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A NEW SPECIES OF *ELEUTHERODACTYLUS* (ANURA: LEPTODACTYLIDAE) FROM TOBAGO, WEST INDIES AND ITS MORPHOMETRIC AND CYTOGENETIC CHARACTERIZATION

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Abstract. Populations of *Eleutherodactylus* on the island of Tobago, hitherto regarded as conspecific with *E. terraebolivaris* Rivero, differ from that species and from all other Eastern Caribbean and northern South American members of the genus by their vocalizations, morphology, sexual size dimorphism, and chromosome complement. These populations thus comprise a new species, endemic to Tobago and the putative sister taxon of *E. terraebolivaris*. The new species occurs abundantly in the forests of the Main Ridge, at elevations ranging from sea level to above 500 m. Our data indicate that this species is most likely the contemporary terminus of a lineage that may have diverged since the Late Miocene. The presumed ancestral species probably inhabited a then continuous mountain range extending from northern Venezuela via the Trinidadian Northern Range to Tobago. Given the geological time frame, this species could assist in determining divergence time in a phylogenetic context. It is yet another example for the faunal link between the Eastern Caribbean and northern South America.

Resumen. Las poblaciones de *Eleutherodactylus* de la isla de Tobago, consideradas hasta ahora conespecíficas con *E. terraebolivaris* Rivero, difieren de esta especie y de todos los demás miembros del género en el Caribe oriental y norte de Sudamérica por sus vocalizaciones, morfología, dimorfismo sexual en tamaño, y complemento cromosómico. Estas poblaciones comprenden así una nueva especie, endémica a Tobago y taxón hermano putativo de *E. terraebolivaris*. La nueva especie ocurre abundantemente en los bosques de la Cordillera Principal en elevaciones que van desde el nivel del mar hasta más de 500 m. Nuestros datos indican que esta especie es muy probablemente el término contemporáneo de un linaje que puede haber divergido desde el Mioceno tardío. La presunta especie ancestral probablemente habitó una cadena montañosa entonces continua extendiéndose desde el norte de Venezuela vía la Cordillera del Norte en Trinidad hasta Tobago. Conocida la edad geológica, esta especie ayudaría a determinar la divergencia dentro del contexto filogenético. Es este otro ejemplo de la conexión entre la fauna del Caribe oriental y la del norte de Sudamerica.

Key Words. Amphibia; Anura; Tobago; Caribbean; Biogeography; Systematics; Morphometrics; Cytogenetics; *Eleutherodactylus*; New species.

Tobago is a small (301 km^2) continental-shelf island in the southeastern Caribbean (Fig. 1). It is the easternmost extension of a historically (Mesozoic) continuous rock formation that included the Cordillera de la Costa and the Península de Paria in present-day Venezuela, as well as the Northern Range of Trinidad (Frost and Snoke 1989; Hardy 1982; Stéphan et al. 1980). Previous studies or checklists of the anuran fauna on Tobago have been scarce and none are comprehensive (e.g., Barbour 1916; Brongersma 1956; Mertens 1970, 1972, 1974), with the notable exception of the detailed review by Hardy (1982). To date, 12 species of frogs are known from Tobago (Table 1), including a single endemic species (*Colostethus olmonae*) and several taxonomic uncertainties (Cannatella and Lamar 1986;

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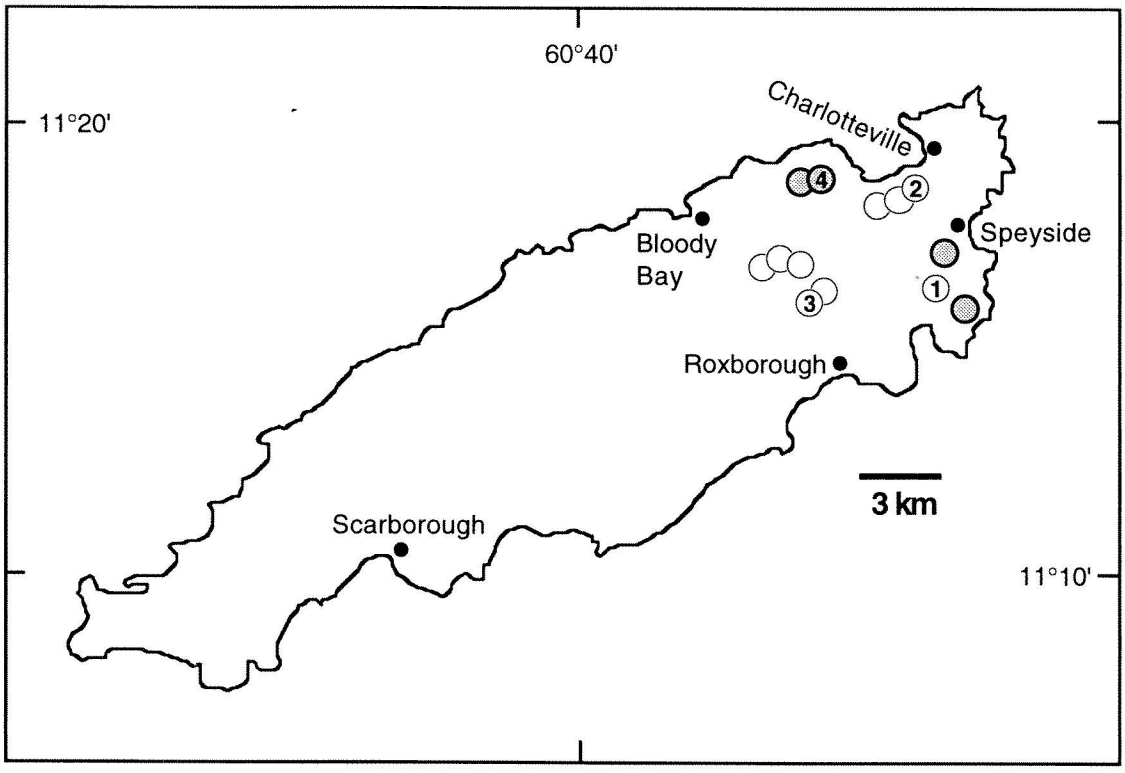

Figure 1. Map of Tobago with numbered localities for *Eleutherodactylus charlottevillensis* n. sp.: 1—Kings Bay Waterworks, 2—1.5 km SW Charlotteville, 3—Mileage marker 3.5 on Roxborough - Bloody Bay Road, 4—Hermitage. Specific collection localities are indicated by circles. Historical localities not verified by us are shaded.

Hardy 1982, 1983*a*,*b*, 1984).

Frogs of the genus *Eleutherodactylus* are ubiquitous in the Caribbean and throughout much of Central and South America. *Eleutherodactylus* is the most speciose vertebrate genus (512 spp.; Duellman 1993), and several new species are described annually. Neither the systematic relationships within the genus nor its detailed taxonomy have been satisfactorily resolved. This is partly because of a relatively conservative morphology which has made identification and polarization of morphological characters problematic (Kaiser 1993; Kaiser et al. 1994*a*; Lynch and La Marca 1993). Thus, Hardy (1982) was careful in listing the three species of *Eleutherodactylus* on Tobago (*E.* cf *rozei* Rivero, *E. terraebolivaris* Rivero, *E. urichi* Boettger), and he discussed their taxonomy and biogeography to prevent misunderstanding or misidentification. During a recent investigation of frogs on Tobago, we realized that those *Eleutherodactylus* previously considered conspecific with *E. terraebolivaris* differ from that species by their vocalizations. Following the suggestion of Hardy (1982:97), we conducted a closer inspection of morphology, vocalizations, and chromosome complement which unequivocally confirmed this assessment. Thus, we herein recognize these Tobago populations as a new species.

MATERIALS AND METHODS

Specimens were collected on Tobago at three localities during 1990–93 (Appendix I). A conscious effort was made to survey dissimilar habitats (i.e., montane forest, coastal forest, plantations) to survey for possible habitat-related variation. All procedures with animals, including captive care, conformed to guidelines established by the Canadian Council on Animal Care (1980–84). Institutional abbreviations follow Leviton et al. (1985).

Seven length measurements (snout–vent length — SVL, head width — HW, tibia length — TIB, eye diameter — EYE, eye–naris distance — E–N, tym-

panum diameter — TYM, interorbital distance — IOD) were taken from 90 specimens to the nearest 0.1 mm, either using a dissecting microscope with camera lucida and digitizer attachments (Numonics 2200 digitizing tablet) supported by Jandel Scientific Sigma Scan (version 3.10) software on an IBM personal computer, or with metal calipers. All measurements were taken by HK to ensure consistency. Independent sample *t*-testing and multiple discriminant function analysis was performed using Systat 5.2 software on a Macintosh LC computer, according to the recommendations and terminology of Hair et al. (1992) and Wilkinson et al. (1992).

Twenty-three females and 21 males of *Eleutherodactylus* from the type locality, Rancho Grande, Henri Pittier National Park, Venezuela, and eight females and 12 males from Tobago were available for chromosome preparations. Mitotic chromosomes were prepared directly from bone marrow after in vivo colchicine treatment. All techniques used for the preparation of cell suspensions, hypotonic treatment, fixation of the cells, as well as for the demonstration of C-bands and nucleolus organizer regions (NORs) have been described previously (Schmid 1978). Five karyotypes for each banding procedure were prepared from each animal.

Sound recordings were made near Charlotteville on Tobago (October 1993) and at the Rancho Grande field station, Estado Aragua, Venezuela (July 1989), using a SONY TCM-74V walkman. Several of the recorded animals were collected and identified by their morphological, morphometric, and cytogenetic characteristics. Audiospectrograms were created with SoundEdit version 2.0.3 (Farallon Computing, Inc.) software on a Macintosh LC personal computer. Terminology for vocalizations follows Duellman and Trueb (1986), and all means reported are for $n=10$ calls unless otherwise indicated.

The format of the species diagnosis follows Lynch (1979), with the addition of the *m. depressor mandibulae* condition (see Lynch 1993). Descriptions of snout shape, structure of vocal sac, and tongue shape follow the definitions given by Duellman (1970). Terminology for finger disks follows Savage (1987). The definition of skin texture used here is that of Kaiser et al. (1994a). Measurements included are ranges, means ± one standard deviation, and sample size for both females and males.

TABLE 1. Checklist of the anuran fauna of Tobago. This assemblage includes single-island endemics (S), regional endemics (R), and widespread or widely introduced taxa (W).

Bufo marinus	W
Colostethus olmonae	S
Eleutherodactylus urichi	R
E. charlottevillensis	S
E. sp. A*	S
Flectonotus fitzgeraldi	R
Hyalinobatrachium orientalis	R
Hyla crepitans	W
Leptodactylus fuscus	W
L. validus	R
Phrynohyas venulosa	W
Physalaemus pustulosus	W
Scinax rubra	W

*This species has been listed previously as *E.* cf. *rozei* (e.g., Hardy 1982).

Eleutherodactylus ***charlottevillensis*** sp. nov.
Figs. 2–3

Holotype.—KU 222409, an adult male from 7 km N Roxborough along the Roxborough–Bloody Bay road, St. John Parish, Tobago, West Indies, collected on 31 August 1992 by H. Kaiser and T. F. Sharbel.

Paratypes.—Three female topotypes (NMC 35064-5, 35064-7, 35064-9), collected on 14 August 1991 by H. Kaiser and H. M. Gray. Two males (ZMB 53746-47), from King's Bay Waterworks, collected on 18 October 1993 by W. Feichtinger, H. Kaiser, and M. Schmid. Three males (KU 222373, 222410, MCZ A-116275) and one female (ZMB 53745) from 1.5 km SW Charlotteville, collected on 17 October 1993 by W. Feichtinger, H. Kaiser, and M. Schmid.

Etymology.—The specific name *charlottevillensis* was chosen in reference to the town of Charlotteville in northeastern Tobago, whose friendly populace has aided herpetologists during their surveys for many decades, and where WF and MS first realized that the animals they heard were distinctly not *Eleutherodactylus terraebolivaris*.

Diagnosis.—A forest-dwelling member of the *Eleutherodactylus conspicillatus* group with the following diagnostic features: (1) Skin on dorsum finely shagreen (Kaiser et al. 1994*a*), without ridges

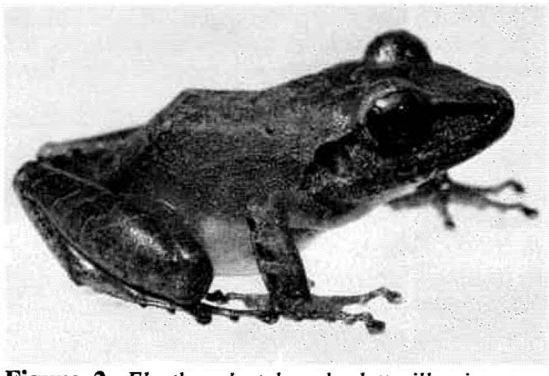

Figure 2. *Eleutherodactylus charlottevillensis* n. sp. (NMC 35060-1), an adult female from near mileage marker 3.5 on Roxborough-Bloody Bay Road, Tobago. Snout-vent length 48.1 mm.

or dorsolateral folds; venter and groin region smooth, anal region strongly areolate; (2) tympanum oval, distinct, widest dorsoventrally, about one-quarter size of orbit; weak supratympanic fold present; (3) snout trapezoid in dorsal view, truncate to rounded in profile; E—N distance greater than or equal to length of eye; canthus rostralis very sharply angled, canthal ridge straight and forming dorsal boundary of dark loreal region; (4) IOD 1.5–2 times width of upper eyelid; a few fine supraocular tubercles present; cranial crests absent; (5) dentigerous processes of vomers triangular and anterolaterally to posteromedially inclined, positioned medial to choanae, each with a single row of teeth; choanae ovoid; (6) males with external vocal slits and a single subgular vocal sac; nuptial pads absent; (7) size of fingers II<I=IV<III, III about 1.3 times longer than I; finger disks III and IV about 1.5 times wider than digits, more expanded than disks I and II; subarticular tubercles oval and raised; a single cordiform (= bifid) palmar tubercle; thenar tubercle elongate, covering base of finger I; one tubercle present at base of each finger; supernumerary palmar tubercles absent (Fig. 3A); (8) fingers with weak lateral fringes; (9) several small tubercles on forearm and elbow; a few small post-tympanic tubercles present; (10) several small heel and knee tubercles; inner tarsal fold absent; (11) two metatarsal tubercles, inner large, raised and elliptical, outer very small, one-fifth size of inner, conical; a single tubercle present at base of toe IV; supernumerary plantar tubercles absent (Fig. 3B); (12) toe disks oval and wider than digits, IV slightly larger than the others; weak lateral fringes; webbing absent; (13)

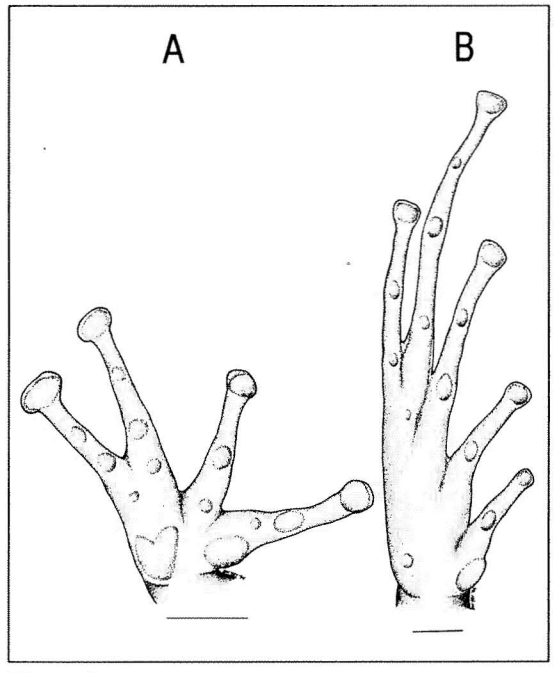

Figure 3. (A) Right hand and (B) foot of *Eleutherodactylus charlottevillensis* n. sp. (KU 222376 and KU 222381, respectively). Scale=2 mm.

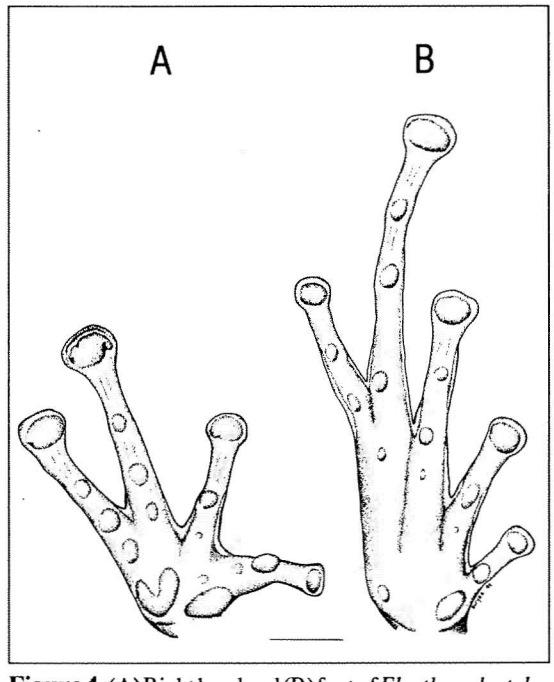

Figure 4. (A) Right hand and (B) foot of *Eleutherodactylus terraebolivaris* (UMMZ 113953). Scale=2 mm.

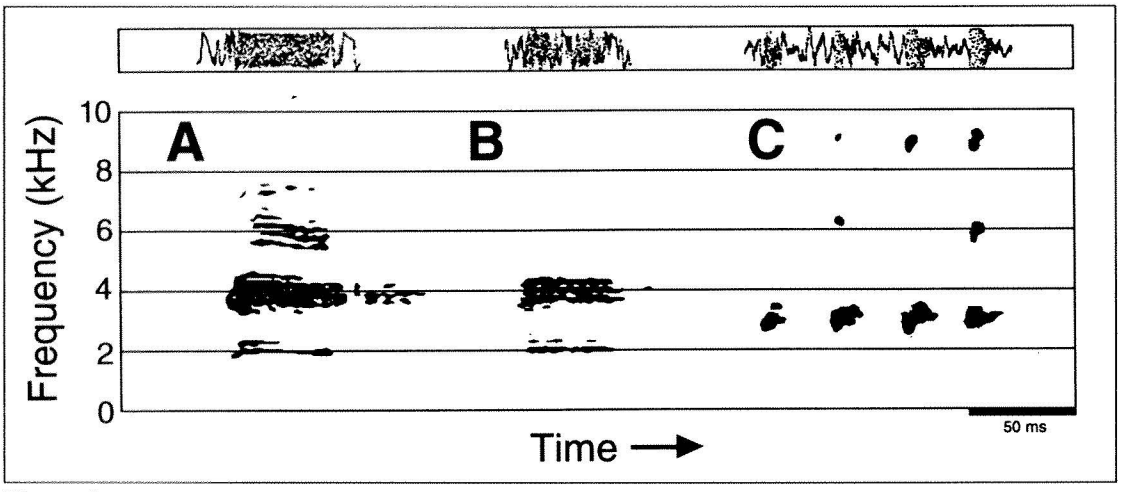

Figure 5. Vocalizations of *Eleutherodactylus charlottevillensis* n. sp. (A, B), and *E. terraebolivaris* (C). Oscillograms showing the wave form of each call are provided above the audiospectrograms.

dorsum brown to dark-brown, with one or two weakly outlined chevrons; venter white without melanophores; concealed surfaces of hind limbs faint orange to light-brown in life; labial area brown, with dark canthal stripe and two broad dark stripes extending from eye to lip; dark interorbital bar present; solid dark supratympanic stripe curving around dorsal and posterior part of tympanum, reaching from corner of eye to back of tympanum; upper iris color golden bronze in life; (14) SVL of females 37.0–48.1 mm ($\bar{x}$=43.1±4.2, n=8), of males 23.1–30.5 mm ($\bar{x}$=26.9±1.7, n=22); (15) (dfsq*at) condition of *m. depressor mandibulae*; (16) 2N=36 acrocentric chromosomes.

The species most similar to *Eleutherodactylus charlottevillensis* is *E. terraebolivaris*, with which it has so far been considered conspecific. These species are readily distinguished from each other by their vocalizations (Fig. 5; see above). Syntopically occurring adults of *E. urichi*, a small (SVL≤25 mm) ground-dwelling species (Kaiser et al. 1994*b*), generally can be differentiated from *E. charlottevillensis* by size alone, but the most striking differences between the two species are the blue upper portion of the iris and the reddish coloration of the hidden portions of the thigh in *E. urichi*.

Although it is possible to separate specimens of *Eleutherodactylus charlottevillensis* from *E. terraebolivaris* using morphological details (e.g., hands and feet; Figs. 3, 4) or morphometrics (Fig. 6), these methods are impractical when only a few specimens are available. However, differentiation is easily accomplished by the position of the NORs in the karyotype (Fig. 7). The position of the NOR on the largest chromosome is diagnostic of *E. terraebolivaris* (Fig. 7A), whereas it is located on chromosome 9 of *E. charlottevillensis* (Fig. 7B). Furthermore, there is significantly more heterochromatin present in the karyotype of *E. charlottevillensis* (Fig. 7B) than in *E. terraebolivaris* (Fig. 7C), and the latter also possesses a C-band heteromorphism on chromosome 14 in both males and females (Fig. 7C, arrow). These data clearly suggest that the two tested groups represent independent evolutionary lineages.

Basic statistics (Table 2), of the type employed by Schwartz (1967), show subtle and statistically significant differences ($P < 0.005$) between *Eleutherodactylus charlottevillensis* and *E. terraebolivaris*, but none of these differences are striking or would assist in identification when only a few specimens are available. Morphological differences are evident in a variety of features (Table 3), but these are sometimes very detailed and may not be reliable in old or poorly preserved specimens.

Description.—Eight adult females, 22 males. Head wider than body, longer than wide; HW 37.4–42.3% ($\bar{x}$=39.3±1.6) of SVL in females, 36.2–41.0% ($\bar{x}$=39.0±1.4) in males; marginally rounded snout, trapezoid in shape in dorsal view, truncate to rounded in lateral profile; mouth slightly subterminal; lower lip bearing a small well-defined papilla; E–N 86.5–133.9% ($\bar{x}$=114.1±1.9) of EYE in females, 73.3–125.0% ($\bar{x}$=90.3±1.5) in males; eyes large, promi-

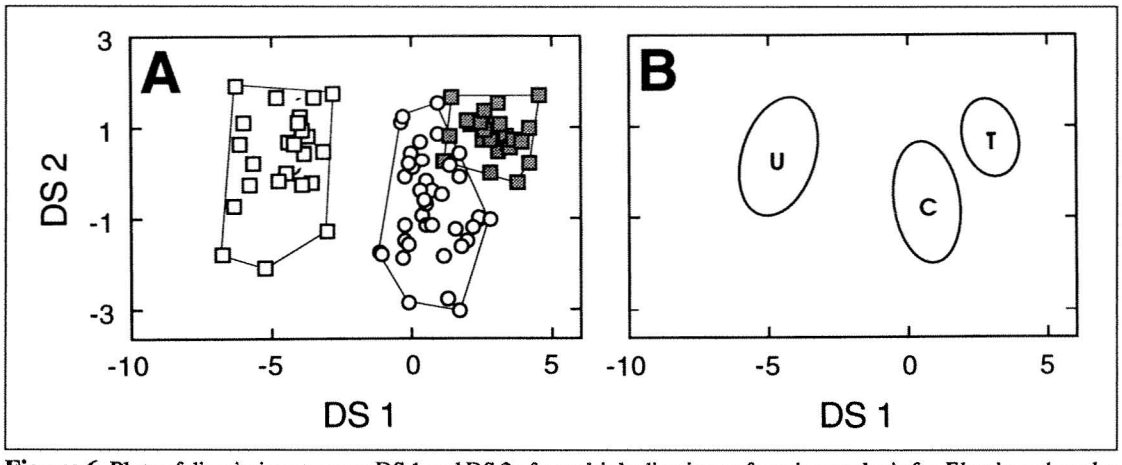

Figure 6. Plots of discriminant scores DS 1 and DS 2 of a multiple discrimant function analysis for *Eleutherodactylus charlottevillensis* n. sp. (open circles, C), *E. terraebolivaris* (stippled squares, T), and *E. urichi* (open squares, U). (A) Direct plot indicating actual morphospaces occupied by all analysed specimens for each species. (B) Ellipsoids (50% centroids) calculated from the same data as in (A), but reducing the data to allow visualization of the statistical differences.

nent; upper eyelid with a few fine supraocular tubercles; IOD approximately 25.3–32.6% ($\bar{x}$=29.0±0.3) of HW in females, 22.0–31.5% ($\bar{x}$=26.8±2.3) in males. Top of head flat; cranial crests absent; canthus rostralis straight, very sharply angled; loreal region slightly concave in anterior two-thirds, flat posteriorly, lacking tubercles; lips not flared; internarial area not depressed; nares oval, protruding laterally. Supratympanic fold weak, describing a posteroventral curve from anterodorsal edge of tympanum near corner of orbit, slightly obscuring dorsal part of tympanic annulus; tympanum oval and medium-sized, in females 31.9–44.7% ($\bar{x}$=37.1±4.7) of EYE, 21.5–48.1% ($\bar{x}$=32.8±6.8) in males; separated from eye by a distance about two-thirds TYM. Choanae ovoid, widely separated, unobscured by palatal shelf of maxillary arch in ventral profile; dentigerous processes of vomers prominent, triangular, aligned in a posteriorly elevated, anterodorsally to posteroventrally inclined position, each bearing a single row of teeth; separated by a distance greater than width of individual process. Tongue nearly round, approximately as long as wide, shallowly notched posteriorly, free posteriorly for approximately one-quarter of its length; vocal slits in males elongate, extending from midlateral base of tongue towards angle of jaw; single, subgular, internal vocal sac.

Skin on dorsum finely shagreen; a few small, low tubercles on forearm, elbow, knee, and heel, tubercles absent on tarsus; posteroventral surface of thighs coarsely areolate. Anal opening unmodified, directed posteriorly at upper level of thighs.

Forearms moderately robust; fingers long, slender, bearing subtruncate (III, IV) to rounded (I, II) disks with round (I, II) to elliptical (III, IV) pads, relative disk sizes II<I<III<IV; relative lengths of fingers II<I=IV<III; number of subarticular tubercles 1-2-2-2 for fingers I–IV, respectively, subarticular tubercles oval and raised; one tubercle at base of each finger; supernumerary palmar tubercles absent; single, bifid palmar tubercle; thenar tubercle elongate, covering base of finger I laterally; nuptial pads absent. Hindlimbs moderately robust, long; heels broadly overlapping when hindlimbs flexed at right angles to body axis; TIB in females 54.1–63.7% ($\bar{x}$=58.8±3.5) of SVL, 52.7–65.1% ($\bar{x}$=59.3±2.9) of SVL in males. Inner tarsal fold absent; two metatarsal tubercles, inner large, raised, and elliptical, outer conical, one-fifth size of inner; toes long, slender, bearing rounded (I, II, III, V) and oval (IV) disks, disk IV larger than others; with narrow lateral fringes, and lacking webbing; relative length of toes I<II<V<III<IV; number of subarticular tubercles 1-1-2-3-2 for toes I–V, respectively, subarticular tubercles conical; one tubercle present at base of toe IV; supernumerary plantar tubercles absent.

Color in preservative.—Dorsum of head and body grayish brown ground; one or two faintly

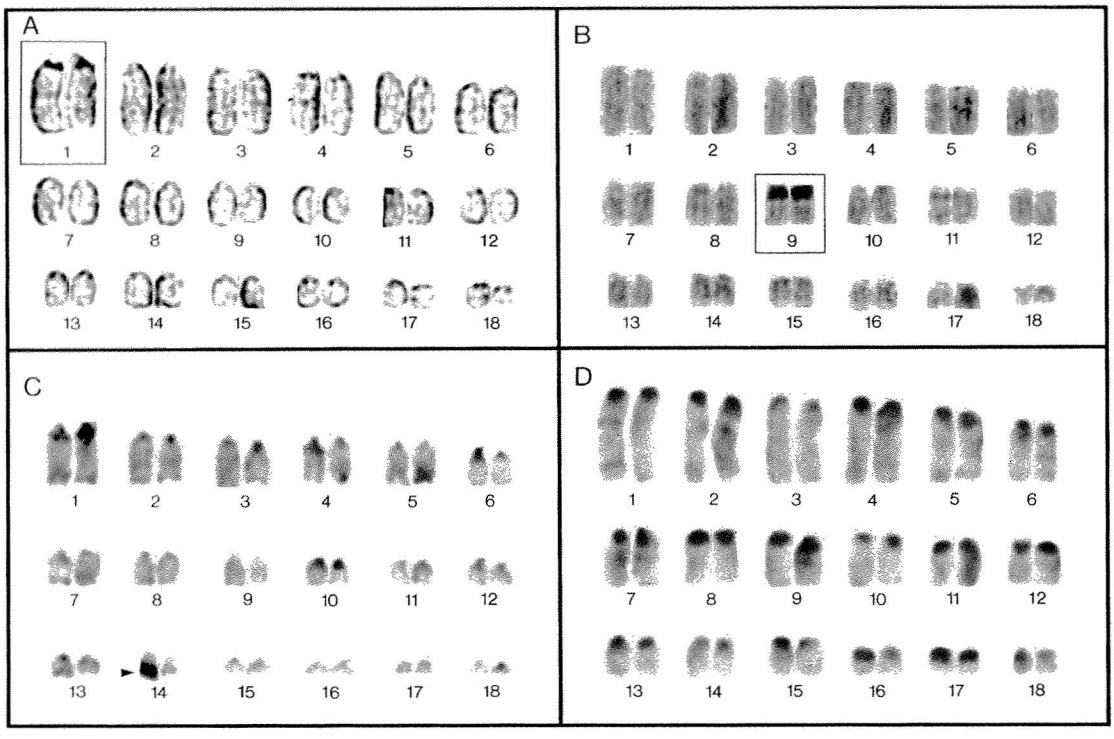

Figure 7. Karyotypes of *Eleutherodactylus terraebolivaris* (A, C) and *E. charlottevillensis* n. sp. (B, D). (A, B) Silver-stained karyotypes identifying positions of nuceolus organizer regions (NORs) of *E. terraebolivaris* (A) on the largest chromosome, and of *E. charlottevillensis* (B) on chromosome pair 9. (C, D) C-banded karyotypes demonstrating the presence of more constitutive heterochromatin in the centromeric and pericentromeric regions of *E. charlottevillensis* (D) than in *E. terraebolivaris* (C). A sex-unspecific C-band heteromorphism exists in the chromosome complement of *E. terraebolivaris* (arrow).

outlined middorsal chevrons may be present; broad dark interorbital bar and canthal stripe present; dark supratympanic stripe present; color of flanks changing from dark grayish brown dorsally to light-gray ventrally. Dorsal surfaces of limbs dark grayish brown, with 3 dark crossbars, fading in some long-preserved specimens; anterior surface of thighs tan and mottled, posterior surfaces tan. Venter white; ventral surface of palm mottled, finger disks white with darker pigmented proximal areas, disk covers brown; plantar surface, toe disks, and disk covers dark-brown; plantar surfaces dark-brown, sometimes offset by a medial cream hairline.

Color in life.—Dorsum light-brown; venter white; groin brownish with a faint, orange tinge; upper iris color golden-bronze.

Measurements (mm).—Values given are for the holotype, followed by ranges with means in parentheses for females and males, respectively. SVL 27.0, 37.0–48.1 (43.1±4.2), 23.1–30.5 (26.9±1.7); TIB 16.1, 22.1–28.4 (25.3±2.5), 14.8–18.3 (15.9±0.9); HW 10.5, 14.6–20.3 (17.0±2.0), 9.3–12.1 (10.5±0.8); IOD 2.8, 3.7–6.2 (4.9±0.9), 2.2–3.7 (2.8±0.3); E–N 3.8, 5.0–6.9 (6.1±0.6), 3.3–4.2 (3.7±0.2); EYE 4.1, 4.5–6.6 (5.4±0.6), 3.1–4.9 (4.1±0.4); TYM 1.3, 1.4–2.6 (2.0±0.3), 0.9–1.8 (1.3±0.2).

Morphometrics.—Statistical classification of specimens by species using the calculated multiple discriminant functions (DF) was successful for 86 of 90 specimens (95.6%). Specimens of the reference species, *Eleutherodactylus urichi,* were classified correctly (100%), whereas three specimens (7.7%) and one specimen (3.7%) of *E. charlottevillensis* and *E. terraebolivaris*, respectively, were statistically misaligned with the other species. The posterior probabilities leading to misalignment had an average of 0.640, indicating that these values were

TABLE 2. Means, standard deviations, and extremes (in mm) of snout–vent length (SVL), head width (HW), tibia length (TIB), eye diameter (EYE), eye–naris distance (E–N), tympanum diameter (TYM), interorbital distance (IOD), and two ratios indicative of body proportions for male and female specimens of *Eleutherodactylus charlottevillensis* n. sp. and *E. terraebolivaris*. Values for the two ratios were *t*-tested at $p<0.5$. The ratio of TIB/SVL was significantly different (*) between taxa, whereas the HW/SVL was not (NS).

Females	*n*	SVL	HW	TIB	EYE	E–N	TYM	IOD	TIB/SVL*	HW/SVL^{NS}
E. charlottevillensis	8	43.1±4.2 (37.0-48.1)	17.0±2.0 (14.6-20.3)	25.3±2.5 (22.1-28.4)	5.4±0.6 (4.5-6.6)	6.1±0.6 (5.0-6.9)	2.0±0.3 (1.4-2.6)	4.9±0.9 (3.7-6.2)	0.588±0.035 (0.541-0.637)	0.393±0.016 (0.374-0.423)
E. terraebolivaris	20	40.7±5.8 (29.4-49.3)	16.1±2.6 (11.1-19.9)	26.1±4.1 (17.3-31.1)	5.9±0.8 (4.2-7.0)	5.7±0.9 (4.1-7.2)	2.2±0.2 (1.8-2.6)	4.8±0.8 (3.3-6.1)	0.640±0.037 (0.531-0.707)	0.395±0.013 (0.375-0.417)
Males	***n***	**SVL**	**HW**	**TIB**	**EYE**	**E–N**	**TYM**	**IOD**	**TIB/SVL***	**HW/SVL^{NS}**
E. charlottevillensis	26	26.9±1.6 (23.1-30.5)	10.5±0.7 (9.3-12.1)	16.0±0.8 (14.8-18.3)	4.1±0.4 (3.1-4.9)	3.7±0.3 (3.1-4.2)	1.4±0.2 (0.9-1.8)	2.8±0.3 (2.2-3.7)	0.594±0.028 (0.527-0.651)	0.389±0.013 (0.362-0.410)
E. terraebolivaris	6	31.6±4.0 (26.9-36.1)	12.1±1.9 (10.0-14.4)	20.0±3.8 (15.7-25.1)	4.9±0.5 (4.3-5.5)	4.7±0.6 (4.0-5.4)	1.7±0.1 (1.5-1.9)	3.7±0.5 (3.1-4.4)	0.629±0.047 (0.563-0.703)	0.380±0.019 (0.358-0.403)

TABLE 3. List of diagnostic morphological differences between *Eleutherodactylus charlottevillensis* n. sp. and *E. terraebolivaris* Rivero. For frogs of the genus *Eleutherodactylus*, these are among the characters that have been considered reliable in delineating morphotypes (Lynch 1979; Lynch and Duellman 1980). However, individual variation has not been accounted for in all hitherto described species, and the role of parallelisms and convergence is as yet uncertain (see Kaiser et al. 1994*a*). It is therefore recommended that alternative data sources be considered in addition to morphology to ensure a taxonomic stability. Numbered characters are in the diagnosis, the others from the description.

Diagnosis Number	Diagnostic Feature	*E. charlottevillensis* n. sp.	*E. terraebolivaris*
2	Supratympanic fold	Weak Short Obscures tympanic annulus	Pronounced Reaching axillary region Does not obscure annulus
4	Interorbital distance in terms of upper eyelid width	1.5–2 times	2–2.5 times
7	Size of fingers	II < I = IV < III	I = II < IV < III
	Length of fingers I and III	III = 1.3 x I	III = 1.5 x I
	Disks III and IV, digit width	Disks = 1.5 x digits	Disks = 2.5 x digits
	Shape of thenar tubercle	Narrow, elongate	Thick, oval
11	Size of metatarsal tubercles	Outer 1/5th size of inner	Outer 1/3th size of inner
13	Paired dark scapular spots	Absent	Present
	Dark stripe from eye to lip	Present	Absent
	Tongue shape	Nearly round As long as wide Shallowly notched	Oval Longer than wide Deeply notched
	Number of tubercles on hands	4 of uniform size	5 of two sizes
	Number of tubercles on feet	1	3

relatively close to the cutoff value of 0.500. The type specimens of *E. charlottevillensis* (KU 222409) and *E. terraebolivaris* (MCZ 31062) achieved posterior probability values of 0.990 and 0.903, respectively. The greatest loading in discriminating species by size (DF1) was TIB (0.634), whereas shape discrimination (DF2) was mainly achieved by high loadings of IOD (0.494) and TYM (0.468).

Plots of discriminant score (DS) 2 against DS1 for the three investigated taxa showed good separation of species morphospaces and very little overlap (Fig. 6). According to the size axis (DS1), *Eleutherodactylus terraebolivaris* was the largest species among the three, closely followed by *E. charlottevillensis*. Values indicative of shape (DS2) for *E. terraebolivaris* occupied only the upper portion of that axis, and the range of DS2 values for *E. terraebolivaris* was only about one-half that of either *E. charlottevillensis* or *E. urichi*. However, there was overlap of DS2 values for all three species (Fig. 6A). Calculation and graphic representation of 50% morphospace centroids (Fig. 6B) allowed easy distinction between the three species, with *E. terraebolivaris* and *E. charlottevillensis* in a phenetically closer position.

TABLE 4. Detailed characteristics of the advertisement call of *Eleutherodactylus terraebolivaris* from the type locality at Rancho Grande, Venezuela. Calls of this species were always intermittent and never given by any individual with any regularity. No bouts of intense calling were heard. Duration time is given in milliseconds. Means were calculated from n=10 calls.

	Note				Interval			Total length
	1st	2nd	3rd	4th	1st	2nd	3rd	
Range	11–17	13–26	17–26	15–21	23–32	21–30	15–30	128–160
Mean	15	19	21	19	28	26	23	138

NATURAL HISTORY

Distribution and Ecology

Eleutherodactylus charlottevillensis is found only on the island of Tobago, West Indies. It seems that the population is continuous throughout the forests of the Main Ridge, and that its range does not extend much beyond the forest perimeter. Frogs were very abundant on the northern slopes of the Main Ridge, part of which is a forest reserve and has greater annual rainfall compared to the southern slopes (Hardy 1982). Large females (>40mm) were encountered on sturdy leaves, balanced on branches close to ground level, or sitting on the substrate (decaying foliage). Males appeared to have a more variable substrate preference, and were seen vocalizing in grassy meadows along the road, perched on thin branches higher off the ground, as well as in the same situations as females. We never found egg clutches or hatchlings. These frogs are known to be parasitized by nematodes of the genus *Rhabdias*, by *Ortleppascaris* larvae, and by seratoid larvae (Moravec and Kaiser 1995).

Vocalizations

The call of *Eleutherodactylus charlottevillensis* (Fig. 5A, B) is a seven-part trill built upon what may be considered very closely spaced primary harmonics. The primary call has a dominant frequency of 4000 Hz which demonstrates sidebands due to trilling (Fig. 5B). It incorporates 6–7 individual trill segments distributed almost evenly over the duration of a trill (Fig. 5B). The fundamental frequency centers on 2000 Hz, with the higher harmonic at 6000 Hz noticeable only in recordings of great intensity (Fig. 5A). The total length of measured calls ranged from 43–68 ($\bar{x}$=51, n=13) milliseconds (ms). Individual frogs generally did not produce calls in rapid succession or with any measurable regularity. Successive calls were given at mean intervals of 1.64 s. Males did not seem to join in calling bouts or "ramping patterns." These are series of chorusing events usually initiated by a single individual. More and more males subsequently join the chorus until a high chorusing activity is achieved. Cessation of all calling activity occurs abruptly and simultaneously and lasts until the initiation of the next bout of ramping (Drewry and Rand 1983). Calling activity was strongest during approximately one hour around dusk, and irregular calling occurred throughout the night. Calling activity near dusk was minimal during a strong rain but increased shortly after the rain stopped.

Although nearly indistinguishable to the human ear, the call structure of *Eleutherodactylus terraebolivaris* from the type locality (Fig. 5C; Table 4) is very different from *E. charlottevillensis*. The call consists of four short notes which together sound like a trill. The dominant (= fundamental) frequency is at 3000 Hz, with harmonics at 6000 and 9000 Hz. Total length of recorded calls ranged from 128–160 ($\bar{x}$=138) ms. The first note of the trill was always without any harmonics, whereas each of the other three notes had a harmonic when recorded at high intensity. The call of the sympatric *E. urichi* is composed of single clicks and quite distinct (Kaiser et al. 1994*b*).

DISCUSSION

The great similarity of *Eleutherodactylus charlottevillensis* and *E. terraebolivaris* at the morphological and chromosomal level may be indicative a close phylogenetic relationship. Considering the geological history of the region, it is most likely that the range of a widespread ancestral form became fragmented during the formation of Trinidad and Tobago. Furthermore, it has been suggested that Tobago separated from the mainland well before Trinidad (Hardy 1982); thus, a peripheral isolate was formed on Tobago. No species resembling either *E. charlottevillensis* or *E. terraebolivaris* occurs on Trinidad (Hardy 1982; Kenny 1969). The greater anuran species diversity on Trinidad, particularly of *Hyla* species, and the ensuing competition for forest habitats, may have driven any ecologically and morphologically similar *Eleutherodactylus* species to extinction. We propose that several shared chromosomal and external morphological characteristics allow the tentative placement of *E. charlottevillensis* and *E. terraebolivaris* as each other's closest relatives, pending a cladistic study of the *E. conspicillatus* group. Any further suggestions about relationships for these two species would be premature.

The preceding species description is a further example of the difficulty in making informed taxonomic decisions for *Eleutherodactylus* based entirely on morphology. Even with detailed comparisons of museum specimens, Hardy (1982) could not separate *E. charlottevillensis* from *E. terraebolivaris* with confidence, although slight differences in vocalizations were apparent (Hardy 1982:97). Traditional morphology remains a very important data source, but morphological characters may only be of limited value in a genus with a highly conserved morphology such as *Eleutherodactylus*. Among such taxa, it is a challenge to determine at which level intra- or interspecific character variability becomes evolutionarily (and taxonomically) important (see Kaiser et al. 1994*a*). In pursuit of a more stable taxonomy, recent studies combining morphological, morphometric, biochemical, and/or cytogenetic data are slowly beginning to reform the classification of *Eleutherodactylus* and many other anuran genera.

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APPENDIX 1

Material Examined

Eleutherodactylus charlottevillensis (36). TOBAGO: Hills above Man-of-War Bay, 1.5–3.5 km ENE Charlotteville, AMNH 87408, 87412, 87427–28, 87431; Parish of St. John, mile marker 27 3/4 on Charlotteville-Bloody Bay road, USNM 167609–11, 167613; ca. 7 km N Roxborough, KU 222409 (holotype), NMC 35056, 35060-1–5, 35064-1–3, 35064-5–11, KU 222381; 1.5 km SW Charlotteville, KU 222370-80, 222410; Kings Bay Waterworks, ZMB 53746-47.

Eleutherodactylus terraebolivaris (26). VENEZUELA: Estado Aragua, Rancho Grande, MCZ 31062 (holotype), UMMZ 113950, 113951 (2 specimens), 113952–54, 113955 (4 specimens), 113956, 113957 (2 specimens), 113958 (2 specimens), 113960–64; Miranda State, Los Canales, Planta Electrica de Naiguata, USNM 128807-08, 128812-14 (paratypes).

Eleutherodactylus urichi (24). TOBAGO: Main Ridge, ca. 7 km N Roxborough, NMC 35059, 35063-1–2, KU 265456. TRINIDAD: N Arima Valley, NMC 35057-1–2, 35061-1–8, 35065-1–5, KU 265457–58, KU 222382.