



# Clutches of Two Bromeliad-dwelling Frogs (*Eleutherodactylus guantanamera* and *E. ionthus*) from Cuba

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The seven species of Cuban frogs in the *Eleutherodactylus varians* species-group, which spend most of their lives in bromeliads, are characterized by moderate size (28–40 mm SVL), large and rounded digital discs, and a tan, brown, or greenish-brown dorsum, some with mottling or bold patterns (Hedges et al. 2008). Ecologically classified as arboreal and bromeliad-dwelling species by Díaz and Cádiz (2008, p. 19 and their Fig. 6), these frogs take refuge in bromeliads and reproduce and lay eggs in them, often high above the ground, and males call from bromeliads and leaves in the canopy (Díaz and Cádiz 2008; Henderson and Powell 2009).

The Oriente Bromeliad Frog (*Eleutherodactylus ionthus*) is endemic to southeastern Cuba (Díaz and Cádiz 2008), where it occurs in evergreen, pine, cloud, and rainforests (Díaz and Cádiz 2008) and in coastal semideciduous forests and xeromorphic scrub (Fong 2005). Males call throughout the night and occasionally during the day (Díaz et al. 2005; Díaz and Cádiz 2008). However, very little is known about reproduction in this frog (see the account in Henderson and Powell 2009).

The Guantanamera Frog (*Eleutherodactylus guantanamera*; Fig. 1), with a range restricted to the Sierra del Cobre, Sierra de La Gran Piedra, and Sagua-Baracoa Mountains (Díaz and Cádiz 2008), inhabits both primary (rainforest, semideciduous forest, pine forest, gallery forest) and secondary forests (timber and coffee plantations, roadside vegetation) (Díaz and Cádiz 2008; Fong 2008). Males call at night from high (typically ≥ 3 m) in trees (Hedges et al. 1992; Díaz and Cádiz 2008). Information on reproduction is summarized in Díaz and Cádiz (2008) and Henderson and Powell (2009). Clutches of 5–9 eggs (inferred from the number of ova in two large females; Hedges et al. 1992) are laid in bromeliads and usually attended by males. However, Díaz and Cádiz (2008) included a photograph (plate 24G) of a male guarding a clutch of 12 eggs laid in the axil of a bromeliad.

Note that the account of the Cuban Bromeliad Frog (*E. varians*) in Henderson and Powell (2009) included data published by Estrada (1990) on clutches found at La Melba and La Naza, two localities in the Sagua-Baracoa Range in Holguín Province (near our site in Arroyo Bueno; see below). At the time of Estrada’s paper, *E. guantanamera* had not been described and all bromeliad frogs were assigned to *E. varians*. Because both of Estrada’s study sites are in the range of *E. guantanamera*, as in Díaz and Cádiz (2008) and Rivalta et al. (2014), we consider Estrada’s data applicable to *E. guantanamera* (see below).

Herein, we provide the first data on clutches, neonates, and parental care in *E. ionthus* and we report additional information on clutches and egg-deposition sites, including communal oviposition, in *E. guantanamera*.



**Fig. 1.** Male Guantanamera Frog (*Eleutherodactylus guantanamera*) calling from a leaf at La Gran Piedra, Santiago de Cuba. Photograph by Ansel Fong.



**Fig. 2.** Habitats of Cuban bromeliad-dwelling frogs: The xeromorphic scrub on limestone soil at Siboney-Juticí Ecological Reserve, habitat of the Oriente Bromeliad Frog (*Eleutherodactylus ionthus*) (left), and the montane rainforest at La Gran Piedra, Sierra Maestra Mountain Range, habitat of the Guantanamera Frog (*Eleutherodactylus guantanamera*) (right). Photographs by Ansel Fong (left) and Luis O. Melián (right).

### Oriente Bromeliad Frog (*Eleutherodactylus ionthus*)

In 1996, we found a clutch of *E. ionthus* (undetermined number of eggs) on a leaf axil of the bromeliad *Tillandsia fasciculata* at Siboney-Juticí Ecological Reserve, Santiago de Cuba Province, Cuba (elev. ~90 m asl) (Fig. 2A). The bromeliad was on a bush about 170 cm above the ground within xeromorphic scrub vegetation on limestone soil. An adult in the same axil with the eggs appeared to be a male based on size (18.70 mm SVL) and other external morphological characteristics. Mean SVL of four of several hatchlings found inside the bromeliad was  $5.34 \pm 0.23$  mm (mean  $\pm$  SD; range: 5.00–5.50 mm). The adult male and froglets were deposited in the herpetological collection of BIOECO (no catalogue number assigned).

A second clutch found on 2 August 2001 at the same locality consisted of 14 eggs also deposited on a leaf axil of *T. fasciculata*. The bromeliad was facing north on the trunk of a bush 125 cm above the ground. The eggs were near the base of the leaf and had been recently deposited judging by the stage of development (i.e., stage 1 or 2; Townsend and Stewart 1985). Three days later on 5 August, the mean diameter of the eggs was  $5.31 \pm 0.24$  mm (mean  $\pm$  SD; range: 4.85–5.65 mm;  $n = 12$ ).

These data, the first documented for the species, generally coincide with observations of other Cuban bromeliad frogs. For example, we infer parental care in *E. ionthus* by witnessing a male adjacent to eggs and froglets, and that parental care is a generalized behavior in bromeliad frogs, in which it is now known to occur in four of the seven species: *E. guantanamera* (Estrada 1990; see below), *E. melacara* (Díaz and Cádiz 2008), *E. leberi* (Rodríguez and Alonso 2009), and *E. ionthus* (this paper). Elsewhere in the West Indies, hatchling attendance has been documented in the Puerto Rican Coqui (*E.*

*coqui*), in which males extend care for 1–6 days after neonates emerge (Townsend et al. 1984).

### Guantanamera Frog (*Eleutherodactylus guantanamera*)

We found two clutches of *E. guantanamera* eggs on 24 September 1997 at Arroyo Bueno, Sagua-Baracoa Mountain Range, Holguín Province, Cuba (elev. ~200 m asl). The two clutches were adhered to different leaves of the same bromeliad (undetermined species) on a bush branch within a stand of rainforest. The apparently older clutch (ca. stage 5 of Townsend and Stewart 1985) consisted of 11 eggs, and the more recently-laid clutch (ca. stage 3) consisted of 12 eggs. Four days later (28 September 1997), the more developed clutch had embryos at ca. stage 10 (i.e., dark-gray embryos, black eyes, and white to cream-colored yolk), and the mean diameter of the eggs was  $5.65 \pm 0.29$  mm (5.25–6.05 mm,  $n = 5$ ). The embryos in the younger clutch were at ca. stage 5 or 6, and the mean diameter of the eggs was  $5.11 \pm 0.30$  mm (4.55–5.45 mm,  $n = 8$ ).

We procured data of three additional clutches on 25 September 1998 at Altiplanicie de El Toldo, Sagua-Baracoa Mountain Range, Holguín Province, Cuba (elev. ~880 m asl). We initially encountered an amplexant pair of *E. guantanamera* inside a bromeliad (*Guzmania monostachya*) at 0830 h. The bromeliad was 170 cm above the ground, growing on a bush in submontane rainforest vegetation. The female had laid eight white eggs, which measured  $6.41 \pm 0.23$  mm (6.15–7.00 mm,  $n = 8$ ). We transferred the pair to a plastic container with a piece of the bromeliad leaf and some water to maintain humidity. At 1130 h, we found 13 additional eggs on the wall of the container, for a total of 21 eggs in the clutch. The female was preserved, assigned the field number AFG 1279, and deposited in the herpetological collection of



**Fig. 3.** A clutch of Guantanamera Frog (*Eleutherodactylus guantanamera*) eggs discovered at Altiplanicie de El Toldo, Holguín Province, Cuba (left) and the adult attending the eggs (right). Photographs by Gerardo Begué Quiala.

BIOECO (BSC.H-2018). The observation of a mating pair in amplexus early in the morning coincides with Townsend and Stewart's (1986) observations of prolonged amplexus and diurnal oviposition in *E. coqui* from Puerto Rico and other eleutherodactylids.

At 1117 h on the same day, we found another clutch of 12 eggs on a leaf axil of a bromeliad (*G. monostachya*) on a bush branch 150 cm above the ground, also in rainforest vegetation. The eggs were at ca. stage 6 (Townsend and Stewart 1985), with embryos evident and faintly pigmented eyes. The mean diameter of the eggs was  $6.53 \pm 0.24$  mm (6.00–6.85 mm,  $n = 12$ ).

We detected a third clutch on another bromeliad (*G. monostachya*) under similar conditions, but the clutch was mostly covered by fungus, and its condition limited the counting and measuring of most eggs. Three eggs largely free of fungus had embryos with elongated limbs indicative of ca. stage 8 (Townsend and Stewart 1985).

An additional clutch found at ca. 0800 h on 21 April 2008 at the same location within submontane rainforest on a bromeliad (*Hohenbergia* sp.) 180 cm above the ground on a bush trunk (height of the bush about 500 cm) contained seven eggs attended by an adult (Fig. 3). The embryos were at an advanced developmental stage, most likely stage 12 (Townsend and Stewart 1985).

Additional observations of *E. guantanamera* eggs included two clutches at La Gran Piedra (Fig. 2B), Sierra Maestra Mountain Range, Santiago de Cuba Province (elev. ~1,200 m asl). The first was found on 16 January 2003 in a bromeliad (*Catopsis nitida*) on a tree branch about 3 m above the ground

at the edge of a stand of montane rainforest. The bromeliad had fruits, 12 developed leaves, and held no water. An adult frog emerged from the plant's interior when we manipulated the bromeliad. It escaped before we could determine its sex. The clutch consisted of 18 white eggs, ca. stages 1 or 2 (Townsend and Stewart 1985), that were not measured. The next day (17 January 2003), we found another clutch ( $n = 18$  eggs) on a bromeliad 52 cm above the ground within montane rainforest. The mean diameter of the eggs was  $5.96 \pm 0.32$  mm (5.50–6.45 mm,  $n = 9$ ).

At 1038 h on 20 February 2018, in the vicinity of Pico Cristal, Sagua-Baracoa Mountain Range, Santiago de Cuba Province (elev. ~630 m asl), we found three clutches inside the same bromeliad (*Vriesea ringens*) and on the same leaf axil (Fig. 4A). The bromeliad was on a tree trunk (diameter = 95 mm) 150 cm above the ground within rainforest vegetation. Two clutches at different developmental stages were in close contact, whereas an even more recently laid clutch was separated by only 1–2 cm from the other two (Fig. 4B). This most recent clutch consisted of 15 eggs at ca. stage 3 (or less) (Townsend and Stewart 1985), the intermediate clutch contained 20 eggs (ca. stage 4), and the most-developed clutch consisted of 14 eggs (ca. stage 7; light-pink embryos, gray eyes, and discernable movement of embryos within the eggs).

Estrada (1990) had reported two clutches of *E. guantanamera* eggs at different developmental stages on the same bromeliad leaf. Díaz and Cádiz (2008) noted that finding two clutches deposited in the same breeding site was not rare. Herein, we describe, for what we believe is the first time, three clutches at the same site. Communal oviposition has been

suggested for three other Cuban species, the Cuban Giant Frog (*E. zeus*) (Alonso et al. 2015), Cuban Groin-spotted Frog (*E. atkinsi*) (Alfonso et al. 2017), and Cuban Flat-headed Frog (*E. planirostris*) (Iturriaga and Dugo-Cota 2018). These observations collectively suggest that multiple clutches deposited at the same breeding site may be more common in species of *Eleutherodactylus* than what has been documented (e.g., Townsend et al. 1984; Burrowes 2000; Ríos-López et al. 2016).

The number of *E. guantanamera* eggs per clutch in our observations ranged from 7 to 21 ( $14.8 \pm 4.4$ ), which exceeds the 6–13 eggs ( $10.0 \pm 2.3$ ) per clutch reported for this species by Estrada (1990) and the 12 eggs per clutch in plate 24G of Díaz and Cádiz (2008). Mean egg diameter of our clutches ( $6.01 \pm 0.61$  mm), however, was within the range (4.4–6.6 mm) reported by Estrada (1990), with much of the variation probably reflecting differences in female size (Wells 2007) and varying time intervals after deposition (Estrada 1990). The mean height of nest sites ( $163 \pm 68$  cm; 52–300 cm) coincided with heights ( $150 \pm 80$  cm; 60–300 cm) reported by Estrada (1990). However, as males vocalize in the upper canopy (Hedges et al. 1992), which ranged in heights at our sites from 12 to 20 m (Reyes and Acosta 2005b, 2006), egg clutches of this species are likely to be deposited at greater heights than those that are generally sampled.

The breeding season of terrestrial anurans that do not depend on standing water for reproduction is typically associated with the rainy season (Wells 2007). However, although most of the *E. guantanamera* clutches described herein and by Estrada (1990) were found during the rainy season (May–October) in the eastern Cuban mountains, we found evidence

of reproduction during drier months (January, February, April). Also, at higher elevations (such as those at our study sites), temperature can influence anuran breeding patterns, with most reproductive activity during the hottest periods (Duellman and Trueb 1994). Nonetheless, we discovered clutches during the winter months of January and February. Particularly in La Gran Piedra, our results contrast with those of Bignotte et al. (2021), who found a drop in the reproductive intensity in the November–February period in five species of ground/litter dwelling *Eleutherodactylus*. In general, our data suggest that *E. guantanamera* reproduces year-round, likely facilitated by more-protected microclimatic conditions in large-sized bromeliads that protect against desiccation and cold temperatures (although this species could exploit other breeding sites as suggested by Díaz and Cádiz 2008).

Epiphytic bromeliads of the genera *Catopsis*, *Guzmania*, *Vriesea*, and *Tillandsia* are confirmed nesting sites of Cuban bromeliad-dwelling frogs. These are the most abundant species of bromeliads in the vegetation types we surveyed (Reyes and Acosta 2005a, 2005b, 2006) and also are the species that grow to sufficiently large sizes to allow the frogs to breed and deposit eggs in their axils (pers. obs.). Future studies should test hypotheses suggesting that the species or physical characteristics of the bromeliad (e.g., plant size, number/length of leaves, orientation on a substrate) affect selection of an individual plant as an oviposition site by these ecologically specialized frogs in Cuba and on other Caribbean islands.

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**Fig. 4.** A communal clutch of the Guantanamera Frog (*Eleutherodactylus guantanamera*) from Pico Cristal, Santiago de Cuba Province (left) and a close-up view of the eggs (right). Numbers represent the progressive development of the clutches from the most recently laid (1) to the most developed (3). Photographs by Ansel Fong.

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