

A new species of the *Eleutherodactylus lacrimosus* assemblage (Anura, Brachycephalidae) from the lowland rainforest canopy of Yasuni National Park, Amazonian Ecuador

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Abstract

A new species of the *Eleutherodactylus lacrimosus* assemblage (Anura, Brachycephalidae) from the lowland rainforest canopy of Yasuni National Park, Amazonian Ecuador. A new species of *Eleutherodactylus* from the lowland rainforest canopy in northeastern Amazonian Ecuador is described. It is placed in the *Eleutherodactylus lacrimosus* assemblage of the greater *unistrigatus* group. It is most similar to the sympatric species *Eleutherodactylus lacrimosus*, but differs by the lack of a papilla at the tip of the snout, tubercles on upper eyelids, tubercles on dorsum, dorsal markings, and larger body size, and the presence of lateral fringes on the fingers and a tarsal fold. The new species inhabits tank bromeliads in the upper strata of the rainforest canopy at heights of 23.5–38.0 m. The effects of implementation of canopy surveys on biological diversity are briefly discussed.

Keywords: Anura, Brachycephalidae, “eleutherodactyline”, *Eleutherodactylus lacrimosus*, *E. unistrigatus*, *E. waoranii* sp. nov., *Pristimantis*, bromeliad patch sampling, canopy sampling, Yasuni National Park, Ecuador.

Introduction

Amphibian taxonomy is currently experiencing rapid changes, particularly within eleutherodactyline frogs, as a result of DNA

sequence analyses (Frost *et al.* 2006, Heinicke *et al.* 2007). The Amphibian tree of life by Frost *et al.* (2006) partitioned the former genus “*Eleutherodactylus*” (*sensu lato*) into five genera and placed them in the family Brachycephalidae along with an additional 11 genera, while acknowledging the probable continued nonmonophyletic state of “*Eleutherodactylus*”

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(*sensu stricto*). Heinicke *et al.* (2007) have just published a molecular analysis comprising a large portion of eleutherodactyline diversity (276 species) and recovered three major geographic clades. The South American Clade is assigned to the resurrected anuran genus *Pristimantis* and is the largest group with 393 species currently described (Heinicke *et al.* 2007). “*Eleutherodactylus*” (*sensu lato*) distribution ranges from the southern United States through Mesoamerica and the West Indies to southern South America (Zug *et al.* 2001); while Heinicke *et al.* (2007) find the newly resurrected *Pristimantis* distribution to be centered in the Andes and spreads east through northern South America, with a few species in southern Central America and two species from the southernmost islands of the Lesser Antilles. Heinicke *et al.* (2007) sampled 87 of 393 described species in their South American Clade; while this number greatly increases previously available DNA sequence data, it represents a minority of the taxa in the group. Thus, pending taxonomic acceptance of Heinicke *et al.* (2007) and the review of additional taxa within the South American Clade, we acknowledge the likely placement of the species described herein to the genus *Pristimantis* but maintain the use of *Eleutherodactylus* for this work.

Ecuador is ranked number three in the world for amphibian diversity with more than 447 known species, following Brazil and Colombia, respectively (Young *et al.* 2004). In Ecuador, at least 132 species are members of the genus *Eleutherodactylus*, with 21 residing in the eastern lowlands of the Amazon basin (IUCN *et al.* 2004). Until recently, only minimal investigation of *Eleutherodactylus* in lowland eastern Ecuador has been conducted following Duellman’s exhaustive work at Santa Cecilia in the sixties and seventies (Duellman 1978, Lynch and Duellman 1980). Furthermore, examination of the canopy anuran community has been poorly represented in the study of the Amazonian amphibian fauna (Guayasamin *et al.* 2006).

The *Eleutherodactylus lacrimosus* assemblage currently consists of 15 recognized species, and is considered a phenetic subgroup of the large *E. unistrigatus* species group applied by Lynch and Duellman (1997) (Guayasamin *et al.* 2006, Heyer and Hardy 1991). The 15 species currently considered part of the *E. lacrimosus* assemblage are: *E. apiculatus*, *E. aureolineatus*, *E. boulengeri*, *E. brevifrons*, *E. bromeliaceus*, *E. dorsopictus*, *E. eremitus*, *E. lacrimosus*, *E. mendax*, *E. olivaceus*, *E. petersorum*, *E. prolixodiscus*, *E. schultei*, *E. tayrona*, and *E. zimmermanae* (Guayasamin *et al.* 2006). The *lacrimosus* assemblage is loosely delineated by the morphological characters of a small body size and broad, flat, pointed head; and more inclusively, a niche-specific association with bromeliads, with the exception of *E. apiculatus* (Lynch and Ruíz-Carranza 1985, Heyer and Hardy 1991, Guayasamin *et al.* 2006).

The species description herein resulted from bromeliad patch sampling conducted at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito in the eastern lowlands of Amazonian Ecuador.

Materials and Methods

Morphological terminology, diagnosis, and description follow Lynch and Duellman (1997); the format follows Guayasamin *et al.* (2006). Diagnosis, description, and variation were conducted from the observation of both preserved specimens and a series of high-resolution digital color macro photographs of each specimen consisting of dorsal, ventral, and lateral views in life. Photographs were taken with a Pentax istD digital SLR camera, outfitted with a 50 mm Pentax macro lens, Pentax AF-140c ring flash, and a Pentax AF-360FGZ external flash. Specimens were euthanized and muscle tissue was removed from the left thigh of all specimens for DNA tissue sample and stored in 95% ethanol at -80°C, deposited in the Frozen Tissue Collection of Dr. Michael R. J. Forstner

(MJF) at Texas State University, San Marcos, Texas; then specimens were preserved in 10% formalin and stored in 70% ethanol. All specimens were deposited at the Texas Cooperative Wildlife Collection (TCWC) at Texas A&M University, College Station, Texas. Specimens and field notes were collected by S. F. McCracken between June and August 2004.

Morphological measurements follow Guayasamin (2004) and Fuentes and Barrio-Amorós (2004), taken with a Mitutoyo CD-S6°C digital dial caliper using an Olympus SZH Stereozoom microscope on preserved specimens to the nearest 0.1 mm. Measurements taken include: (1) snout–vent length (SVL); (2) tibia length; (3) foot length; (4) head length; (5) head width; (6) upper–eyelid width; (7); interorbital distance; (8) eye diameter; (9) eye-to-nostril distance; (10) snout–eye distance; (11) tympanum diameter; (12) eye-to-tympanum distance; (13) internarial distance; (14) radioulna length; (15) hand length; (16) Finger I length; (17) Finger II length; (18) Finger III disc width; (19) femur length; (20) Toe IV disc width. Sexual maturity was determined by observation for the presence or absence of vocal slits in males, and examination for the presence of convoluted oviducts in females.

All specimens were collected at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito via bromeliad patch sampling using single-rope climbing technique to access canopy bromeliads. Two trees in each of four quadrat plots were surveyed; five bromeliads ($n = 40$) were removed from each tree at varying vertical heights and placed in large plastic bags to be lowered to the forest floor. Bromeliads were searched by removal of individual leaves to allow collection of all anurans within a screened enclosure to prevent escape. Vertical height of bromeliads was obtained by attaching a 100 m reel tape measure to the tree climber’s harness and positioning the reel at the base of the tree, height was recorded by a climbing assistant on the ground when the climber was level with the bromeliad being

collected. Environmental data (Appendix I) were recorded for collection sites and reported herein for occurrences of the new species and include: temperature (°C) at ground, 1 m and bromeliad collection site; barometric pressure (hPa) at 1 m and bromeliad collection site with a Brunton Sherpa; humidity (% relative humidity) at 1 m with a sling psychrometer; pH of water in bromeliad with a Oakton (pHTestr 30) meter; volume (L) of water in bromeliad with graduated cylinder; and diameter at breast height (DBH) of tree to the nearest 0.5 cm (Appendix I). Spatial distribution of sampled trees is represented by altitude (m.a.s.l.) and the shortest distance (m) to the Tiputini River using the measurement tool in ArcInfo 9.1 (Figure 1). Identifications of plant species were done using Ribeiro *et al.* (1999) and Kreft and Köster (2004).

Species Description

Eleutherodactylus waoranii sp. nov.

(Figures 2 and 3, Appendix II)

Holotype - TCWC 90728, adult male collected near the border of Yasuni National Park along the Tiputini River, Provincia de Orellana, Ecuador; approximately 2.5 km west-northwest of the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, 0°38'18.49" S, 76°08'56.69" W, 217 m a.s.l., collected on 15 August 2004 by Shawn F. McCracken.

Paratopotypes - TCWC 90729 and 90730, adult females, collected 15 August 2004 by Shawn F. McCracken.

Paratypes - TCWC 90731, adult female, collected 31 July 2004; TCWC 90732, adult male, collected 08 August 2004 by Shawn F. McCracken at the type locality.

Etymology - Named in recognition of the Waorani, an ancient indigenous tribe who have inhabited the type locality for centuries. They are excellent tree climbers and continue to persist, along with the local biota, despite

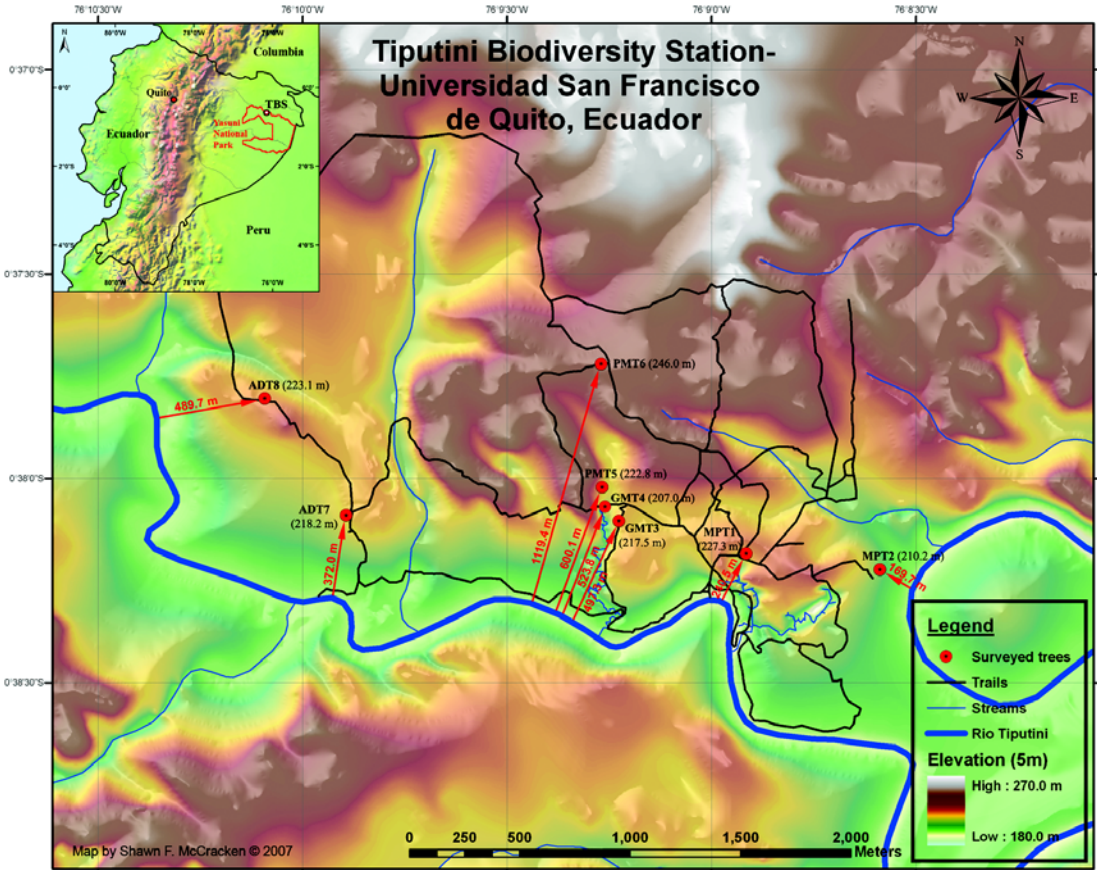


Figure 1 - Map of trees surveyed by bromeliad patch sampling at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, Ecuador. Tree distance shortest to the Tiputini River in red and elevation in black. Inset map shows location of TBS and outline of Yasuni National Park in red.

widespread anthropogenic pressures in the region.

Diagnosis - A member of the *Eleutherodactylus lacrimosus* assemblage contained within the greater *E. unistrigatus* group (Lynch and Duellman 1980, Lynch and Ruiz-Carranza 1985, Lynch and Duellman 1997) having (1) skin on dorsum finely shagreen, that on venter areolate; discoidal fold low; dorsolateral folds absent; (2) tympanic membrane weakly differentiated in males and slightly evident in females; tympanic annulus evident, with

supratympanic fold obscuring upper and posterodorsal edges; horizontal diameter of tympanum 39–49% of eye diameter; (3) snout subacuminate in dorsal view (truncated by protruding nostrils), truncate in profile; (4) upper eyelid lacking tubercles, 50–74% of interorbital distance; cranial crests absent; (5) dentigerous process of the vomer triangular, each bearing 3–6 teeth; (6) males with vocal slits and median subgular vocal sac; white, nonspinous nuptial pads present; and white testes (mesorchium); (7) first finger shorter than the second; Fingers III–IV bearing expanded

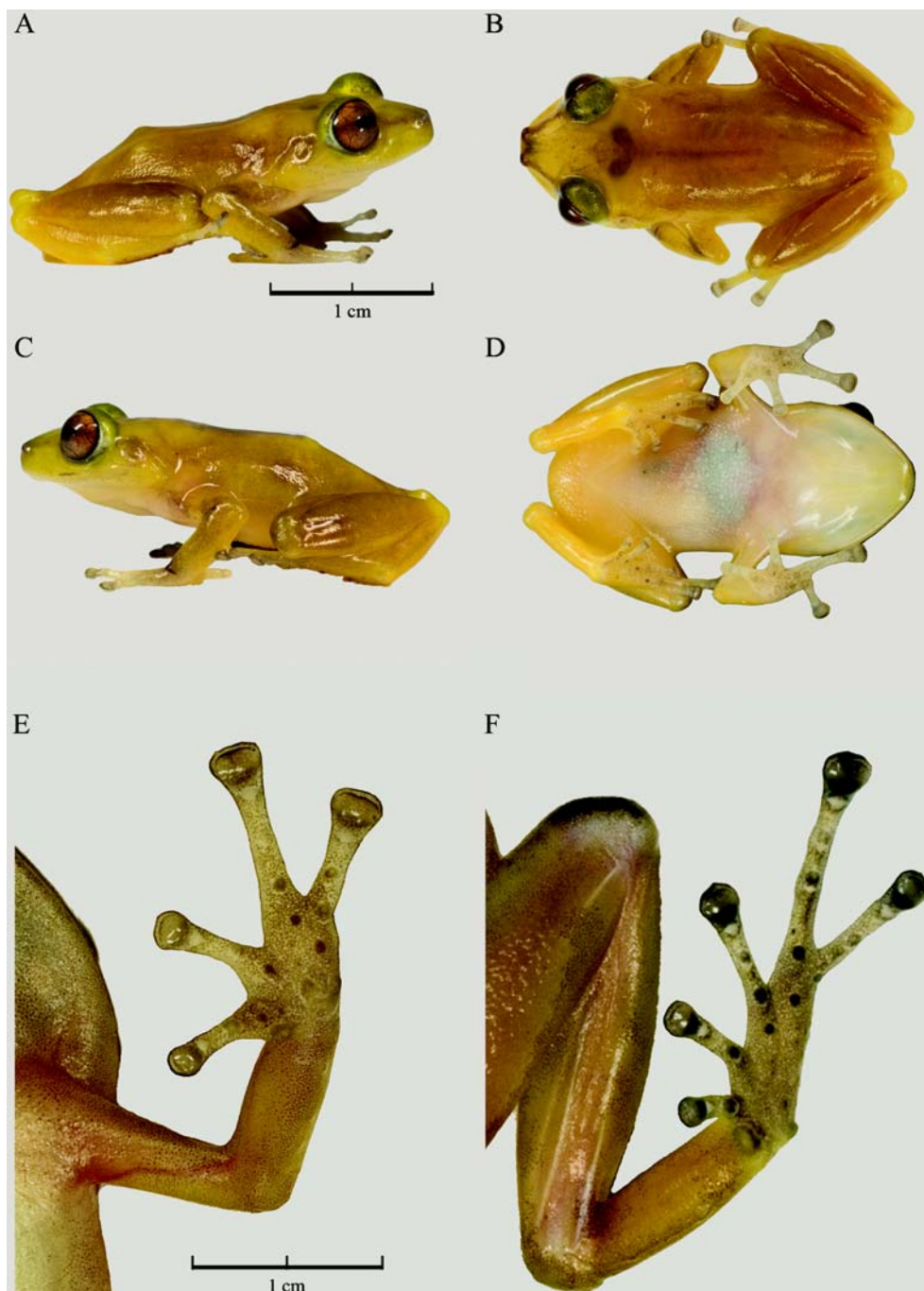


Figure 2 - *Eleutherodactylus waorani* (TCWC 90728, holotype in life, SVL = 21.2 mm, male) in lateral (A, C), dorsal (B), and ventral (D) views; and plantar view of left hand (E) and left foot (F) of *E. waorani* (TCWC 90731, paratype, SVL = 27.5 mm, female). Photographs by Bejat A. McCracken.

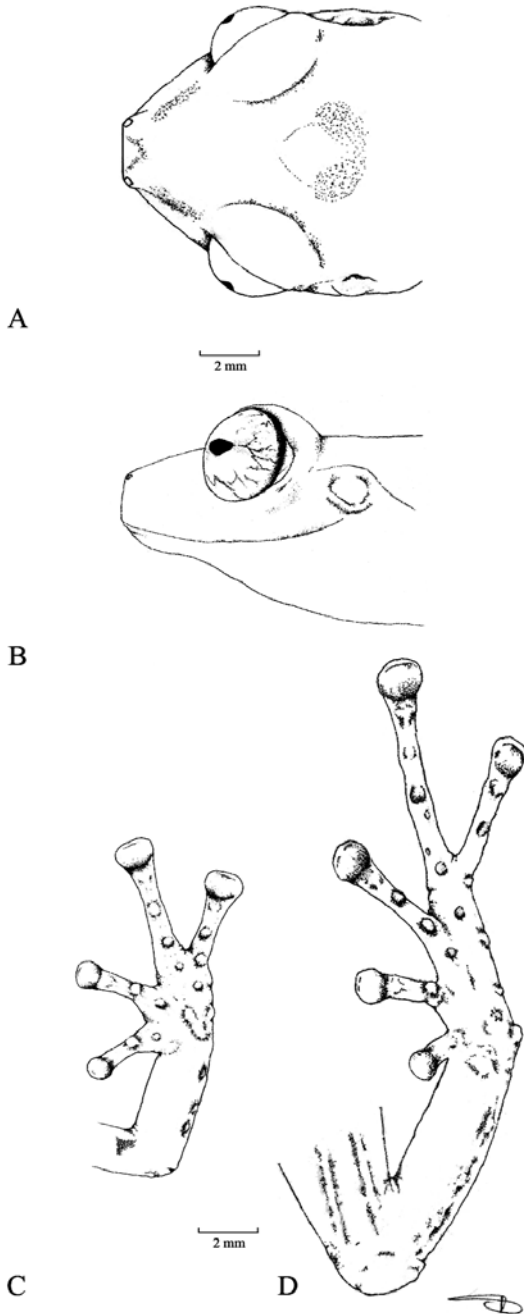


Figure 3 - Dorsal (A) and lateral (B) views of head, and ventral views of hand (C) and foot (D) for the holotype of *Eleutherodactylus waoranii* (TCWC 90728, holotype, SVL = 21.2 mm, male). Drawings by Tana Ryan.

and rounded discs about twice as wide as digits; (8) fingers with narrow lateral fringes; (9) ulnar tubercles low, antebrachial largest; (10) tubercles on heel and outer edge of tarsus absent; inner tarsal fold present; (11) inner metatarsal tubercle oval, 2–3x as long as round outer metatarsal tubercle; supernumerary plantar tubercles small and low, at the base of Toes III and IV; (12) toes with narrow lateral fringes; webbing absent; fifth toe much longer than third; (13) in life, dorsum of males bright golden-brown, that of females golden-brown with greenish tint to dark brown; venter of males translucent white, that of females white to creamy white; (14) adults small, SVL in males 19.7–21.2 mm ($\bar{x} = 20.4 \pm 1.12$, $n = 2$), in females 27.5–31.1 mm ($\bar{x} = 29.7 \pm 1.97$, $n = 3$).

Comparison with similar species - *Eleutherodactylus waoranii* is most similar to *E. lacrimosus*, both of which occur in the Yasuni region. *Eleutherodactylus waoranii* differs from *E. lacrimosus* by the lack of a papilla at tip of snout (variably reported in literature for *E. lacrimosus*), tubercles on upper eyelids, tubercles on dorsum (few tubercles in *E. lacrimosus*), and dark brown markings on dorsum and hind limbs (Lynch and Schwartz 1971, Lynch and Duellman 1980, Rivero and Serna 1987, Heyer and Hardy 1991, Rodríguez and Duellman 1994, Guayasamin *et al.* 2006). It also differs by the presence of narrow lateral fringes on the fingers, series of tubercles on the ulnar, inner tarsal fold, and marginally larger body size than *E. lacrimosus* (Lynch and Schwartz 1971, Lynch and Duellman 1980, Rivero and Serna 1987, Heyer and Hardy 1991, Rodríguez and Duellman 1994, Guayasamin *et al.* 2006). Lynch and Duellman (1980) reported smaller sizes for lowland (< 800 m) populations of *E. lacrimosus* compared to those on Amazonian slopes. For lowland populations they recorded a SVL for males 16.1–20.0 mm ($\bar{x} = 18.5 \pm 0.5$, $n = 18$) and for females 20.6–24.4 mm ($\bar{x} = 22.5 \pm 0.5$, $n = 18$); in *E. waoranii*, we recorded a SVL for males 19.7–

21.2 mm ($\bar{x} = 20.4 \pm 1.1$, $n = 2$), and for females 27.5–31.1 mm ($\bar{x} = 29.7 \pm 2.0$, $n = 3$). *Eleutherodactylus waoranii* differs from the sympatric species *Eleutherodactylus acuminatus* by lacking a green dorsum, pointed snout, and a dark stripe from the tip of the snout extending through the eye to above the arm or along the flank. *Eleutherodactylus waoranii* has a prominent tympanum whereas the tympanum is concealed in *E. acuminatus* (Duellman 1978, Rodríguez and Duellman 1994). *Eleutherodactylus waoranii* differs from the syntopic *E. aureolineatus* by lacking both a papilla on tip of the snout and a creamy-yellow interorbital stripe extending above the eyes and dorsolaterally to the sacrum (Guayasamin *et al.* 2006). *E. waoranii* differs from all other members of the *E. lacrimosus* assemblage by its unmarked, bright to dark golden-brown dorsal coloration and absence of any tuberculation of the upper eyelid (Guayasamin *et al.* 2006).

Description of Holotype - Adult male (TCWC 90728), 21.24 mm. Head slightly wider than body, wider than long; head width 41.4% SVL; snout subacuminate in dorsal view (truncated by protruding nostrils), truncate in lateral view, comparatively short (snout–eye distance 16.0% SVL); eye–nostril distance 96.3% diameter of eyes; nostrils protuberant, weakly directed dorsolaterally; canthus rostralis angular, straight to slightly sinuous; loreal region weakly concave, sloping abruptly to lips; upper lips not flared; lacking upper eyelid tubercle; interorbital space flat, wider than upper eyelid (upper eyelid width 63.2% interorbital distance); cranial crests absent; upper eyelid lacking tubercles; temporal region slightly angled; supratympanic fold weakly defined, obscuring upper and posterodorsal edge of annulus, extending posteroventrally to just above insertion of arm; tympanic membrane weakly differentiated from surrounding skin; tympanic annulus, round, uppermost portion slightly obscured by supratympanic fold, tympanum diameter 38.7% of eye diameter,

separated from eye by distance 70.0% of tympanum diameter; postrectal tubercles posteroventral to tympanic annulus, compressed and fused, forming short ridge extending ventrolaterally from tympanum; choanae round, small, not concealed by palatal shelf of maxillary arch, separated by distance approximately twice the width of single choanae; vomerine odontophores triangular, postereomedian to choanae, separated medially by distance less than width of odontophore, with four vomerine teeth right and three left in transverse row at base of triangle (bilateral variation); tongue as wide as long, posterior border not notched, posterior 25% not adherent to floor of mouth; vocal slits elongate, posterolateral to tongue; vocal sac single, subgular.

Skin on the dorsum, head, flanks and upper surface of limbs smooth to finely shagreen; dorsolateral folds absent; skin on throat smooth; skin on venter areolate; ventral surfaces of limbs smooth, except skin of thigh below and ventrolateral to vent areolate; discoidal fold well anterior to groin; cloacal opening directed posteriorly above dorsal plane of thighs, short transverse fold or flap dorsally; cloacal sheath and enlarged tubercles absent.

Forearm slender; forearm length 24.0% SVL; three low ulnar tubercles in row extending to just distal of elbow, unevenly spaced, low, antebrachial largest; hand length longer than forearm length (hand length 24.6% SVL); thenar tubercle oval, twice as long as wide; palmar tubercle large, incompletely bifid, about 2x larger than thenar; four distinct supernumerary palmar tubercles present on each hand, low, round; subarticular tubercles elevated, rounded; rest of palmar surface areolate; narrow lateral fringes present on fingers; webbing absent; finger length I<II<IV<III, first finger slightly shorter than second; discs on fingers broad, round on Fingers I and II, expanded and rounded on Fingers III and IV, on Finger I slightly wider than width of digit, on Finger II less than 1.5x width of digit, those on Fingers III and IV at least 2x width of digit (about equal to

tympanic annulus in life); broad ventral pads with complete circumferential grooves on all discs; white nonspinous nuptial pad evident on medial surface at base of Finger I.

Hind limbs slender; tibia length 50.6% SVL; foot length 136.1% of tibia length; heels overlap considerably when flexed hind limbs held perpendicular to sagittal plane; heel tubercle absent; outer edge of tarsus lacking tubercles; inner surface of tarsus bearing row of low tubercles with a short ridge between tubercles forming an inner tarsal fold, extend from proximal inner metatarsal tubercle to just distal of heel; inner metatarsal tubercle oval, about 2x as long as wide; outer metatarsal tubercle round, subconical, about one-fourth size of inner metatarsal tubercle; supernumerary plantar tubercles small and low at bases of Toes III–IV; subarticular tubercles subconical; rest of plantar surface areolate; toes bearing narrow lateral fringes coalescing at base of toes; webbing absent; toe length $I < II < III < V < IV$; tip of Toe V reaching just beyond distal border of distal subarticular tubercle of Toe IV; tip of Toe III reaching distal border of medial subarticular tubercle of Toe IV; disc on Toe I round, slightly broader than digit proximal to disc; discs on Toes II and III broad and rounded increasingly larger; disc on Toe IV large, significantly broadened, slightly smaller than discs on Fingers III and IV; disc on Toe V slightly smaller, broadened.

Coloration of holotype in life - Dorsum uniformly bright golden-brown, dark outline of optic lobes clearly visible through skin; upper eyelids a slightly darker golden-green; flanks pale golden-yellow with minute brown specks; faint pale salmon color ventrolaterally and across chest, most prominent at insertion of forearm. Venter translucent white; outline of heart, liver and intestines visible; throat pale yellow; limbs uniformly bright golden-brown, with the exception of a transverse narrow brown bar on the upper surface of the wrist and the upper surface of the heel being pale golden-

yellow. The iris is bright reddish-copper with a median, horizontal reddish-brown streak (Figure 2).

Color of holotype in ethanol - Dorsum light tan; canthus and nostrils brown; flanks white; venter translucent white; throat and ventral surfaces of limbs white.

Measurements of holotype and paratypes - See Table 1.

Variation - The ridge formed by the compressed and fused postriotal tubercles is variable; in one male (TCWC 90732) and one female (TCWC 90730) there is a break in the line of the ridge, forming what appears as two elongate tubercles. The number of teeth on the vomerine odontophores is variable; bilateral variation is evident in two other specimens, one female (TCWC 90731) has 3 and 5 teeth, one male (TCWC 90732) has 4 and 6 teeth, whereas two females (TCWC 90729, 90730) do not show bilateral variation, with 4 or 5 teeth on each odontophore. Tongue length longer than wide in TCWC 90729, 90730, and 90732; small notch in posterior border of TCWC 90730 and 90732. Ulnar tubercles marginally evident in TCWC 90729. In life, males have a bright golden-brown dorsum by night and pale golden-brown dorsum by day. Females have a darker coloration, one female (TCWC 90731) was golden-brown with a greenish tint by night and dark golden-brown by day, whereas the other two females (TCWC 90729, 90730) were dark golden-brown by night and a darker reddish-brown by day. Flanks pale golden-yellow with minute brown specks and faint pale salmon color ventrolaterally, pale salmon color absent in TCWC 90732; venter translucent white to creamy white, throat pale yellow in males and white in females. Posterior surface of thighs is variably lighter than dorsum of individuals. Iris lacking median, horizontal reddish-brown streak in TCWC 90729, 90730, and 90732. In preservative, the dorsum is creamy brown

Table 1 - Measurements (in mm) of adult *Eleutherodactylus waoranii*.

	TCWC 90728 (H) ♂	TCWC 90732 ♂	TCWC 90729 ♀	TCWC 90730 ♀	TCWC 90731 ♀
SVL	21.2	19.7	30.7	31.1	27.5
Tibia length	10.7	11.0	15.8	16.3	15.2
Foot length	14.6	13.4	21.3	22.7	20.1
Head length	7.0	7.2	11.7	12.0	11.2
Head width	8.8	8.6	14.4	14.1	12.2
Upper–eyelid width	1.8	1.9	2.9	2.9	2.0
Interorbital distance	2.9	2.6	3.9	4.0	4.1
Eye diameter	2.7	2.5	3.8	4.2	3.2
Eye-to-nostril distance	2.6	2.7	3.7	4.3	3.0
Snout-to-eye distance	3.4	3.5	4.8	5.0	4.2
Tympanum diameter	1.0	1.1	1.8	1.7	1.6
Eye-to-tympanum distance	0.7	0.6	1.5	1.5	1.3
Internarial distance	1.6	1.5	2.1	2.1	1.9
Forearm length	5.1	5.2	7.4	7.5	6.7
Hand length	5.2	5.6	8.6	8.6	7.0
Finger–I length	3.5	3.6	5.8	5.6	4.8
Finger–II length	3.6	3.7	6.0	5.9	5.1
Finger–III disc width	0.9	0.8	1.6	1.6	0.9
Femur length	9.5	10.0	13.4	13.2	12.5
Toe–IV disc width	0.8	0.8	1.3	1.6	1.0

(TCWC 90732) or reddish brown (TCWC 90729, 90730); soles and palms pale tan (TCWC 90731, 90732) or pale brown (TCWC 90729, 90730).

Distribution and natural history - *Eleutherodactylus waoranii* is known only from the type locality in the upper Amazon Basin of eastern Ecuador at the Tiputini Biodiversity Station–Universidad San Francisco de Quito. The region is classified as lowland evergreen broadleaf rain forest, with an annual mean temperature of 25°C, annual mean relative humidity of 88% and annual rainfall between 2425–3145 mm (Iremonger *et al.* 1997, Balslev *et al.* 1987; Blandin 1976, Duellman 1978). Tiputini Biodiversity Station is on the north

shore of the Río Tiputini at an elevation of 217 m, with low rolling hills up to an elevation of 310 m. The habitat consists of terra firme forest (non-flooded forest), varzea (flooded forest), seasonally flooded forest, palm swamp, and igapó forest (black-water tributary associated forest) similar to that described by Jaramillo and de Vries (2002) for the adjacent oil concession block 31 of Yasuni National Park. On 15 August 2004, the holotype and two adult females (TCWC 90729, 90730) were collected by day in a tank bromeliad (*Aechmea zebrina*), at 29.5 m height, on the trunk of a tree in terra firme forest approximately 2.5 km west-northwest of the TBS camp. On 31 July 2004, an adult female (TCWC 90731) was collected by day in a tank bromeliad (*Aechmea zebrina*), at 38.0 m height,

on the trunk of a tree (*Dimorphandra* sp.) in terra firme forest approximately 1.0 km northwest of the TBS camp. On 08 August 2004, an adult male (TCWC 90732) was collected by day in a tank bromeliad (*Aechmea zebrina*), at 23.5 m on a branch of a tree in terra firme forest approximately 1.25 km northwest of the TBS camp. All individuals were found in the water-filled outer bracts of bromeliads. Vertical heights and environmental data collected for bromeliads are listed in Appendix I. All trees and bromeliad sites were above the surrounding forest canopy, *Eleutherodactylus waoranii* is unmistakably an inhabitant of the sub- to upper canopy strata.

Eleutherodactylus waoranii lives syntopically with *E. aureolineatus*, both have been observed in the same bromeliad during bromeliad patch sampling conducted at TBS. *Eleutherodactylus waoranii* and *E. lacrimosus* are considered to exist sympatrically at TBS, no *E. lacrimosus* have been collected during the sampling effort of 80 canopy bromeliads to date.

Remarks - Only one *E. lacrimosus* (SFM 0371) was found at TBS on May 24, 2004 on the upper surface of a palm leaf at 1.2 m during nocturnal terrestrial quadrat surveys. Representative specimens of *E. waoranii* collected in the initial study and after the writing of this manuscript during the June–August 2006 field season will be deposited at the Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador, after examination by the senior author.

Discussion

Eleutherodactylus waoranii is considered to be a member of the *E. lacrimosus* assemblage, a group that has experienced considerable historic taxonomic confusion potentially as a result of limited confirmed specimens and difficulties associated with definitive morphological characters among vouchers. The placement is based upon its niche-specific association with bromeliads, and morphological characters of a

small body size, and broad, flat head, with the fifth toe much longer than third (Lynch and Ruíz-Carranza 1985, Heyer and Hardy 1991, Guayasamin *et al.* 2006).

Eleutherodactylus waoranii is recognized as a new species, differentiated from *Eleutherodactylus lacrimosus* based upon comparison to cumulative descriptions within the literature, preserved specimens, and high-resolution digital macro photographs in life of an adult male (specimen/photo voucher, SFM 0371), found at the type locality, matching the descriptions of *E. lacrimosus*. Lynch and Schwartz (1971) redescribed and figured *E. lacrimosus* in lieu of absence of the original holotype collected and described by Jiménez de la Espada (1875). Heyer and Hardy (1991) designated the specimen KU 110782, examined and figured by Lynch and Schwartz (1971), as the neotype for *E. lacrimosus* in agreement with Lynch and Schwartz's (1971) description, "...with the minor exceptions that the upper eyelids, although flattened in preservative, appear to be moderately tuberculate and moderately developed antibrachial tubercles are present". *E. lacrimosus* (SFM 0371) from the type locality of *E. waoranii* conforms to these descriptions, with the exception of possessing a low inner tarsal fold most evident in the macro photographs. The most obvious morphological differences between the species are the lack of a papilla at the tip of the snout (variably reported in literature for *E. lacrimosus*), tubercles on upper eyelids, tubercles on dorsum (few tubercles in *E. lacrimosus*), and dark brown markings on dorsum and hind limbs, larger size, and the presence of lateral fringes on the fingers and a tarsal fold in *E. waoranii* (Lynch and Schwartz 1971, Lynch and Duellman 1980, Rivero and Serna 1987, Heyer and Hardy 1991, Rodríguez and Duellman 1994, Guayasamin *et al.* 2006).

The inclusion of sampling methods directed at little known habitats are necessary to ensure the accuracy of biodiversity estimates for a given study site. Terrestrial amphibian surveys

are the historical method of sampling in tropical rainforest, only encompassing a small stratum (~2 m vertical height) of rainforest diversity. Investigations into the amphibian fauna of the canopy has historically only been represented by a few surveys of felled trees conducted during forest clearing operations, limiting collection of substantial data on habitat strata occupancy and utilization (Duellman 1978). Yet, where the upper strata have been examined the results are very productive. Annual canopy surveys conducted from 1998 to 2002 along a 100 m-long system of canopy bridges at TBS, and sporadic canopy surveys conducted during one week in May 2002 at the Yasuni Scientific Research Station–Universidad Católica del Ecuador by students from the respective universities resulted in the collection of 15 species (see Guayasamin *et al.* [2006] for a detailed report on the results). The current study has also shown glimpses of additional diversity in these habitats. We found 17 specimens from three species of metamorphosed anurans and 11 individuals of unidentified larval amphibians. Two of those species are now described as new taxa in the genus *Eleutherodactylus*. Of the total 15 species revealed by canopy searches, the majority have not been collected during typical terrestrial surveys. It is hypothesized that the reported diversity, community structure, and abundance of amphibians within the Ecuadorian Amazon rainforest system is biased towards the terrestrial strata (Guayasamin *et al.* 2006). Given the rapid rate of deforestation throughout Amazonia canopy surveys are crucial for the documentation of their vertebrate taxa to facilitate the conservation of these important “wetlands in the sky” (McCracken and Forstner 2006).

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Appendix I - Vertical heights and environmental data collected for bromeliads with occurrences of *Eleutherodactylus waoranii*

Environmental parameters	Bromeliads		
	PMT5B1	PMT6B2	ADT8B2
Vertical height (m)	38.0	23.5	29.5
Water volume (L)	1.240	1.195	1.830
Temp.(°C) @ 0m	24.5	23.8	26.5
Temp.(°C) @ 1m	25.0	24.0	24.5
Temp.(°C) @ bromeliad	24.0	23.0	25.0
hPa @ 1m	1015	1017	1018
hPa @ bromeliad	1015	1014	1018
% relative humidity @ 1m	92.0	95.0	99.0
Water pH	5.45	4.88	4.19
DBH (cm) of tree	289.0	47.0	56.5

Appendix II - Specimens Examined

Eleutherodactylus acuminatus – ECUADOR: **Orellana**: Tuputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, SFM 0039, 0040, 0051, 0126, 0409, 0410, and 0687. PERU: **Loreto**: Moropon, TCWC 41521.

Eleutherodactylus aureolineatus – ECUADOR: **Orellana**: Tuputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, TCWC 90334–90342 (paratypes).

Eleutherodactylus lacrimosus – ECUADOR: **Napo**: Isla Anaconda, KU 202406; Rio Alpayacu, KU 180294. ECUADOR: **Orellana**: Tuputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, SFM 0371. ECUADOR: **Sucumbios**: Lago Agrio, KU 126210; Limoncocha, KU 104623 and 106967; Santa Cecilia, KU 148895, 148897, 148905–148908, 152385, and 153022. PERU: **Loreto**: Explornapo, KU 220426.