	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.02	0.18 ± 0.02	0.16 ± 0.01	0.19
TL/SVL	0.90 ± 0.12	0.74 ± 0.27	0.89 ± 0.14	0.83 ± 0.06	0.97 ± 0.07	0.64
Webbing in digit III of hand	Lacking	Lacking	Lacking	Two phalanges free of webbing	Two phalanges free of webbing	Extensive
Webbing in digit III of feet	Lacking	Lacking	Lacking	One phalange free of webbing	Two phalanges free of webbing	Extensive

TABLE 3. Morphological measurements of the paratype (UVG-A7016) and eight topotypes (A, UTADC 9633; B, UTADC 9634; C, UTADC 9635; D, UTADC 9636; E, UTADC 9637; F, UTADC 9638; G, UTADC 9639; H, UTADC 9640) of *Bolitoglossa qeqom*.

Variable	Paratype	Topotypes							
		A	B	C	D	E	F	G	H
Sex	F	F	F	F	F	M	M	M	F
SVL (mm)	63.4	68.7	75.4	80.0	87.0	49.8	73.1	58.8	71.8
TL (mm)	53.8	57.0	60.0	62.9	66.1	41.8	56.2	56.6	63.5
HW/SVL	0.08	0.10	0.08	0.08	0.05	0.10	0.09	0.10	0.07
TL/SVL	0.85	0.83	0.80	0.79	0.76	0.84	0.77	0.96	0.88
Limb interval	1	1	1	1	1	1	1	1	1

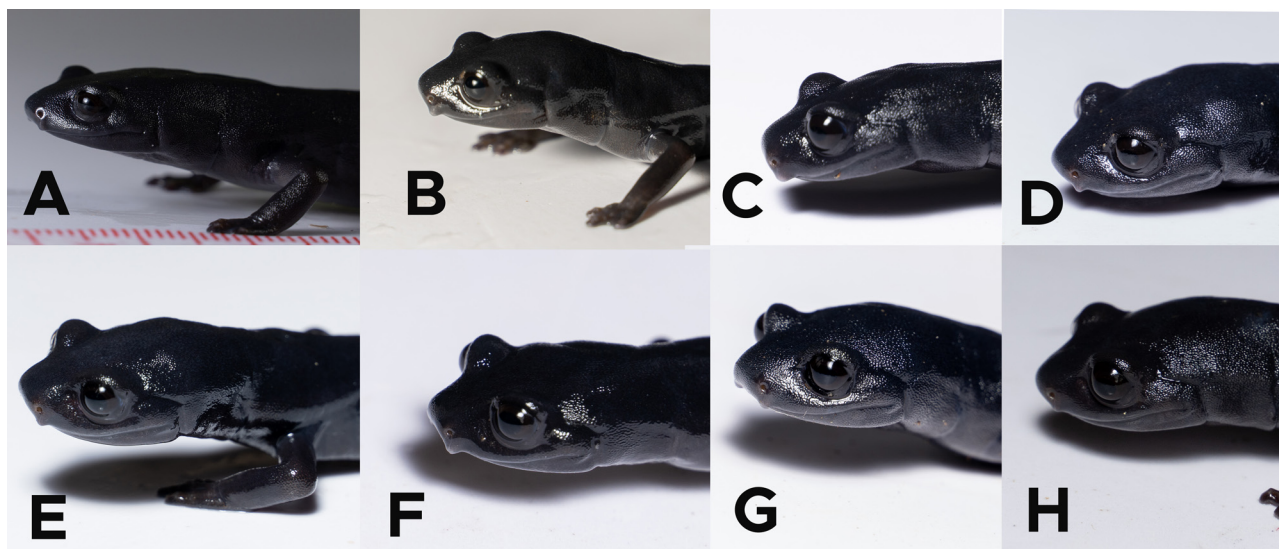


FIGURE 2. Lateral view of the head of *Bolitoglossa qeqom* topotypes (UTADC 9633–40). Identification letters correspond to the codes shown in Table 3.

Coloration in life. The color description is based on high-resolution photographs taken of specimens. The ground color of the dorsal surface of the head, trunk, and tail was a dark uniform black tone. When exposed to direct light, the coloration seemed more purplish black-toned, as uniform as previously described. The patch around the nostrils had a pinkish coloration. The upper surfaces of forelimbs and hind limbs presented the same dark purplish-black color, except that under direct light there were pinkish colored areas specifically in the elbows and knees. The hands and feet were a pale translucent dark gray, being almost translucent at the fingertips and darker in the inter-

digital webbing. The lateral surfaces of the head, body, and tail had this same uniform dark black coloration. The iris was a dark brown with some darker spots. The ventrolateral surface of the head, the ventral surface of the body, and limbs were lighter grayish black. Numerous very fine circular brown and grayish chromatophores were evident in all the trunk, base skin of limbs to insertion of limbs and the head. The chromatophores were more condensed in the gular region making it notably paler than the rest of the ventral surfaces. The ventral surface appearance of the tail is the same as the ventral surface of the trunk.

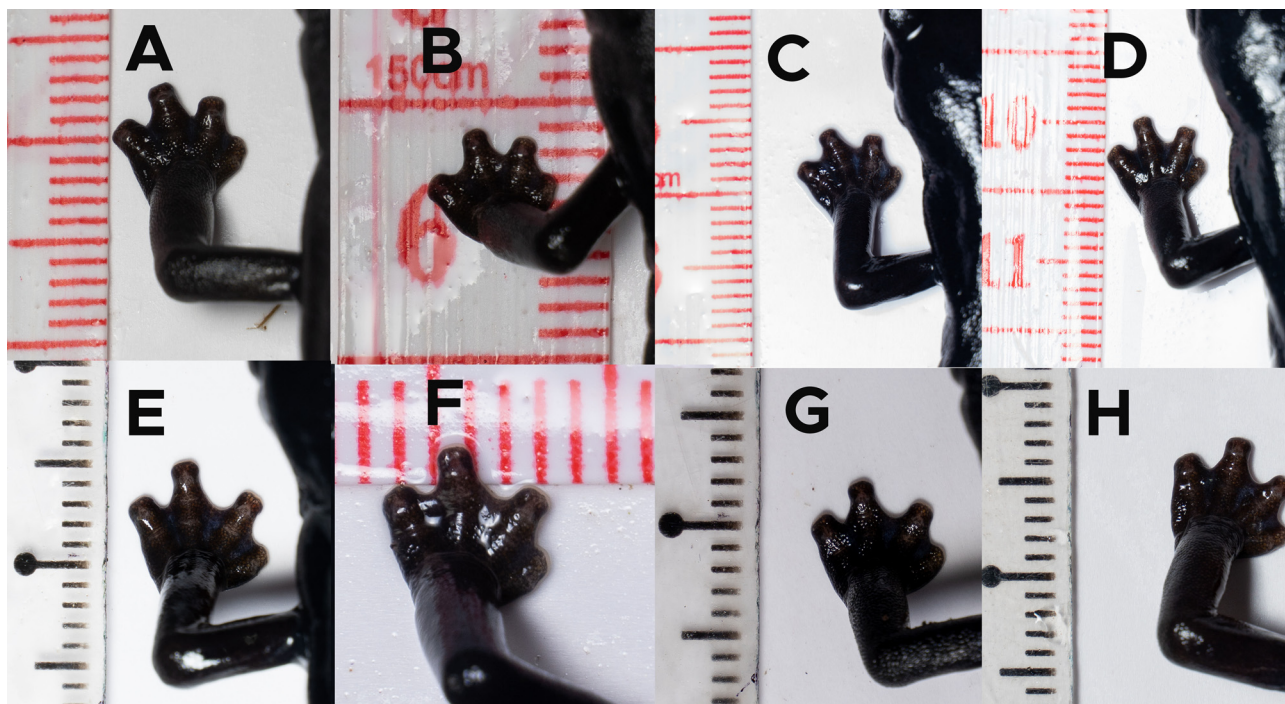


FIGURE 3. Dorsal view of left hands of *Bolitoglossa qeqom* topotypes (UTADC 9633–9640). Identification letters correspond to the codes shown in Table 3.

Description of the holotype. A medium sized male (SVL=49.9mm); TL 42.7, AG 28.9, FLL 13.9, HLL 12.4, SGF 13.1, PECW 8.1, HWD 6.5, FW 7.6, LiiiF 7.8, LiiiT 7.8, LvT 6.6, HW 10.3, DBE 2.6, EN 2.4, DN 3.6. The head was broad (HW/SVL = 0.21) with a large and bulging mental gland (LMG= 4.47mm). Maxillary teeth numerous (28 on the left side and 25 on the right side), 3 premaxillary teeth (enlarged), 10 right and 8 left vomerine teeth. Premaxillary teeth anterior to the line of maxillary teeth, and the vomerine teeth extending in a row past the outer edge of internal nares. Limbs relatively long with adpressed limbs separated only by one costal fold. Tail relatively long, with a TL/SVL ratio of 0.86. Webbing reduced, digit III with the first two phalanges free of webbing on hands and with the first phalange free of webbing on feet. Phalangeal formulae are 1-2-3-2 for hands and 1-2-3-3-2 for feet. The tips of the toes are broad and blunt.

Color after preservation. Dorsal surface of body, head, tail, and limbs, and lateral surface of body and tail, uniform black purplish. The upper surface of the feet is light brown. Ventral surface of body black-purplish with some light grey at the insertion of forelimbs, pectoral area, and mental gland. Gular region light black purplish with a faint band of light grey extending across gular region anterior to gular fold, the chromatophores are prominent with light brown coloration. The underside of the tail is black purplish, with a white line at the center. The underside of limbs, hands, and feet are light grey.

Etymology. The specific epithet is taken from the Poqomchi Mayan language word *q'eqom* that means dark, in allusion to its uniformly purplish-black coloration and the overwhelming darkness of the nights within the restricted patch of forest where we found this species. The species name is a noun in apposition to the genus name.

Distribution and habitat. The species is known only from Cerro Guachmalén, San Cristóbal Verapaz, Alta Verapaz (Fig. 6) at around 1,900 m elevation in a cloud forest. The vegetation is characterized by the presence of the following species: *Cojoba arborea*, Fabaceae; *Quercus* spp., Fagaceae; *Alfaroa guatemalensis*, Juglandaceae; *Dendropanax arboreus*, Araliaceae; *Amphitecna montana*, Bignoniaceae; various species of Lauraceae and the recently described *Magnolia poqomchi* (Serrano *et al.* 2020), Magnoliaceae. The broadleaf cloud forest in this area is

isolated from other montane forests by lower elevations surrounding it, where the habitat is highly fragmented by subsistence maize crops and migratory farming.

Life history and ecology. *Bolitoglossa qeqom* seems to be a mainly terrestrial salamander as most individuals (including the holotype and paratype) were found in very low understory vegetation or coming out from the debris and loose bark of fallen logs. This species seems to be restricted to a very small area at the type locality where a water spring emerges, as we were unable to find other specimens of this species in the rest of Cerro Guachmalén after several field trips. This salamander is relatively abundant at this site, with usually 10–15 salamanders being found on every trip of different age classes and sexes in this tiny forest patch (~ 5 ha). A large gravid female was found during August at the site.

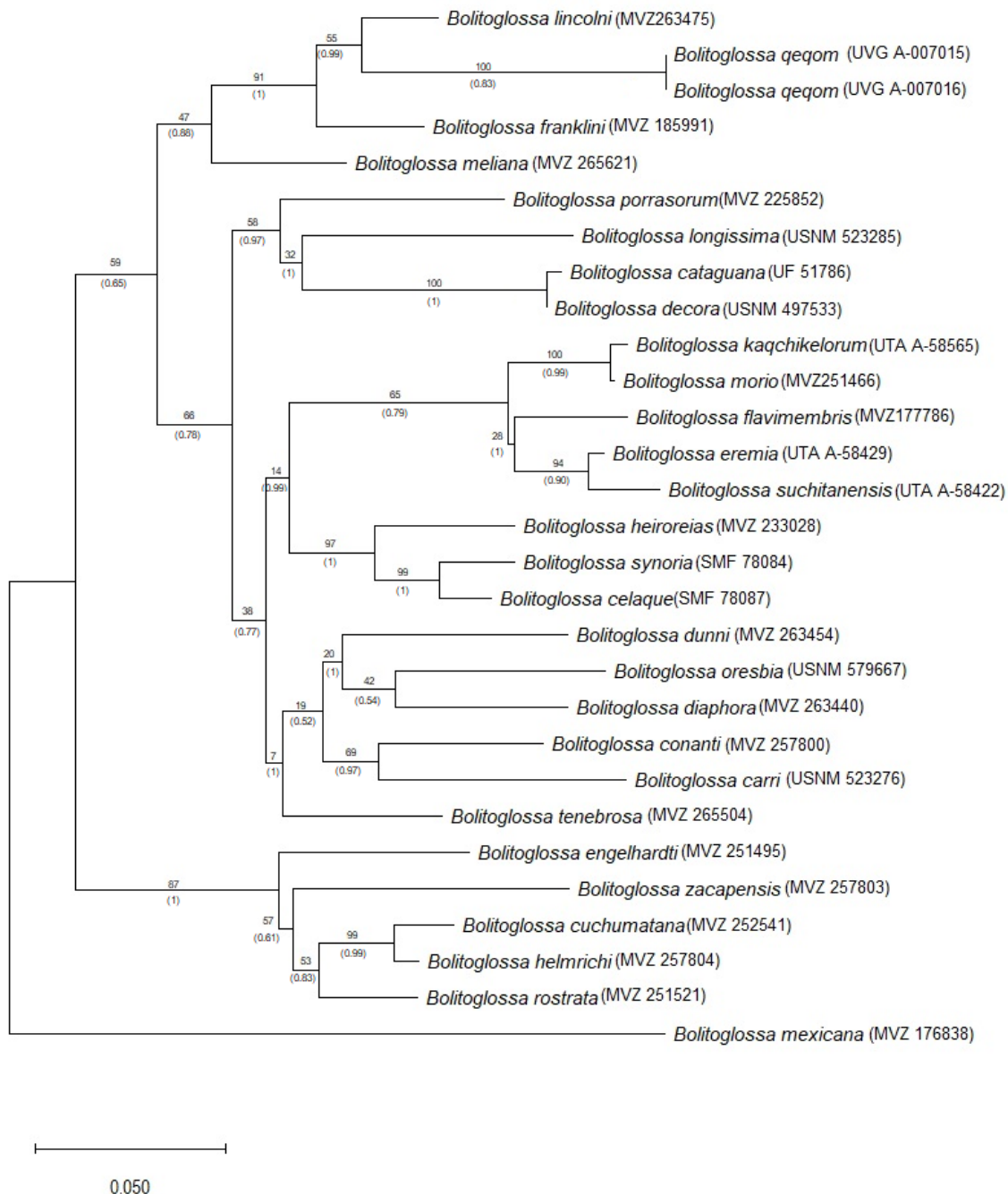


FIGURE 4. Mitochondrial gene tree from Maximum Likelihood analysis of *16S* and *cytb* genes (concatenated sequence of 1078bp) from several salamander species including *Bolitoglossa qeqom*. Branch lengths are proportional to substitutions. Bootstrap (ML) proportions (%) from RAxML analysis are given outside parentheses and posterior probabilities from MrBayes analysis are inside parentheses.

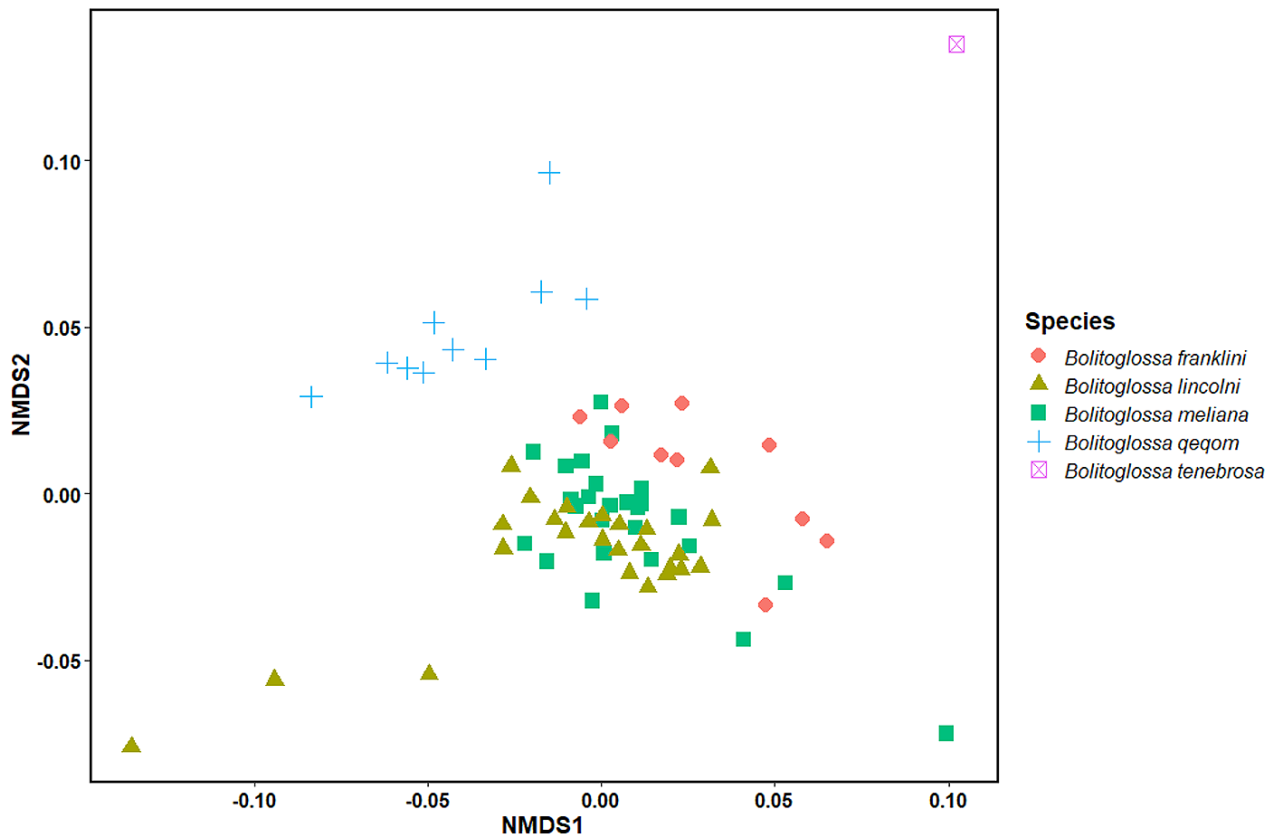


FIGURE 5. Non-metric multidimensional scaling (NMDS) plots detailing the differences between *Bolitoglossa franklini*, *B. lincolni*, *B. meliana*, *B. qeqom*, and *B. tenebrosa* based on morphological measurements. Measurements of the holotype (UVG-A7015), paratype (UVG-A7016), and topotypes (UTADC 9633–9640) are included in the analysis for *B. qeqom*. Measurements of *B. tenebrosa* correspond to specimen USAC 2114.

Discussion

The complex tectonic background of Guatemala, with multiple events of fragmentation and isolation overall its geological history, has been reported as a plausible promotor of species divergence for the isolated cloud forests in the mountain ridges of Nuclear Central America region (Castoe *et al.* 2009, Ornelas *et al.* 2013). This has produced disjunct distributions of several taxa such as passalid beetles *Proculus* (Schuster *et al.* 2003) and *Ogyges* (Cano *et al.* 2018), *Cryptotriton* salamanders (Vásquez-Almazán *et al.* 2009, Rovito *et al.* 2015), *Abronia* lizards (Campbell & Frost 1993, Gutiérrez-Rodríguez *et al.* 2021), *Bothriechis* pit vipers (Mason *et al.* 2019) and shrews (Woodman *et al.* 2012).

An overlooked biogeographical barrier that seems to isolate several related species between the northwestern and northeastern highlands of Guatemala is the Chixoy river canyon. This canyon causes a steep decrease in elevation from 2,000 to 500 m in its eastern section and from 1,500 to 500 m in its western section in less than 3 km in a straight line on both sides. This forms a low elevation seasonally dry forest that separates the eastern part of Sierra de los Cuchumatanes from Cerro Guachmalén and Sierra de Yalijux (Stuart 1950; Ariano- Sánchez 2017). The Chixoy river canyon has been reported as a biogeographic barrier for hardwood trees such as *Magnolia* (Serrano *et al.* 2020). In this context, the Chixoy river canyon appears to be the major biogeographic barrier that separated *B. qeqom* from its sister taxon *B. lincolni*. Besides, the Chixoy river canyon may be another factor, along with competitive exclusion, useful to explain the distribution pattern of *Dendrotriton* salamanders, which have not reached the mountain ridges of Sierra de Yalijux or Sierra de las Minas, in northeastern Guatemala.

Cerro Guachmalén in San Cristóbal Verapaz, Alta Verapaz, Guatemala is in an isolated mountain ridge between Sierra de los Cuchumatanes and Sierra de Yalijux, a site without previous herpetological surveys. The nearest loca-

tion with previous herpetological surveys is the pine-oak forest of Finca La Primavera, located at a lower elevation (1,100 m) in the gorge of the Chixoy river canyon (Stuart 1948), a different habitat compared to Cerro Guachmalén. In 2018, a geographic range extension of *Nyctanolis pernix* was reported from the site, which filled a gap between the western and eastern localities for its Guatemalan distribution and represents the higher elevation report for this species (Pineda-Schwarz *et al.* 2018). Cerro Guachmalén can be considered a relevant site for biodiversity conservation as a new species of a hardwood tree, *Magnolia poqomchi* was described recently from this area (Serrano *et al.* 2020). Also, in early 2020, a geographic range extension for the orchid *Sobralia amabilis* was made from the southeastern border of Guatemala to the study site (Grajeda *et al.* 2020).

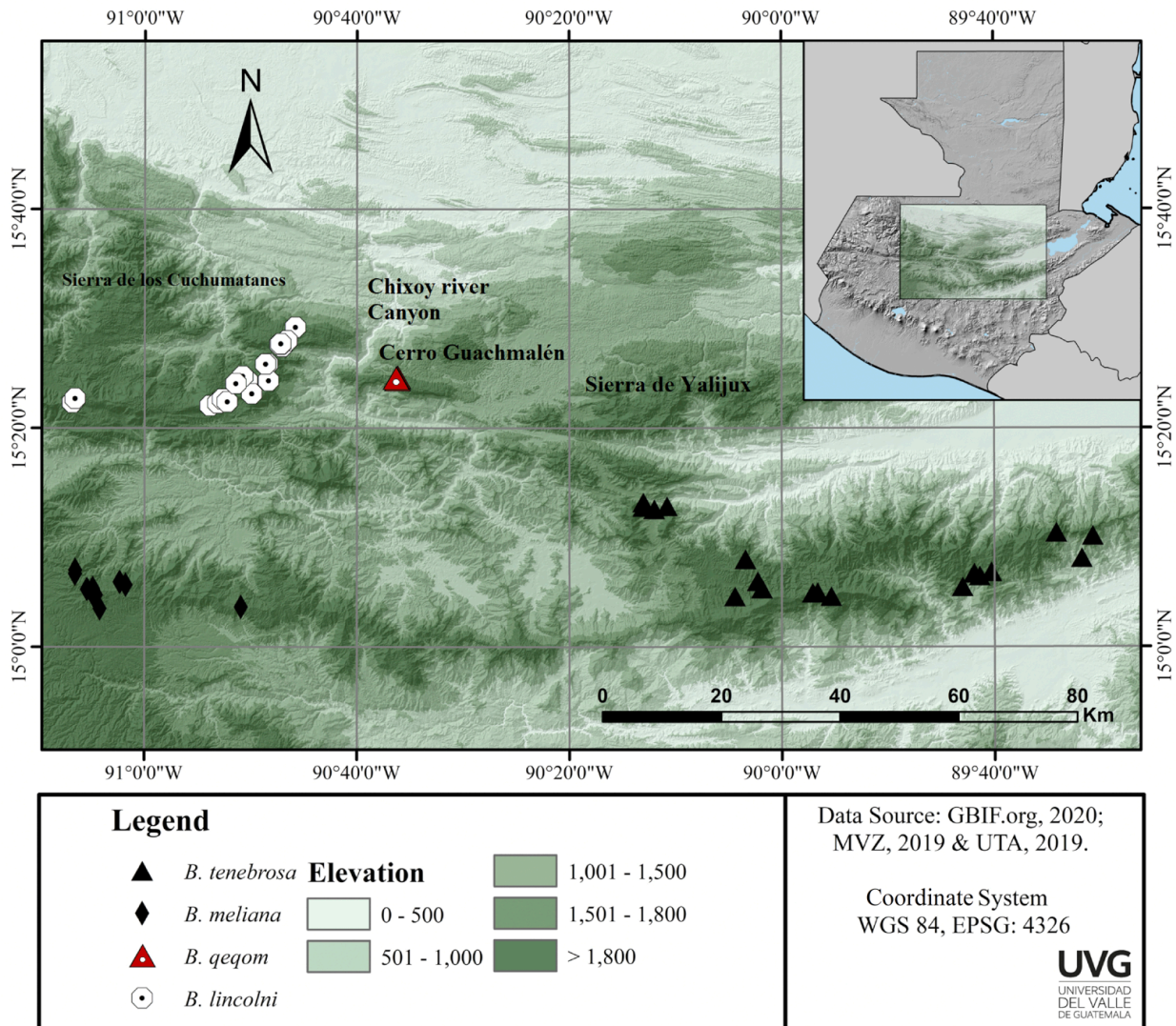


FIGURE 6. Map of Sierra de los Cuchumatanes, Cerro Guachmalén, Chixoy river canyon, and Sierra de Yalijux, Guatemala, showing the distribution of *Bolitoglossa lincolni* (white circles), *B. meliana* (black diamonds), *B. qeqom* (red triangle), and *B. tenebrosa* (black triangles).

Additionally, taking into account that we have conducted around 500 person-hours in our direct herpetological sampling effort at the site (from 2018 to 2020) and that we have covered extensive areas adjacent to Cerro Guachmalén, it seems likely that *B. qeqom* is a restricted endemism to the type locality within Pamac II natural reserve. Interestingly, all salamanders we found were within a small area of less than 0.05 km² inside Pamac II natural reserve, where a water spring sprouts to the surface. At this specific site, *B. qeqom* seems to be quite abundant, as several individuals of different age classes were usually found in every survey conducted at the site.

Despite intense field efforts completed in other areas of Cerro Guachmalén, salamanders were only found within this small area. Our behavioral observations of the type series and the topotypes at the study site suggest that this species is likely a ground-dwelling species that emerge at night from leaf litter or underground crevices to climb

on vegetation. Almost all individuals were found less than 0.5 m above ground, and most were observed climbing up understory stems or emerging from rotten logs. Further research on the behavioral ecology of this cloud forest inhabitant is needed. Unfortunately, the cloud forest in Guatemala has undergone severe fragmentation and destruction, due to the expansion of subsistence agriculture followed by extraction of fuelwood and larger-scale logging operations (Pope *et al.* 2015). Based on our current knowledge, *B. qeqom* meets the criteria for being included in the Critically Endangered (B1ab[iii]+B2ab[iii]) category of the IUCN Red List, because it is restricted to a single known locality, with an extent of occurrence of less than 10 km², and we expect a continuing decline in the quality of habitat within the region.

A positive note gives hope for the endemic biodiversity that inhabits Cerro Guachmalén. In 2018, research and conservation actions began at the site, due to the interest of private landowners in the area toward creating a private natural reserve with the involvement of the surrounding Poqomchi Mayan villages, intending to preserve the cloud forest remnant. This current movement offers an opportunity for new biodiversity discoveries to be made in that isolated region along with the preservation of the environmental services that this mountain ridge provides to the neighboring rural communities.

Acknowledgments

We dedicate this work to the memory of the people of San Cristobal Verapaz who have died because of the massive landslides produced in the area by Eta and Iota hurricanes during November 2020, in Guatemala. We thank Felipe Lem, Diegopablo Pineda, and Juan Pablo Gudiel for their help during the field trips that led to the discovery of this species. Thanks to Prem family and the local Poqomchi communities for permitting us to access Pamac II natural reserve. We thank two anonymous reviewers and Miguel Vences for reading through the different versions of the manuscript and providing valuable suggestions and constructive criticisms to improve it. Thanks to Alejandra Zamora and the Ecological Genetics and Conservation Laboratory at Manchester Metropolitan University, and Miguel Vences for their help and valuable suggestions for the genetic analysis. Thanks to Elena Dardón, Juan Pablo Rustrián, Edgar Rosales, Maria Solé Guzmán Luis Rios, and Miguel Morales for their help in the initial steps of the molecular analyses. Special thanks to Erick Smith for giving access to conduct the measurements of the salamander specimens kept at the University of Texas at Arlington. Thanks to Gregory Pandelis, at the Amphibian and Reptile Diversity Research Center of the University of Texas at Arlington for assigning ID numbers to the photographic vouchers of the holotype, paratype, and topotypes. Thanks to Zabdi López, from Colecciones Biológicas of Universidad del Valle de Guatemala for receiving and assigning the reference numbers to the holotype and paratype of the new species. We thank Priscila Juárez and Gabriela Alfaro from the Biology Department of Universidad del Valle de Guatemala for the support with the equipment, reactive, and materials used in this research. Thanks to Melissa Villatoro, Andrés Novales, and Juan Pablo Pinto for permitting us access to some other specimens for comparison. We thank Consejo Nacional de Areas Protegidas (CONAP) of Guatemala for providing research permit # 0492014. This research was conducted under animal care and use protocol reviewed and approved by Comité de Ética, Uso y Cuidado Animal (CEUCA) from Universidad del Valle de Guatemala according to permit D-2018-1.

References

- Ariano-Sánchez, D. (2017) Introducción al bosque seco: distribución y ecología. In: Yoshimoto, J. & Ariano-Sánchez, D. (Eds.), *El Bosque Estacionalmente Seco de Guatemala: Flora, Fauna y Cultura*. Pronatura Japan, Guatemala, pp. 9–23.
- AVMA (2018) Guidelines for the euthanasia of animals. American Veterinary Medical Association, Schaumburg, Illinois, 121 pp.
- Brodie, E.D., JR., Acevedo, M. & Campbell, J.A. (2012) New salamanders of the genus *Oedipina* (Caudata: Plethodontidae) from Guatemala. *Journal of Herpetology*, 46, 233–240.
<https://doi.org/10.1670/11-106>
- Campbell, J.A., Smith, E.N., Streicher, J., Acevedo, M.E. & Brodie Jr, E.D. (2010) New salamanders (Caudata: Plethodontidae) from Guatemala with miscellaneous notes on known species. *Miscellaneous Publications of the Museum of Zoology University of Michigan*, 200, 1–60.
- Cano, E.B., Schuster, J.C. & Morrone, J.J. (2018) Phylogenetics of *Ogyges* Kaup and the biogeography of Nuclear Central America (Coleoptera, Passalidae). *Zookeys*, 737, 81–111.

<https://doi.org/10.3897/zookeys.737.20741>

- Castoe, T.A., Daza, J.M., Smith, E.N., Sasa, M.M., Kuch, U., Campbell, J.A., Chippindale, P.T. & Parkinson, C.L. (2009) Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American highland biogeography. *Journal of Biogeography*, 36, 88–103.
<https://doi.org/10.1111/j.1365-2699.2008.01991.x>
- Edler, D., Klein, J., Antonelli, A., Silvestro, D. (2020) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution*, 12, 373–377.
<https://doi.org/10.1111/2041-210X.13512>
- Elias, P. (1984) Salamanders of the northwestern highlands of Guatemala. *Contributions in Science, Los Angeles*, 348, 1–20.
- Grajeda-Estrada, R., Rustríán-López, J.P., Serrano, M.J., Villalobos-Soberanis, A., Maldonado, M.L., Álvarez-Ruano, M.R. & Dix, M.A. (2020) The elusive *Sobralia amabilis* (Orchidaceae): a range extension of its distribution to Alta Verapaz, Guatemala, with notes on its habitat. *Lankesteriana*, 20, 1–6.
<https://doi.org/10.15517/lank.v20i1.40754>
- Gutiérrez-Rodríguez, J., Zaldívar-Riverón, A., Solano-Zavaleta, I., Campbell, J.A., Meza-Lázaro, R.N., Flores-Villela, O. & Nieto-Montes de Oca, A. (2021) Phylogenomics of the Mesoamerican alligator-lizard genera *Abronia* and *Mesaspis* (Aniguidae: Gerrhonotinae) reveals multiple independent clades of arboreal and terrestrial species. *Molecular Phylogenetics and Evolution*, 154, 106963.
<https://doi.org/10.1016/j.ympev.2020.106963>
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.
<https://doi.org/10.1093/bioinformatics/17.8.754>
- Jaramillo, A., De la Riva, I., Guayasamin, J., Chaparro, J., Gagliardi-Urrutia, G., Gutiérrez, R., Brcko, I., Vilà, C. & Castroviejo-Fisher, S. (2020) Vastly underestimated species richness of Amazonian salamanders (Plethodontidae: *Bolitoglossa*) and implications about plethodontid diversification. *Molecular Phylogenetics and Evolution*, 149, 106841.
<https://doi.org/10.1016/j.ympev.2020.106841>
- Jain, S., Brahmabhai, M., Rank, D., Joshi, C. & Solank, J. (2007) Use of cytochrome b Gene variability in detecting meat species by multiplex PCR assay. *Indian Journal of Animal Sciences*, 77, 880–881.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.
<https://doi.org/10.1093/molbev/msy096>
- Mason, A.J., Grazziotin, F.G., Zaher, H., Lemmon, A.R., Moriarty, E. & Parkinson, C.L. (2019) Reticulate evolution in nuclear Middle America causes discordance in the phylogeny of palm-pitvipers (Viperidae: *Bothriechis*). *Journal of Biogeography*, 46, 833–844.
<https://doi.org/10.1111/jbi.13542>
- Oksanen, J., Guillaume, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H., Szoecs, E. & Wagner, H. (2019) vegan: community ecology package. R package. Version 2.5-6. Available from: <https://CRAN.R-project.org/package=vegan> (accessed 7 April 2021)
- Ornelas, J.F., Sosa, V., Soltis, D.E., Daza, J.M., González, C., Soltis P.S., Gutiérrez-Rodríguez, C., Espinosa, A., Castoe, T.A., Bell, C. & Ruiz-Sánchez, E. (2013) Comparative phylogeographic analyses illustrate the complex evolutionary history of threatened cloud forests of northern Mesoamerica. *PLoS ONE*, 8, e56283.
<https://doi.org/10.1371/journal.pone.0056283>
- Palumbi, S. (1996) Nucleic acids II: the polymerase chain reaction. In Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematics*, Sinauer Associates, Sunderland, Massachusetts, pp. 204–247.
- Parra-Olea, G., García-París, M. & Wake, D.B. (2004) Molecular diversification of salamanders of the tropical American genus *Bolitoglossa* (Caudata: Plethodontidae) and its evolutionary and biogeographical implications. *Biological Journal of the Linnean Society*, 81, 325–346.
<https://doi.org/10.1111/j.1095-8312.2003.00303.x>
- Pineda-Schwarz, D., Alonso-Asencio, M., Arrivillaga-Cano, E., Cruz-Font, J.J., Dahinten-Bailey, H., Rosito-Prado, I. & Ariano-Sánchez, D. (2018) *Nyctanolis pernix*: Geographic distribution notes. *Herpetological Review*, 49, 499–450.
- Pope, I., Bowe, D., Harbor, J., Shao, G., Zanotti, L. & Burniske, G. (2015) Deforestation of montane cloud forest in the Central Highlands of Guatemala: contributing factors and implications for sustainability in Q’eqchi’ communities. *International Journal of Sustainable Development & World Ecology*, 22, 201–212.
<https://doi.org/10.1080/13504509.2014.998738>
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available from: <https://www.r-project.org/> (accessed 23 March 2021)
- Rovito, S.M., Vásquez-Almazán, C.R. & Papenfuss, T.J. (2010) A new species of *Bolitoglossa* (Caudata: Plethodontidae) from the Sierra de las Minas, Guatemala. *Journal of Herpetology*, 44, 516–525.
<https://doi.org/10.1670/09-205.1>
- Rovito, S.M., Vásquez-Almazán, C.R., Papenfuss, T.J., Parra-Olea, G. & Wake, D.B. (2015) Biogeography and evolution of Central American cloud forest salamanders (Caudata: Plethodontidae: *Cryptotriton*), with the description of a new species. *Zoological Journal of the Linnean Society*, 175, 50–166.
<https://doi.org/10.1111/zoj.12268>

- Schuster, J.C., Cano, E.B. & Reyes-Castillo, P. (2003) *Proculus*, giant Latin-American passalids: Revision, phylogeny and biogeography. *Acta Zoologica Mexicana*, New Series, 90, 281–306.
- Serrano, M.J., Grajeda-Estrada, R., Villalobos, A., Álvarez-Ruano, M.R. & Vásquez-García, J.A. (2020) *Magnolia poqomchi*, a new species of subsection *Magnolia* (Magnoliaceae) from San Cristóbal Verapaz, Alta Verapaz, Guatemala. *Phytotaxa*, 454 (4), 231–243.
<https://doi.org/10.11646/phytotaxa.454.4.1>
- Stamatakis, A. (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
<https://doi.org/10.1093/bioinformatics/btu033>
- Stuart, L.C. (1943) Taxonomic and geographic comments on Guatemalan salamanders of the genus *Oedipus*. *Miscellaneous Publications Museum of Zoology, University of Michigan*, 56, 1–34.
- Stuart, L.C. (1948) The amphibians and reptiles of Alta Verapaz Guatemala. *Miscellaneous Publications Museum of Zoology, University of Michigan*, 69, 1–109.
- Stuart, L.C. (1950) A geographic study of the herpetofauna of Alta Verapaz, Guatemala. *Contributions from the Laboratory of Vertebrate Biology, University of Michigan*, 45, 1–77.
- Taylor, E.H. (1944) The genera of plethodont salamanders in Mexico. Part 1. *The University of Kansas Scientific Bulletin*, 30, 189–232.
<https://doi.org/10.5962/bhl.part.6508>
- Universidad del Valle de Guatemala (2020) Universidad del Valle de Guatemala - Colecciones Biológicas—Anfibios. Available from <https://doi.org/10.15468/3du34x> (accessed 23 March 2021)
- Vásquez-Almazán, C.R., Rovito, S.M., Good, D.A. & Wake, D.B. (2009) A new species of *Cryptotriton* (Caudata: Plethodontidae) from eastern Guatemala. *Copeia*, 2009, 313–319.
<https://doi.org/10.1643/CH-08-086>
- Vásquez-Almazán, C.R. & Rovito S.M. (2014) A new species of black *Bolitoglossa* (Caudata: Plethodontidae) from Guatemala. *Journal of Herpetology*, 48, 518–524.
<https://doi.org/10.1670/13-170>
- Wake, D.B. & Elias, P. (1983) New genera and a new species of Central American salamanders, with a review of the tropical genera (Amphibia, Caudata, Plethodontidae). *Los Angeles County Museum Contributions in Science*, 345, 1–19.
- Wake, D.B. & Lynch, J.F. (1976) The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. *Natural History Museum of Los Angeles County Science Bulletin*, 25, 1–65.
- Wake, D.B. & Lynch, J.F. (1982) Evolutionary relationships among Central American salamanders of the *Bolitoglossa franklini* group, with a description of a new species from Guatemala. *Herpetologica*, 38, 257–272.
- Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, 213 pp.
https://doi.org/10.1007/978-3-319-24277-4_9
- Woodman, N., Matson, J.O., McCarthy, T.J., Eckerlin, R.P., Bulmer, W. & Ordoñez-Garza, N. (2012) Distributional records of shrews (Mammalia, Soricomorpha, Soricidae) from northern Central America with the first record of *Sorex* from Honduras. *Annals of the Carnegie Museum*, 80, 207–237.