



## A new species of palustrine *Eleutherodactylus* (Anura: Leptodactylidae) from Puerto Rico

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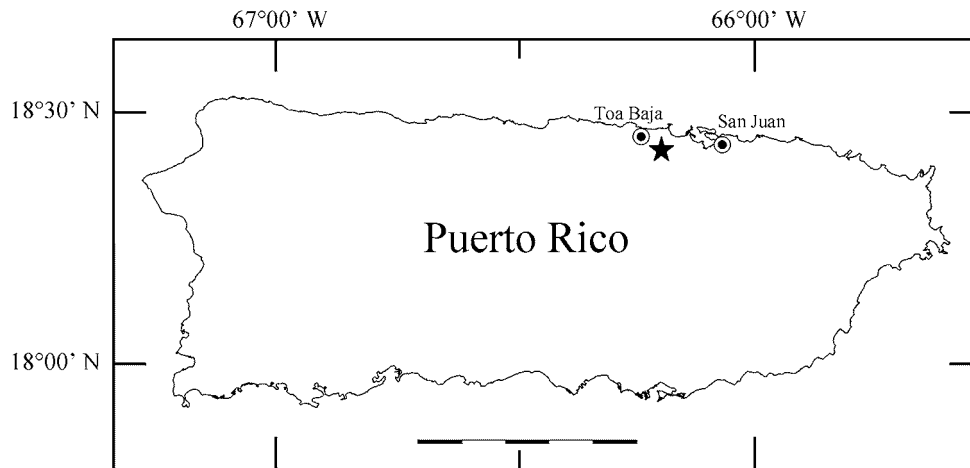
### Abstract

We describe adult morphology, advertisement call, and natural history diagnostic of a new species of *Eleutherodactylus* from a fresh water (palustrine) herbaceous wetland of northern coastal Puerto Rico. The new species is apparently the smallest Puerto Rican *Eleutherodactylus* and is morphologically most similar to *E. gryllus*, which inhabits high-elevation humid forests and cloud forests. Although both species have well-developed glands throughout the body, morphological ratios, body coloration, frequency of calls, call structure, and habitat association indicate that it is a well-differentiated species. The new species and *E. gryllus* may have diverged from an ancestral wetland-dwelling species.

**Key words:** coastal ecosystems, Coqui Llanero, freshwater, herbaceous, karst, NMDS

### Introduction

THE anuran genus *Eleutherodactylus* (Duméril & Bibron) represents the most species-rich genus of vertebrates with more than 600 recognized species in Central, South America, and the West Indies (Frost, 2002; Frost *et al.*, 2006). Although herpetology in Puerto Rico began formally in 1820 (Thomas & Joglar, 1996), it was not until 1863 that the first *Eleutherodactylus*, *E. antillensis* (Reinhardt & Lutken), was described. By 1976, the addition of *E. jasperii* (Drewry & Jones), the first New World anuran reported to be ovoviparous (Drewry & Jones, 1976), comprised 15 *Eleutherodactylus* on the main island of Puerto Rico. The native anuran fauna of Puerto Rico has been nomenclaturally stable for 30 years and no additional species has been described since. Most of these descriptions were based, however, on studies in forest remnants in mid–high elevation areas, while herpetological studies in lowland wetlands were virtually nonexistent. The vast majority of these lowland wetlands were heavily altered mostly for agriculture since 1500's and for urban development since the 1930's, which may partly suggest that these areas were considered of little herpetological interest. On the other hand, ~94% of forested areas were cleared from 1930 to 1950 during the peak of agriculture activity (Birdsey & Weaver, 1987; López del Mar *et al.*, 2001; Lugo, 2004). Consequently, several ecologically specialized *Eleutherodactylus* like *E. jasperii* and two more Puerto Rican *Eleutherodactylus* from forested and high elevation areas are now presumably extinct (Joglar, 1998), mostly due to habitat destruction. Then, it was with great surprise that one of us (NRL) discovered a small, unknown *Eleutherodactylus* in a palustrine herbaceous habitat on the northern coastal plain, not far west from the capital city of San Juan (Fig. 1).



**FIGURE 1.** Map of Puerto Rico showing type locality (star) of *Eleutherodactylus juanariveroi* along with the capital city of San Juan and the Toa Baja municipality. Scale bar 50 km.

## Material and methods

Specimens were collected, fixed in 10% formalin, and transferred to 70% ethanol for storage. Two specimens of the new species and two of *Eleutherodactylus gryllus* (Schmidt) were cleared and stained following Dingerkus and Uhler (1977) and Rosa-Molinar *et al.* (1999) for cartilage and osteological data. The following measurements were taken, to the nearest 0.01 mm, using a digital slide-caliper: Snout-vent length (SVL), Femur length (FL), Tibio-fibula length (TiL), and Tarsal length (TL, from the tibio-tarsal articulation to the inner base of metatarsal tubercle). The following measurements were also taken, but to the nearest 0.001 mm using a digimatic micrometer: HW (head width, at the angle of jaws), HL (head length, from the rear of mandible to tip of snout), UEW (maximum upper eyelid width), IOD (inter orbital distance, that is, the shortest distance between the upper eyelids), TYW (tympanum width, maximum horizontal width), IN (inter-narial distance or distance between internal border of nostrils), EL (eye length or orbital length, which is the horizontal distance between orbital borders of eye), EN (distance from nostril to anterior orbital border of eye),  $FD_{III}$  (disk width on finger III),  $TD_{IV}$  (disk width on toe IV), and  $FDB_{III}$  (width of finger III at the base of disk;  $FDB_{III}$  was taken by measuring tracings made using a camera lucid attached to a dissecting microscope). Angular measurements were taken with a vernier protractor on camera lucid tracings of the relevant structures. Sex was determined by examining gonads. At first glance, several morphological and acoustic affinities of the new species with *E. gryllus* lead to focus our comparison to the small Puerto Rican *Eleutherodactylus* showing affinities with *E. gryllus* (*E. eneidae* [Rivero] and *E. locustus* [Schmidt]) and/or those small-bodied species that occur in the same type locality (*E. brittoni* [Schmidt] and *E. cochranæ* [Grant]). We used Non-metric Multidimensional Scaling (NMDS) to compare body proportions of the new species with those of *E. brittoni*, *E. cochranæ*, *E. eneidae*, *E. gryllus*, and *E. locustus*. NMDS was selected, because most of the morphological variables are correlated and do not conform to the normality assumptions required by parametric multivariate analyses (e.g., PCA, CCA; McCune & Grace, 2002). The NMDS was performed using PC-ORD 4 software package (McCune & Mefford, 1999). The data matrix consisted of 97 rows that represented one individual from each species and columns represented 91 relative body proportions. The NMDS analysis resulted in groups of individuals, in ordination space, representing every taxon characterized by the combination of morphological variables. The spatial arrangement of these groups allowed detecting those species that showed a greater morphological affinity with the new species. Indicator Species Analysis (Legendre & Legendre, 1998) was then used to detect those morphological variables/ratios that could be useful in distinguish-

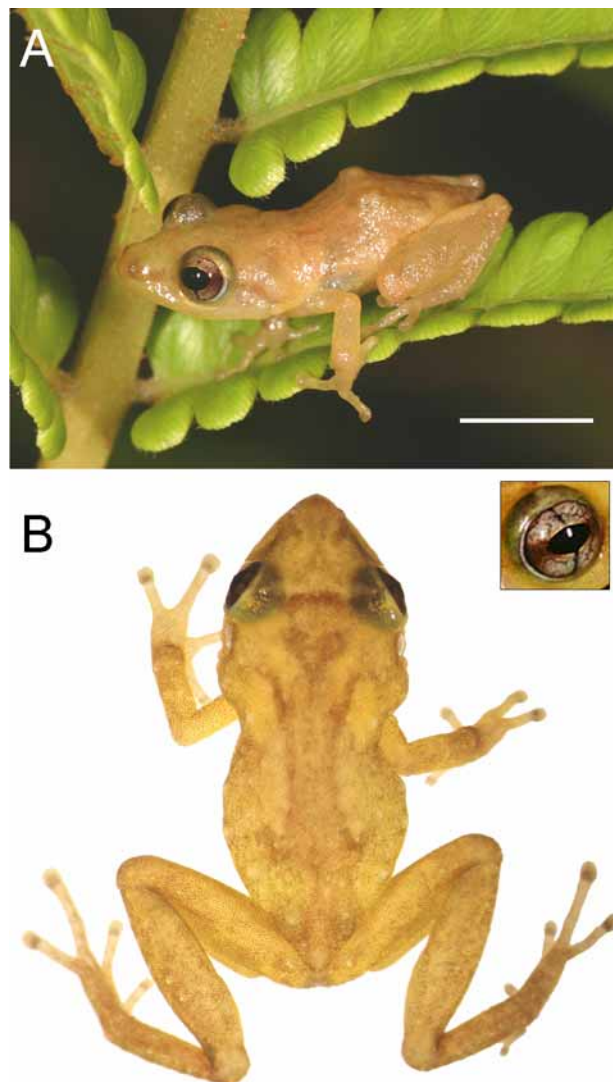
ing the new species from the most similar species. The main diagnostic attributes of the new species are qualitative characters not included in the multivariate matrix with morphological data.

We recorded calls in the field using a Sony stereo microphone (Model ECM-719) and a Sony cassette recorder (Model WM-D6C). Call parameters were analyzed using Adobe® Audition™ 1.5 software package (© Adobe Systems Incorporated) and Raven 1.0 software package (© Cornell Lab of Ornithology). The analyses were performed using Butterworth filter, which allows for the specification of the width of the transition band. A narrow 20 Hz transition band was selected for all analyses.

***Eleutherodactylus juanariveroi*, n. sp.**

(Fig. 2; Table 1)

*Holotype*: KU 306997, an adult female collected at Sabana Seca, Toa Baja Municipality (Fig. 2), in a seasonally flooded herbaceous wetland in the vicinity of the US Naval Security Group Activity Sabana Seca (USNS-GASS) and the Caribbean Primate Research Center, Puerto Rico (18°26.127'N, 66°12.092'W), 10–20 m elevation, by N. Rios and R. Thomas on 2 August 2005.



**FIGURE 2.** Live *Eleutherodactylus juanariveroi* (female) on a willdenow's maiden fern (*Thelypteris interrupta*) from the type locality (A) (specimen number unavailable). Scale bar 5 mm. Dorsal view of *E. juanariveroi* showing reversed comma pattern (B) (KU 306997, holotype). Pupil shape with '+' pattern (insert, specimen number unavailable).

*Paratypes*: 45 (26 females, 18 males, 1 juvenile). Females (KU 306998–99, USNMS 563626–28, UPRRP 6340–2, UPRRP 6343, UPRRP 6348–9, UPRRP 6351–4, UPRRP 6356–7, UPRRP 6359, UPRRP 6360–2, UPRRP 6365, UPRRP 6367–9, and RT 14535 [Richard Thomas, private collection]), males (KU 307000–02, USNM 563623–25, UPRRP 6344–7, UPRRP 6350, UPRRP 6355, UPRRP 6358, UPRRP 6363–4, UPRRP 6366, UPRRP 6370, and RT 14501), juvenile (UPRRP 6371), all from the vicinity of the USNSGASS, the Caribbean Primate Research Center, and the public lands in the Toa Baja Municipality, 18°26.049'N, 66°12.209'W, 18°26.127'N, 66°12.092'W, N. Rios and R. Thomas, 30 July 2005, 2 August 2005, 3 August 2005, and 23 August 2005.

**Diagnosis.** A member of the West Indian subgenus *Eleutherodactylus*, *auriculatus* section, *martinicensis* series (sensu Hedges, 1989) having extensive dorsal skin glandularity (Fig. 3), minute vomerine teeth, a distinctive carpal element (see description), a unique high-pitched call, and a palustrine habitat. Males have an external single subgular vocal sac, absent in females; nuptial pads absent. Pupil horizontally elliptical with two thin, sharp vertical slits, black colored below and above the mid portion of pupil, resulting in a '+', sometimes only the inferior slit is clearly visible resulting in a 'T' (Fig. 2). The digital disks are small and spatulate as opposed to widening abruptly from the base of the pad. The terminal phalanges are nearly T-shaped, clearly visible in finger III and Toe IV, but only with minute lateral projections of the terminal phalanges; rest of phalanges knobbed clearly visible in finger I of holotype (KU 306997), although terminal transverse groove across the tip of the digital pad visible in external view. Large carpal element (fused carpals 2+3) with pronounced ventral spine and emargination (the equivalent structure in *E. gryllus* being more solid and rounded with a minute spine). Eyelid tubercles absent; dorso-lateral folds absent. Ulnar tubercles absent; thenar tubercle elevated, elliptical, about the same size as pad in finger I; subarticular tubercles rounded; few minute supernumerary tubercles on proximal segments of fingers; a few minute centrally grouped supernumerary palmar tubercles; fingers lacking lateral fringes; relative length of fingers 1=2<4<3; disk of fingers small, spatulate not widening abruptly (Fig. 4A). Heel tubercles few, small, rounded, elevated; tarsal fold and lateral fringes of the toes absent; metatarsal tubercle rounded; subarticular tubercles rounded and slightly larger than palmar; supernumerary plantar tubercles present on proximal segments of toes; sole with a few minute irregularly scattered plantar tubercles; relative lengths of toes 1<2<3=5<4 (Fig. 4B). Finger webs and toe webs absent.

The closest relative of *E. juanariveroi* is *E. gryllus* with which it agrees in size and proportions (Fig. 3, Tables 1 and 2), skin glandularity and fundamental call note. However, *E. juanariveroi* is smaller than *E. gryllus* (SVL in mm: males, 14.7 females 15.8 vs. *E. gryllus* males 15.7 and females 16.4) (Table 1 and 2). The nares are prominent, and a ridge connects them behind the snout tip, giving it a somewhat squared-off appearance (Fig. 3A). On the other hand, the nares are less prominent in *E. gryllus*; the loreal surface is steeper on the average (65–79°) in *E. juanariveroi* compared with *E. gryllus* (51–67°); the loreal surface has a mid-indentation, which is not so pronounced in *E. gryllus*. The vomerine teeth are minute (2–3 teeth in UPRRP 6358) or absent (UPRRP 6341); the vomerine teeth of *E. gryllus* are small, but always visible (2–3 teeth in UPRRP 6373 and UPRRP 6374). The large carpal element (fused 2+3) is sculptured and emarginated ventrally, much more so than in *E. gryllus*. Glands in *E. juanariveroi* are clearly discernible on both external and internal examination (KU 306998) while glands in *E. gryllus* can only be verified by examining the inner surface of the skin (UPRRP 6372); glands are heavier and more extensive over the posterior angle of the head (supra-axillary gland, parotoid glands sensu Lynch [1971]), body (flanks-lumbar-inguinal) and hind leg (dorsal side of thighs, tibiae, and tarsi) in *E. juanariveroi* than in *E. gryllus* (Fig. 3B); gular glands prominent and more extensive in females of *E. juanariveroi* than in *E. gryllus* (Fig. 5A); the hyoid plate is narrower in *E. juanariveroi* than in *E. gryllus* (Fig. 5b); left lobe of liver short and rounded, significantly smaller than right lobe compared with *E. gryllus* (Fig. 5C); the ventral skin is unpigmented or with a very light stippling of melano- phores and is smooth to weakly areolate in *E. juanariveroi* (pigmented, slightly glandular, and with more prominent areolae in *E. gryllus*). The relationship between tympanum width and inter-narial distance and

between tibio-fibula length and tarsal length are significantly smaller than in *E. gryllus* (see beyond, *Multivariate comparison of morphological ratios*, for a detailed explanation on how we identified diagnostic morphological ratios and assessed the statistical significance).

**TABLE 1.** Range of measured characters (in mm) followed by mean and standard deviation in parentheses. For abbreviations, see Materials and methods.

	<i>Eleutherodactylus juanariveroi</i>		<i>Eleutherodactylus gryllus</i>	
	Males (N = 18)	Females (N = 27)	Males (N = 11)	Females (N = 10)
SVL	12.9–16.0 (14.66 ± 0.74)	12.1–17.3 (15.76 ± 1.21)	14.0–17.0 (15.65 ± 0.89)	14.1–17.9 (16.41 ± 1.18)
HW	5.1–6.1 (5.61 ± 0.23)	4.7–6.5 (5.98 ± 0.39)	5.4–6.5 (6.07 ± 0.33)	5.7–7.1 (6.39 ± 0.44)
HL	5.6–6.4 (5.95 ± 0.22)	5.4–7.8 (6.33 ± 0.48)	5.8–9.3 (6.83 ± 0.88)	6.3–7.4 (7.03 ± 0.41)
UEW	0.9–1.4 (1.21 ± 0.11)	1.0–1.7 (1.27 ± 0.14)	1.1–1.7 (1.38 ± 0.17)	1.2–1.5 (1.38 ± 0.10)
IOD	1.7–2.0 (1.82 ± 0.08)	1.4–2.3 (1.97 ± 0.18)	1.7–2.2 (2.00 ± 0.20)	1.8–2.4 (2.17 ± 0.21)
TYW	0.6–1.0 (0.81 ± 0.09)	0.5–1.2 (0.95 ± 0.14)	0.8–1.1 (1.00 ± 0.09)	0.8–1.3 (1.09 ± 0.13)
IN	1.3–1.5 (1.43 ± 0.08)	1.2–1.7 (1.51 ± 0.10)	1.3–1.7 (1.51 ± 0.12)	1.4–1.7 (1.60 ± 0.10)
EL	2.1–2.6 (2.27 ± 0.14)	2.0–2.8 (2.36 ± 0.19)	2.3–3.0 (2.60 ± 0.25)	2.3–3.0 (2.73 ± 0.24)
EN	1.3–1.7 (1.52 ± 0.09)	1.4–2.2 (1.70 ± 0.15)	1.6–2.1 (1.78 ± 0.15)	1.7–2.1 (1.93 ± 0.12)
FD <sub>III</sub>	0.6–0.7 (0.67 ± 0.05)	0.5–0.9 (0.73 ± 0.08)	0.6–1.0 (0.76 ± 0.11)	0.6–0.9 (0.77 ± 0.08)
FDB <sub>III</sub>	0.4–0.5 (0.44 ± 0.04)	0.4–0.6 (0.45 ± 0.04)	0.4–0.5 (0.44 ± 0.04)	0.4–0.5 (0.43 ± 0.03)
TD <sub>IV</sub>	0.5–0.8 (0.67 ± 0.07)	0.5–0.9 (0.74 ± 0.09)	0.6–1.0 (0.78 ± 0.12)	0.6–0.9 (0.77 ± 0.10)
FL	5.7–7.3 (6.59 ± 0.42)	5.4–7.5 (6.99 ± 0.46)	6.4–7.6 (7.00 ± 0.44)	6.5–8.9 (7.71 ± 0.63)
TiL	5.9–7.2 (6.45 ± 0.33)	5.5–7.6 (6.99 ± 0.51)	6.6–8.7 (7.26 ± 0.60)	6.7–8.6 (7.70 ± 0.56)
TaL	3.5–4.0 (3.84 ± 0.14)	3.3–4.6 (4.10 ± 0.28)	3.9–5.5 (4.48 ± 0.46)	3.9–5.1 (4.71 ± 0.35)

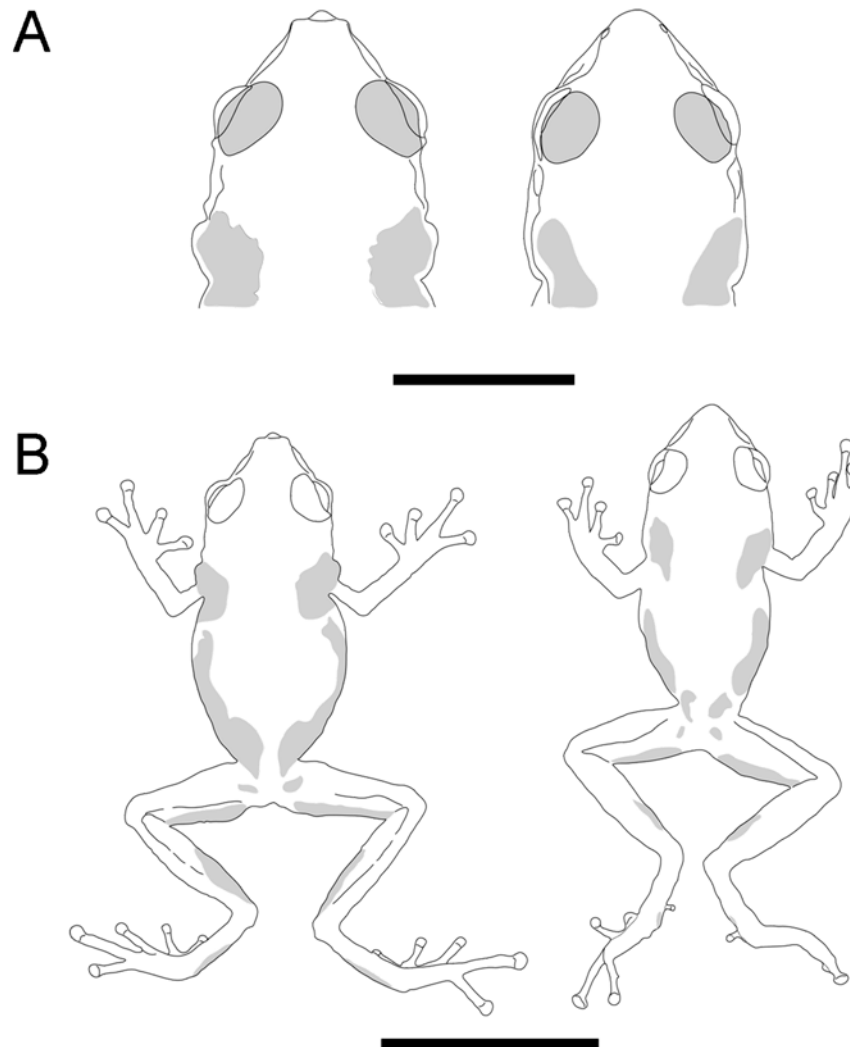
**TABLE 2.** Body proportions (mean ± SD) of *Eleutherodactylus juanariveroi* and *Eleutherodactylus gryllus* that showed significant differences at  $\alpha = 0.05$  and  $\alpha = 0.10$ . Significant variables (TYW/IN and TiL/TaL) were extracted from NMDS multivariate analysis. Data for males and females were analyzed separately; Mann-Whitney U-Test.

	Males				Females			
	<i>E. juanariveroi</i> (N = 18)	<i>E. gryllus</i> (N = 10)	Z	P	<i>E. juanariveroi</i> (N = 27)	<i>E. gryllus</i> (N = 9)	Z	P
TYW/IN	0.56 ± 0.06	0.66 ± 0.07	3.03	<0.01	0.63 ± 0.07	0.68 ± 0.06	1.60	0.11
TiL/TaL	1.68 ± 0.06	1.64 ± 0.09	1.66	0.09	1.70 ± 0.06	1.65 ± 0.07	1.34	0.18

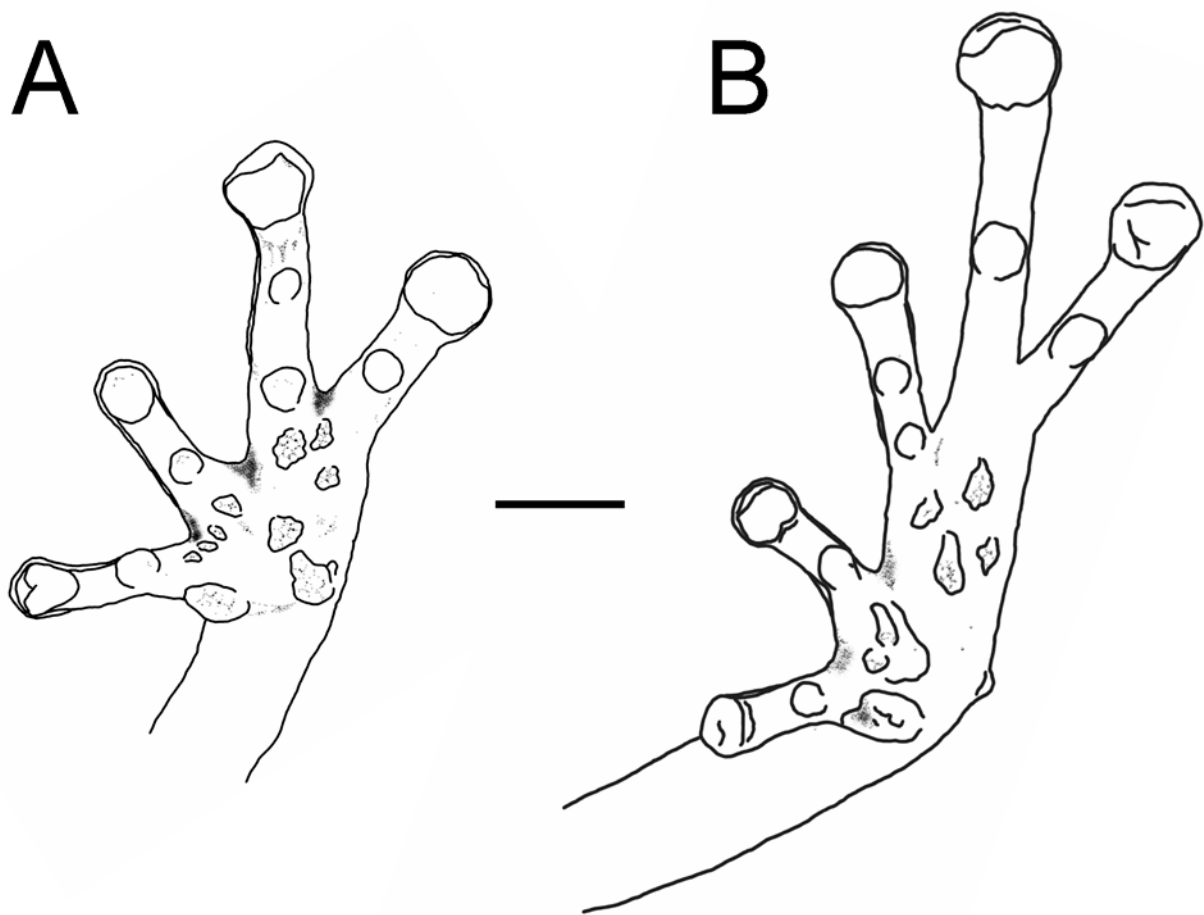
The dorsal coloration of *Eleutherodactylus juanariveroi* in life is yellow to yellowish brown (Fig. 2B) with a light, longitudinal, reversed comma mark on each side, lateral to the dorsal pigmented zone, with the head of the comma lying over the supra-axillary (parotoid) gland; the dorsal pigmented zone itself bifurcates, each branch being broad and fading, usually defining part of the cephalic triangle and not forming the sharp, well-defined “tines” of *E. gryllus*; the pattern is generally non-contrasting, versus a strongly contrasting dorsal pattern in *E. gryllus* that may include reversed parenthesis marks and occasionally a bold yellow mid-dorsal stripe (Fig. 2B; Joglar, 1998, Figs. 4.11–4.12; Rivero, 1998, Pl. XXV). *Eleutherodactylus juanariveroi* can be distinguished from other Puerto Rican species by an overall yellowish, non-contrasting coloration with a reversed comma pattern and a broadly bifurcated mid-dorsal zone (Figs. 2B and 6).

The marks that we call inverted commas in *E. juanariveroi* may be homologues of the inverted parenthesis marks of *E. gryllus*, but they could also be a modification of the entire lateral zone (Fig. 6). However, these

inverted commas may be a modification of the basic pattern elements of related species. Thus, in order to describe the color pattern of *E. juanariveroi* we relate it to the patterns of the other small Puerto Rican species (*E. cochranæ*, *E. eneidae*, *E. gryllus*, and *E. locustus*) by identifying similar pattern elements (Fig. 6): (1) a dark mid-dorsal zone extending from roughly the sacral region to the head, usually narrowing gradually along the trunk and expanding again near the head, thus having curved indentations on either side, (2) a pair of light, narrow reversed parenthesis marks that closely follow the indentation in the mid-dorsal zone, (3) a bifurcation of the mid-dorsal zone near the head, producing two dark, diverging extensions that approach the postero-medial edge of each eyelid (in *E. juanariveroi*, these are pale and broad), (4) a median head triangle, an apex of which projects caudad, fitting between the diverging extensions of the divided mid-dorsal zone, with the other two angles at each eye and side extending between the eyes (another pattern element that occurs in some of these species is a light midline stripe, but it is an occasional variant and is not found in *E. juanariveroi*). These pattern elements have been variously modified by adding or lessening of pigment in the different species (and are variable within species), but reversed parentheses are frequent. A further modification occurs when pigment bridges form between the lower parts of the “tines” of the fork to the caudad apex of the cephalic triangle, which results in a dark scapular ‘W’ (Fig. 6).



**FIGURE 3.** Comparative morphology of holotype of *E. juanariveroi* (left, KU 306997) and *E. gryllus* (right, UPRRP 6372): canthus rostralis in dorsal view, scale bar 5 mm (A), distribution and shape of glands in dorsal view, scale bar 10 mm (B).



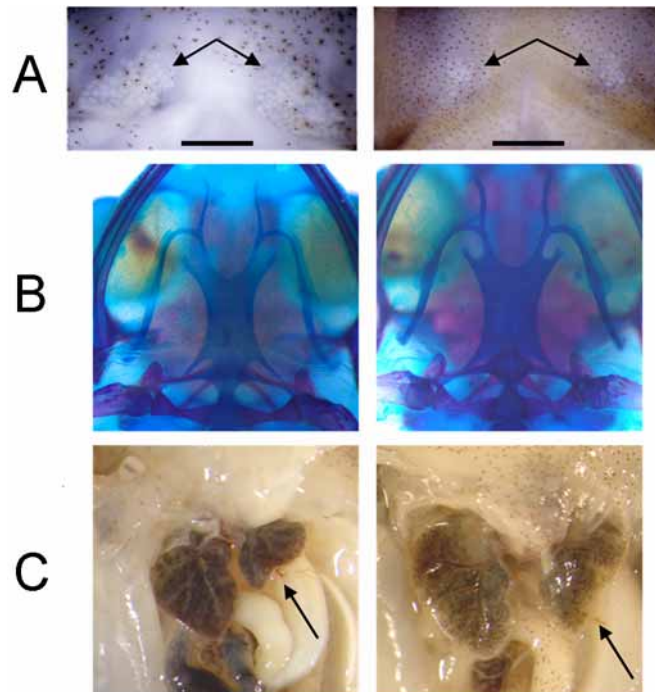
**FIGURE 4.** Holotype of *Eleutherodactylus juanariveroi*, KU 306997. Palmar view of hand (A) and plantar view of foot (B). Scale bar 1 mm.

On some individuals of these related small species, particularly *E. locustus*, the scapular 'W' and the light reversed parentheses may be the only pattern readily seen. Some of the components occur in frog species we have not mentioned; for example, *E. hedricki* (Rivero) and *E. eneidae* may have the reversed parentheses. In *E. juanariveroi* the pattern is much reduced in contrast; the reversed parenthesis marks are very wide; and the median dark zone is relatively light and the anterior branches broad; a well-developed scapular 'W' is not found. The result is a pair of light, longitudinal marks that curve laterally onto each side of the occiput, terminating with the large posterior head glands (Figs. 2B and 6). Interior to these marks on many specimens is another pair of diverging, light longitudinal marks (the altered median dorsal zone and branches). Some individuals show only the light heads of the commas, the rest of the pattern being obscure; this results in a pattern of two ocelli over the area of the scapula on the back of the head (Fig. 6), representing the most divergent pattern among this group of small frogs.

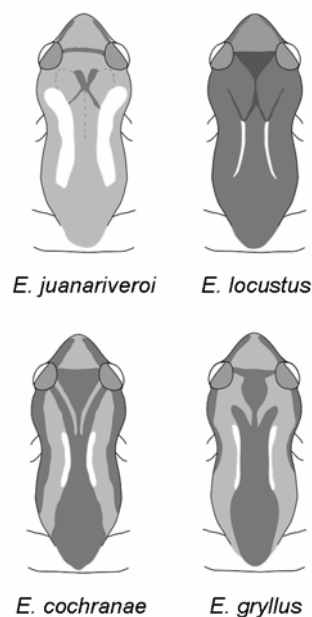
A light-brown inter-orbital band that extends to the tip of the snout is present in 16.3% of the *E. juanariveroi* (clearly defined in KU 306999) and absent or rare in *E. gryllus* (some specimens are so faded that it is difficult to discern the pattern). The legs of *E. juanariveroi* do not have any cross bands; those of *E. gryllus* have 1–2 hazy bands on the thigh and two on the shank. Although its call is related to that of *E. gryllus*, it is distinctive in its patterning and higher pitch (see *Advertisement call* for a detailed explanation on call parameters).

A Puerto Rican *Eleutherodactylus* that may have morphological affinities with *E. juanariveroi* and *E. gryllus* is *E. jasperi*. *Eleutherodactylus jasperi* also has two pairs of lateral sub-dermal glands well developed on flanks and posterior surfaces of thighs (Drewry and Jones, 1976). However, although *E. jasperi* has a yellow to yellow-brown body coloration in life, it differs dramatically from *E. juanariveroi* and *E. gryllus* in repro-

duction (*E. jasperi* is ovoviviparous, the other species are oviparous), body size (19–20 mm in *E. jasperi*, 14–15 mm in *E. juanariveroi*, 15–16 mm in *E. gryllus*), acoustics (frequency of calls: 5 kHz in *E. jasperi*, ~7 kHz in *E. gryllus*, ~8 in *E. juanariveroi*), habits (*E. jasperi* is an obligate bromeliad-dwelling in somewhat xeric conditions in highlands, *E. juanariveroi* is an obligate herbaceous-dwelling in palustrine lowland wetland [N. Rios, in prep.], *E. gryllus* is arboreal in highland wetland conditions), and geographic distribution. Additionally, *E. jasperi* lacks prevomerine teeth while *E. juanariveroi* and *E. gryllus* have them.

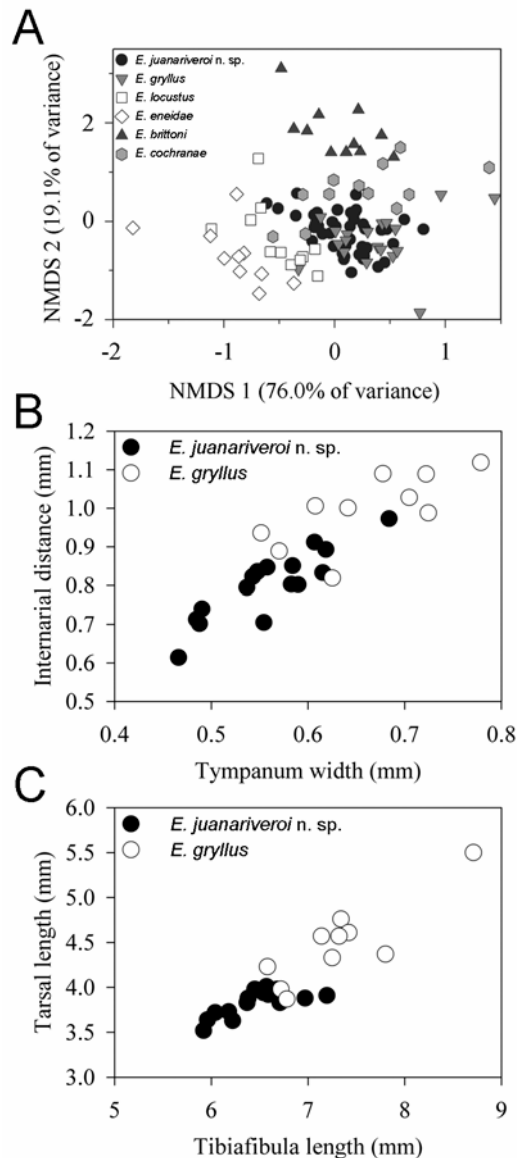


**FIGURE 5.** Comparative morphology of *E. juanariveroi* (left, holotype, KU 306997) and *E. gryllus* (right, UPRRP 6374): glands in gular region (arrows), scale bar 1 mm (A), cleared-stained hyale apparatus (UPRRP 6341 and UPRRP 6374) (B), and relative liver size and shape of liver (*E. juanariveroi* UPRRP 6358 and *E. gryllus* UPRRP 6373) with arrows highlighting small-sized left lobe of liver (C).



**FIGURE 6.** Comparison of dorsal color pattern of *E. juanariveroi* with related species showing reversed comma pattern and basic similar coloration elements.





**FIGURE 7.** Ordination scores of NMDS axis II plotted against axis I (A) and comparative morphometry of males of *Eleutherodactylus juanariveroi* and *E. gryllus*: IN plotted against TYW (B); TaL plotted against TiL (C).

**Measurements (in mm) and description of the holotype.** Adult female; SVL 16.7; head width 6.1; head length 6.7; upper eye lid width 1.1; inter-ocular distance 2.1; tympanum width 1.0; inter-narial distance 1.5; eye length 2.3; eye-tympanum distance 0.3; eye-naris distance 1.7; fingertip (III) width 0.8; toe tip (IV) width 0.9; femur length 7.5; tibia-fibula length 7.2; tarsal length 4.2. Inter-orbital band absent; gular glands prominent; supra-axillary gland (parotoid), body glands (flanks-lumbar-inguinal), and hind limb glands (dorsal side of thighs, tibiae, and tarsi) clearly visible; well-expressed pale reversed comma heads and a pale dorsal zone bifurcation occur in dorsum. Mature eggs undetected externally. Dorsal coloration in life yellow to light brown; light brown in preservative.

**Variation.** SVL of adult males 12.9–16.0 mm (mean = 14.66, n = 18), of adult females 12.1–17.3 mm (mean = 15.76, n = 27), and juvenile (SVL 5.82 mm) of moderate habitus, typical of scansorial species of *Eleutherodactylus* (Table 1); head around 40% of SVL; snout tip pointed in dorsal view, sides forming an angle of about 72°; nares distinctly protuberant, canthus rostralis not distinct, lores sloping, slightly indented; eyes prominent, EL into HW around 2.7; tympanic membrane present and tympanic annulus distinct but with

roughly the dorso-posterior third of the rim obscured by supra-tympanic fold, fitting tightly between the ventro-posterior edge of the orbit and the angulus oris, TYW into HW around 6; 2–3 minute vomerine teeth barely visible on weakly defined patches postero-medial to choanae (of two cleared and stained specimens UPRRP 6358, a male, with 3 tiny vomerine teeth on one patch, 2 or 3 in the other patch, teeth absent in UPRRP 6341, a female); tongue rounded posteriorly with a weak notch; there are well-developed glands on the following regions (Fig. 3): (1) supra-axillary (parotoid), extending ventrally to glenoid region, (2) flanks to sacral region, (3) posterior surfaces of thighs, shank, tarsi and (4) postero-lateral parts of the gula (sexually dimorphic: present in 96.3% in females, which also have larger glands, compared with 23.5% in males). Digital disks moderate, fitting within the tympanum; hyoid plate narrow (Fig. 5B); medial element of anterior process of the hyale long and straight; hyale without free flanges; antero-lateral process angled anteriorly; postero-lateral process long and thin; thyrohyal process narrow. Venter nearly smooth with weak, rounded areolations and with a sparse stippling of melanophores; testes pigmented. Measurements (range, mean, and standard deviation) of the type series are given in Table 1 along with measurements of *E. gryllus* for comparative purposes.

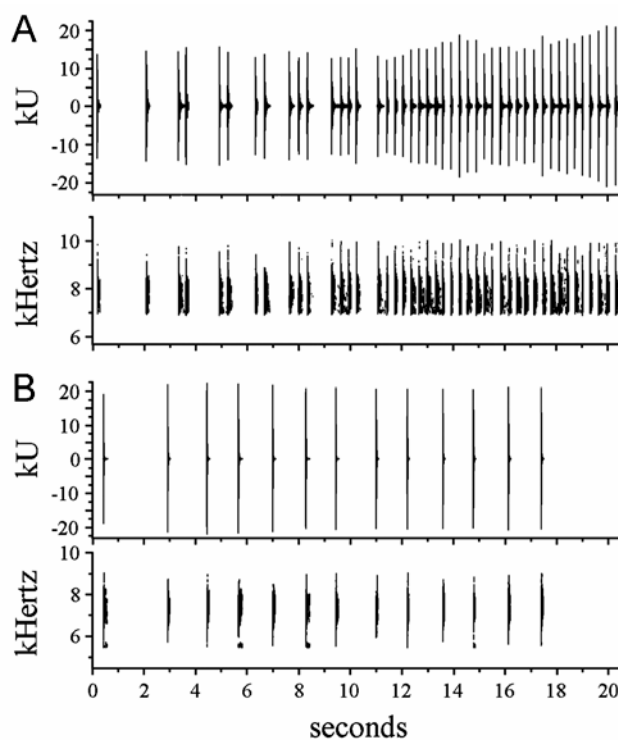
The limbs are irregularly pigmented with varied intensities of pigment but without a distinct patterning; the post-femoral surfaces of some specimens are darker; the digit tips of manus and pes are darkly pigmented. In some individuals of *E. juanariveroi*, a horizontally elliptical pupil, and a thin, dark vertical slit below the mid portion of pupil result in a ‘T’ (Fig. 2A) similar to the condition in *E. gryllus*, *E. eneidae*, and *E. locustus*. In other individuals of *E. juanariveroi* the pupil resembles that in *E. cochranæ*, which has thin, vertical slits below and above pupil resulting in a ‘+’. Pupil shape in *E. juanariveroi*, however, differs from *E. brittoni*, which lacks vertical slits.

**Multivariate comparison of morphological ratios.** Non-metric Multidimensional Scaling (NMDS) of morphological ratios of adult specimens extracted two axes that explained 95.1% of the variance in ordination space (final stress = 11.3 and instability = 0.052 after 500 iterations, orthogonality = 94.8%) (Fig. 7A). A Multi-Response Permutation Procedure (MRPP, a non-parametric analysis used to test the hypothesis of six species in the data matrix) revealed six groups that had similar within-group homogeneity ( $A = 0.27$ ,  $p < 0.0001$ ), which corroborates the hypothesis of six nomenclaturally and taxonomically stable species in the data matrix. However, the overlap between *E. juanariveroi* and *E. gryllus* in ordination space suggests a strong affinity in morphological ratios between species. An Indicator Species Analysis (ISA) (Legendre & Legendre, 1998) that could be helpful in distinguishing *E. juanariveroi* from other species resulted in four potential diagnostic morphological ratios (morphological ratio and Indicator Value from Monte Carlo tests with 10,000 permutations): SVL:HW, 17.1 ( $p = 0.067$ ); TYD:IND, 18.9 ( $p = 0.004$ ); TYD:EN, 18.2 ( $p = 0.028$ ); TiL:TaL, 17.1 ( $p = 0.054$ ). In ordination space, only TYD:IN and TiL:TaL differed significantly between males of *E. juanariveroi* and the most similar *E. gryllus* (Table 2), which justified the use of these two morphological ratios to distinguish *E. juanariveroi* from *E. gryllus* (Figs. 7B and 7C).

**Distribution and natural history.** *Eleutherodactylus juanariveroi* is known only from the type locality (Fig. 1), which lies within the subtropical moist forest life zone (Ewel & Whitmore, 1973). This locality consists of a palustrine herbaceous wetland at 17 m elevation. The wetland is seasonally flooded with fresh water; the soil consist of swamp and marsh organic deposits from Pleistocene and/or recent origin (Briggs & Akers, 1965). Herbaceous vegetation in this habitat consists of the toothed midsorus fern (*Blechnum serrulatum*), willdenow’s maiden fern (*Thelypteris interrupta*), bulltongue arrowhead (*Sagittaria lancifolia*), flatsedges (*Cyperus* sp.), spike rushes (*Eleocharis* sp.), and vines and grasses. The habitat at the type locality occupies approximately 180 ha; we were unable to find the frog at other wetland sites in the northern coastal plain. All specimens were collected between 1900–2200 h while perching, sitting, or calling on herbaceous vegetation, mainly on ferns, between 0.4 m and 1.2 m above water level. Calling sites for males were 0.6 to 1.2 m above the ground. Egg clutches comprised 1–5 eggs and were found on leaf axils (21 egg clutches) or leaf surfaces (3 egg clutches) of only *S. lancifolia* (N. Rios, unpubl. data). Like *E. gryllus*, the calling activity of *E. jua-*

*nariveroi* started at approximately 1630 h and decreased significantly before midnight. The single locality from which *E. juanariveroi* is known is adjacent to the coast at 17 m elevation, while *E. gryllus* is only known from between 305 m to 1189 m elevation (Joglar, 1998), although we have not recently found *E. gryllus* below 600 m elevation. This species is mostly arboreal, calling from branches and bromeliads, which contrasts with *E. juanariveroi*.

**Advertisement call.** The call consists of a series of short high-pitched notes (Fig. 8A). At 26 C, call duration varies from 4–21 sec ( $10.9 \pm 6.2$  sec,  $n = 6$ ). Call structure consists of one to three one-note series, followed by one or more series of two notes each, one (or more) series of three-four notes each, and a series that varies from 5–30 notes at the end of the call. Notes per call was 8–45 ( $23.0 \pm 13.4$  notes,  $n = 6$ ) (Fig. 8A). Frequency was 6.03–9.02 kHz; the dominant frequency was 7.38–8.28 kHz ( $7.69 \pm 0.18$  kHz,  $n = 92$ ) (Fig. 8A). Note duration was 15–31 ms ( $24.1 \pm 3.1$  ms,  $n = 92$ ). Note repetition rate varies between consecutive notes within a series of two or more notes ( $341 \pm 22$  ms,  $n = 58$ ) and between the final note of a series and the note in the following series ( $819 \pm 99$  ms,  $n = 30$ ) (Fig. 8A). The advertisement call of *E. juanariveroi* differs from that of *E. gryllus* in notes per call (range 5–29,  $15.3 \pm 10.0$  notes,  $n = 4$ ) (Fig. 8B), dominant frequency (range 6.31–7.79 kHz,  $7.44 \pm 0.28$  kHz,  $n = 61$ ) (Fig. 8B), note duration (range 21–32,  $26.8 \pm 2.4$  ms,  $n = 61$ ), and note repetition rate (consecutive notes within a series  $1.23 \pm 0.13$  sec,  $n = 53$ ; between the first note of the call and the note in the following series in the call  $1.97 \pm 0.67$  sec,  $n = 4$ ) (Fig. 8B). Among sympatric *Eleutherodactylus*, the call of *E. juanariveroi* has the highest frequency and the greatest structural complexity compared with *E. brittoni*, *E. cochranæ*, and *E. coqui* (Thomas), but probably among all Puerto Rican *Eleutherodactylus* as well (for comparison see Drewry & Rand, 1983 and Narins, 1995).



**FIGURE 8.** The advertisement calls of *Eleutherodactylus juanariveroi* (A) and *E. gryllus* (B). Oscillogram (relative amplitude in kU vs. time) and audiospectrogram (sonogram: frequency in kHz vs. time).

**Etymology.** The specific name honors Juan A. Rivero, distinguished Puerto Rican herpetologist and naturalist.

## Discussion

Among the West Indian *Eleutherodactylus*, *E. juanariveroi* is most similar to *E. gryllus* based on (1) its small size, (2) morphological ratios in multivariate analyses, and (3) gland distribution. Although it is premature to conclude that this similarity is a reflection of phenetic or phylogenetic relationship, DNA sequencing data suggests that *E. juanariveroi* belongs to the Puerto Rican species radiation of *Eleutherodactylus* (S. B. Hedges, pers. comm.). Hedges (1989) included *E. gryllus* in the genus *Eleutherodactylus*, subgenus *Eleutherodactylus*, *auriculatus* section, *martinicensis* series, based on liver shape, testis coloration, and protein data. *Eleutherodactylus juanariveroi* and *E. gryllus* share liver shape and testis coloration, which justifies the inclusion of *E. juanariveroi* in the *auriculatus* section, most likely as a sister species of *E. gryllus* under Hedges (1989) classification.

*Eleutherodactylus juanariveroi* appears to be an obligate marsh-dweller (N. Rios, unpubl. data) and is thus nearly unique among its West Indian congeners; the only other species known to have a similar habitat requirement is *E. caribe* (Hedges & Thomas) of Haiti (Hedges & Thomas, 1992). We have noted that *E. juanariveroi* is the smallest of the Puerto Rican frogs; however, it is not much smaller than *E. unicolor* (Stejneger), a fossorial species of high elevations, in which the largest recorded size is 17.3 mm SVL (identical to the largest female of *E. juanariveroi*); *E. unicolor* has a rounded body and much shorter legs, however, and the average SVL for both sexes is also larger (males 15.0 mm, females 16.1 mm; Joglar, 1998). A partial consequence of small size is a high-pitched call; *E. juanariveroi* has a dominant frequency between 7.38 and 8.28 kHz, which is among the highest pitched call of frogs calling in the sonic range of humans (Estrada & Hedges, 1996). It is now known that *Amolops tormotus* (Wu) of China communicates in the ultrasonic range (Feng *et al.*, 2006).

### Land-use history and conservation

The discovery of a Puerto Rican *Eleutherodactylus* with such an apparently restricted and small range may be a surprise to the uninitiated, but it may reflect a remnant population of a once wide-distributed herbaceous wetland specialist whose habitat was decimated during the Puerto Rican land-use history. For example, the vast majority of coastal wetlands in Puerto Rico were drained and destroyed for agriculture and cattle ranching since the settlement by the Spaniards in the early 1500's (Picó, 1990). During the mid 1900's, however, many agriculture lands were abandoned due to a shift in the Puerto Rican economy, from agriculture to industry (Grau *et al.*, 2003). As a result, most of these lands were destroyed and invaded by grasses and other herbaceous vegetation or converted for urban development, dramatically reducing these wetlands' cover area (Lugo & Brown, 1988). Not surprisingly, the type locality of *E. juanariveroi* has many plant species that have been recorded from sites now destroyed for urban development (F. Axelrod, pers. comm.).

Aerial photographs from 1937 show, however, that a significant portion of the type locality in the north-eastern side was largely protected from agriculture and/or wetland drainage. This portion of the type locality was occupied by the USNSGASS since late 1940's for military purposes after World War II. Since then, access to people has been much restricted, and this portion has not been developed. This contrasts with the land-use history of the rest of the wetlands in eastern and western Puerto Rico and that were transformed dramatically through drainage and landfill for local socio-economic reasons (Lugo & Brown, 1988; Pico, 1990). Thus, the type locality of *E. juanariveroi* has experienced little disturbances partly due to restricted access of local people by the USNSGASS and the limited development of military installations in this wetland. The restricted access to this wetland site, and the fact that few herpetological studies have been conducted in Puerto Rican fresh water wetlands, may have contributed to the late discovery of *E. juanariveroi* more than 30 years since the discovery of the last *Eleutherodactylus* from mainland Puerto Rico.

Unfortunately, *E. jasperi*, the last discovered species, has not been found since 1981 (R. Thomas, pers. obs.). Its historic habitat has been dramatically fragmented for agriculture and urban development and

destroyed by fires. This species has possibly become extinct before effective conservation actions were taken to protect its habitat (Joglar & Burrowes, 1996). Similarly, recent surveys conducted in nearby wetlands failed to locate populations of *E. juanariveroi* (N. Rios, unpubl. data) and apparently, there are few or no wetlands with plant composition similar to that in the type locality. This suggests that the type locality could represent a relict palustrine herbaceous wetland now rare in Puerto Rico. Also, the high-pitched call of *E. juanariveroi* is, to a human perceiver, overwhelmed by the loud and lower chorus of *E. coqui*, *E. brittoni*, *E. cochranæ*, and *Leptodactylus albilabris* (Günther) at the type locality, which makes its detection more difficult. The type locality, however, is now threatened by private and governmental housing, industrial, and recreational projects that are spreading rapidly through the region after the USNSGASS has ceased operations in 2005. These immediate threats on the ecological integrity of this wetland and on the survival of this species require prompt conservation efforts.

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## Appendix 1. Specimens examined

- Eleutherodactylus brittoni*: Puerto Rico: Utuado-Arecibo: Bosque Estatal de Río Abajo, <464 m, RT 554; Toa Baja: Sabana Seca, 17 m, RT 14524, 14528, 14531–32; RT 3835, 3998–99, 8454, 8773–74 [no data].
- Eleutherodactylus cochranæ*: Puerto Rico: Isla Vieques: east of Ensenada Sombe, <5 m, RT 9306–9308, 9310; Guayama-Santa Isabel: Bosque Estatal de Aguirre, <10 m, RT 10909–11; Toa Baja: Sabana Seca, RT 14525, 14529, 14551; RT 7154, 8735 [no data].
- Eleutherodactylus eneidae*: Puerto Rico: Río Grande: El Yunque, La Mina Recreation Area, ~670 m, RT 145–148; Jayuya: Toro Negro Forest, 8.8 km [by road] E La Pica, 1182 m, RT 2220–21, 2223–24; RT 3845, 3847, 6461 [no data].
- Eleutherodactylus gryllus*: Puerto Rico: Cayey: Bosque Estatal de Guavate, 8 km [by road] SE Las Cruces, ~630 m, RT 592; Río Grande: El Yunque, 2 km [by road] E Pico del Este, UPRRP 6372–4, RT 14466–68; Río Grande: El Yunque, trail to Pico del Este, ~650 m, UPRRP 20–21; La Mina Recreation Area, ~560 m, UPRRP 41; southern slopes Luquillo forest, ~758 m, UPRRP 2157–59, 2215–16; ~803 m, UPRRP 2275; El Yunque, University of Puerto Rico-Biology Station, ~636 m, UPRRP 2856, 2864; Maricao: near Jct. roads PR-105 and PR-128, UPRRP 3411; RT 3968, RT 8398 [no data].
- Eleutherodactylus juanariveroi*: Puerto Rico: Toa Baja: Sabana Seca, 17 m, KU 306997 (holotype), KU 306998–02; USNM 563623–8; UPRRP 6340–71; RT 14501, RT 14535.
- Eleutherodactylus locustus*: Puerto Rico: Río Grande: El Yunque, La Mina Recreation Area, RT 138, RT 140, RT 142; Cayey: Bosque Estatal de Guavate, 8 km [by road] SE Las Cruces, ~630 m, RT 574, RT 576, RT 578, RT 581, RT 590; Río Grande: El Yunque, 2 km [by road] E Pico del Este, RT 14558–60.