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A beautiful new yellow salamander, genus *Bolitoglossa* (Caudata: Plethodontidae), from the northeastern slopes of the Cordillera de Talamanca, Costa Rica

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Abstract

A new yellow salamander belonging to the genus *Bolitoglossa,* subgenus *Eladinea,* is described from a premontane rainforest in the vicinity of Moravia de Chirripó, on the northeastern slopes of the Cordillera de Talamanca in Costa Rica at an elevation of ca. 1300 m. This new taxon is distinguished from its congeners by its chromatic and morphological characteristics, and by differentiation in DNA sequences of the mitochondrial 16S rRNA and cytochrome *b* genes.

Key words: Amphibia, Central America, *Bolitoglossa aurae* **sp. nov**., *Bolitoglossa robinsoni* clade, caudate, *Eladinea*, 16S rRNA, cytochrome *b*

Resumen

Se describe una nueva salamandra amarilla perteneciente al género *Bolitoglossa,* subgénero *Eladinea*, del bosque lluvioso premontano en las proximidades de Moravia de Chirripó en la vertiente noreste de la Cordillera de Talamanca en Costa Rica, a una elevación aproximada de 1300 m.s.n.m. Esta nueva especie se diferencia de sus congéneres por sus características cromáticas, morfológicas y su diferenciación molecular en los genes mitocondriales 16S rARN y citocromo *b*.

Palabras clave: América Central, Amphibia, *Bolitoglossa aurae* **sp. nov**., caudado, clado *Bolitoglossa robinsoni, Eladinea*, 16S rARN, citocromo *b*

Introduction

Ongoing research on the Bolitoglossini tribe of lungless salamanders (family Plethodontidae) now reveals an impressive diversity, both on the beta and alpha level, in tropical America with 14 genera and nearly 300 species currently recognized (Rovito *et al.* 2015; Frost 2016). Costa Rica, a tiny country with a highly variable topography and corresponding climatic diversity, has the fifth highest total diversity of salamanders on the planet. A total of 49 species of salamanders, among three genera, are known from within Costa Rica's 51,000 km^2 national territory, resulting in it being the country with the highest diversity density of caudates in the world, with 0.96 species per 1000 km^2 (Boza-Oviedo *et al.* 2012; AmphibiaWeb 2016).

The genus of salamanders with the highest representation of species within Costa Rica is *Bolitoglossa*; currently 26 species are known for the genus within the tiny republic (Boza-Oviedo *et al.* 2012). The Caribbean slopes of the Talamanca mountain range (from the continental divide to the coastal lowlands) has the highest concentration of *Bolitoglossa* species, where 19 of the 26 species have been documented (AmphibiaWeb 2016). Despite the known richness of *Bolitoglossa* in this region, the majority of the area has been studied poorly, due to the steep topography and very limited and difficult access. Undoubtedly, as more sites within the extensive forest on the Caribbean slopes of Talamanca are explored further, additional species will continue to be discovered and

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described. Herein we describe a new species of *Bolitoglossa* from a remote site within the premontane rainforest of the northeastern Caribbean slopes of Talamanca.

Material and methods

The holotype was found during fieldwork by BK in the vicinity of Moravia de Chirripó (N 9.82 W 83.46), in the northeastern section of the Cordillera de Talamanca (Fig. 1). The holotype was fixed in a 10% formalin solution and processed over to 70% ethanol for long-term storage. The tissue sample used for the genetic analyses was preserved in 96% ethanol. All morphological characteristics reported herein are from the adult holotype, which is deposited at the Museo de Zoología (UCR), Universidad de Costa Rica, San José, Costa Rica. Additional specimens examined during this study are listed in Appendix I. The provided coordinates are WGS84 datum.

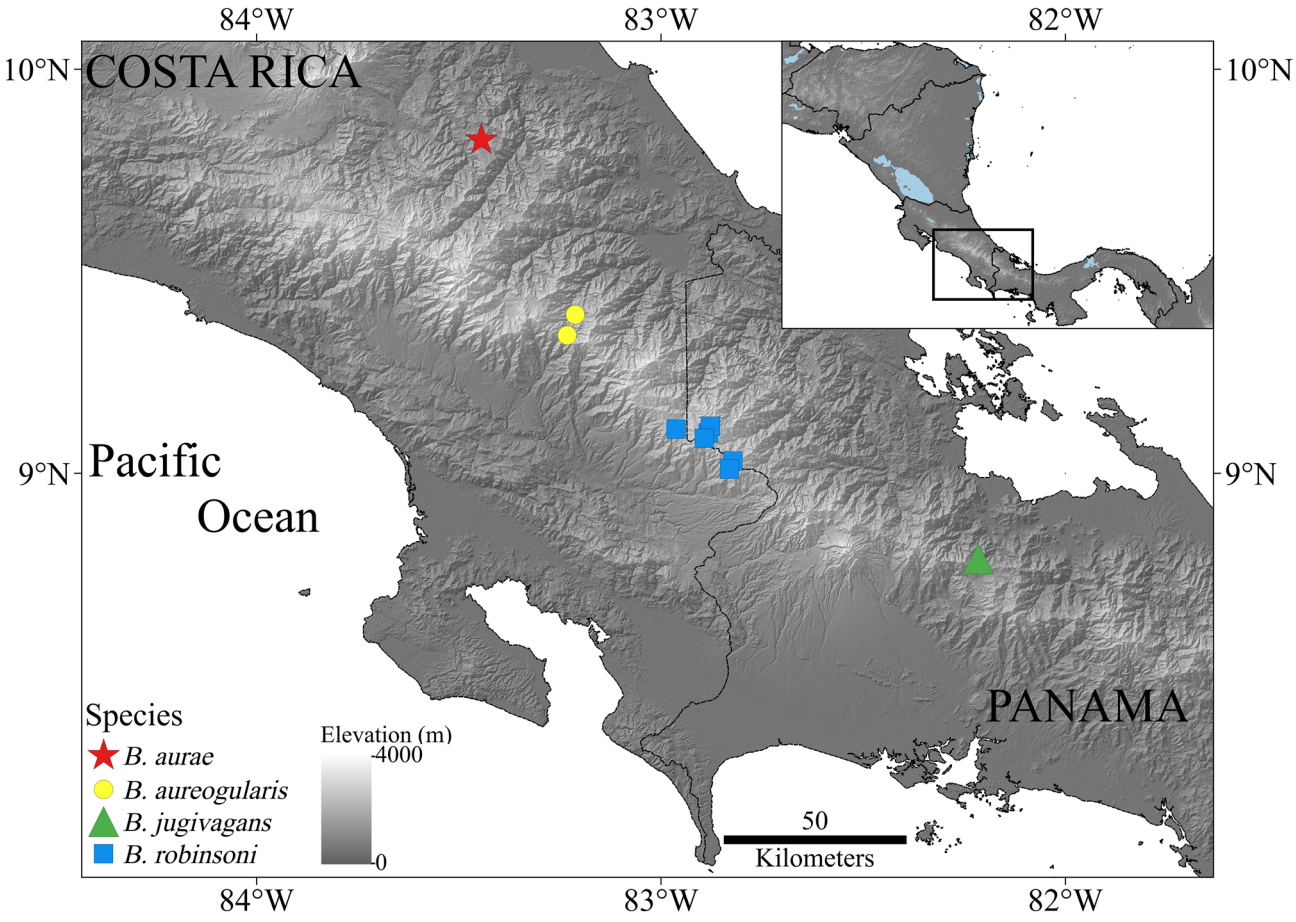

FIGURE 1. Map showing the type locality for *Bolitoglosssa aurae* **sp. nov.** (red star) in the Cordillera de Talamanca. The localities for the other known members of the *B. robinsoni* species group are shown for comparison.

Morphometrics. Measurements were taken by BK with a dissecting scope fitted with an ocular micrometer, or with measurements exceeding 8 mm a ROHS NORM 2002/95/EC digital caliper, and rounded to the nearest 0.1 mm. Morphological measurements taken and the abbreviations used herein are as follows: standard length (SL), internarial tip of snout to posterior margin of cloacal opening; tail length (Tal), posterior tip of tail to posterior margin of cloacal opening; total length (TL), internarial tip of snout to posterior tip of tail; shoulder width (ShW), width of shoulders at center axis of upper arms; head width (HeW), greatest width of head at jaw articulation; neck width (NeW), width of neck at gular fold crease on lateral portions of neck; eye width (EW), horizontal distance between anterior/inner and posterior/outer corners of right eye opening; snout length (SnL), distance between anterior/inner corner of right eye opening and tip of snout; jaw to snout length (JSL), distance between posterior corner of mouth on right side of head to tip of snout; lateral gular fold to tip of snout (LGFS), distance between most posterior margin of gular fold on right side of body to tip of snout; nostril height (LNH), greatest vertically

oriented distance of left narial opening; nostril width (RNW), greatest width of right narial opening; internarial distance (IND), distance between inner margins of narial openings; naris to lip distance (NLP), distance between inferior margin of left narial opening and margin of upper lip; intercanthal distance (ICD), distance between canthal ridges at anterior/inner corners of eyes; hind limb length (HLL), distance from right hind limb's juncture with body to tip of Toe III; front limb length (FLL), distance from right front limb's juncture with body to tip of Finger III; trunk width (TW), width of trunk at mid-way point between groin and axilla; mid-ventral gular fold to snout length (VGS), distance between mid-ventral edge of gular fold to tip of snout; front limb to snout distance (FSL), distance between anterior margin of left front limb's juncture with body and tip of snout; ulna and hand length (UHL), distance from external margin of ventral elbow crease to tip of Finger III on left arm; axilla to groin length (AGL), distance between posterior margin of front limb–trunk juncture and anterior margin of hind limb–trunk juncture of left side of body; vent length (VL), distance between anterior and posterior margins of cloacal opening; hand width (HaW), distance between distal exterior margin of Finger I and distal exterior margin of Finger IV on right hand; hand length (HaL), distance from center proximal margin of palmar surface to tip of Finger III on right hand; length of Finger II (LF2), distance from baseline along most distal margin of Finger I to tip of Finger III on right hand; length of Finger III (LF3), distance from baseline along most proximal interdigital margin between fingers II and III and fingers III and IV to tip of Finger III on right hand; width of Finger III (WF3), distance between widest external margins of tip of Finger III on right hand; foot width (FoW), distance between distal exterior margin of Toe I and distal exterior margin of Toe V on right foot; foot length (FoL), distance from center proximal margin of plantar surface to tip of Toe III on right foot; length of Toe II (LT2), distance from baseline along most distal margin of Toe I to tip of Toe III on right foot; length of Toe III (LT3), distance from baseline along most proximal interdigital margin between toes II and III and toes III and IV to tip of Toe III on right foot; width of Toe III (WT3), distance between widest external margins of tip of Toe III on right foot. The following are also expressed as percentage of standard length (SL): mid-ventral gular fold to snout length (VGS); head width (HeW); axilla to groin length (AGL); left nostril height (LNH); right nostril width (RNW); right hind limb length (HLL); right front limb length (FLL). The following are expressed as a percentage of mid-ventral gular fold to snout length (VGS): right hand length (HaL); right foot length (FoL). The following are expressed as a percentage of head width (HeW): internarial distance (IND); snout length (SnL); left nostril height (LNH); right nostril width (RNW); right hand width (HaW); right foot width (FoW); length of Toe III on right foot (LT3). The following is expressed as a percentage of axilla to groin length (AGL): head width (HeW). The following is expressed as a percentage of right foot width (FoW): length of Toe III on right foot (LT3). Tooth counts are based on number of clearly visible teeth, including premaxillary teeth (PMT), maxillary teeth (MT), and vomerine teeth (VT). The number of maxillary and vomerine teeth also are indicated according to the count on the corresponding right and left sides of the mouth in the following manner: R/L. 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, 4.5). Phenotypic comparisons to other members of the *Bolitoglossa robinsoni* clade are based on the examination of the holotypes and/or original species descriptions (i.e., *Bolitoglossa aureogularis* Boza-Oviedo, Rovito, Chaves, García-Rodríguez, Artavia, Bolaños, and Wake, 2012, *B. jugivagans* Hertz, Lotzkat, and Köhler, 2013, and *B. robinsoni* Bolaños and Wake, 2009). The capitalized colors and their corresponding color codes (code in parentheses) used in the color in life and color in ethanol descriptions of the holotype follow Köhler (2012).

Amplification and sequencing. The total genomic DNA was extracted from the preserved tissues using the phenol-chloroform standard protocol (Sambrook & Russell 2006). EA sequenced the holotype (UCR 22842) for the 16S rRNA (16S) and cytochrome *b* (cyt *b*) mitochondrial genes. The primers 16Sar y 16Sbr (Palumbi *et al.* 1991) were used for 16S and primers MVZ15 and MVZ16 (Moritz *et al.* 1992) for cyt *b*. The PCR conditions consisted of an initial cycle of 5 min (16S) or 2 min (cyt *b*) at 94ºC, followed by 35 (16S) or 38 (cyt *b*) cycles of 45 s (16S) or 30 s (cyt *b*) at 94ºC, 30 s at 55ºC for 16S or 1 min at 48ºC for cyt *b*, 45 s (16S) or 1 min (cyt *b*) at 72ºC, plus a final cycle of 3 min (16S) or 8 min (cyt *b*) at 72ºC. 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). After cycle sequencing, the products were column-purified using a Sephadex G-50 (GE Healthcare) and were run on an ABI PRISM 3100 DNA Analyzer (Applied Biosystems). Consensus sequences for each individual were constructed using SEQUENCHER 5.3 (Genes Codes Corp.). The resulting sequences were deposited in GenBank (Table 1).

TABLE 1. Institutional voucher numbers, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses.

Species	Voucher	GenBank 16S	GenBank cyt *b*
Bolitoglossa aurae **sp. nov.**	UCR: 22842	KX779527	KX779528
Bolitoglossa aureogularis	UCR: 19858	JQ899151	JQ899182
Bolitoglossa aureogularis	UCR: 19859	JQ899152	-
Bolitoglossa aureogularis	UCR: 19892	JQ899153	-
Bolitoglossa aureogularis	UCR: 19893	JQ899154	JQ899183
Bolitoglossa jugivagans	SMF: 94467	KC428634	-
Bolitoglossa robinsoni	UCR: 20489	JQ899161	JQ899191
Bolitoglossa bramei	MVZ: 225893	-	AF212066
Bolitoglossa bramei	UCR: 20483	JQ899159	JQ899189
Bolitoglossa bramei	UCR: 20484	JQ899160	JQ899190
Bolitoglossa bramei	UCR: 20851	JQ899142	JQ899172
Bolitoglossa pesrubra	MVZ: 210360	EU448105	-
Bolitoglossa pesrubra	UCR: 12068	AY526132	AF212069
Bolitoglossa kamuk	UCR: 20852	JQ899143	JQ899173
Bolitoglossa kamuk	UCR: 20853	JQ899144	JQ899174
Bolitoglossa kamuk	UCR: 20854	JQ899145	JQ899175
Bolitoglossa splendida	UCR: 19835	JQ899150	JQ899181
Bolitoglossa gracilis	MVZ: 229170	AY526121	AF212067
Bolitoglossa gracilis	MVZ: 229171	AY526122	AF212068
Bolitoglossa subpalmata	MVZ: 194828	EU448107	AF212091
Bolitoglossa subpalmata	MVZ: 194889	-	AF212095
Bolitoglossa subpalmata	MVZ: 229172	AF416697	AF212094
Bolitoglossa tica	UCR: 12065	AY526137	AF212089
Bolitoglossa tica	UCR: 20514	JQ899162	JQ899192
Bolitoglossa gomezi	UCR: 20843	JQ899140	JQ899170
Bolitoglossa gomezi	UCR: 20848	JQ899139	JQ899169
Bolitoglossa gomezi	UCR: 20849	JQ899141	JQ899171
Bolitoglossa sooyorum	MVZ: 190847	EU448108	-
Bolitoglossa minutula	MVZ: 225870	AY526124	AF212098
Bolitoglossa marmorea	MVZ: 210286	AF218493	U89627
Bolitoglossa cerroensis	MVZ: 233516	AF199233	AF199195
Bolitoglossa epimela	MVZ: 181260	AY526120	AF212097
Bolitoglossa colonnea	SMF: 97136	KM527326	-
Bolitoglossa colonnea	No voucher	AY526119	AY526162
Bolitoglossa nigrescens	CH: 7478	JQ899165	JQ899168
Bolitoglossa nigrescens	MVZ: 225875	AY526135	AY526173
Bolitoglossa schizodactyla	USNM: 572791	FJ784482	-
Bolitoglossa schizodactyla	No voucher	AY526133	AY526171
Bolitoglossa compacta	UCR: 20532	JQ899163	JQ899193
Bolitoglossa robusta	MVZ: 190830	EU448109	EU448110
Bolitoglossa chucantiensis	MHCH: 2665	KM527324	-

.....continued on the next page

TABLE 1. (Continued)

Species	Voucher	GenBank 16S	GenBank cyt *b*
Bolitoglossa taylori	MHCH: 2663	KM527331	-
Bolitoglossa taylori	SMF: 97140	KM527323	-
Bolitoglossa biseriata	MHCH: 2668	KM527334	-
Bolitoglossa biseriata	MHCH: 2658	KM527322	-
Bolitoglossa sima	MVZ: 163575	AY526134	AY526172
Bolitoglossa leandrae	MCNUP: 63	KC257102	-
Bolitoglossa leandrae	MCNUP: 64	KC257103	-
Bolitoglossa nicefori	PAG: 926	KC257105	-
Bolitoglossa mucuyensis	CVULA: 7100	JN635335	-
Bolitoglossa orestes	CVULA: 7107	JN635340	-
Bolitoglossa tamaense	MCNUP: 51	KC257100	-
Bolitoglossa tamaense	MCNUP: 53	KC257101	-
Bolitoglossa peruviana	KU: 217421	AY526131	AY526170
Bolitoglossa equatoriana	QCAZ: 25448	-	DQ353842
Bolitoglossa medemi	No voucher	AY526123	AY526163
Bolitoglossa paraensis	LSUMZH: 13735	AY526129	AY526168
Bolitoglossa palmata	KU: 217422	AY526125	AY526164
Bolitoglossa palmata	KU: 217423	AY526126	AY526165
Bolitoglossa adspersa	MVZ: 158485	AF218492	AF212984
Bolitoglossa mexicana	MVZ: 176838	GU725457	GU725470
Bolitoglossa morio	MVZ: 257825	GU725452	GU725465

Phylogenetic analyses. Sequences obtained from the holotype were compared with 59 sequences available on GenBank for *Bolitoglossa* (subgenus *Eladinea*), in addition to two outgroups. Comparisons were concentrated on holotypes or *sensu stricto* (topotypic) representatives for each species whenever possible. Individuals alignments were performed using the MUSCLE 3.7 software (Edgar 2004). We used PartitionFinder v1.1.1 (Lanfear *et al.* 2012) and the Bayesian Information Criterion (BIC) to select an appropriate model of the DNA sequence evolution. The following substitution models were selected: GTR+I+G for 16S, HKY+I+G for cyt *b* codon position 1, K80+G for cyt *b* codon position 2, and GTR+G for cyt *b* codon position 3. Analyses were performed using both the maximum likelihood (ML) and Bayesian analyses (BA). The ML analyses were performed using RAxML 8.1.11 (Stamatakis 2014) and run on the CIPRES portal (Miller *et al.* 2010), including 1000 bootstrap replicates to evaluate nodal support. The Bayesian phylogenetic analyses were performed using MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001) and 4 heated MCMC samples of every 1000 generations for 50 million generations. We examined a time-series plot of the likelihood scores of the cold chain to check stationarity using Tracer 1.6 software (Rambaut *et al*. 2014). We discarded the first 25% of trees as burn-in and used the remaining trees to estimate the consensus tree along with the posterior probabilities for each node and each parameter. Estimates of pairwise evolutionary genetic divergence between and within groups were computed using MEGA6 (Tamura *et al.* 2013), assuming corrected distances based on the Tamura 3-parameter model (Tamura 1992), with rate variation among the sites modeled as a gamma distribution with the shape parameter = 4 as the default of the software.

Results

Molecular genetics. The resulting data matrix had a total sequence length of 1334 bp, including gaps; 527 bp for 16S and 807 for cyt *b.* The phylogenies inferred using ML and BA were concordant in supporting the tree shown in Fig. 2. The phylogeny shows that the specimen from Moravia de Chirripó is divergent from other members of the

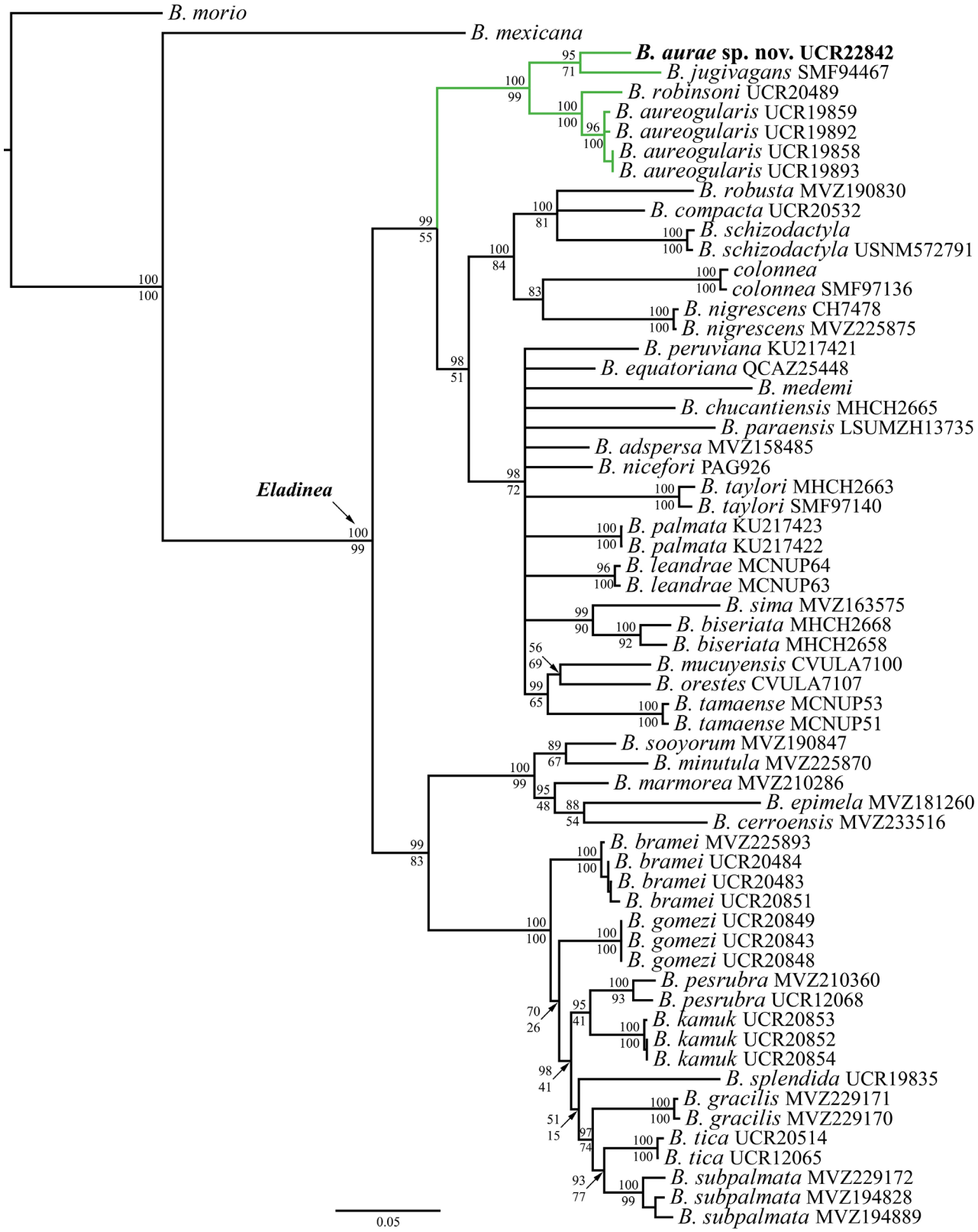

FIGURE 2. Bayesian phylogenetic inference of the relationships of *Bolitoglossa aurae* **sp. nov.** within the *B. robinsoni* species group (green) based on the 16S and cyt *b* mitochondrial DNA genes. Bayesian posterior probabilities (multiplied by 100) are shown above the branch; maximum likelihood bootstrap values from the RAxML analysis are shown below the branches. The scale bar refers to the estimated substitutions per site.

subgenus *Eladinea* that were analyzed, but forms part of a clade that includes *Bolitoglossa aureogularis*, *B. jugivagans*, and *B. robinsoni* (Fig. 2). According to our results, the *Bolitoglossa* specimen from Moravia de Chirripó is separated by a mean-corrected genetic distance of: *B. aureogularis* 2.0 % (16S) and 11.0 % (cyt *b*); *B. jugivagans* 2.6 % (16S); *B. robinsoni* 2.4 % (16S) and 10.3 % (cyt *b*). Sequences of cytochrome *b* mtDNA were not available for *B. jugivagans* so we are unable to report herein the amount of divergence between the *Bolitoglossa* specimen from Moravia de Chirripó and *B. jugivagans* for that gene. The combination of the above-mentioned genetic distances and distinct phenotypic characteristics (comparisons presented here below) of the specimen from Moravia de Chirripó provide us sufficient evidence to recognize it as a separate evolutionary unit and propose the following as a new species.

***Bolitoglossa aurae* sp. nov.**
Aura's golden salamander
(Figures 3–9)

Holotype. UCR 22842, an adult female from Costa Rica: Provincia de Limón: Cantón de Turrialba: Distrito de Chirripó: in the vicinity of Moravia de Chirripó, ca. 1300 m a.s.l., collected by Brian Kubicki, in the company of Maximo Flores and Aura Reyes on 22 March 2013.

Generic Placement. Assigned to the genus *Bolitoglossa* due to having fewer than 14 costal grooves and lacking a sublingual fold, and to the subgenus *Eladinea* based the molecular evidence presented herein.

Diagnosis. The combination of the following characteristics can be used to distinguish *Bolitoglossa aurae* from other described species in the genus: (1) unique coloration consisting of a light yellow ground color of the body, limbs, and tail, and having a dark brown middorsal stripe on the head and body and a pair of thin dark brown lateral stripes running from behind the eye posteriorly onto the tail (Fig. 3); (2) iridescent green chromatophores scattered throughout the dorsal surfaces of the head, body, and tail, at times resulting in an evident greenish hue; (3) relatively long prehensile tail, 57.9 % of total length, or 137 % of SL; (4) few premaxillary teeth, [3 total] in relation to SL; (5) numerous vomerine teeth [32] and maxillary teeth [60] in relation to SL; (6) relatively short truncate digits with moderate to extensive webbing on the hands and feet.

FIGURE 3. *Bolitoglossa aurae* **sp. nov.** Photograph taken by BK.

Comparisons. *Bolitoglossa aurae* is differentiated from members of the subgenus *Eladinea* by its 16S and cyt *b* mtDNA distances. Since *B. aurae* is only known to occur in Costa Rica and molecular evidence strongly supports it forming part of the *Bolitoglossa robinsoni* clade within the subgenus *Eladinea*, phenotypic comparisons are presented here only with respect to members of that clade (i.e., *B. aureogularis*, *B. jugivagans*, and *B. robinsoni*), which are endemic to southeastern Costa Rica and northwestern Panama.

Contrasting characteristics for *Bolitoglossa aurae* are presented in parentheses. *Bolitoglossa aureogularis* Boza-Oviedo *et al.,* 2012 can be distinguished from *B. aurae* by having a tan to reddish-brown dorsum with numerous dark brown streaks, black flanks and a pair of dirty white patches along the ventrolateral surface of the ventrum, and a dark midventral stripe (dorsum uniform light yellow with a thin dark brown middorsal stripe on the trunk, flanks light yellow to light brown [subject to metachrosis], ventrum uniform translucent yellow and lacking any evident or contrasting light or dark dermal pigmentation or midventral stripe); 6.5 costal folds between adpressed limbs [accuracy of costal folds count confirmed in Hertz *et al*. 2013] (4.5 costal folds between adpressed limbs); relatively short broadly rounded snout, SnL = 27.9 % of HeW (relatively longer and narrow truncate snout, SnL = 31.0 % of HeW); relatively fewer maxillary teeth, MT/SL = 1.2 (MT/SL = 1.5); more premaxillary teeth, 6 PMT (fewer premaxillary teeth, 3 PMT); and longer digits, LT3 = 32.6 % of FoW (shorter digits, LT3 = 24.4 % of

FoW). *Bolitoglossa jugivagans* Hertz *et al*., 2013 has a heavy concentration of darker pigmentation on the dorsal surfaces of the head, body, tail, and limbs that is reddish-brown with lighter streaks (dorsal surfaces of the head, body, tail, and limbs having a uniform light yellow coloration lacking any contrasting darker dermal pigmentation with the exception of a thin broken dark brown middorsal stripe on the body, which forms two thin separating lines between the base of the neck and the posterior/inner margins of the orbits); tips of the digits on the hands and feet are rounded (tips of the digits on the hands and feet truncate); relatively shorter tail, TaL = 124 % of SL (TaL = 137 % of SL); narrower internarial distance, IND = 23.3 % of HeW (wider internarial distance, IND = 27.6 % of HeW); longer snout, SnL = 41.9 % of HeW (shorter snout, SnL = 31 % of HeW); relatively shorter front limbs, FLL = 15.4 % of SL (relatively longer limbs, FLL = 20.5 % of SL); narrower feet, FoW = 65.1 % of HeW (slightly wider feet, FoW = 70.7 % of HeW); longer digits, LT3 = 46.4 % of FoW (shorter digits, LT3 = 24.4 % of FoW); relatively smaller eyes, EW = 72.2 % of SnL (relatively larger eyes, EW = 116.7 % of SnL). *Bolitoglossa robinsoni* Bolaños and Wake, 2009 has a heavy concentration of dark pigmentation on the dorsal surfaces of the head, body, tail, and limbs (dorsal surfaces of the head, body, tail, and limbs having a uniform light yellow coloration lacking any contrasting darker dermal pigmentation, with the exception of a thin broken dark brown middorsal stripe on the body, which forms two thin separating lines between the base of the neck and the posterior/inner margin of the orbits); large robust species with a tail shorter than SL, TaL = 94 % of SL (slender species with a longer tail, TaL = 137 % of SL); relatively fewer maxillary and vomerine teeth, MT/SL = 1.0, VT/SL = 0.3 (MT/SL = 1.5, VT/SL = 0.8); shorter trunk, AGL = 53.5 % of SL, with 2 costal folds between adpressed limbs (relatively longer trunk, AGL = 58 % of SL, with 4.5 costal folds between adpressed limbs); wide head, HeW = 30 % of AGL (narrower head, HeW = 24.7 % of AGL); longer limbs, HLL = 26.1 % of SL and FLL = 25.4 % of SL (relatively shorter limbs, HLL = 21.5 % of SL and FLL = 20.5 % of SL); wider hands and longer digits on the feet, HaW = 58.8 % of HeW and LT3 = 36.5 % of FoW (relatively narrower hands and shorter digits, Haw = 51.7 % of HeW and LT3 = 24.4 % of FoW); and relatively smaller eyes, EW = 82.3% of SnL (relatively larger eyes, EW = 116.7% of SnL).

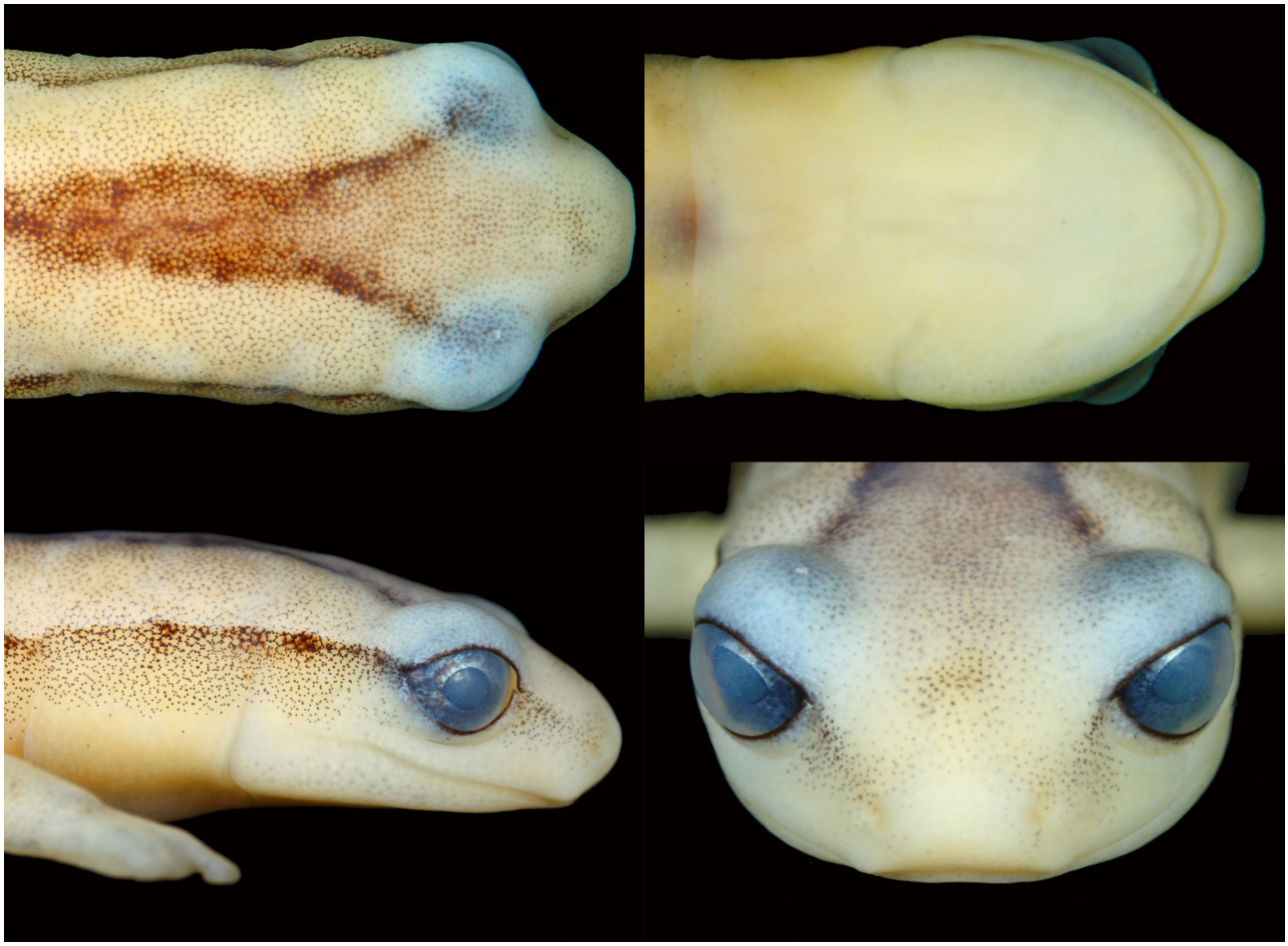

FIGURE 4. Images demonstrating the different aspects of the head of the holotype of *Bolitoglossa aurae* **sp. nov.** Photographs taken by BK.

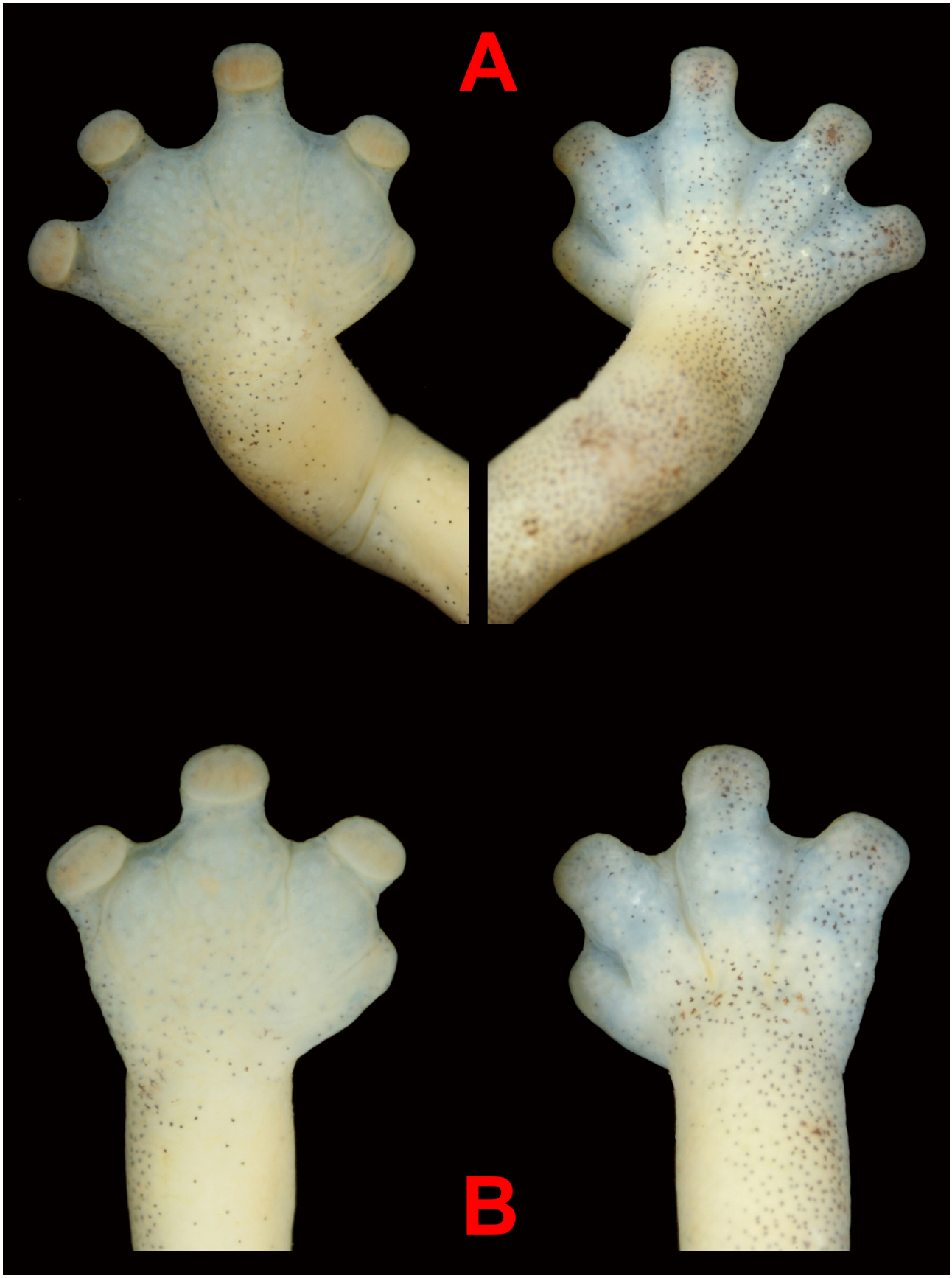

FIGURE 5. Dorsal and ventral views of the right foot (**A**) and right hand (**B**) of the holotype of *Bolitoglossa aurae* **sp. nov.** Photographs taken by BK.

Description of holotype. Adult female having a SL of 40.5 mm. Head slightly wider than neck and shoulders (Hew 5.8 mm, NeW 5.2 mm, ShW 5.6 mm), with greatest width of head just posterior to the articulation of the jaws; snout raised anterodorsally, truncate in dorsal outline, but rounded in profile; snout relatively long (SnL 1.8 mm, 4.4 % of SL), with nearly terminal non-protruding tiny nostrils (LNH 0.09 mm, RNW 0.03 mm) directed laterally; internarial area slightly convex. Eyes relatively large (EW = 116 % of SnL), protruding beyond dorsal and ventral outline of head, directed anterolaterally, with a distinct suborbital groove. Top of head flat and smooth, lacking any contrasting interorbital dermal structure; *canthus rostralis* distinct and rounded; intercanthal area slightly convex, loreal region slightly concave. Poorly developed, but discernible rounded cirri (nasolabial protuberances) present on the tip of the snout, protruding slightly beyond the margin of the upper lip. Gular fold well-defined, starting on the lateral portion of the neck, below the dark lateral stripe, curving slightly posteriorly initially and continuing down onto the ventrum, crossing as an overall straight crease but with slight anterior curve in midventral region. In life a red heart was visible beneath the skin at the midventral gular fold, lying mostly posterior to gular fold. In ventral view, tip of snout protruding markedly beyond edge of lower lip. No mental gland visible under skin of anterior intermandibular region. Nuchal groove well defined, starting just posterior to jaw articulation, about level with lower margin of eye; groove has very slight posterior orientation on side of head, extending onto ventrum just medial to posterior edge of mandibular bones, terminating as an anteriorly oriented curve (Fig. 4).

Sublingual fold absent; tongue circular in shape, lacking distinct notches. Vomerine teeth numerous (14/18), present in two arching rows, starting just posterior to choanae, curving postero-medially towards center of roof of mouth; rows of vomerine teeth not in contact medially; maxillary teeth numerous (29/31), present in two long arching single rows on the opposite/outer margins of the upper mouth, out of maxillary bones; premaxillary teeth relatively few (3), present as a short row at anterior tip of upper mouth, out of premaxillary bones. Choanae moderately large, teardrop shaped, with narrow groove emerging from each opening laterally, curving posteriorly beneath eye. On the medial roof of the mouth, lying posterior to the vomerine tooth rows, there is a triangular patch of paravomerine teeth, which are numerous.

Arms relatively short (FLL 8.3 mm, 20.5 % of SL), with forearm slightly hypertrophied compared to upper arm. Hands moderate-sized (HaL 2.7 mm, 30.7 % of VGS; HaW 3.0 mm, 51.7 % of VGS). Fingers II, III, and IV short and robust with truncate tips nearly equal in width (WF3 0.7 mm); terminal pads present on ventral tips of fingers II, III, and IV; Finger I not protruding beyond interdigital webbing; fingers II and IV with only distal phalanx free of webbing (LF2 0.6 mm), Finger III appearing to have at least 1.5 phalanges free of webbing (LF3 0.9 mm). Palmar surface smooth overall, but with evident dermal creases extending centrally from margins of interdigital webbing. Dorsal surfaces of all fingers with well defined interdigital grooves extending from margins of webbing to metacarpal region. Interdigital webbings on the hands are moderate to extensive, with distal margins of webbing concave in outline. Estimated webbing formula of right hand: **I** 0^{-} – 1 1/2 **II** 1 1/2 – 2^{-} **III** 2^{-} – 1^{+} **IV**. Relative lengths of fingers on right hand I < II < IV < III (Fig. 5 B).

Legs relatively short (HLL 8.7 mm, 21.5 % of SL), with little perceivable difference between thickness of upper and lower leg. Feet moderate-sized (FoL 3.3 mm, 37.5 % of VGS; FoW 4.1 mm, 70.7 % of VGS). Toes II, III, IV, and V short and robust with truncate tips nearly equal in width (WT3 0.7 mm), Toe III slightly longer than others (LT2 0.5 mm, LT3 1.0 mm); well-defined terminal pads present on ventral tips of toes II, III, IV, and V, weakly defined and smaller pad present on ventral tip of Toe I; tip of Toe I barely protruding beyond margin of interdigital webbing; toes II and V with only distal phalanx free of webbing, Toes III and IV appearing to have at least 1.5 phalanges free of webbing. Plantar surface smooth overall, but with evident dermal creases extending centrally from margins of interdigital webbing. Dorsally all digits of feet well defined with interdigital grooves traveling inward from webbing margins to metatarsals. Interdigital webbing on feet moderate to extensive, with the distal margins concave in outline. Estimated webbing formula of right foot: **I** 0^{-} – 1 1/2 II 1 – 2^{-} **III** 2 – 1 1/2 **IV** 1 1/2 – 1^{+} **V**. Relative lengths of toes on right foot I < II < V < IV < III (Fig. 5 A).

Body smooth, subcylindrical (wider than high) in cross section, relatively slender (TW 6 mm). Adpressed limbs separated by 4.5 costal folds, with 13 weakly discernable costal grooves between axilla and groin; costal grooves most visible on ventral and lateral portions of the body. Slight middorsal depression traveling longitudinally along length of body, starting at base of head, and becoming indiscernible on anterior third of tail length. Slight constriction at base of tail, between hind limbs and posterior edge of cloacal opening (body 5.2 mm in width at anterior margin of hind limbs, 4.1 mm at midsection of cloacal opening, and 4.4 mm just posterior to

cloacal opening). Tail long, cylindrical in cross section, evenly tapering to pointed terminus; 27 caudal grooves discernible on anterior 3/4^{ths} of tail, no grooves discernible on posterior 1/4th of tail.

Skin on all surfaces of head, body, limbs, and tail smooth.

FIGURE 6. Dorsal (**A**) and ventral (**B**) color in life views of the holotype of *Bolitoglossa aurae* **sp. nov.** Photographs taken by BK.

FIGURE 7. Images demonstrating the range of metachrosis that was observed in the holotype of *Bolitoglossa aurae* **sp. nov.** Photographs taken by BK.

Coloration in life. The holotype was kept alive in captivity for six months by BK to allow for natural history observations prior to being euthanized and preserved. During the months in captivity only very minimal metachrosis was witnessed, being limited to minor tonal shifts in the intensity of the yellow ground color of the head, body, limbs, and tail, and the shift in the flanks and limbs from light yellow to light to medium brown. The tonal shift of the background coloration of the dorsum of the holotype varied from Pale Greenish Yellow (86) to Medium Greenish Yellow (88). The only contrasting coloration on the dorsal surfaces of the head, body, or tail was the middorsal stripe, which started out as a pair of dark brown lines at the posterior/inner margins of the orbits and converge to meet as a single middorsal stripe just posterior of the gular fold, traveling along the mid-dorsum of the body and initiating a curve laterally towards the right side at about the anterior margin of the cloacal opening and connecting to the lateral line on the right side of the body just posterior of the vent (Fig. 6 A). The middorsal stripe consisted of a mixture of fine pigments between Brick Red (36) to Warm Sepia (40). Scattered evenly throughout

much of the ventral and dorsal surfaces of the head, body, limbs, and tail were minute dark melanophores, either in the form of very fine dots or a very fine reticulation; these dark melanophores were visible under magnification.

The highest degree of metachrosis was observed on the lateral margin of the head, body, tail, and limbs, where there was either a thin dorsolateral line starting at the posterior margin of the eyes and extending along the body and onto the tail nearing the tip, or at times appearing as a wider band of pale brown that started at the posterior margin of the nares, extended along the canthal ridge to the anterior corner of the eye, and continued as a wider, better defined pale brown band from the posterior corner of the eye, extending posteriorly along the body and onto the tail almost to the tip; when the wider lateral band was present there also was a pale brown coloration on the dorsal surfaces of the limbs (Fig. 7). The thin lateral lines varied with metachrosis from Brick Red (36) to Warm Sepia (40), indistinguishable in color from that of the mid-dorsal line; when present, the thicker lateral bands and dorsal surfaces of the limbs were Orange-Rufous (56) to Amber (51).

Light iridescent green chromatophores were observed scattered throughout the intense yellow dorsal surfaces of the head, body and tail, and at times resulted in an overall greenish hue to those surfaces; these pigments were especially concentrated and more consistently observed on the eyelids (Fig. 8). When the holotype was first discovered in the field it was immediately noteworthy due to the strong greenish-yellow tone of the dorsum.

FIGURE 8. Images showing the iridescent green chromatophores in the skin above the eyes and lower eyelids of the holotype of *Bolitoglossa aurae* **sp. nov.** Photographs taken by BK.

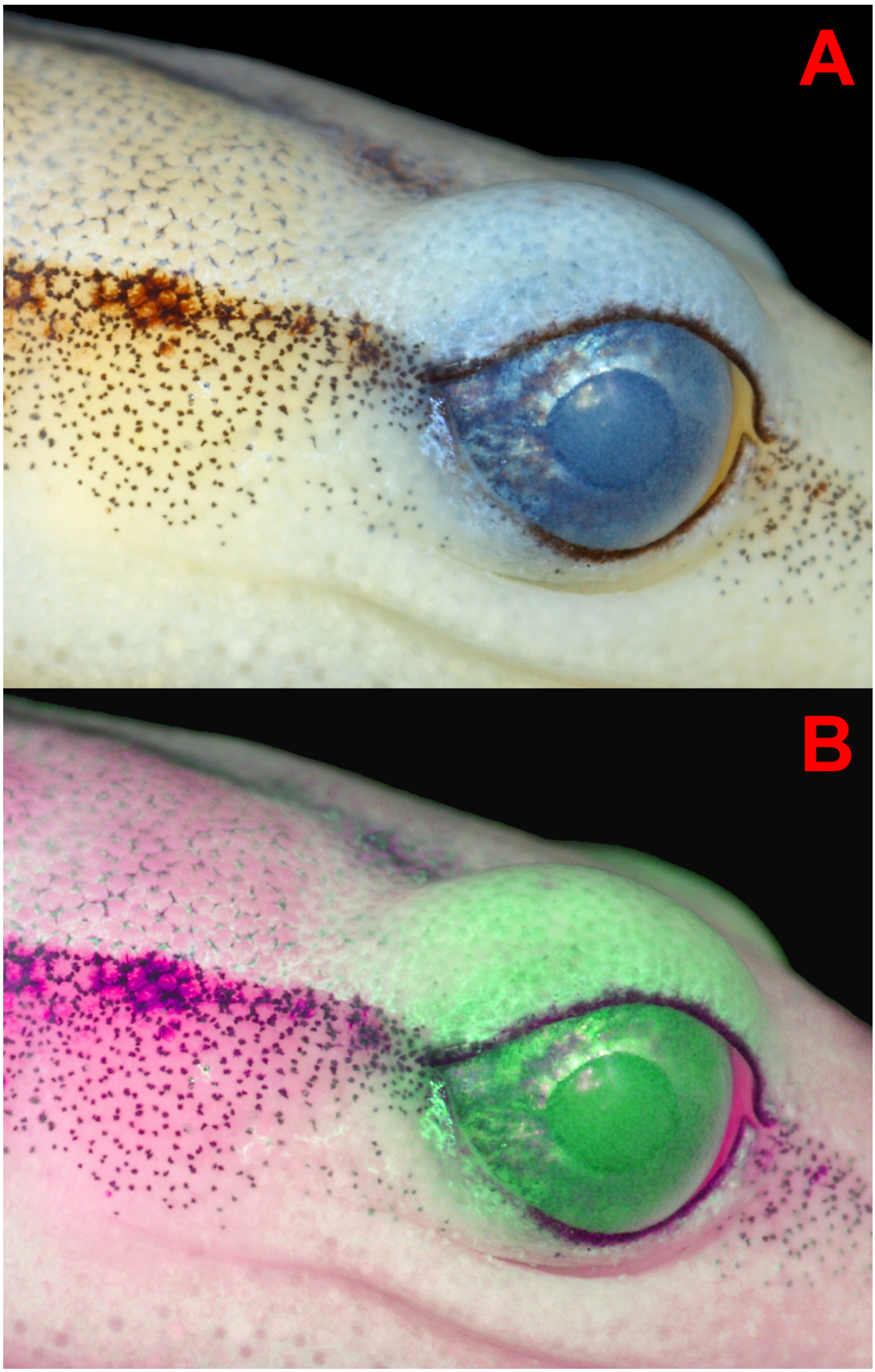

FIGURE 9. Images showing the light iridescent green chromatophores after three years of storage in 70% ethanol in the skin above the eyes and lower eyelids of the holotype. **a**) Natural colors and **b)** highlighting the presence of the chromatophores through hue modification. Photographs taken by BK.

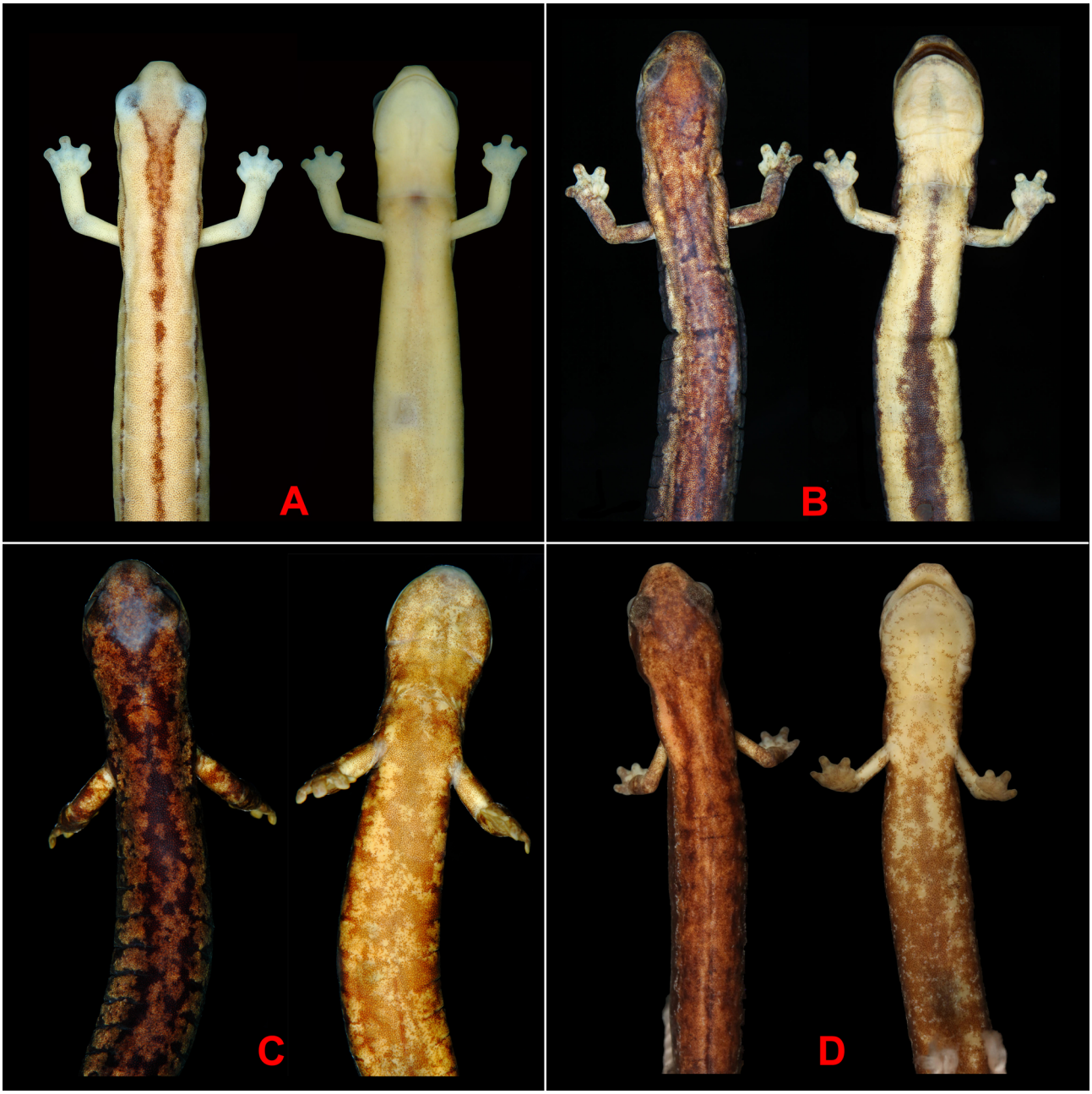

FIGURE 10. Anterodorsal and anteroventral images of the holotypes of the members of the *Bolitoglossa robinsoni* species group. **a)** *B. aurae* **sp. nov.** [UCR 22842], **b)** *B. aureogularis* [UCR 19893], **c)** *B. robinsoni* [UCR 11216], and **d)** *B. jugivagans* [SMF = 94467]. Photographs in plates A, B, and C taken by BK; photographs in plate D courtesy of Gunther Köhler.

The hands and feet typically had a Trogon Yellow (81) coloration, but at times would also present scattered concentrations of Orange-Rufous (56) pigmentation on the proximal dorsal region. The tips of the digits would also vary in coloration from Trogon Yellow (81) to Spectrum Orange (9).

The iris had a golden background color with darker pigments in the form of fine to moderate-sized spots; the dark spotting of the iris was Brick Red (36) to Warm Sepia (40). Additionally, there were also often heavy concentrations of Chartreuse (89) pigments in the iris; the concentration and intensity of the chartreuse pigments were subject to metachrosis.

The ventral skin of the head and body was uniformly translucent yellow, ranging in tone from Cream Color (12) to Light Orange Yellow (77); no contrasting lighter or darker pigmentation in the form of spots or other markings was ever observed (Fig. 6 B). Longitudinally, along the mid-ventral line, some scattered concentrations of iridophores were observed beneath the skin. Beneath the skin at the midventral gular fold, lying principally

posterior of the gular fold, the bright red corpus of the heart was visible. The heart appears to lack pigmentation in the pericardial sac, such as iridophores, thus the red coloration is caused by oxygenated blood. At times heavy concentrations of blood red capillaries were observed throughout the ventral surfaces of the head, body and limbs.

Coloration in ethanol. After three years in ethanol (70%), overall bright yellow coloration has faded to Light Buff (2) and Pale Pinkish Buff (3). The only noticeable contrasting colors on the entire specimen are the dark middorsal stripe, lateral stripes, and minute melanophores scattered throughout the skin surfaces, either in the form of tiny dots or as extremely fine reticulations; the minute melanophores are best visible under magnification. The middorsal stripe and lateral stripes are all constituted by diffuse Pratt's Rufous (72) pigmentation. The minute melanophores are Dark Grayish Brown (284). In the external layer of the skin above the eyes there is a contrasting pale bluish-white hue, which is likely in relation to the green iridescent pigments that were visibly concentrated in this region in life. Upon close inspection, this bluish-white hue is definitely present as a type of pigmentation in the dermal tissue and not reflective artifact from any pale coloration on the dorsal surface of the orbit itself (Fig. 9).

Measurements (in mm), tooth counts, limb interval, and percentages of the holotype. SL 40.5; Tal 55.6; TL 96.1; ShW 5.6; HeW 5.8; NeW 5.2; EW 2.1; SnL 1.8; JSL 4.1; LGFS 9.0; LNH 0.09; RNW 0.03; IND 1.6; NLP 0.7; ICD 3.2; HLL 8.7; FLL 8.3; TW 6.0; VGS 8.8; FSL 10.6; UHL 5.9; AGL 23.5; VL 3.0; HaW 3.0; HaL 2.7; LF2 0.6; LF3 0.9; WF3 0.7; FoW 4.1; FoL 3.3; LT2 0.5; LT3 1.0; WT3 0.7. Limb interval 4.5. Number of teeth PMT 3, MT 29/31, VT 14/18. Measurements in related percentages: VGS/SL 21.7 %; IND/HeW 27.6 %; AGL/SL 58 %; HeW/SL 14.3 %; Hew/AGL 24.7 %; SnL/ HeW 31 %; LNH/HeW 1.6 %; LNH/SL 0.2 %; RNW/HeW, 0.5%; RNW/SL 0.001 %; HLL/SL 21.5 %; FLL/SL 20.5 %; HaL/VGS 30.7 %; FoL/VGS 37.5 %; Haw/HeW 51.7 %; FoW/HeW 70.7 %; LT3/HeW 17.2 %; LT3/FoW 24.4 %.

Etymology. The name "*aurae"* is in dedication to Aura Reyes, the wife of BK, who co-discovered the holotype and has supported and encouraged BK's research and conservation efforts with the amphibians of Costa Rica for many years, this in addition to her own contributions to increase the knowledge of Costa Rica's amphibians made possible through dedicating her time to accompanying BK on numerous field trips, many of which consisted of enduring prolonged periods of cold conditions while being soaking wet within the cloud forests of Costa Rica to search for elusive anuran and caudate species. The name also alludes to the Latin *aureus,* meaning golden*,* for the yellow coloration the holotype possessed in life.

Habitat and natural history observations. The site where *Bolitoglossa aurae* was found is cloud forest, with an abundance of bryophytes and other epiphytes covering the arboreal structures. *Bolitoglossa aurae* was discovered within moss and decomposing organic material surrounding and intertwined in a root mass of a large orchid attached to a thin rotten tree that was still standing in the forest understory. The rotten tree fell to the ground as soon as a light amount of pressure was applied to it. The orchid, within which the salamander was hiding, had been growing at approximately 3 meters above the ground. *Bolitoglossa aurae* was found during a diurnal exploration. When the holotype was discovered it had a very evident and impressive greenish hue to the entire dorsal surface, a tone and intensity that was never witnessed again within the six months it was kept alive in captivity. It is possible that the green tone that was observed the day the holotype was collected was not seen again due to the individual being housed in a terrarium with a thick loose and humid layer of dead sphagnum moss, that was pale tan in color. Had the holotype of *B. aurae* been housed in a terrarium with an abundant supply of live green moss it is possible that the green hue observed when collected might have been observed again.

Inasmuch as *Bolitoglossa aurae* is only known from a single specimen fortuitously found curled up within an orchid root mass during the day, any assumptions about its natural history are speculative. With that said, however, it is probable that this species is given to living within thick moss mats and other epiphytic growth in arboreal habitats in the cloud forest.

The holotype was kept alive in captivity for six months prior to being euthanized and preserved; it was housed in a small terrarium (50 cm x 30 cm x 25 cm). The enclosure had a thick bed (ca. 10 cm thick) of dead sphagnum moss; no other structures were present in the enclosure to facilitate easy observation of the holotype and its activity and behavior. The individual showed a definite nocturnal behavior, often being observed crawling on the surface of the sphagnum moss or even the walls of the enclosure at night. During the day the holotype would burrow deeply within the loose moist sphagnum moss, only emerging after dusk. The holotype was fed a diet consisting of wild fruit flies (Family: Drosophiliidae). The holotype adapted very well to captivity, maintaining a relatively robust body and tail during the time it was in a terrarium, and would aggressively feeding on numerous fruit flies immediately following their introduction to the enclosure.

Distribution. *Bolitoglossa aurae* is known only from a single site within the Tropical Premontane Rain Forest life zone (Holdridge 1967) along the mid-elevation slopes of northeastern Cordillera de Talamanca, in the vicinity of Moravia de Chirripó, ca.1300 m (Fig. 1).

Remarks. With the addition of *Bolitoglossa aurae*, the number of salamanders known from Costa Rica is now elevated to 50, of which 27 belong to the genus *Bolitoglossa.* The relationships within *Bolitoglossa* were revised by Parra-Olea *et al.* (2004), who defined subgenera and species groups; however, all the species currently associated with the *B. robinsoni* clade (Bolaños & Wake 2009; Boza-Oviedo *et al*. 2012; Hertz *et al*. 2013) were described after the efforts by Parra-Olea *et al.* (2004). All known species of the *B. robinsoni* clade have been found at remote sites in the Cordillera de Talamanca in eastern Costa Rica and Cordillera Central of western Panama; we propose that the members of this clade be recognized distinctly as the *Bolitoglossa robinsoni* Species Group, currently containing four species: *B. aurae, B. aureogularis, B. jugivagans,* and *B. robinsoni* (Fig. 10).

Given the large expanses of remote and unexplored forest along the Caribbean slopes of the Talamancan mountains in eastern Costa Rica and northwestern Panama, it is likely that in future explorations more information will be made available regarding the distributional ranges of the members of this group, this in addition to the potential discovery and description of additional species. Furthermore, it is important evaluate the taxonomic status of all populations currently considered to belong to *B. robinsoni. Bolitoglossa robinsoni* was described from a type series of four individuals (holotype and two paratopotypes from Cerro Echandi, and one paratype from Cerro Burú), but Bolaños & Wake (2009) included additional referred specimens from four other sites in the Cordillera de Talamanca as tentatively belonging to this taxon. Bolaños & Wake (2009) noted that the different populations represented by their referred materials potentially might represent separate undescribed species. The DNA sequence of *B. robinsoni* that was included in our molecular analysis is from one of these dubious populations, Valle del Silencio. Nevertheless, as this was the only sequence available to represent this species as currently recognized, we included it for tentative comparison purposes. In order to obtain more clarity surrounding the actual taxonomic status of certain populations currently assigned to *B. robinsoni*, topotypic DNA sequences are needed.

The genetic distances among members of the *Bolitoglossa robinsoni* species group are relatively low in comparison to those suggested by Fouquet *et al.* (2007) for Neotropical frogs, in which those authors recommend 3% 16S divergence as threshold to define candidate species. Nevertheless, this threshold possibly underestimates the real diversity of Neotropical salamanders. In a recent phylogenetic study of the minute salamanders of the genus *Thorius* from the Mexican Highlands, two mitochondrial and two nuclear genes efficiently defined the species within the genus, however the 16S divergence among several species were equal or lower than 2% (Rovito *et al.* 2013). Despite the relatively narrow genetic distances among the members of the *B. robinsoni* species group we are confident that these distances are significant, especially when combined with their geographic proximities and robust phenotypic characteristics that define each species even further. Other members of the genus *Bolitoglossa* from within Costa Rica are also known to have similar narrow genetic distances among congeners, one example of a species well recognized for its phenotypic distinctness, but presenting a relatively low genetic divergence among some of its congeners (as low as 2.0 % 16S) is *Bolitoglossa splendida* Boza *et al*., 2012. Genetic distances of less than 2.5 % are actually common among several members of the *Bolitoglossa subpalmata* species group within Costa Rica (Boza *et al*. 2012), and despite these narrow genetic distances, the species of this group are well recognized as being distinct.

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APPENDIX I.

Additional Specimens Examined. UCR voucher number refers to the Museo de Zoología, Universidad de Costa Rica. CRARC refers to the Costa Rica Amphibian Research Center private collection.

Bolitoglossa aureogularis: COSTA RICA: Limón: UCR 19893 (holotype); Cartago: CRARC 0062.

Bolitoglossa gracilis: COSTA RICA: Cartago: UCR 9378 (holotype), CRARC 0219, CRARC 0210, CRARC 0213; Limón: CRARC 0072, CRARC 0073.

Bolitoglossa subpalmata: COSTA RICA: Cartago: CRARC 0007, CRARC 0008, CRARC 0009, CRARC 0157.

Bolitoglossa robinsoni: COSTA RICA: Puntarenas: UCR 11216 (holotype).