

**Natural History Notes on the Reproductive Biology of the Melodious Coqui, *Eleutherodactylus wightmanae* (Schmidt, 1920), the Whistling Coqui, *E. cochranae* (Grant, 1932), and the Mountain Coqui, *E. portoricensis* (Schmidt, 1927) (Anura: Eleutherodactylidae), from Puerto Rico<sup>1</sup>**

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**Abstract:** We document the first account of a triple egg clutch in the Melodious Coqui, *Eleutherodactylus wightmanae*, and in the Whistling Coqui, *E. cochranae*, along with the first record of amplexus in the Mountain Coquí, *E. portoricensis* in the wild. These species are abundant in their current natural distribution, and the low frequency of similar observations from these and other species documented from the literature, reflects the rarity of encountering these events in the wild. These reports expand our knowledge on multiple breeding events and reproductive behavior in the most diverse group of frogs in the Caribbean.

**Key Words:** amplexus, coquis, coquíes, *Eleutherodactylus*, multiple egg clutch.

The Melodious Coqui, *Eleutherodactylus wightmanae*, is a small-sized frog (mean snout-vent length [SVL] = 20.2 mm [Stewart and Woolbright 1996]; range 19.1 mm [males]–20.3 mm [females] [Joglar 1998]) frequently found close to the ground and in the leaf litter of close-canopy forests throughout all major upland mountain ranges in Puerto Rico (Joglar 1998 and references within; Ríos-López and Dávila-Casanova 2014; Ríos-López et al. 2015d). Information on single egg-mass clutches for the species comes from studies by Joglar et al. (2005) and Ríos-López and Dávila-Casanova (2014), while a description of double egg-mass clutches comes from Ríos-López et al. (2015d). Currently, the International Union for the Conservation of Nature (IUCN) lists the species as Endangered (Angulo 2008b). The Whistling Coqui, *Eleutherodactylus cochranae*, is also a small-sized frog (range mean SVL = 19.4 mm [males]–21.8 mm [females] [Joglar 1998]), but frequently found in herbaceous vegetation in lowland wetlands at sea level, in xeric to mesic forests, anthropogenic ecosystems, and in ground and arboreal bromeliads. The species has an altitudinal distribution of up to 600 m above sea level (reviewed in

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Henderson and Powell 2009; NRL personal observation). Currently, the IUCN lists the species as Least Concern (Ríos-López 2010). In general, body size of individuals in both species rarely exceeds 23 mm of snout-vent length (SVL) (reviewed in Joglar 1998; Ríos-López et al. 2015d; NRL unpublished data).

The Mountain Coqui, *Eleutherodactylus portoricensis*, is a medium-size frog, that rarely exceeds 38 mm in SVL (reviewed in Joglar 1998; Ríos-López et al. 2015b) and it is a forest-interior species with individuals frequently found near or at the ground level up to 3 m on vegetation surfaces above the ground (NRL, unpublished data). Distribution of extant populations includes mountain forests above 650 m in elevation in the Sierra de Cayey and the Sierra de Luquillo in southeastern and northeastern Puerto Rico, respectively (reviewed in Ríos-López et al. 2015b). The first record of amplexus behavior was described in captivity by Ríos-López et al. (2015b), but it has been suggested that reproductive behavior in captivity may differ under natural conditions. To date, amplexus in *E. portoricensis* has not been observed in the wild. Currently, the IUCN lists the species as Endangered (Angulo 2008a).

In this report, we document the first account of a triple egg clutch in *Eleutherodactylus wightmanae* and in *E. cochranæ*, and we contrast these accounts with similar data from other Puerto Rican *Eleutherodactylus*. In addition, we described for the first time amplexus behavior in *E. portoricensis* under natural conditions.

### **Triple Egg Clutch in a Single Nest in *Eleutherodactylus wightmanae***

On March 26, 2016 at 1608h, we found the first triple egg clutch of *E. wightmanae* in a single nest, with masses of eggs with embryos in different stages of development—judging by their external appearance and coloration (Figure 1A). All masses were found in direct contact from each other and inside a plastic tube (approximate diameter = 23.5 mm; length between 15 and 17 cm) placed on the surface of the leaf litter in our study site at the Sierra de Cayey mountains (Cayey-Guayama municipalities, southern Puerto Rico: 18.054405°N, -66.123019°W, datum: WGS84, elev. = 817 m above sea level; for a brief description of study site and sampling design refer to Ríos-López and Dávila-Casanova 2014). The egg masses in the nest were found with a guarding male and a female (the female was in front on the recently-laid egg mass), and the male was behind her (Figure 1A). This suggests that the mating pair just finished oviposition after amplexus, and that they were the parents of this egg mass. The species does not show communal nesting (Ríos-López et al. 2015d), and males do not call in groups, but spatially isolated from each other while calling (NRL, personal observation). As these observations are consistent with territorial behavior, the presence of the male in this nest suggests that he was the “father” of all egg masses found there.

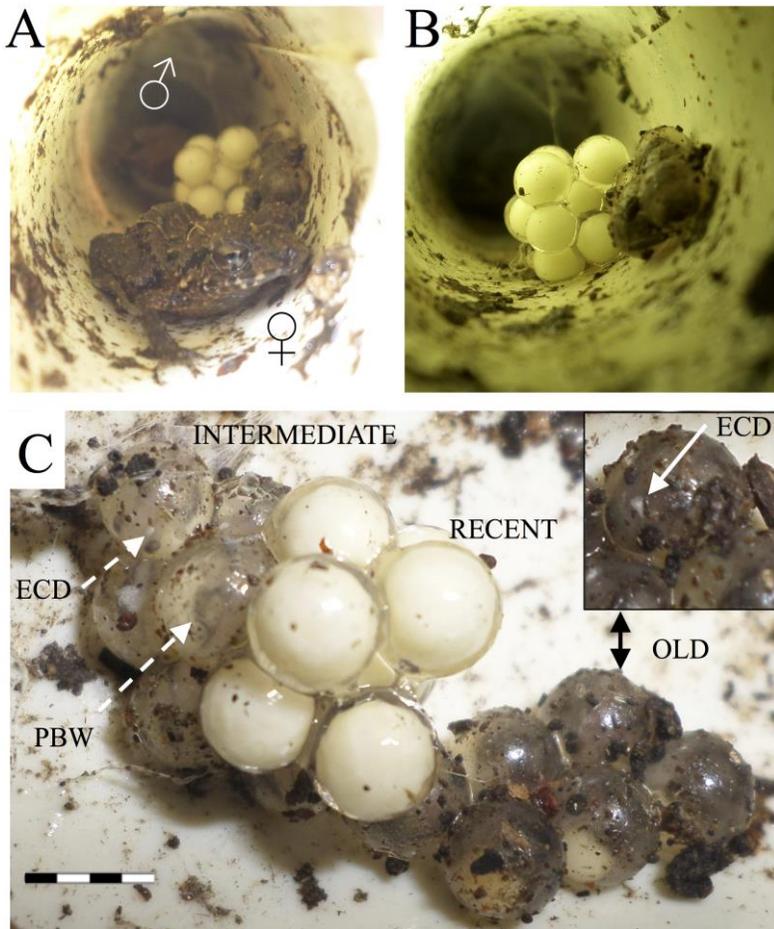


Figure 1. In panel A, a panoramic view inside the plastic tube with female in the foreground, two of the three egg masses visible in the median plane, and male in the background. In panel B, adults removed to highlight a generalized view of egg masses inside the plastic tube. In panel C, detail of the triple egg clutch with egg masses of different developmental stages: an egg mass (labeled INTERMEDIATE) with embryos at developmental stages 7 or 8 based on Townsend and Stewart's (1985) staging table; a recently-laid egg mass at developmental stages 1 or 2 (labeled RECENT); an egg mass with embryos at older developmental stages 9 or 10 (labeled OLD). Arrows highlight prominent characteristics used for staging embryos: ECD = endolymphatic calcium deposits as patches quadrangular (INTERMEDIATE) to triangular (OLD) in shape with forward extensions partially to rear medial area of eyes (inset of the same embryo in OLD; superior right corner); PBW = pigmented body wall appears as an expanded disc with border even and flanking the trunk between the front and hind limbs (INTERMEDIATE). Bottom left: scale bar = 4 mm.

The triple egg clutch consisted of a recently-laid mass of 9 eggs, an old mass of 7 eggs, and an intermediate mass of 6 eggs (Figure 1B illustrates a **recently-laid egg mass**, whitish coloration without a discernible embryo and in developmental stages between 1 and 2 following Townsend and Stewart's [1985] staging table, with mean  $[\pm \text{SD}]$  egg diameter =  $3.99 \pm 0.26$  mm, range: 3.67–4.33 mm,  $N = 6$  eggs measured; an **old egg mass** with distinguishable embryos, dorsal coloration light gray with pink undertones and exposed yolk white-cream, endolymphatic calcium deposits evident as symmetric triangular patches with forward extensions to rear medial area of eyes and posterior areas expanding medially, and in developmental stages between 9 and 10, with mean  $[\pm \text{SD}]$  egg diameter =  $4.27 \pm 0.23$  mm, range: 3.97–4.63 mm,  $N = 6$  eggs measured; and a younger, **intermediate egg mass**—based on appearance of disk flanking trunk [future pigmented body wall enclosing the egg yolk]—with the border of this disk even, with a darker pigmented line at the edges of it and extending from tips of front to hind limbs, endolymphatic calcium deposits evident as somewhat quadrangular patches from dorsal view and without forward extension, and in developmental stages between 7 and 8, with mean  $[\pm \text{SD}]$  egg diameter =  $3.90 \pm 0.23$  mm, range: 3.65–4.06 mm,  $N = 3$  eggs measured).

#### **Triple Egg Clutch in a Single Nest in *Eleutherodactylus cochranae***

On March 28, 2009 at 1615h, NRL found the first triple egg clutch of *E. cochranae* in a single nest within the leaf axil of a Bulltongue Arrow Head, *Sagittaria lancifolia* (Linnaeus 1759). The locality is a small palustrine herbaceous wetland (ca. 260 ha;  $18^{\circ}26.127'N$ ,  $66^{\circ}12.092'W$ ), at 1–6 m above sea level (asl) located at the northernmost limestone belt in the Toa Baja municipality, northern Puerto Rico (refer to Ríos-López et al. 2014 for a description of the study site). This triple egg clutch (Figure 2A) consisted of a **recently-laid mass** of 5 eggs (whitish coloration without a discernible embryo, in developmental stages between 1 and 2 following Townsend and Stewart's [1985] staging table), and **two distinct older masses** of 5 eggs each. The embryos between these **older egg masses** were in somewhat similar developmental stages—between stages 7 and 9—judging by their external appearance and coloration of the eye. The external similarity of embryos between these **older egg masses** suggests that egg masses were deposited a few days apart by different females (e.g., see Ríos-López et al. 2015d for similar observations on *E. wightmanae*). In addition, the **older egg masses** were characterized by well-developed embryos of pink dorsal coloration, white-cream exposed yolk, and visible endolymphatic calcium deposits with forward extensions somewhat partially (or completely) to rear medial area of eyes. Oviposition was not observed, but we found these tree egg masses along with a (guarding) male and a female, which was on physical contact with the **recently-laid egg mass**.

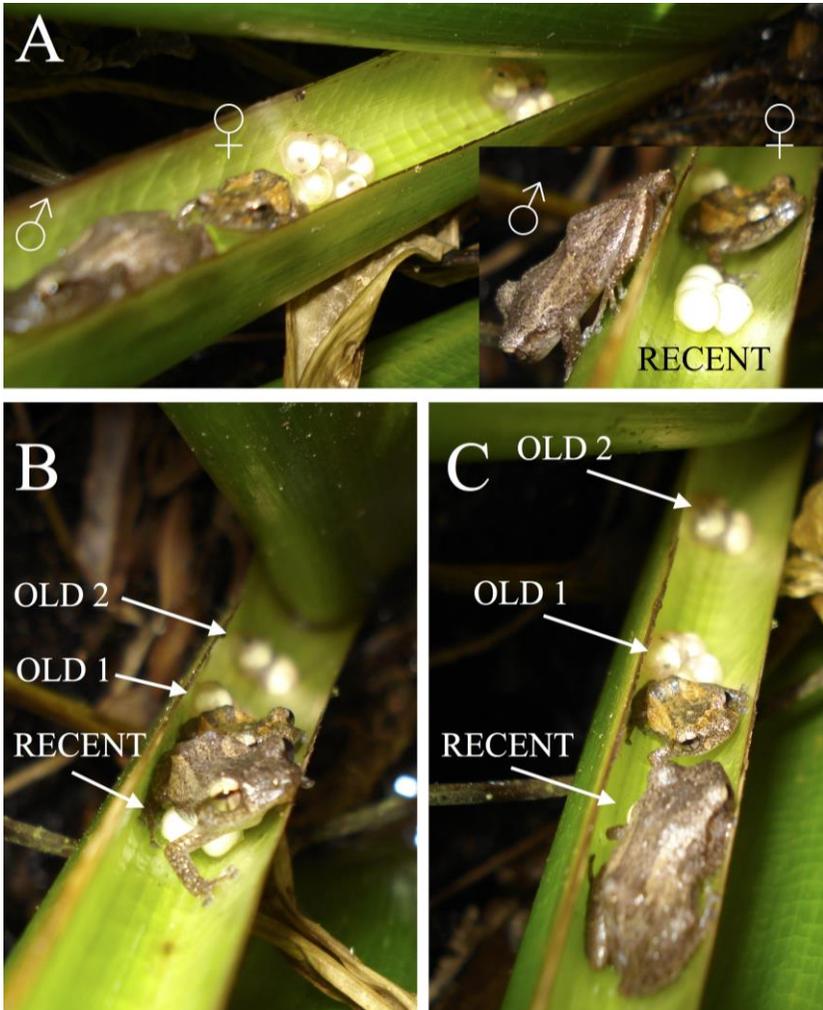


Figure 2. In A, panoramic view of the triple egg clutch deposited in a leaf axil of the Bulltongue Arrow Head, *Sagittaria lancifolia* Linnaeus (Alismataceae). the recently-laid egg mass beneath the larger female in the foreground, the smaller male in the background behind the female; inset in A highlights the recently-laid egg mass (labeled RECENT) once we disturbed the female for visual clarity and she revealed the egg mass beneath her. In B and C, two views of all three egg masses in the nest, along with their location in the leaf axil relative to the location of adults.

Communal nesting has not been reported for *E. cochranæ*, and males engage in territorial and aggressive behavior while attempting amplexus and defending their retreat/breeding sites when threatened by neighboring and

intruder conspecifics (Michael 1995). Consequently, the fact that the male was behind the female and in close contact with the **recently-laid egg mass**, may suggest that the adults represent a mating pair that had finished oviposition after being engaged in amplexus; that the female was the “mother” of the **recently-laid egg mass**; and that the male was the “father” of all egg masses found in this leaf axil (Figure 2B).

In Puerto Rican *Eleutherodactylus* frogs, multiple egg clutches have been found in the Common Coqui (up to double clutches: *E. coqui* Thomas 1966; Townsend et al. 1984), the Cave-Dwelling Coqui (up to quadruple clutches: *E. cooki* Grant 1932; Burrowes 2000), the Plains Coqui (up to triple clutches: *E. juanariveroi* Ríos-López and Thomas 2007; Ríos-López et al. 2014), and Hedrick’s Coqui (up to double clutches: *E. hedricki* Rivero, 1963; L. J. Villanueva-Rivera, personal communication). Presumably, production of multiple egg clutches in single nests may represent a strategy that benefits male fitness by reducing missed opportunities for mating while increasing their offspring per unit of time-effort (Townsend et al. 1984; Woolbright 1985; Townsend 1986; Burrowes 2000; for a review, see Wells 2007). While the observations described throughout this report expand our knowledge on multiple clutches in Puerto Rican *Eleutherodactylus*—with up to triple egg clutches in the Melodious Coqui (*E. wightmanae*) and in the Whistling Coqui (*E. cochranae*)—future studies should assess the consequences of costs of calling on male fitness in these and other *Eleutherodactylus* frogs. For example, Stewart and Pough (1983) showed experimentally that an intrinsic population increase in the closely-related *E. coqui* resulted from an increase of suitable retreat/breeding sites in the wild: retreat/breeding sites are limited resources that regulate population size in this terrestrial frog. Therefore, future studies may examine how the availability of suitable retreat/breeding sites may relate to production of multiple clutches, and hence, examine the consequences of the costs of calling on male-fitness once a limited retreat/breeding site has been secured by the male of *E. wightmanae* and *E. cochranae* (and in other *Eleutherodactylus* with male-parental care).

### **First record of amplexus in *Eleutherodactylus portoricensis* in the wild**

On March 26, 2016 at 1615h we found a pair of *E. portoricensis* in amplexus inside a plastic tube placed on the surface of the leaf litter in our study site at the Sierra de Cayey mountain range (see description above for *E. wightmanae*). The male was on top of the female, and the female had engaged in a reverse hind-leg clasp around the waist of the male during oviposition (Figure 3). In this species, oophagy frequently occurs shortly after oviposition in field and captive conditions, and especially when guarding male is disturbed by conspecifics or an observer (Ríos-López et al. 2015b). Consequently, after having handled the tube briefly for photographing the event, we did not disturb the pair any further and returned the tube where we found it on the ground.

The appearance of the reverse hind-leg clasp in *Eleutherodactylus portoricensis* observed under natural conditions was similar to that described under captive conditions (e.g., Ríos-López et al. 2015b). This reverse hind-leg clasp during amplexus has been suggested as a mechanism that facilitates internal fertilization in eleutherodactylids (Townsend and Stewart 1986; Townsend et al. 1990), a reproductive strategy known only for *E. coqui* and the viviparous Golden Coqui, *E. jasperi* (Drewry and Jones 1976), but presumed for *E. portoricensis* (Townsend 1996). Consequently, this is the second *Eleutherodactylus* species for which a reverse hind-leg clasp has been documented (the first being *E. coqui*, Townsend et al. 1990), and the evidence presented here and in Ríos-López et al. (2015b) further suggests that internal fertilization is a reproductive strategy that may also be present in *E. portoricensis*.

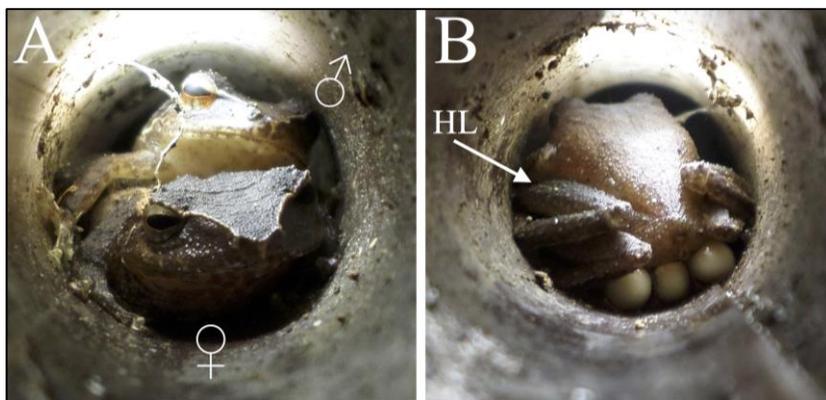


Figure 3. A mating pair of *Eleutherodactylus portoricensis* with the male on top of the larger female while inside a plastic tube in the field (A) and the female engaged in a reverse hind-leg clasp around the waist of the male (B). The arrow highlights the hind leg (HL) of the female clasp the male by his waist.

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#### Literature Cited

- Angulo, A. 2008a. *Eleutherodactylus portoricensis*. The IUCN Red List of Threatened Species 2008: e.T56875A11547757. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T56875A11547757.en>. Downloaded on 09 April 2016.
- Angulo, A. 2008b. *Eleutherodactylus wightmanae*. The IUCN Red List of Threatened Species: e.T57056A11575451. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T57056A11575451.en>. Downloaded on 09 April 2016.
- Burrows, P. A. 2000. Parental care and sexual selection in the Puerto Rican Cave-Dwelling frog, *Eleutherodactylus cooki*. *Herpetologica* 56:375–386.

- Henderson, R. W., and R. Powell. 2009. *Natural History of West Indian Reptiles and Amphibians*. University Press of Florida, USA. 528 pp.
- Joglar, R. L. 1998. *Los Coquies de Puerto Rico: Su Historia Natural y Conservación*. Editorial de la Universidad de Puerto Rico, San Juan, USA. 232 pp.
- Joglar, R. L., P. A. Burrowes, D. Dávila, A. Rodríguez, A. López, A. Longo, and P. Medina. 2005. *Eleutherodactylus wightmanae* (Coquí Melodioso), Reproduction. *Herpetological Review* 36(4):433-434.
- Michael, S. F. 1995. Captive breeding of two species of *Eleutherodactylus* (Anura: Leptodactylidae) from Puerto Rico, with notes on behavior in captivity. *Herpetological Review* 26(1):27-29.
- Ríos-López, N. 2010. *Eleutherodactylus cochraeae*. The IUCN Red List of Threatened Species 2010: e.T56515A11488661. <http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T56515A11488661.en>. Downloaded on 09 April 2016.
- Ríos-López N. and D. Dávila-Casanova. 2014. *Eleutherodactylus wightmanae* (Melodious Coqui), Reproduction, Parental Care, and Calling Sites. *Herpetological Review* 45(4):678-679.
- Ríos-López, N., Reyes-Díaz, M., Ortíz-Rivas, L., Negrón-Del Valle, J. E., and de Jesús-Villanueva, C. N. 2014. Natural History and Ecology of the Critically Endangered Puerto Rican Plains Coquí, *Eleutherodactylus juanariveroi*, (Amphibia: Anura: Eleutherodactylidae). *Life: The Excitement of Biology* 2(2):69-93.
- Ríos-López, N., E. Agosto-Torres, R. M. Hernández-Muñiz, C. Vicéns-López, A. Bernardi-Salinas, W. N. Tirado-Casillas, and Y. M. Flores-Rodríguez. 2015b. Conservation Efforts for the Puerto Rican Mountain Coqui (Anura: Eleutherodactylidae: *Eleutherodactylus portoricensis* Schmidt, 1927): Reproductive Biology in Captivity. *Life: The Excitement of Biology* 3(2):61-82.
- Ríos-López, N., Y. M. Flores-Rodríguez, E. Agosto-Torres, C. Vicéns-López, and R. M. Hernández-Muñiz. 2015d. Life History Observations on the Melodious Coqui, *Eleutherodactylus wightmanae* (Anura: Eleutherodactylidae), from Puerto Rico: Double Clutches and Adult Predation by the Yellow-Chinned Anole, *Anolis gundlachi* (Squamata: Dactyloidae). *Life: The Excitement of Biology* 3(2):137-148.
- Stewart, M. M. and F. H. Pough. 1983. Population density of tropical forest frogs: relation to retreat sites. *Science* 221(4610):570-572.
- Stewart, M. M. and L. L. Woolbright. 1996. Amphibians. pp. 273-320. In, D. P. Reagan and R. W. Waide (Editors). *The Food Web of a Tropical Rain Forest*. University of Chicago Press. Chicago, Illinois, USA. 616 pp. <http://dx.doi.org/10.1126/science.221.4610.570>
- Townsend, D. S. 1986. The costs of male parental care and its evolution in a Neotropical frog. *Behavioral Ecology and Sociobiology* 19:187-95. <http://dx.doi.org/10.2307/1445011>
- Townsend, D. S. 1996. Patterns of parental care in frogs of the genus *Eleutherodactylus*. pp. 229-239. In, Powell, R. and R. W. Henderson (Editors). *Contributions to West Indian Herpetology: A Tribute to Albert Schwartz*. Contributions to Herpetology. Volume 12. Society for the Study of Amphibians and Reptiles. Ithaca, New York, USA. 457 pp.
- Townsend, D. S. and M. M. Stewart. 1985. Direct development in *Eleutherodactylus coqui* (Anura:Leptodactylidae): a staging table. *Copeia* 1985:423-436. <http://dx.doi.org/10.2307/1444854>
- Townsend, D. S. and M. M. Stewart. 1986. The effect of temperature on direct development in a terrestrial-breeding Neotropical frog. *Copeia* 1986:520-523. <http://dx.doi.org/10.2307/1445011>
- Townsend, D. S., M. M. Stewart, and F. H. Pough. 1984. Male parental care and its adaptive significance in a Neotropical frog. *Animal Behaviour* 32:421-431. [http://dx.doi.org/10.1016/S0003-3472\(84\)80278-X](http://dx.doi.org/10.1016/S0003-3472(84)80278-X)
- Townsend, D. S., R. J. Wassersug, V. A. King, and G. C. B. Elder. 1990. Sexual dimorphism in the hindlimb of *Eleutherodactylus coqui*: does behavior predict morphology? *The Journal of Experimental Zoology* 255:350-354.
- Wells, K. D. 2007. *The ecology and behavior of amphibians*. The University of Chicago Press. Chicago, Illinois, USA. 1148 pp.
- Woolbright, L. L. 1985. Patterns of nocturnal movement and calling by the tropical frog *Eleutherodactylus coqui*. *Herpetologica* 41:1-9.