Description of a new species of the *Bolitoglossa subpalmata* group (Caudata: Plethodontidae) from Costa Rica

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The Costa Rican plethodontid salamander long known as *Bolitoglossa subpalmata* is a species complex that includes *B. subpalmata* of the Cordillera Central and adjoining areas, *B. pesrubra* of the Cordillera de Talamanca, *B. bramei* and *B. gomezi* from the Pacific slopes of the Panama–Costa Rica border region and a species from the extreme northern portions of the Cordillera de Talamanca and the Cerros de Escazú described here as *Bolitoglossa tica*. The new species is a gracile, semi-arboreal species that has a prominent head well delimited from the neck. It differs from the parapatrically distributed *B. pesrubra* in coloration, and it occupies lower elevations. Allozymes and mitochondrial DNA sequences distinguish it from other species in the complex.

Key words: biogeography, Central America, salamander, taxonomy

INTRODUCTION

The *Bolitoglossa subpalmata* clade in the subgenus *Eladinea* (Parra-Olea et al., 2004) comprises several closely related species from the uplands of Costa Rica and extreme western Panama: *B. subpalmata*, *B. pesrubra*, *B. gracilis*, *B. bramei* and *B. gomezi* and some additional undescribed species from the Costa Rica–Panama border region (García-París et al., 2000a; Wake, 2005). The enigmatic *B. diminuta* may be a member of this group as well (Robinson, 1976). The *subpalmata* clade is sister to a morphologically heterogeneous clade that includes *B. cerroensis*, *B. marmorea*, *B. epimela*, *B. sooyorum* and *B. minutula*, all occurring in mountainous central and eastern Costa Rica and western Panama.

Bolitoglossa pesrubra is by far the most studied of the roughly 230 species that comprise the neotropical plethodontid salamander fauna, although literature references almost exclusively use the name *B. subpalmata*; the independent status of these two species was established by García-París et al. (2000a). The former abundance of *B. pesrubra* in the uplands of the Cordillera de Talamanca in Costa Rica and its relative ease of maintenance under controlled laboratory conditions made possible studies of its ecology (Vial, 1968), reproduction (Houck, 1982), development (Collazo, 1996; Wake & Hanken, 1996) and genetics (Sessions & Kezer, 1991; Hanken & Wake, 1982). The *B. subpalmata* clade includes a diverse array of geographically delimited units located at high elevations (from 1500 to more than 3000 m) over most of the Costa Rican Cordillera Central and Cordillera de Talamanca and some minor upland areas. Extreme local polymorphism in body shape and coloration patterns has made it difficult to characterize these local units, but genetic differentiation is remarkable. Large genetic distances in both allozymes and mtDNA between geographically contiguous groups of populations have been recorded, and the existence of unnamed taxa noted (García-París et al., 2000a).

Analyses of allozyme and mtDNA data led to a new phylogenetic hypothesis, that there are four well-supported clades within the *B. subpalmata* clade (García-París et al., 2000a). A northern clade includes populations in the volcanic Cordillera Central and adjacent ranges and bears the name *B. subpalmata*. Two clades are distributed parapatrically along the Cerro de la Muerte region in the Cordillera de Talamanca. The most widely distributed species of high elevations in the region known as Cerro de la Muerte and extending at least as far as Cerro Chirripó is *B. pesrubra,* and the other, described below (*Bolitoglossa species B* of García-París et al., 2000a), has a more restricted distribution in mid-elevations along the northern slopes of the Cordillera de Talamanca and to the northwest in the neighbouring Cerros de Escazú. The fourth clade includes *B. bramei* and *B. gomezi* (which together correspond to *B. species C* of García-París et al., 2000a) from parts of the Talamancan uplands along the border between Costa Rica and northern Panama and in the Cordillera de la Costa along the Pacific versant, and undescribed species (Wake et al., 2007).

Here we describe a new species that is distinctive in molecular traits and morphology.

MATERIALS AND METHODS

Measurements were made using digital or dial callipers or a dissecting microscope fitted with an ocular micrometer; standard length (SL) was measured from the anterior tip of the snout to the posterior angle of the vent. Limb interval equals the number of costal interspaces between the tips of appressed fore- and hind limbs, measured in one-half

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Table 1. Localities, voucher information and GenBank accession numbers for the sequences and specimens used in this study. Numbers in brackets correspond to sequences used in previous studies: (1) Jackman et al., 1997; (2) García-París & Wake, 2000; (3) García-París et al., 2000a; (4) García-París et al., 2000b; (5) Parra-Olea et al., 2002; (6) Parra-Olea et al., 2004. Abbreviations: DBW: D. B. Wake field number; MVZ: Museum of Vertebrate Zoology collection (USA); UCR: Universidad de Costa Rica collection (Costa Rica).

	Name	Locality	Voucher	Cytb	16S
1	*B. (E.) gracilis*	Costa Rica: Cartago: Reserva Tapantí	MVZ229170	AF212067 (3)	AY526121 (6)
2	*B. (E.) gracilis*	Costa Rica: Cartago: Reserva Tapantí	MVZ229171	AF212068 (3)	AY526122 (6)
3	*B. (E.) pesrubra*	Costa Rica: Cartago: Salsipuedes, 19 km S of El Empalme	UCR12068	AF212069 (3)	AY526132 (6)
4	*B. (E.) pesrubra*	Costa Rica: Cartago: 22.7 Km SE of El Empalme, 2760 m	MVZ190923	AF212074 (3)	EU448104
5	*B. (E.) pesrubra*	Costa Rica: Cartago: Cerro Asunción, 37.1 km S of El Empalme, 3230 m	MVZ210361	AF212080 (3)	–
6	*B. (E.) pesrubra*	Costa Rica: Cartago: Cerro Asunción, 37.1 km S of El Empalme, 3230 m	MVZ210360	–	EU448105
7	*B. (E.) tica*	Costa Rica: Cartago: Macho Gaff	UCR12066	AF212088 (3)	AY526137 (6)
8	*B. (E.) tica*	Costa Rica: Cartago: 1.1 Km S of El Empalme, 2400 m	MVZ194858	AF212086 (3)	EU448106
9	*B. (E.) subpalmata*	Costa Rica: Alajuela: Cerro Pata de Gallo, 8 km S of San Ramón	MVZ194828	AF212091 (3)	EU448107
10	*B. (E.) subpalmata*	Costa Rica: Puntarenas: Monteverde Cloud Forest Preserve	MVZ229172	AF212094 (3)	AF416697 (5)
11	*B. (E.) cerroensis*	Costa Rica: San José: Cuericí, 5 km E of Villa Mills	DBW5123	AF199195 (2)	AF199233 (2)
12	*B. (E.) sooyorum*	Costa Rica: San José: 22.9 km SE of El Empalme	MVZ 190847	–	EU448108
13	*B. (E.) marmorea*	Panama: Chiriquí	MVZ 210286	U89631 (1)	AF218493 (4)
14	*B. (E.) epimela*	Costa Rica: Cartago: Turrialba	MVZ 181260	AF212097 (3)	AY526120 (6)
15	*B. (E.) minutula*	Costa Rica: Puntarenas: Las Tablas, Cerro Pando	MVZ 225870	AF212098 (3)	AY526124 (6)
16	*B. (E.) adspersa*	Colombia: Cundinamarca	MVZ 158485	AF212984 (4)	AF218492 (4)
17	*B. (E.) robusta*	Costa Rica: Alajuela: Salto El Angel	MVZ190830	EU448110	EU448109
18	*B. (B.) mexicana*	Belize: Toledo: Blue Creek	MVZ 191635	AF212099 (3)	AF177588 (4)

increments. Tooth counts are based on direct counts of clearly ankylosed teeth. Numbers of maxillary and vomerine teeth are summed for right and left sides. Institutional abbreviations are as listed in Leviton et al. (1985), except for UCR (Universidad de Costa Rica).

Sequences of 16S (548 bp) were obtained for six specimens representing the following taxa: *Bolitoglossa (Eladinea) pesrubra*, *B. (E.) subpalmata*, *B. (E.) tica sp. nov.*, *B. (E.) sooyorum* and *B. (E.) robusta*. An additional short fragment of cyt b (376 bp) was obtained for one specimen of *B. (E.) robusta*. These sequences were compiled together with previously analysed sequences from the *B. subpalmata* group (Table 1) to reconstruct a phylogenetic hypothesis of relationships for *B. tica sp. nov*. Extraction, amplification, sequencing and alignment of the gene products was performed as in Parra-Olea et al. (2004). Phylogenetic analyses were performed using the combined data set, which included 17 samples of *Bolitoglossa* subgenus *Eladinea*, plus *B. (Bolitoglossa)* *mexicana* as an outgroup. Treatments of data follow Parra-Olea et al. (2004).

Description of new species

Bolitoglossa tica, new species
Tico salamander, salamandra tica

Holotype. MVZ 203684, an adult male from 0.8–0.9 km SSW El Empalme junction on InterAmerican Highway 2, Prov. Cartago and San José, Costa Rica; 9.7137, –83.9507; 2360–2370 m elevation; collected by D.C. Cannatella, D.B. Wake, M.H. Wake and T.A. Wake, 7 July 1986.

Paratypes. MVZ 114121, 5.1 km N of El Empalme, Prov. Cartago, Costa Rica; MVZ 138895–98, 143836, 173377–78, 190918–19, 190921, 194858–59, all from vicinity of type locality, 2200–2400 m elevation; MVZ 203685, 203687, 203690, 203692, same data as holotype; MVZ 225894, UCR11771–72, 4.5 km NNE of Cañón, below La Damita , Prov. Cartago, Costa Rica, 2380 m elevation; MVZ 221313,

Table 2. Comparison of measurements of three species of *Bolitoglossa* from Costa Rica. Ranges; means in parentheses.

	Size (SL; mm)	Limb interval	SL/HW	Maxillary teeth	Vomerine teeth	Foot width (mm)
Males						
B. pesrubra	42.2–52.8 (46.1)	0–1 (0.5)	6.1–6.8 (6.4)	33–53 (42)	19–22 (20.1)	4.4–5.4 (5.0)
B. subpalmata	45.1–54.4 (48.2)	1–2.5 (1.6)	6.2–7.6 (6.6)	51–60 (48.0)	15–24 (19.8)	4.8–5.8 (5.2)
B. tica	37.7–59.7 (45.1)	0–1.5 (0.8)	6.3–7.1 (6.7)	39–59 (45.8)	19–34 (24,2)	3.6–5.5 (4.6)
Females						
B. pesrubra	41.7–57.8 (51.4)	2–3 (2.3)	6.7–7.5 (7.0)	44–57 (49.2)	17–28 (22.2)	4.4–5.6 (5.1)
B. subpalmata	52.0–58.1 (54.6)	2.5 (2.5)	6.5–7.3 (6.8)	37–65 (53.8)	18–21 (19.5)	5.4–5.8 (5.6)
B. tica	41.3–53.9 (46.8)	1–2.5 (1.9)	6.1–6.7 (6.4)	38–61 (46.0)	22–46 (28.9)	4.3–5.5 (4.8)

along branch of Río Agrés, Cerros de Escazú, approximately 5.5 km S of San Antonio de Escazú, Prov. San José, Costa Rica, 1745 m elevation; UCR 6544–45, Pico Blanco, Escazú, Prov. San José, Costa Rica; UCR 12065–66, 9.6861, –83.8944 near Macho Gaff, 2440 m elevation, Prov. Cartago, Costa Rica.

Diagnosis. A member of the *B. subpalmata* clade distinguished from all other members by differences in allozymes and mtDNA haplotypes, and by having a particularly well-defined head; in addition, distinguished from *B. subpalmata* by having fewer maxillary and more vomerine teeth, and from *B. pesrubra* by having more vomerine teeth (Table 2). It has smaller hands and feet than either *B. pesrubra* or *B. subpalmata*. It differs from *B. gracilis* in having a generally shorter but still long tail, in achieving larger size and in having much darker coloration. It is much larger than *B. diminuta*. It differs from all of these species in having prominent whitish spots on the venter, especially in the vicinity of the tail base.

Description. This is a salamander of moderate size that has long limbs. SL ranges from 37.7 to 59.7 mm (mean 45.1) in ten males, and from 41.3 to 53.9 (mean 46.8) in seven females. Tails are long and generally slender, usually exceeding standard length; SL divided by tail length ranges from 0.76 to 1.1 (mean 0.96) in males and from 0.88 to 1.0 (mean 0.97) in females. The head is moderately broad (Brame & Wake, 1972); SL divided by head width is 6.3–7.1 (mean 6.7) in males and 6.1–6.7 (mean 6.4) in females. The snout is truncate to bluntly rounded. Nostrils are small, and nasolabial protuberances are modest to well-developed (in sexually mature males). Eyes are moderate in size and relatively protuberant; they protrude slightly beyond the lateral margins of the head in ventral view. Teeth are moderate in number. There are 2–5 (mean 3.4) premaxillary teeth in males and 3–5 (mean 3.7) in females. In sexually mature males the teeth are long and anteriorly placed, lying outside the mouth where they protrude from the upper lip. Maxillary teeth of moderate size range from 39 to 59 (mean 45.8) in males, and from 38 to 61 (mean 46.0) in females. Vomerine teeth range from 19 to 34 (mean 24.2) in males, and from 22 to 46 (mean 28.9) in females. The trunk is relatively slender; distance across the shoulders is 3.9–5.2 mm (mean 4.6) in males, and 4.1–5.5 mm (mean 5.2) in females. Limbs are relatively long; limb interval ranges from 0 to 1.5 (mean 0.8) in males, and from 1 to 2.5 (mean 1.9) in females. Hands and feet are relatively large and broad (3.6–5.5 mm, mean 4.6 in males, 4.3–5.5 mm, mean 4.8 in females); digits are moderately webbed (Wake & Brame, 1969), with two or more phalanges of the longest digits free of webbing. Digits are bluntly tipped, and all digits can bear subterminal pads that are especially well developed on the longest digits. Fingers, in order of decreasing length, are 3-4-2-1; toes are 3-4-2-5-1. Postiliac glands are generally obscure. Mental glands (indicating sexual maturity) are present in males larger than 47.4 mm (absent in 4 paratypes between 43.1–47.3 mm); they are
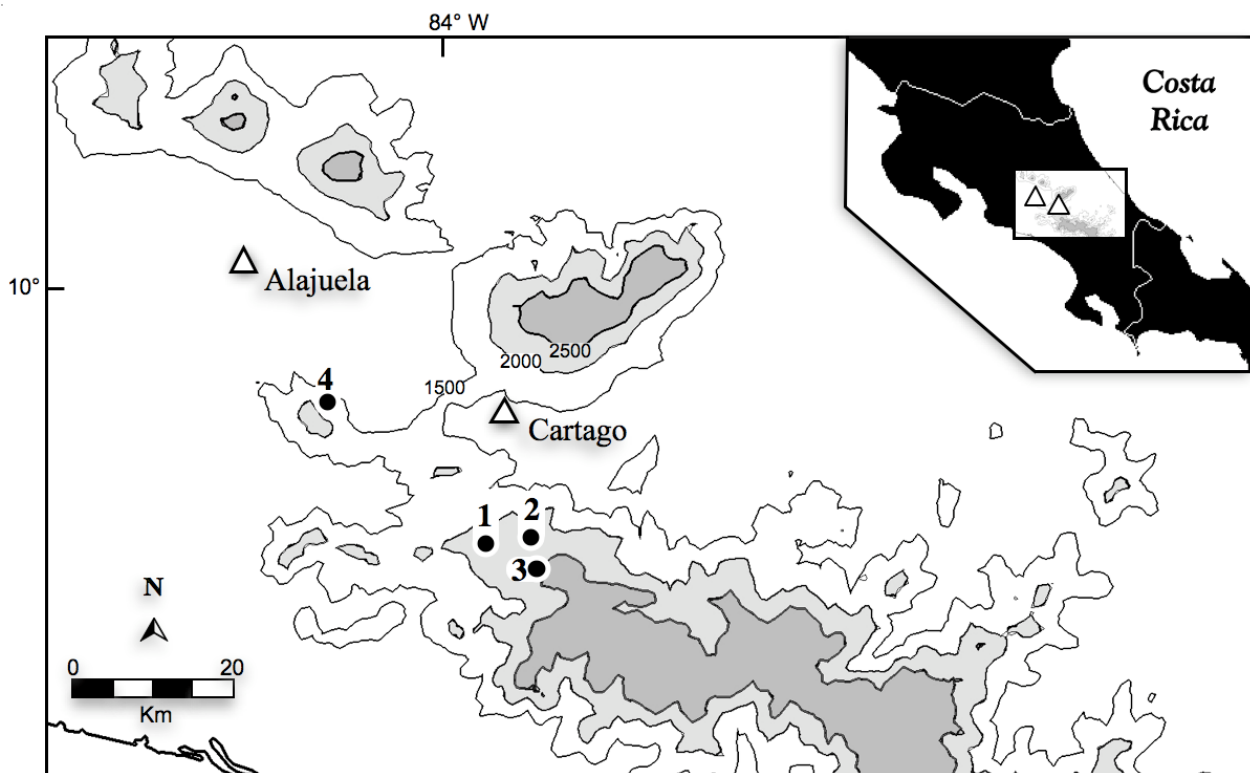

Fig. 1. Habitus in life of *Bolitoglossa tica*. Top: holotype from 0.8–0.9 km SSW of El Empalme. Centre: adult male active at night, photographed in situ, from near Macho Gaff (UCR 12065). Bottom right and left: two juvenile specimens showing diversity of coloration patterns from 4.5 km NNE of Cañón, below La Damita.

Fig. 2. Geographic distribution of *Bolitoglossa tica*. Numbers indicate the following: 1) Type locality: 0.8–0.9 km SSWof El Empalme junction on InterAmerican Highway 2, Prov. Cartago, 2360–2370 m; 2) Génesis II, 4.5 km NNE of Cañón, below La Damita; elevation 2360 m, Prov. Cartago; 3) near Hotel Tapantí; 4) along branch of Río Agres, Cerros de Escazú, approximately 5.5 km S of San Antonio de Escazú; elevation 1745 m, Prov. San José, Costa Rica.

large and oval, with dimensions of 2.3–3.3 mm (mean 2.7) wide and 1.9–3.5 mm (mean 2.5) long.

Measurements of the holotype (in mm) and tooth counts. Head width 7.8; snout to gular fold (head length) 12.5; head depth at posterior angle of jaw 4.0; eyelid width 1.3; eyelid length 3.2; anterior rim of orbit to tip of snout 3.2; horizontal orbit diameter 2.2; interorbital distance between angle of eyes 3.8; interorbital distance between eyelids 2.2; length of groove extending posteriorly from eye 2.6; distance between nuchal groove and gular fold 4.2; snout to forelimb 15.4; distance separating external nares 2.7; distance separating internal nares 2.1; snout projection beyond mandible 1.1; snout to posterior angle of vent (standard length) 52.5; snout to anterior angle of vent 48.1; axilla to groin 27.6; shoulder width 4.7; number of costal interspaces between appressed limbs 0.5; tail length 48.2; tail width at base 2.7; tail depth at base 3.5; forelimb length (to tip of longest finger) 12.1; hind limb length 13.4; hand width 4.5; foot width 5.5; length of fifth toe 1.0; length of third toe 1.9; distance between vomerine teeth and parasphenoid tooth patch 0.4; width of mental gland 3.3; length of mental gland 2.8. Number of teeth: premaxillary 5; maxillary 22–25; vomerine 8–11.

Coloration (in life). Notes for 14 specimens collected at the type locality on 7 July 1986 indicate that the general colour was a flat reddish brown dorsally with a darker tail, especially the tail venter. The largest specimen had an obscure reddish brown stripe over each shoulder that dis-

appeared about midbody. A brownish red mid-dorsal stripe appeared at about the shoulder level and gradually broadened, only to teminate abruptly at the tail constriction. The tail was very dark. The tip of the snout and the area in front of the eyes was pale tan, and the iris was a bright gold-grey. Ventrally the ground colour was dark shiny black, with speckles of bright white concentrated in the pelvic region. The throat was a bit lighter than the rest of the ventral surface. Three of the largest specimens were relatively dark and one was almost solid black dorsally; the others had reddish brown highlights and partial stripes. All were darker ventrally and all had whitish speckles or lichen-like patches, all smaller than the eye, on the venter with the heaviest concentration near the cloaca and on the tail and pre-pelvic region, as well as the gular area. The iris was grey-gold.

There may be some geographic variation (see Fig. 1 for a view of overall diversity within the species). A juvenile (approximately 32 mm SL) from the Cerros de Escazú area was very dark reddish brown, nearly black on the flanks, and was streaked with black dorsally. A dorsolateral reddish brown stripe or band started at the eye and extended over the shoulder along the back and on to the tail. The stripe contained a line of whitish speckles with brassy highlights. The tail was mottled dark reddish brown and black with brassy highlights. The venter was very dark brown-speckled and flecked with very fine brassy pigment and much less numerous coarser guanophores, which made a few bright white spots. The tail venter was
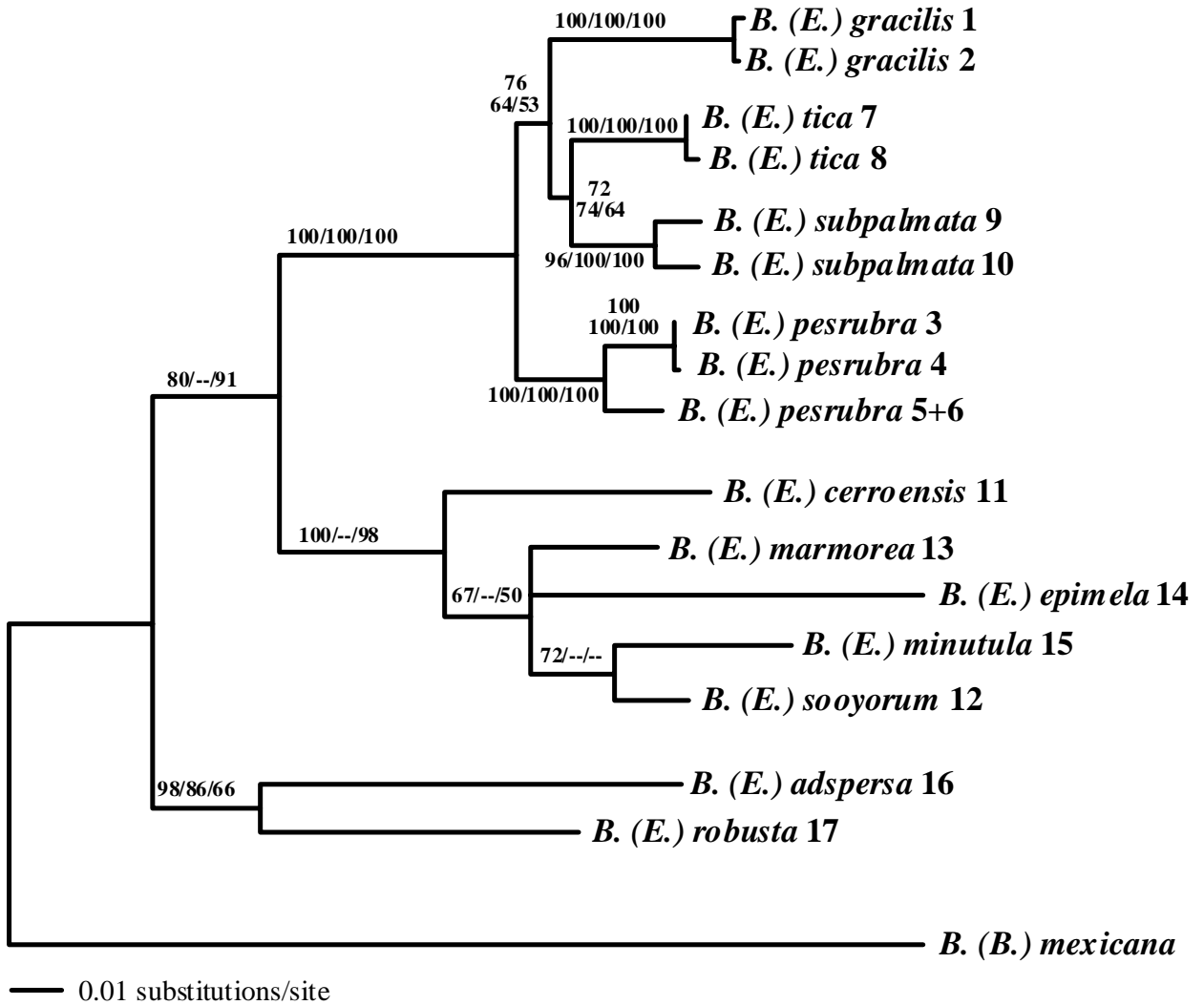
Fig. 3. Phylogenetic relationships of *Bolitoglossa tica* based on Cytb and 16S mtDNA sequence data. One of the two trees obtained using ML (–lnL = 4656.1324), which differ in the internal structure of the *B. epimela* clade. Numbers on branches correspond to non-parametric bootstrap pseudoreplicates (ML: 100, MP: 1000, ME: 1000). See Table 1 for locality information.

nearly black but had more whitish spots than the trunk venter. Tips of the nasolabial protuberances were cream in colour. An adult female from Cañón, below La Damita, observed on 31 May 1994, had a broad reddish brown dorsal band mottled with darker brown. Numerous tiny punctate to semireticulate cream-coloured guanophores were present on the lateral and ventral surfaces, and much larger, bright white spots or patches were present. The facial area was mottled reddish brown, cream and black. The tail venter was dark brown with a streak of bright white.

Coloration (in alcohol). The holotype is dark grey-black dorsally with a narrow tan stripe that extends from the nape of the neck to the end of the body, abruptly terminating at the base of the tail. A pair of similarly coloured broad stripes occurs in the shoulder region, lateral to the main stripe. The stripe is irregularly bordered and interrupted in places by the encroaching ground colour. The head is somewhat lighter than the trunk, and is dark grey-brown, becoming pale grey at the end of the snout and on the relatively pronounced nasolabial protuberances. The

limbs are a little lighter than the body, and the hands and feet are lighter still, pale grey mottled with darker grey. The limbs are mottled with some yellowish spots. The tail is uniformly dark grey-black, dorsally and ventrally. The venter of the trunk is also grey-black, just a little lighter than the flanks, and the gular region is pale grey. The mental gland is large and prominent, and is nearly devoid of pigment.

Habitat and geographic range. This species is known from the northern slopes of the Cordillera de Talamanca between 2200 and 2500 m, as well as from the Cerros de Escazú, isolated to the northwest, which form the southern boundary of the Meseta Central (Fig. 2). In the Cerros de Escazú the species descends to at least 1745 m. The habitat of *B. tica* was discussed by García-París et al. (2000b; as *Bolitoglossa sp. B*). The vegetation in the El Empalme area corresponds to the transition between the lower montane and montane rainforest of Tosi (1969), and between the subtropical montane rainforest and subtropical cloud forest of Gómez (1986). The mean annual temperature is 12–15 °C and between 1825 and 2300 mm of

rain falls each year, with a moderate seasonal deficit between January and April.

The species is relatively arboreal in its habits. It has frequently been found in bromeliads, and has been observed at night climbing on ferns (Lycopodiaceae) and other vegetation from 15 cm to 3 m above the ground. However, it has also been taken under surface litter, under the bark of logs and on road banks at night. In July 1986, 14 individuals were observed in one day at the type locality, a disturbed oak forest with abundant arboreal bromeliads. Of these, eight were obtained from inside bromeliads, one from under a fallen bromeliad, one under the bark of a stump, one in a rotten log and the remainder in leaf litter or under surface debris. Field observations compared specimens of the new species with *B. subpalmata* and *B. pesrubra*, and noted that they look and act differently. Members of this species are generally quick and lively, and they appear large-headed and slender.

Etymology. This species is named in honour of the Costa Rican people, who refer to themselves as "ticos", in recognition of their leadership in habitat preservation and conservation.

Comparisons. Coloration is subject to substantial variation in this species, with patterns that range from nearly solid dark brownish black to light grey or ochre with various dorsal highlights of brighter yellowish, orange or reddish colour. Some individuals have a reddish dorsal band that is highly irregular and much interrupted by encroachment of the darker ground colour. The ground colour of the venter is dark blackish, especially the tail. Most individuals have irregular spots or lichen-like patches of whitish pigmentation on the venter, especially in the vicinity of the hind limb insertions and tail base. Neither of the two commonest colour patterns characteristic of *B. pesrubra* are found. These are the "pesrubra" pattern, consisting of a dark brown dorsum variously mottled or streaked with lighter and darker tan, brown or black and having reddish to red-orange limb insertions, or entire limbs including hands and feet, and the "torresi" pattern, consisting of a black ground colour and a more superficial overlay or "frosting" of silvery, silvery-grey or whitish coloration arranged in patches of varying size and shape (Taylor, 1952).

DISCUSSION

The *Bolitoglossa subpalmata* clade inhabits moderate to high elevations (above 1500 m) in humid cloud forests and more upland forests and paramo-like habitats (to over 3300 m) in the mountains of Costa Rica and western Panama. This distribution encompasses two disjunct geographic units. The northern unit includes populations from Volcán Cacao to Volcán Turrialba along the Cordillera de Guanacaste, Cordillera de Tilarán and Cordillera Central, as well as in the small and isolated Cordillera de Aguacate. All of these populations are assignable to *B. subpalmata* (*sensu stricto*). The second unit includes all populations located in the Cordillera de Talamanca from the isolated northwestern Cerros de Escazú to the mountains east of Volcán Barú in Chiriquí,

western Panama. Based on general morphology, this clade (as the *B. subpalmata* group) was placed in the *alpha* section of *Bolitoglossa* by Wake & Lynch (1976). It is one of four main clade groups in the subgenus *Eladinea* (Parra-Olea et al., 2004), and is a sister-taxon of the *B. epimela* clade, which includes co-occurring species in the Cordillera de Talamanca (*B. epimela*, *B. cerroensis*, *B. compacta*, *B. marmorea*, *B. minutula* and *B. sooyorum*). Photographs of *B. subpalmata*, *B. gracilis*, *B. diminuta*, *B. pesrubra*, *B. epimela*, *B.cerroensis*, *B. minutula* and *B. sooyorum* are published in Savage (2002), and can be compared with our pictures of *B. tica*.

In the past, salamanders of the *subpalmata* clade were abundant. These were long included in a single taxon, *B. subpalmata* (e.g. Dunn, 1926). Taylor (1952) recognized some of the diversity within the complex and described two species, *B. pesrubra* and *B. torresi*, from the high central region of the Cordillera de Talamanca (Cerro de la Muerte). Subsequently Vial (1966) reduced these two to the synonymy of *B. subpalmata*, based on the lack of morphological differentiation. The first genetic studies reported high levels of differentiation in allozymes (Hanken & Wake, 1982). More extensive studies including both allozymes and mtDNA sequences led to the conclusion that several species-level taxa were represented, and the name *B. pesrubra* was resurrected for the high Talamancan populations (García-París et al., 2000a). In the meantime, another member of the complex, *B. gracilis*, was described by Bolaños et al. (1987) from lower elevations (approximately 1280–1500 m) in the Tapantí valley of the Río Reventazón, which drains the northern Talamancan region. This species appears to be truly rare, because it has been encountered infrequently despite intensive sampling in the Tapantí region. A third species, the even rarer, tiny *B. diminuta*, is known from only two adult specimens (as well as two clutches of eggs, one partly hatched). It also occurs in the Tapantí region, between 1300 and 1650 m elevation. Although this species was tentatively placed in the *B. epimela* group by Parra-Olea et al. (2004), molecular data are scanty and its relationships are uncertain.

García-París et al. (2000a) studied 19 proteins from five populations of *B. subpalmata*, one population of *B. tica*, one population of *B. gracilis*, one population of *B. bramei* and seven populations of *B. pesrubra*. In addition, a single specimen of *B. diminuta* was used. We reexamine these data for the first three species and present information that could not be presented because of the condensed nature of their publication. Population sizes for *B. subpalmata* were: Volcán Turrialba 3, Volcán Irazú (Las Nubes) 1, Volcán Barva (Braulio Carillo) 10, Volcán Poás 4, Cordillera de Aguacate (Cerro Pata de Gallo) 10; for *B. tica* 7, and for *B. gracilis* 1. Values of D_N (Nei, 1972) were 0.28–0.45 between *B. tica* and nearby populations of *B. pesrubra* (the smallest geographic distance between samples is to a population approximately 20 km southeast of the *B. tica* type locality). The lowest genetic distance from *B. tica* to any other species is to *B. gracilis* (D_N=0.16), but the latter is a sample of only one so the metric is suspect. There is one "fixed" difference (i.e. no alleles shared between taxa for one protein) between

these two taxa, which are closely parapatric. Genetic distances from *B. tica* to *B. subpalmata* range from 0.21 (to Volcán Irazú, also a sample of only one) to 0.43 (Volcán Turrialba); these are slightly less than the distances to *B. pesrubra*. While *B. tica* was long included in *B. pesrubra* (both as *B. subpalmata*) (Vial, 1968), the species are well differentiated in allozymes (D_N=0.28–0.45; García-París et al., 2000a). Differentiation within both *B. pesrubra* and *B. subpalmata* is great (see García-París et al., 2000a, Fig. 5). The Pata de Gallo population of *B. subpalmata* is more similar to *B. tica* (D_N=0.243) than to other populations of *B. subpalmata* (D_N=0.33–0.47 to the three volcano populations). There are three "fixed" differences between Pata de Gallo and *B. tica*, and from two to three fixed differences between Pata de Gallo and the volcano populations (suggesting that the Pata de Gallo population should be considered a candidate for species status). There are from one to three "fixed" differences between volcano populations of *B. subpalmata* and *B. tica*. Multidimensional scaling of genetic distances shows *B. tica* (referred as "Emp" in Fig. 5 in García-París et al., 2000a) somewhat closer to *B. subpalmata* than to *B. pesrubra*.

The genetic distances between *B. gracilis* and *B. subpalmata* range from 0.23–0.35, with two to four "fixed" differences. Data are available for a single specimen of *B. diminuta*, which differs by D_N=0.29 (four "fixed" differences) from *B. tica*, D_N=0.24 (five "fixed" differences) from *B. gracilis*, and D_N=0.27–0.40 from *B. subpalmata*.

An earlier allozyme study (Hanken & Wake, 1982) including *B. tica* (n=11) and a population of *B. subpalmata* (Volcán Poás, n=7) recorded D_N=0.33, nearly the same (D_N=0.34) as in the newer study using different specimens and some different proteins. The ranges of *B. tica* and *B. subpalmata* are discontinuous, with the latter generally distributed on the north side of the Meseta Central (which is about 1000 m high).

A phylogenetic hypothesis (maximum parsimony) based on sequences of the mitochondrial cytochrome b gene showed support (99 bootstrap, bs) for the clade as a whole (García-París et al., 2000a), and for the monophyly of *B. pesrubra* (nine samples), *B. subpalmata* (five samples) and *B. tica* (four samples) (99–100 bs). Relationships among the species that were studied (*B. subpalmata*, *B. pesrubra*, *B. gracilis*, *B. tica* and *B. bramei*, the last two identified as species B and C, respectively) were unresolved. However, *B. subpalmata* and *B. tica* may be sister taxa (decay index of two but no bootstrap support). A more extensive study of cytochrome b and 16S RNA sequences found strong support for the clade but relationships among *B. subpalmata*, *B. pesrubra*, *B. tica* (identified as *Bolitoglossa sp. 4*) and *B. gracilis* were unresolved (Parra-Olea et al., 2004).

We have added some new 16S mtDNA data for *Bolitoglossa tica*, *B. pesrubra*, *B. subpalmata* and *B. sooyorum*. The analyses (ML, MP, ME) of these data together with previously published sequences for the *B. subpalmata* group provide support for a *B. subpalmata* clade as previously reported (Parra-Olea et al., 2004) (Fig. 3). We find *B. tica* and *B. subpalmata* to be sister taxa (72, 74, 64, 71 bs). In turn, they form a sister group to *B. gracilis* (76, 64, 53 bs), and *B. pesrubra* is the sister taxon to

these three species (100 bs). The sister group to the *subpalmata* group as a whole is the *epimela* group (80, 91 bs) (*B. cerroensis*, *B. epimela*, *B. marmorea*, *B. minutula* and *B. sooyorum*. The *epimela* group is well supported (100, 98 bs) except for the inclusion of *B. sooyorum* in MP analyses (a problem of lack of cytb data for this species), but largely unresolved.

It is not surprising that the species of this group are so similar in general morphology, given their close relationships. However, there are differences in coloration, ecology, behaviour and altitudinal distribution. The only known sympatry is between *B. gracilis* and *B. diminuta*, but *B. tica* might overlap with both (at present there is an altitudinal gap of more than 500 m separating them). We expect *B. tica* and *B. pesrubra* will be found in sympatry, because they are separated by only a few kilometres and by about 100 m in altitude. Closely related species of plethodontid salamanders often hybridize when they come into contact, but if any hybridization occurs it cannot be very much, because *B. tica* and *B. pesrubra* are not clearly sister taxa and they approach each other closely in distribution without showing any signs of genetic interchange. There are two to three "fixed" genetic differences between *B. tica* and any given population of *B. pesrubra* (although only one of 19 loci shows a difference between all samples of the two species), and there are no haplotypes shared by the two species for the cytochrome b gene, sampled in nine populations of *B. pesrubra* and four of *B. tica*. These haplotypes differ by a minimum of 6.6% (K2P).

The northern Cordillera de Talamanca has an unusually large salamander fauna (Wake, 1987, 2005; García-París et al., 2000a; Hanken et al., 2005; Wake et al., 2007). What is unusual about the local assemblages of salamanders in this area is that they include clusters of closely related species that are sympatric or narrowly parapatric, suggesting that this region has been an active centre of species formation. Endemism is high in the area. In the immediate vicinity of the type locality of *B. tica*, two other species occur, *Oedipina altura* and *Oedipina paucidentata*. The former is known from the type series (collected in 1959; Brame, 1968) and a couple of additional specimens, while the latter is known only from the type series (also collected in 1959; Brame, 1968) (DBW, unpublished data). In the Tapantí region, immediately downslope (to an altitude of 1200 m) to the north and east of the El Empalme sites, other endemic species occur: *B. gracilis*, *B. diminuta*, *B. obscura*, *Nototriton tapanti* and *N. picadoi*. Also at Tapantí is the rare *B. epimela*. Just upslope from the El Empalme area occur the endemic species *B. cerroensis* and *B. sooyorum*. A rare species that occurs from the Cerros de Escazú to the Cerro de la Muerte area is *B. nigrescens*. The only relatively widespread species in the region are the formerly abundant *B. pesrubra*, which is a high elevation endemic, *Oedipina uniformis*, which is present in the Cerros de Escazú and Tapantí region, and *B. robusta*, which is widespread at elevations generally below 1500 m to the northwest, north and southeast (Savage, 2002).

Taxonomic work on Costa Rican *Bolitoglossa* is not completed. Savage (2002) drew attention to populations

assigned to *B. subpalmata* from Cerro Cacao and the Cordillera de Tilarán, suggesting that they might represent undescribed species. A population at Monteverde, in the Cordillera de Tilarán, studied by García-París et al. (2000a), clusters with other samples of *B. subpalmata* with high support (bs 99), but it is sister to the remaining samples. The divergence is relatively low (K2P distances range from 0.023 to 0.033 to the other samples). Accordingly, we assign the Cordillera de Tilarán populations to *B. subpalmata*. Members of this complex that occur in the Cordillera de Guanacaste (one adult from Volcán Cacao in MVZ, one juvenile from Volcán Rincón de la Vieja in UCR) lack tissue samples. The MVZ specimen is a long-tailed, slender, arboreal animal. We tentatively assign these specimens to *B. subpalmata*.

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