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A New Species of Black *Bolitoglossa* (Caudata: Plethodontidae) from Guatemala

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ABSTRACT.—We describe a new species of large black *Bolitoglossa* from the Sierra de las Minas and adjacent regions of Guatemala based on both morphological and molecular data. The new species, specimens of which were formerly assigned to *Bolitoglossa meliana* (Melanistic Salamander), is distinguished by its dark coloration, numerous teeth, and large size. We present molecular data to support the distinctiveness of this species and estimate its phylogenetic placement within the subgenus *Magnadigita*. Molecular data do not support a sister taxon relationship between the new species and *B. meliana*, despite their outward morphological similarity. This species adds to the already high salamander species diversity of the Sierra de las Minas, much of which has been described recently.

RESUMEN.—Describimos una nueva especie de *Bolitoglossa* grande y negra de la Sierra de las Minas y regiones cercanas en Guatemala, basados en datos morfológicos y moleculares. La nueva especie, anteriormente asignada a *Bolitoglossa meliana*, se distingue por su coloración oscura, dientes numerosos y gran tamaño. Presentamos datos moleculares para apoyar el reconocimiento de esta especie y para estimar su posición filogenética dentro del subgénero *Magnadigita*. Los datos moleculares no apoyan una relación de grupo hermano entre la especie nueva y *B. meliana*, a pesar de su similitud morfológica externa. Esta especie aumenta la alta diversidad de especies de salamandras de la Sierra de las Minas, mucha de la cual se ha descrito recientemente.

Salamanders of the family Plethodontidae are represented in the Neotropics by a single lineage, the supergenus *Bolitoglossa*. While many areas in Mesoamerica are rich in salamanders, one of the areas of highest diversity is the highlands of Guatemala (Wake and Lynch, 1976). Guatemala is rich in both species and genera of bolitoglossine salamanders, and new species continue to be described on a regular basis (Vásquez-Almazán et al., 2009; Campbell et al., 2010; Rovito et al., 2010; Brodie et al., 2012). This high salamander diversity is most likely a product of the great geological age of many of these highlands (Marshall, 2007) as well as the complex topography of the region. Guatemala is geologically complex because it lies at the intersection of the North American and Caribbean plates. Nuclear Central America, the area between the Isthmus of Tehuantepec and the Nicaraguan Depression (Schuchert, 1935), is divided into two major geological blocks: the Maya block (on the North American plate) and the Chortís block (on the Caribbean plate) (Marshall, 2007). These two blocks meet along the Motagua–Polochic fault zone creating a rugged, uplifted area that extends roughly east-west through the center of Guatemala. The Sierra de las Minas, reaching over 3,000 m in elevation, and the Sierra de Chuacús are between the Motagua and Polochic faults, and valleys along either fault zone separate the Sierra de las Minas from other highland areas to the north and south. The Motagua Valley in particular, an area of low elevation with semiarid vegetation (Stuart, 1954), is a major biogeographic barrier for multiple taxa (Castoe et al., 2009; Daza et al., 2010) and divides higher-elevation forest areas on either side. These strong geographic barriers have led to high endemism in the Sierra de las Minas and Sierra de Chuacús, both in salamanders (Campbell et al., 2010; Rovito et al., 2010) and other taxa (Campbell and Smith, 1998; Schuster and Cano, 2006).

Earlier herpetological explorations of the Sierra de Chuacús and Sierra de las Minas resulted in the description of a large black species, *Bolitoglossa meliana* (Melanistic Salamander), with a generalized morphology (Wake and Lynch, 1982). Allozyme analysis of *B. meliana* revealed relatively high levels of genetic

differentiation between populations in the western Sierra de Chuacús (Departamento El Quiché), in the Purulhá area at the eastern end of the Sierra de Chuacús (Deptos. El Progreso and Zacapa) (Wake and Lynch, 1982). This allozyme study had very small sample sizes, (two individuals from the Sierra de las Minas and only one from Purulhá), and there was no further molecular work on the species for many years. As a result of recent survey expeditions to document the biodiversity of Nuclear Central America, we acquired additional specimens and tissues from both the Sierra de Chuacús and the Sierra de las Minas. We used both morphological and molecular data to test the hypothesis that *B. meliana* contains more than one species, as suggested by the high genetic distances found in the allozyme study.

METHODS

Morphological Analyses.—We measured 35 adult specimens of black *Bolitoglossa* from nine sites in the Sierra de Chuacús and the Sierra de las Minas (Fig. 1 and Appendix 1) all of which were previously assigned to *B. meliana*. We took eight measurements of external morphology to the nearest 0.1 mm using dial calipers; distance from tip of snout to posterior angle of vent (snout–vent length, SVL), tail length (TL), axilla–groin distance (AG), distance from snout to gular fold (SG), head width at widest point (HW), forelimb length (FLL), hind limb length (HLL), and right foot width (RFW). We counted ankylosed maxillary and premaxillary teeth (MT) and vomerine teeth (VT), with counts summed for the right and left sides.

We conducted all morphological analyses separately for males and females because sexual dimorphism in size and other characters is present (Wake and Lynch, 1982), as occurs in many species of *Bolitoglossa*. We tested for normality of each measurement using a Shapiro-Wilk test and could not reject the null hypothesis of normality for any variable. We excluded tooth counts for two males from El Quiché that had very low numbers of teeth compared to all other specimens examined, perhaps indicating a developmental abnormality. We used *t*-tests to compare means for all other measurements for each species. We calculated mean,

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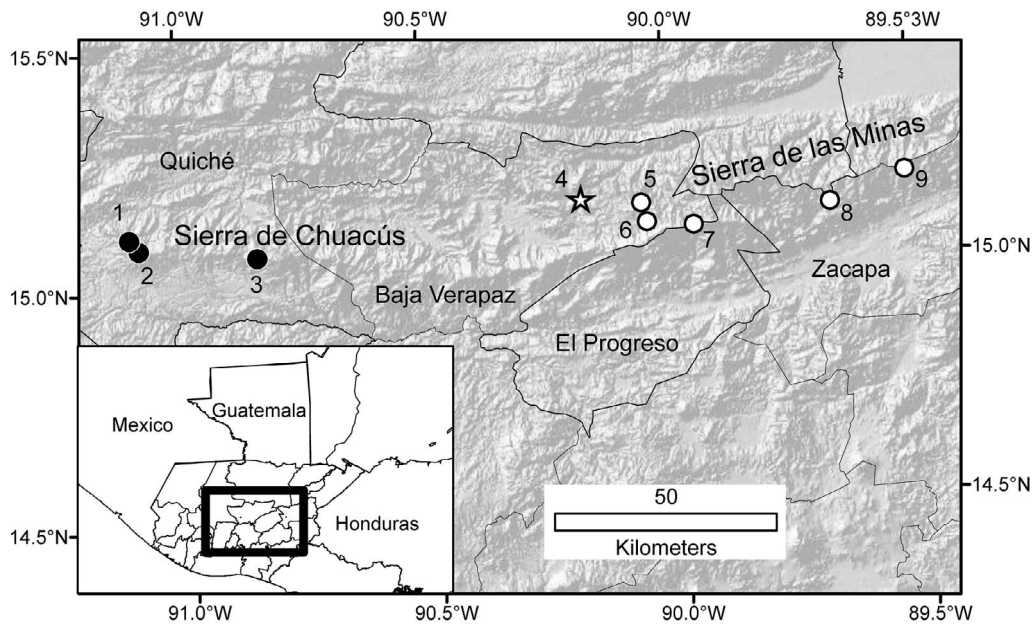


FIG. 1. Map of the Sierra de Chuacús and Sierra de las Minas, Guatemala, showing distribution of *B. meliana* (black dots) and *B. tenebrosa* (white dots). The type locality of *B. tenebrosa* is indicated by a white star. *Bolitoglossa meliana* localities (all in El Quiché): 1) 5 km ENE San Pedro Jocopilas, 2) Santa Rosa Pass, 3) Chuxilic, 9.6 km NE Zacualpa; *B. tenebrosa* localities: 4) vicinity of Purulhá, Baja Verapaz, 5) Finca San Jorge, Chilascó, Baja Verapaz, 6) San Antonio, Chilascó, Baja Verapaz, 7) La Bella Crest/cabañas de Albores, El Progreso, 8) Volcán de los Monos, 11–12 km N Santa Cruz, Zacapa, 9) Finca Planada, Zacapa.

standard deviation, and range of all measurements. All statistical analyses were done in R ver. 2.14 (R Core Development Team, 2013).

Molecular Analyses.—Liver tissue from three field-collected specimens of *Bolitoglossa* from El Quiché and the Sierra de las Minas was flash-frozen in liquid nitrogen or preserved in 95% ethanol. Specimens and tissues were catalogued in the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley and the Museo de Historia Natural, Universidad de San Carlos (USAC), Guatemala. We sequenced fragments of the 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). For one tissue that had been ground for allozyme analysis, we sequenced only a short fragment of *16S* using primers MVZ15Bol and Pleth16SiR1 (Rovito et al., 2013). We obtained sequences of other species of *Bolitoglossa* (*Magnadigita*) and one outgroup (*Bolitoglossa mexicana*; Mexican Mushroom-tongue Salamander) from GenBank; accession numbers and voucher information are given in Table 1. We edited sequences using Sequencer 4.8 (GeneCodes, Ann Arbor, MI, USA) or Geneious v 5.1.7 (BioMatters, Auckland, NZ). We aligned sequences using Muscle v.3.8 (Edgar, 2004) and trimmed alignments to the point where a majority of taxa had sequence data. Final alignment lengths were 517 base pairs (bp) for *16S* and 772 bp for *cytb*.

We concatenated both alignments and estimated the mitochondrial gene tree using both maximum likelihood and Bayesian inference. We partitioned sequence data by gene, with *cytb* further partitioned by codon position (four partitions) following Rovito et al. (2010). We used MrModeltest ver. 2.2 (Nylander, 2004) to select a model of nucleotide substitution for each partition. The following substitution models were selected: *16S*—GTR+I+G; *cytb* codon position 1—GTR+G; *cytb* codon position 2—HKY+I+G; *cytb* codon position 3—HKY+I. We inferred the mitochondrial gene tree using MrBayes v3.1.4 (Huelsenbeck and Ronquist, 2001). Bayesian analysis consisted of two runs of four chains each (one

cold, three heated) with a Markov chain Monte Carlo (MCMC) of 2×10^7 generations. The chain was sampled every 1,000 generations and the first 5,000 samples were discarded as burn-in. Maximum likelihood analysis was done using RAxML v7.1.4 (Stamatakis, 2006) with the GTR+I+G model for all partitions (RAxML does not implement models less complex than GTR) and 1,000 bootstrap replicates.

RESULTS

Specimens of black *Bolitoglossa* from the Purulhá area (Baja Verapaz) and Sierra de las Minas (El Progreso, Zacapa) differed from those from El Quiché in a number of morphological characteristics including size and numbers of teeth (Table 2). We found that our sample of *B. meliana* from relatively near the type locality in El Quiché was the sister taxon of *Bolitoglossa lincolni* + *Bolitoglossa franklini* (Lincoln's Salamander + Franklin's Salamander) with moderate to high support (bootstrap proportion [BS] = 68, posterior probability [PP] = 0.98) while samples from Baja Verapaz and El Progreso were placed in a clade with species from eastern Guatemala and Honduras and members of the *Bolitoglossa morio* (Cope's Mushroom-tongue Salamander) species complex (Fig. 2) with high support (BS = 71, PP = 0.99). Relationships between the Baja Verapaz/El Progreso samples and other taxa in the clade were not resolved in our gene tree. Based on the morphological differences we found, as well as the polyphyly of *B. meliana* in our mitochondrial gene tree, we describe the populations from the departments of Baja Verapaz, El Progreso, and Zacapa as a new species.

SYSTEMATIC ACCOUNT

Bolitoglossa (*Magnadigita*) *tenebrosa* sp. nov.

Figure 3A–C

Guatemalan Black Salamander, Salamandra Negra Guatemalteca.

TABLE 1. GenBank numbers and voucher information for specimens of *Bolitoglossa* used in phylogenetic analyses.

Species	Voucher	Country: State/Province	16S GenBank accession	Cytb GenBank accession
<i>B. carri</i>	USNM 523276	Honduras: Francisco Morazán	AY526138	AY526175
<i>B. cataguana</i>	UF 51786	Honduras: Francisco Morazán	KJ628089	KJ628090
<i>B. celaque</i>	SMF 78087	Honduras: Lempira	AY526140	AY526177
<i>B. conanti</i>	MVZ 257800	Guatemala: Zacapa	GU725445	GU725458
<i>B. cuchumatana</i>	MVZ 251541	Guatemala: El Quiché	GU725454	GU725467
<i>B. decora</i>	USNM 497533	Honduras: Olancho	AY526143	AY526180
<i>B. diaphora</i>	MVZ 263440	Honduras: Cortés	GU725447	GU725460
<i>B. dunni</i>	MVZ 263454	Honduras: Cortés	GU725446	GU725459
<i>B. engelhardti</i>	MVZ 251495	Guatemala: San Marcos	GU725448	GU725461
<i>B. eremia</i>	UTA A-58429	Guatemala: Jalapa	—	HQ009992
<i>B. flavimembris</i>	MVZ 177786	Mexico: Chiapas	GU725449	GU725462
<i>B. franklini</i>	MVZ 185991	Mexico: Chiapas	AY526147	AY526184
<i>B. heiroreias</i>	MVZ 233028	El Salvador: Santa Ana	AY526154	AY526191
<i>B. helmrichi</i>	MVZ 257804	Guatemala: Zacapa	GU725450	GU725463
<i>B. kaqchikelorum</i>	UTA A-58565	Guatemala: Sacatepéquez	—	HQ010020
<i>B. lincolni</i>	MVZ 263475	Guatemala: San Marcos	GU725451	GU725464
<i>B. longissima</i>	USNM 523285	Honduras: Olancho	AY526149	AY526186
<i>B. meliana</i>	MVZ 265621	Guatemala: El Quiché	KJ175100	KJ175105
<i>B. mexicana</i>	MVZ 176838	Mexico: Chiapas	GU725457	GU725470
<i>B. morio</i>	MVZ 251466	Guatemala: Chimaltenango	KJ175098	KJ175106
<i>B. oresbia</i>	USNM 579667	Honduras: Comayagua	KJ175101	KJ175108
<i>B. porrasorum</i>	MVZ 225852	Honduras: Atlántida	AY526151	AY526188
<i>B. rostrata</i>	MVZ 251521	Guatemala: Totonicapán	KJ175099	KJ175107
<i>B. suchitanensis</i>	UTA A-58422	Guatemala: Jutiapa	—	HQ010001
<i>B. synoria</i>	SMF 78084	Honduras: Ocotepeque	AY526156	AY526193
<i>B. tenebrosa</i>	MVZ 264289	Guatemala: El Progreso	KJ175103	KJ175110
<i>B. tenebrosa</i>	MVZ 265504	Guatemala: El Progreso	KJ175102	KJ175109
<i>B. tenebrosa</i>	MVZ 169038	Guatemala: El Progreso	KJ175104	—
<i>B. tenebrosa</i>	UTA A-58565	Guatemala: Baja Verapaz	—	HQ010004
<i>B. zacapensis</i>	MVZ 257803	Guatemala: Zacapa	GU725455	GU725468

Bolitoglossa meliana (Lynch and Wake, 1982) in part; *Bolitoglossa meliana* (Campbell et al., 2010) in part.

Holotype.—MVZ 108854, an adult female, from Rta. Nacional 5, ca. 3 mi S (by road) of Purulhá, Municipio Purulhá, Departamento Baja Verapaz, Guatemala, 1,650–1,675 m elevation, approximately 15°12'58"N, 90°12'49"W, WGS84 datum, collected by Lynne D. Houck, James F. Lynch, and David B. Wake on 21 August 1972.

Paratypes.—Fifteen specimens, all from Guatemala. Ten males: MVZ 113160, 3.9 km SE Purulhá on Rta. Nacional 17, Municipio Purulhá, Departamento (Depto.) Baja Verapaz; MVZ 264289 (Fig. 3A–B), 250 m SW (by air) of las cabañas de Albores and 3.0 km W (by air) Cerro Pinalon, Municipio San Agustín Acasaguastlán, Depto. El Progreso, 2422 m; MVZ 150789, 150805–150807, 150812, Finca Planada, 15 km NNE Rio Hondo, Municipio Rio Hondo, Depto. Zacapa, 1700 m; and MVZ 150808, 150809, 150811, Volcán de los Monos, 11 km N Santa Cruz, Depto. Zacapa, 1981 m. Four females: MVZ 150813, Finca San Jorge, 5 km ENE Chilascó, Municipio Salamá, Depto. Baja Verapaz, 1829 m; MVZ 169038–169039, La Bella Crest, 20 km NNW San Agustín Acasaguastlán, Municipio San Agustín Acasaguastlán, Depto. El Progreso, 2725 m; and USAC 2114 (Fig. 3C), 250 m SW (by air) of las cabañas de Albores and 3.0 km W (by air) Cerro Pinalon, Municipio San Agustín Acasaguastlán, Depto. El Progreso, 2422 m.

Referred Specimens.—All from Guatemala. KU 186138, 186139, El Volcancito, Depto. Baja Verapaz; MVZ 113161, 3.9 km SE Purulhá on Rta. Nacional 17, Depto. Baja Verapaz; MVZ 150519, 2.3 mi S (via CA-14) Purulhá, Depto. Baja Verapaz; MVZ 169069, 6.5 mi ESE Purulhá, Depto. Baja Verapaz; MVZ 150804, San Antonio, 8 km ESE Chilascó, Depto. Baja Verapaz; UTA-A 58565, Depto. Baja Verapaz; UTA A-32877, S of Cerro Pinalon, Depto. El Progreso; MVZ 150810, Volcán de los Monos, 11 km N Santa Cruz, Depto. Zacapa; MVZ 160772, 12 km N (by air) km 126 on

CA-9 at Santa Cruz, Sierra de Las Minas, Depto. Zacapa; and MVZ 265504, Peña del Angel, 1 km W (by air) of Albores cabins, Sierra de las Minas Biosphere Reserve, Depto. Zacapa.

Diagnosis.—A large species of *Bolitoglossa* (maximum SVL 79.9 mm; males: 55.0 ± 3.88 mm [mean ± s.d.], $n = 10$; females 69.1 ± 12.27 mm, $n = 5$) of the subgenus *Magnadigita* (Parra-Olea et al., 2004). Assigned to *Bolitoglossa* because it lacks a sublingual fold and to *Magnadigita* based on its reduced interdigital webbing and on mtDNA sequence data. Differs from members of the subgenera *Bolitoglossa*, *Mayamandra*, *Nanotriton*, and *Pachymandra* by its lack of fully webbed feet and from *Eladinea* and *Oaxakia* by having a first caudal vertebra with branched transverse processes (Wake and Lynch, 1982) and by large differences in mtDNA sequences. Differs from all members of its subgenus except *B. meliana* by its uniform black coloration, with no speckling or spots of other colors, and from all species except *B. meliana* (Table 2), *Bolitoglossa omniunsanctorum* (Todos Santos Salamander) (up to 76.6 mm SVL [Campbell et al., 2010]), *B. lincolni* (maximum SVL 79 mm [Elias, 1984]), and *B. franklini* (Wake and Lynch, 1982) by its larger size. Distinguished from *Bolitoglossa tzultacaj* (*Tzultacaj Salamander*), which also occurs in the western Sierra de las Minas, by larger size, reduced foot webbing, and having a relatively longer tail (Campbell et al., 2010). Distinguished from *B. meliana* by having more maxillary teeth (Fig. 4; males: 59.9 ± 8.89 vs. 44.3 ± 8.79 in *B. meliana*, $t = 3.59$, $df = 13.17$, $P = 0.0032$; females: 61.8 ± 5.02 vs. 51.3 ± 10.81 in *B. meliana*, $t = 2.66$, $df = 13.91$, $P = 0.019$) and vomerine teeth (males: 24.5 ± 2.17 vs. 19.0 ± 4.04 in *B. meliana*, $t = 3.28$, $df = 18.44$, $P = 0.010$; females: 25.0 ± 3.24 vs. 18.1 ± 5.15 in *B. meliana*; $t = 3.25$, $df = 12.08$, $P = 0.0069$). The differences in maxillary tooth number are still significant when size is accounted for in males (MT/SVL: $t = 3.66$, $df = 12.34$, $P = 0.003$), as is the difference in vomerine tooth number (VT/SVL: $t = 2.68$, $df = 10.13$, $P = 0.046$); both

TABLE 2. Mean and standard deviation, with range in parentheses, of measurements used in morphological comparisons for *B. tenebrosa* and *B. meliana*. Numbers of individuals used to calculate mean and variation noted if different from number in first row.

Measurement	<i>B. tenebrosa</i>		<i>B. meliana</i>	
	Males (n = 10)	Females (n = 5)	Males (n = 9)	Females (n = 11)
SVL	55.0 ± 3.88 (49.1–61.4)	69.1 ± 12.27 (50.5–79.9)	53.6 ± 5.76 (48.1–65.1)	60.8 ± 11.7 (45.5–75)
TAL	54.4 ± 9.73 (33.5–63.3) (n = 8)	66.7 ± 15.49 (43.7–76.9)	43.6 ± 8.24 (33.0–61.1)	46.9 ± 11.72 (35.6–68.4) (n = 10)
AG	28.7 ± 2.26 (25.3–32.3)	37.4 ± 6.98 (26.9–44.4)	28.0 ± 3.05 (24.5–34.0)	32.2 ± 7.14 (23.3–41.3)
SG	13.4 ± 0.86 (11.8–14.4)	16.0 ± 2.34 (12.1–17.8)	12.6 ± 1.44 (11.4–15.4)	13.6 ± 2.1 (10.9–16.7)
HW	9.5 ± 0.62 (8.8–10.6)	10.9 ± 1.67 (8.5–12.7)	8.7 ± 0.98 (7.8–10.8)	9.6 ± 1.32 (7.9–11.5)
FLL	13.5 ± 1.02 (11.9–15.1)	15.9 ± 2.09 (12.7–17.6)	12.7 ± 1.77 (11.0–16.0)	13.9 ± 2.44 (10.6–17.2)
HLL	14.8 ± 1.21 (12.6–16.4)	17.8 ± 2.53 (13.7–20.2)	14.0 ± 1.93 (12.3–18.1)	15.2 ± 2.53 (11.4–18.1)
RFW	6.2 ± 0.66 (5.0–7.4)	7.1 ± 1.76 (4.9–9.3)	5.5 ± 0.9 (4.5–7.3)	6.1 ± 1.18 (4.3–7.6)
MT	59.9 ± 8.89 (48–77)	61.8 ± 5.02 (54–68)	44.3 ± 8.79 (28–55) (n = 7)	51.3 ± 10.81 (40–74)
VT	24.5 ± 2.17 (20–27)	25 ± 3.24 (21–30)	19.0 ± 4.04 (13–25) (n = 7)	18.1 ± 5.15 (10–26)

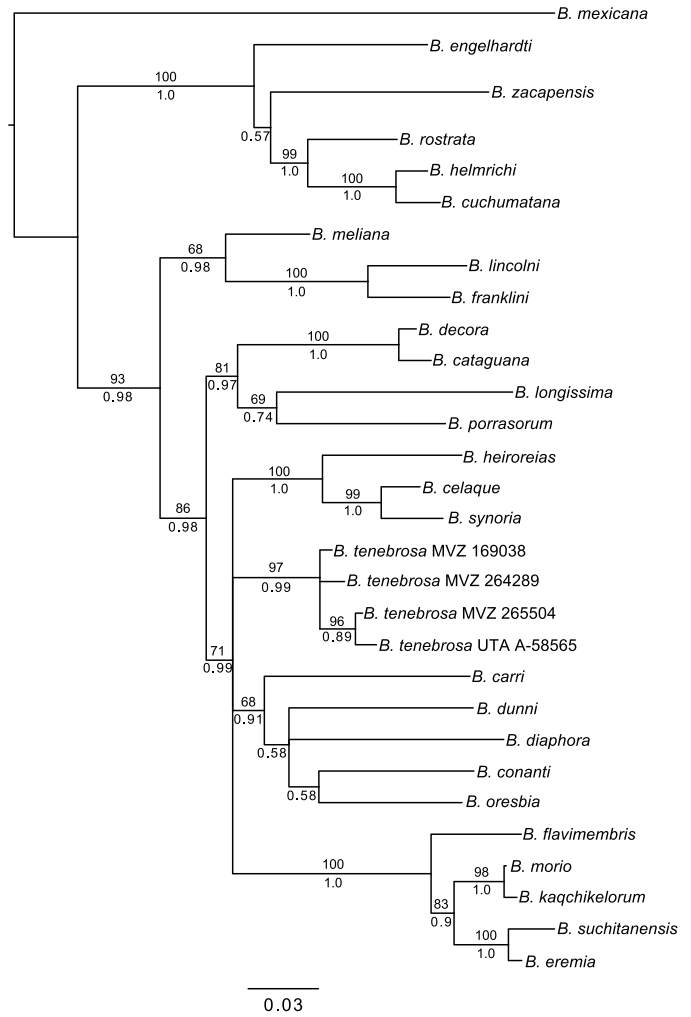


FIG. 2. Mitochondrial gene tree from Bayesian analysis of 16S and *cytb* genes. Bootstrap proportions from RAxML analysis are given above branches and posterior probabilities from MrBayes analysis are below branches.

differences in MT/SVL and VT/SVL are not significant for females (MT/SVL: $t = 0.74$, $df = 11.54$, $P = 0.48$; VT/SVL: $t = 1.31$, $df = 10.06$, $P = 0.22$). Males of *B. tenebrosa* differ from those of *B. meliana* in having a longer tail (TL 54.4 ± 9.73 vs. 43.6 ± 8.24 in *B. meliana*, $t = 2.45$, $df = 13.85$, $P = 0.028$) and wider head (HW 9.5 ± 0.62 vs. 8.8 ± 0.98 in *B. meliana*; $t = 2.15$, $df = 13.36$, $P = 0.050$). Using residuals from a regression against SVL to remove the effect of body size, both males and females of *B. tenebrosa* have relatively longer tails (males: residuals of TL vs. SVL, $t = 2.27$, $df = 14.93$, $P = 0.039$; females, $t = 3.63$, $df = 11.63$, $P = 0.0036$).

Description of the Holotype.—A large female (SVL = 79.4 mm). Head relatively broad (HW/SVL = 0.16). Maxillary teeth numerous (61 maxillary teeth, seven premaxillary teeth), premaxillary teeth anterior to line of maxillary teeth. Vomerine teeth relatively numerous (30) extending in a row past outer edge of internal nares. Labial protuberances not well developed. Limbs relatively short; adpressed limbs separated by approximately two costal folds. Tail relatively long, approximately same length as body; TL/SVL = 0.97. Webbing reduced with two phalanges of digit III free of webbing on both hands and feet. Digits in order of

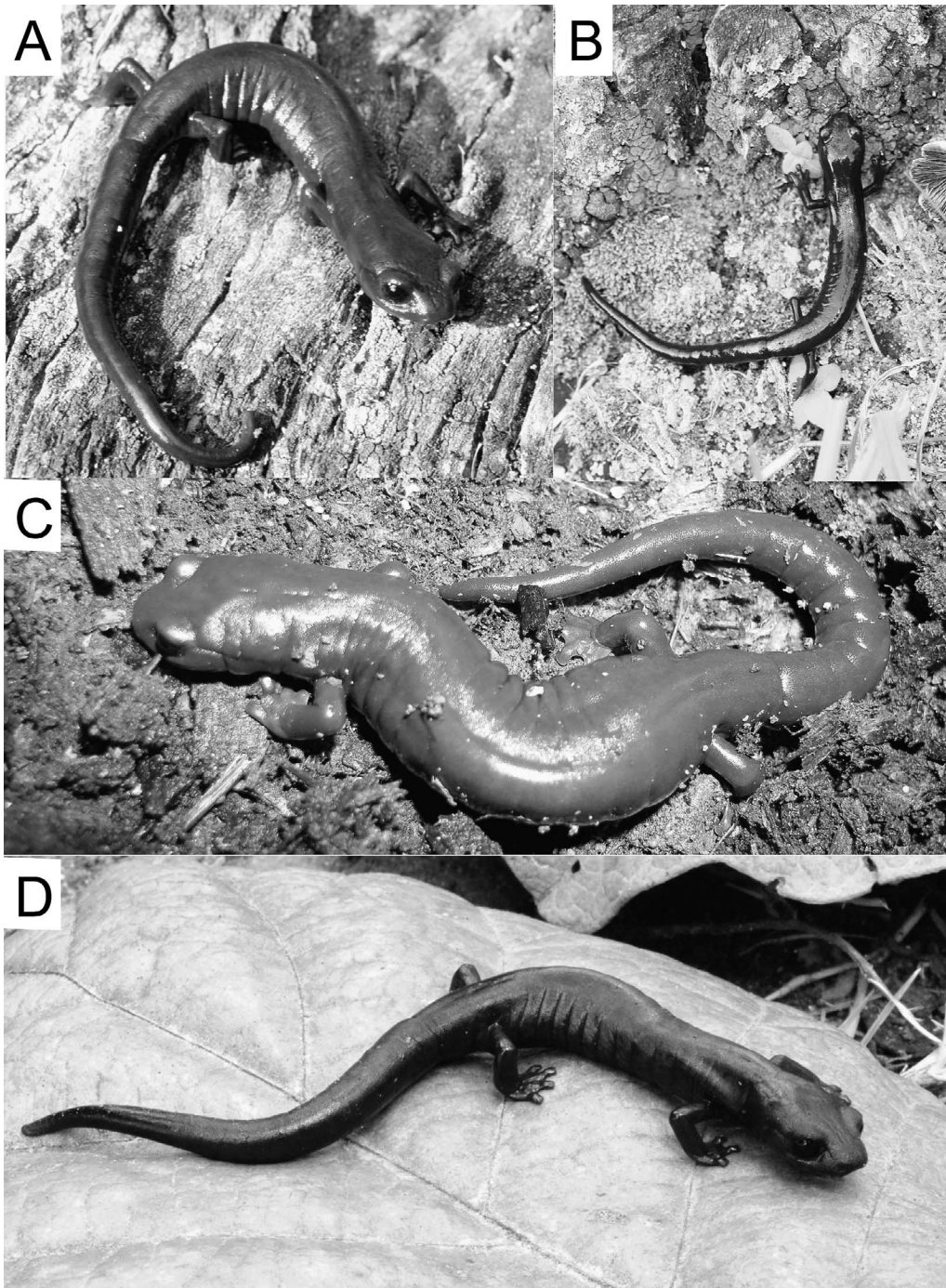


FIG. 3. (A,B) Paratype of *B. tenebrosa* (MVZ264289) in life. (C) Paratype of *B. tenebrosa* (USAC 2114) in life. (D) *Bolitoglossa meliana* (MVZ 265621) from near Zacualpa, El Quiché, in life.

increasing length I-II>IV-III on hands and I-II-V-IV-III on feet. Phalangeal formulae 1-2-3-2 for hands and 1-2-3-3-2 for feet.

Measurements (mm) and Tooth Counts of the Holotype.—SVL 79.4, TL 76.9, AG 42.3, SG 17.8, HW 12.7, FLL 17.4, HLL 19.5, RFW 8.4, head depth at angle of jaw 6.0, eyelid length 5.1, eyelid width 2.5, anterior rim of orbit to snout 4.8, horizontal orbital diameter 4.0, interorbital distance 4.6, snout to forelimb distance 23.1, distance separating internal nares 3.2, distance separating external nares 3.7, maximum nostril diameter 0.2, snout projection beyond mandible 1.0, shoulder width 10.2, snout to anterior angle of vent 72.9, tail width at base 6.0, width of right hand 7.3, length of longest (third) toe 3.7, length of fifth toe 1.8. Maxillary teeth 61

(32/29), premaxillary teeth 7, vomerine teeth 30 (15/15). Adpressed limbs separated by approximately 2 costal folds.

Color (in Life) of Holotype (from Field Notes).—Dorsal surface of body, head, and tail solid black. Venter light grey.

Color (in Alcohol) of the Holotype.—Dorsal surface of body, head, tail, and limbs, and lateral surface of body and tail, uniform dark grey. Upper surface of feet light grey-brown. Ventral surface of body light grey with some light brown at insertion of forelimbs. Gular region light brown with faint band of light grey extending across gular region anterior to gular fold. Underside of tail uniform light grey. Underside of limbs, hands, and feet light grey-brown.

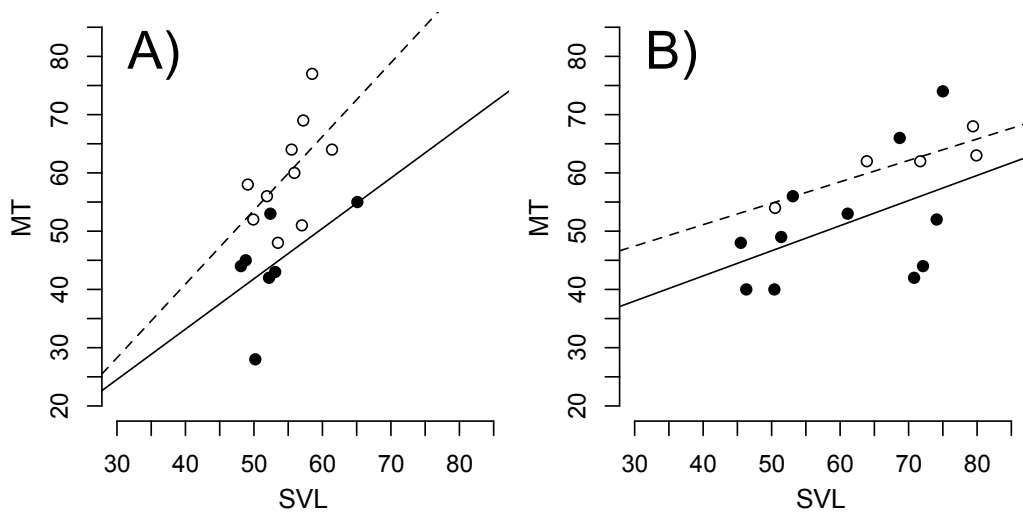


FIG. 4. Maxillary tooth number vs. SVL for (A) males and (B) females of *B. tenebrosa* (open circles) and *B. meliana* (closed circles), with least squares linear regression lines fit to points (dotted line for *B. tenebrosa*, solid line for *B. meliana*).

Distribution and Ecology.—*Bolitoglossa tenebrosa* is known from the highlands of the eastern portion of the Depto. Baja Verapaz, in the vicinity of Purullhá and Chilascó as well as from the southern slopes and crest of the Sierra de las Minas in the Depto. El Progreso and Zacapa (Fig. 1). All specimens were found between 1,585 and 2,725 m elevation in broadleaf cloud forest habitat. Specimens were collected by day, and on road banks at night, from under and inside rotten logs, under bark, and inside a fallen bromeliad. One specimen was collected approximately 2 m off the ground in a jumble of logs. Its prehensile tail suggests that it is capable of being highly arboreal.

Etymology.—The specific epithet is an adjective meaning “dark” or “shrouded in darkness” in Latin, making reference both to the dark color of this species and to the fact that its distinctiveness was hidden for many years by its external morphological similarity to *B. meliana*.

DISCUSSION

Wake and Lynch (1982) proposed that *B. meliana* was the sister taxon to all other species in the *B. franklini*–*lincolni* species complex based on allozyme data and the generalized morphology of this species. They also found relatively large genetic distances between populations of *B. meliana* (which included *B. tenebrosa*), especially between the population at Purullhá and those in the Sierra de las Minas and El Quiché. Campbell et al. (2010) included samples from Baja Verapaz and Zacapa, which we assign to *B. tenebrosa*, in their phylogenetic analysis and found these sequences to be in a clade with *B. franklini*, *B. lincolni*, and *Bolitoglossa heiroreias* (Holy-mountain Salamander), with low support for the 12S mitochondrial gene and as the sister taxon to the *B. morio* species complex for *cytb*. Our study includes sequences of *B. meliana* from El Quiché for phylogenetic analysis as well as those from Baja Verapaz and the Sierra de las Minas. Our results agree with those of Wake and Lynch (1982) in placing *B. meliana* as the sister species of *B. franklini* + *B. lincolni* and broadly with those of Campbell et al. (2010) in the placement of *B. tenebrosa*. The fact that salamanders in different parts of the phylogenetic tree of *Bolitoglossa* (*Magnadigita*) were placed within a single species highlights how closely these salamanders resemble one another in general external morphology, especially in color pattern. Taxonomy of black *Bolitoglossa* continues to be challenging in general; in Costa Rica and Panama,

multiple species that are similar in external morphology have been described in recent years (Hanken et al., 2005; Wake et al., 2005).

Wake and Lynch (1982) found relatively large genetic differences among the three populations of black *Bolitoglossa* included in their allozyme study. The largest distances were between the population from Purullhá (type locality of *B. tenebrosa*) and the population from the Sierra de las Minas (now assigned to *B. tenebrosa*; Nei's $D = 0.314$). The Purullhá population also had a large Nei's D from the type locality of *B. meliana* ($D = 0.256$) while the distance between the Sierra de las Minas and Purullhá populations was smaller ($D = 0.117$). These results conflict somewhat with our mtDNA gene tree, which shows a close relationship between the Purullhá and Sierra de las Minas populations of *B. tenebrosa* and a more-distant relationship of both of these populations to *B. meliana*. The mtDNA results agree with the morphological differences, particularly maxillary and vomerine teeth numbers. We suspect that the discrepancy between our results and those from the allozyme analysis might be because of the small sample sizes of the Purullhá and Sierra de las Minas populations in the allozyme analysis (one and two individuals, respectively). The use of multiple nuclear loci in future phylogenetic analyses will reveal whether the relationships seen in our mitochondrial gene tree are reflective of the true species relationships.

The Sierra de las Minas and Purullhá areas contain a larger number of endemic species of salamanders than was previously known. When Wake and Lynch (1982) described *B. meliana*, only that species and *Bolitoglossa helmrichi* (Coban Mushroom-tongue Salamander) were known from the Sierra de las Minas, and *Cryptotriton veraepacis* (Baja Verapaz Salamander) was known from Purullhá, Baja Verapaz. Subsequently, the enigmatic species *Nyctanolis pernix* (Nimble Long-limbed Salamander) was found at Purullhá, bringing the number of species there to four. The descriptions of *Cryptotriton sierraminensis* (Sierra de las Minas Hidden Salamander) (Vásquez-Almazán et al., 2009) and three *Bolitoglossa* from the Sierra de las Minas (*Bolitoglossa daryorum* [Dary's Salamander], *B. tzultacaj*, *Bolitoglossa zacapensis* [Zacapa Salamander]) (Campbell et al., 2010; Rovito et al., 2010), one of which (*B. daryorum*) also occurs at Purullhá, greatly increased both the known salamander species diversity and the level of endemism of this region. It is surprising that new species continue to be described from the Purullhá area, given that it is

easily accessible and is the site of a much-used reserve of the Universidad de San Carlos, the Biotopo del Quetzal. By contrast, the northern slope of the Sierra de las Minas and the eastern portions of the range remain more-poorly explored than the southern slopes or western part of the range and might yield additional undescribed species of salamanders.

Bolitoglossa tenebrosa has never been found in great abundance, making it difficult to determine the status of populations. While much of the Sierra de las Minas is protected as a biosphere reserve, illegal activities and fires still threaten salamander habitat in the region. Similarly, although habitat within the Biotopo del Quetzal near Purulhá is well protected, nearly all of the surrounding forest has been felled or is severely disturbed by human activity. Based on our current knowledge of its distribution and population status, *B. tenebrosa* does not currently qualify for any of the IUCN threatened categories (Critically Endangered [CR], Endangered [EN], Vulnerable [VU]). While it is known from fewer than 10 localities and has an extent of occurrence of approximately 600 km², much of its habitat (such as at the Biotopo del Quetzal) is protected. Continuing habitat destruction in the Sierra de las Minas or Purulhá region, however, could lead to *B. tenebrosa* being classified as VU under criterion B1a,b(iii) (www.iucnredlist.org). We suggest that *B. tenebrosa* be classified as Near Threatened (NT) to reflect the fact that it might be threatened in the future.

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APPENDIX 1

Specimens Examined for Morphological Analyses

Bolitoglossa tenebrosa ($n = 15$), Guatemala, Depto. Baja Verapaz, Municipio Purulhá, Rta. Nacional 5, ca. 3 mi S (by road) Purulhá: MVZ 108854; 3.9 km SE Purulhá on Rta. Nacional 17: MVZ 113160; Municipio Salamá, Finca San Jorge, 5 km ENE Chilascó: MVZ 150813; Depto. El Progreso, Municipio San Agustín Acasaguastlán, Sierra de las Minas, La Bella Crest, 20 km NNW San Agustín Acasaguastlán: MVZ 169038, 169039; Sierra de las Minas, 250 m SW (by air) of las cabañas de Albores and 3.0 km W (by air) Cerro Pinalon: MVZ 264289, USAC 2114; Depto. Zacapa, Municipio Rio Hondo, Sierra de las Minas, Finca Planada, 15 km NNE Rio Hondo: MVZ 150789, 150805–150807, 150812; Sierra de las Minas, Volcán de los Monos, 11 km N Santa Cruz: MVZ 150808, 150809, 150811. *Bolitoglossa meliana* ($n = 20$), Guatemala, Depto. El Quiché, Municipio Santa Cruz del Quiché, 4 km E of Santa Rosa Chujuyub: MVZ 160395, 160397; Santa Rosa Pass, 9 km NE (by air) Santa Cruz del Quiché: MVZ 160736–160739, 160741–160744, 160746, 160748 160750, 160755, 160753, 160755, 160760, 160763; Municipio San Pedro Jocopilas, 5 km ENE San Pedro Jocopilas: MVZ 160384, 160398; Municipio Zacualpa, Chuxilic, 9.6 km NE (by rd) of center of Zacualpa on road to Chojlomquiej: MVZ 265621.