Harmonics: Rediscovering *Eleutherodactylus* Vocalizations[1]

Alejandro Ríos-Franceschi[2,3,4] and Rafael L. Joglar[2]

Abstract: There are 17 described species of *Eleutherodactylus* in Puerto Rico, some more common than others. The use of harmonics as a means of vocalization has been little studied in the Caribbean. As part of a long-term study of the evolution of sonic communication in Puerto Rican *Eleutherodactylus* frogs, we document the use harmonics in three common but distinct Puerto Rican endemic species: *E. coqui*, *E. antillensis*, and *E. brittoni*. This study focuses only on the description of these vocalizations using computer software not on the behavioral implications of these vocalizations.

Key Words: *Eleutherodactylus*, Puerto Rico, vocalizations, harmonics

Except for *Eleutherodactylus coqui* Thomas, 1966, relatively little is known about the biology and natural history of *Eleutherodactylus* frogs in Puerto Rico (Joglar 1998). While it is common knowledge that these frogs vocalize by channeling air from their lungs through the larynx (or voice box, Deka et al. 2015), amplifying the sound in their vocal sacs, detailed quantitative analyses of their spectrograms in a comparative spatial sense are scarse. Herein, we briefly discuss the calling pattern of three common endemic Puerto Rican species of *Eleutherodactylus*: *E. coqui*, *E. antillensis* Reinhardt and Lutken, 1863, and *E. brittoni* Schmidt, 1920) (Figure 1).

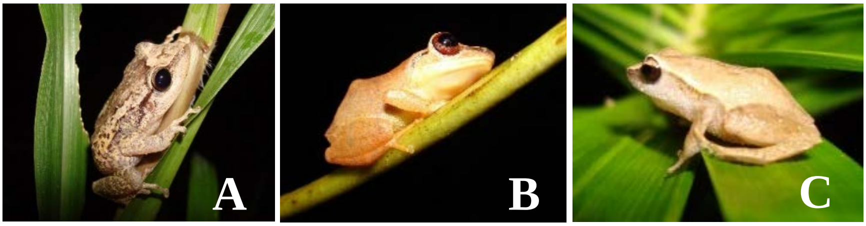

Figure 1. Images (location of vocalization and recording) of the three endemic species of Puerto Rican *Eleutherodactylus* mentioned in this paper. A. *Eleutherodactylus coqui* (Utuado, https://blaypublishers.files.wordpress.com/2018/07/eleutherodactylus_coqui_zoom0009_lr.wav) B. *E. antillensis* (Arecibo, https://blaypublishers.files.wordpress.com/2018/07/eleutherodactylus_antillensis_zoom0013_lr.wav), C. *E. brittoni* (Utuado, https://blaypublishers.files.wordpress.com/2018/07/eleutherodactylus_brittoni_utuado_casa-2015-09-09_18-30.wav. Photos by Alejandro Ríos-Franceschi©.

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[2] Department of Biology, University of Puerto Rico, Río Piedras, Puerto Rico 00664 USA. Corresponding authors' e-mail: alejandro.rios1@upr.edu (ARF); rjoglar@gmail.com (RJ).
[3] School of Health Sciences, Sistema Universitario Ana G, Méndez, Universidad Metropolitana, Jayuya, Puerto Rico 00664 USA.
[4] School of Natural Sciences, Sistema Universitario Ana G. Méndez, Universidad del Turabo, Barceloneta, Puerto Rico 00617 USA.

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Methods

Study Sites. Data were collected during the wet season (May–November) in 2017. Two study sites were selected due to their distinct locations (montane vs. coastal) and the three species occur sympatrically. The first study site was in Utuado; this area is mountainous with an elevation of 600 meters above sea level with an average night temperature and humidity of 25.5°C and 95%, respectively. The second study site was in Arecibo, located near the coast with an elevation of only 12 meters above sea level and an average night temperature and humidity of 29.4°C and 78%, respectively.

Vocalizations. To collect vocalizations, we used two different recorders. For automated field recordings, we used *ARBIMON II Bio-acoustics Analysis Platform* (*https://arbimon.sieve-analytics.com/*). The equipment was set to record for three nights (1800–0600). Additionally, we collected sound data from 50 individuals using a *Zoom H6*® recorder (Hauppauge, New York, USA) equipped with a shotgun microphone. Data was collected using 1 meter as the distance between the microphone and the calling frog (Meenderink et al. 2010, Narins and Meenderink 2014). From 721 recordings, the best 150 were selected for analysis using *Raven Pro Interactive Sound Analysis Software*® by Cornell Lab of Ornithology (Ithaca, New York, USA). By using two different recording methods we can reduce likelihood of errors caused by microphone overstimulation and creating false harmonics.

Results and Discussion

While analyzing the sound spectrogram, we noticed something special. These species can use harmonics, or notes whose pitches have a higher pitch than the lowermost or base note. Although some females such as the Túngara frog, *Engystomops pustulosus* (Cope, 1864), formerly known as *Physalaemus pustulosus*, can react to such harmonies (Ryan et al. 2010), studies in which coqui frogs are submitted to behavioral studies in which harmonics are represented are not common. True harmonic structures are not common in frog calls (Straughan and Heyer 1976). In frogs, the presence of harmonics has been shown in *Odorrana tormota* Wu, 1977 (Ranidae) (Narins et al. 2004, Shen et al. 2008); *Adenomera hylaedactyla* Cope, 1868, formerly known as *Leptodactylus hylaedactylus* (Leptodactylidae); *Hyla gratinosa* LeConte,1857 (Hylidae) (Bodnar, 1996), *Leptodactylus bolivianus* Boulenger, 1898 (Leptodactylidae) (Straughan and Heyer 1976). More recently, a study by Benevides and Mautz (2013), showed harmonics in the calls of the Puerto Rican common coqui, *Eleutherodactylus coqui*, in Hawaii. Until now, these harmonizing capabilities on *E. coqui* had only been mentioned by Narins 1982. However, recent studies such as Galvis et al. (2016) suggest that harmonics in the Caribbean frogs of the genus *Eleutherodactylus* are not that rare and can be used to shed some light into their evolutionary relationships.

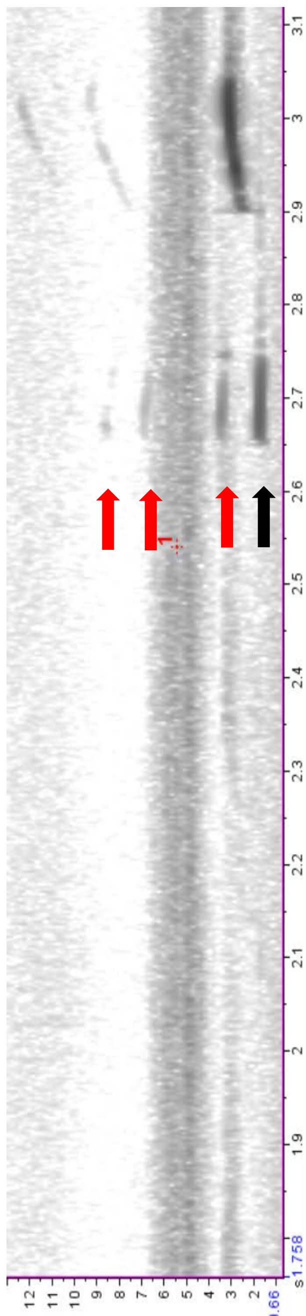

Figure 2. Harmonics from *Eleutherodactylus coqui* represented by the red arrows. Recorded at the Arecibo beach, Puerto Rico. Sound spectrogram generated by Raven Pro®.

The common coqui, *Eleutherodactylus coqui*, produces harmonics up to approximately 12.5 kHz (Figure 2). These signals are not in the ultrasound range (>20 kHz, the human ear tends to hear sounds in the range of 20 Hz, low pitches, to 20 kHz, high pitches) but they are quite high considering that *E. coqui* males produce vocalizations between 1.8 kHz–3.5 kHz to communicate with females and to defend their territory.

Eleutherodactylus antillensis, commonly known as the Red-eyed coqui, tends to be abundant in disturbed forested areas, which can facilitate data collection. This frog has two major vocalizations: (1) the two-note call "chu-rrí" (Figure 3); and (2) the aggressive trill call ("ki, ki, ki…") (Ovaska and Caldbeck 1997). Both vocalizations have their own specific harmonics, the two-note call has a shorter frequency range up to 6.0kHz, Similarly, the trill call can go up to 13.1kHz, which is almost the same frequency *E. coqui* has (Figure 4).

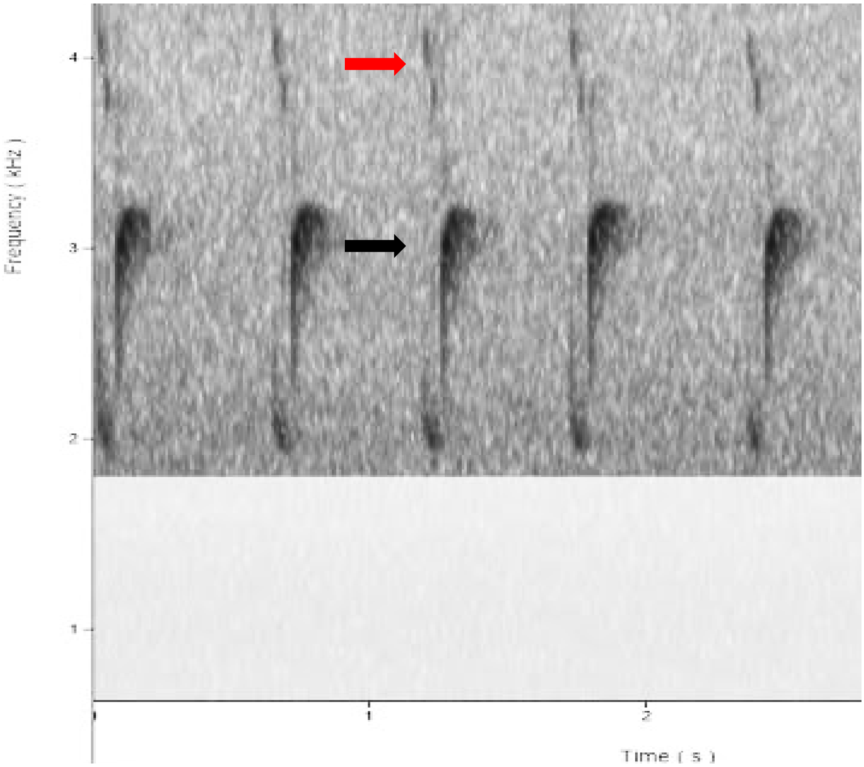

Figure 3. *Eleutherodactylus antillensis* vocalizations. Two-note call ("chu-"rrí", black arrow) with one harmonic (red arrow).

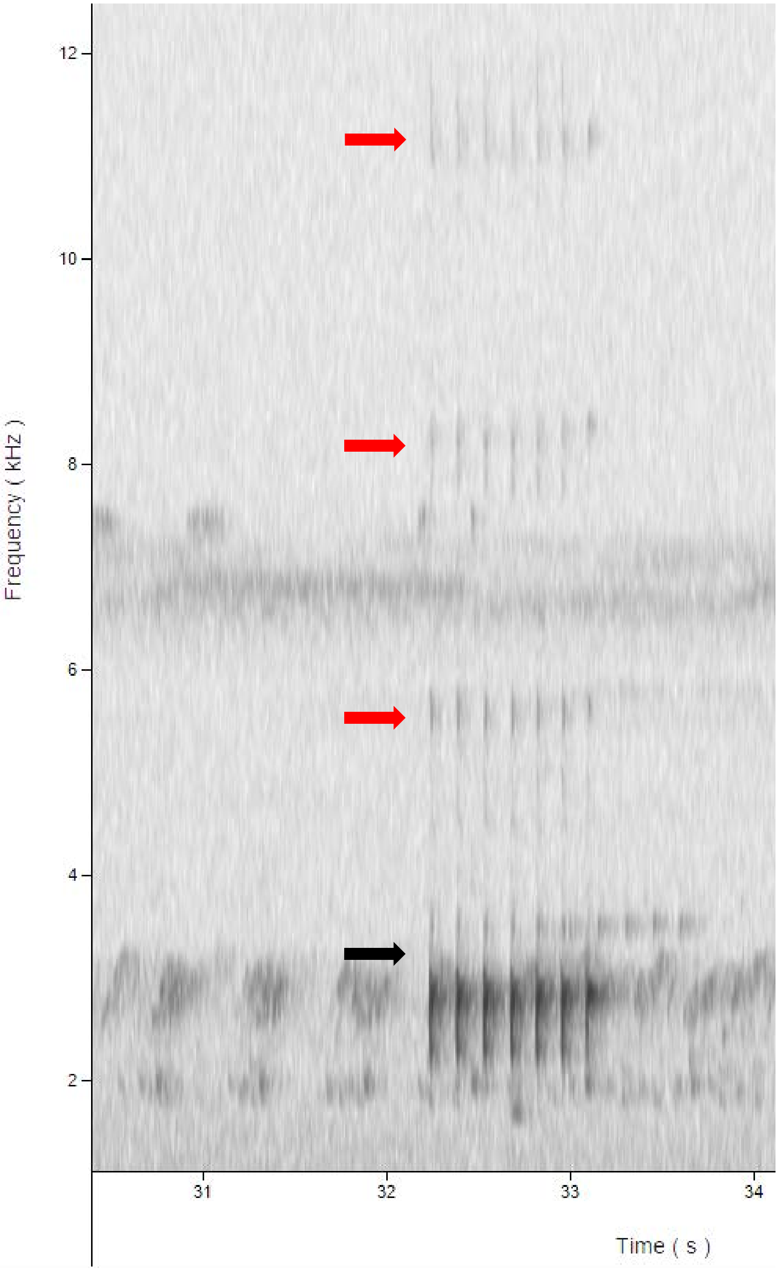

Figure 4. *Eleutherodactylus antillensis* vocalizations. Agresive trill call (black arrow) with three harmonics (red arrows). Sound spectrogram generated by ARBIMON II.

Eleutherodactylus brittoni, the smallest and the least studied of the three species, has the most interesting communication system of all. This tiny frog produces pulses in a specific pattern between 3.0kHz and 6.3kHz (N=10), repeating itself two to three times before starting with the two-pulse call (Figure 5). Even though Ríos-López and Villanueva-Rivera, 2013, showed this species spectrogram, they focused in partitioning of acoustic environment, rather than their behavioral pattern component. These patterns were not known until now and their functional significance, if any, is unknown.

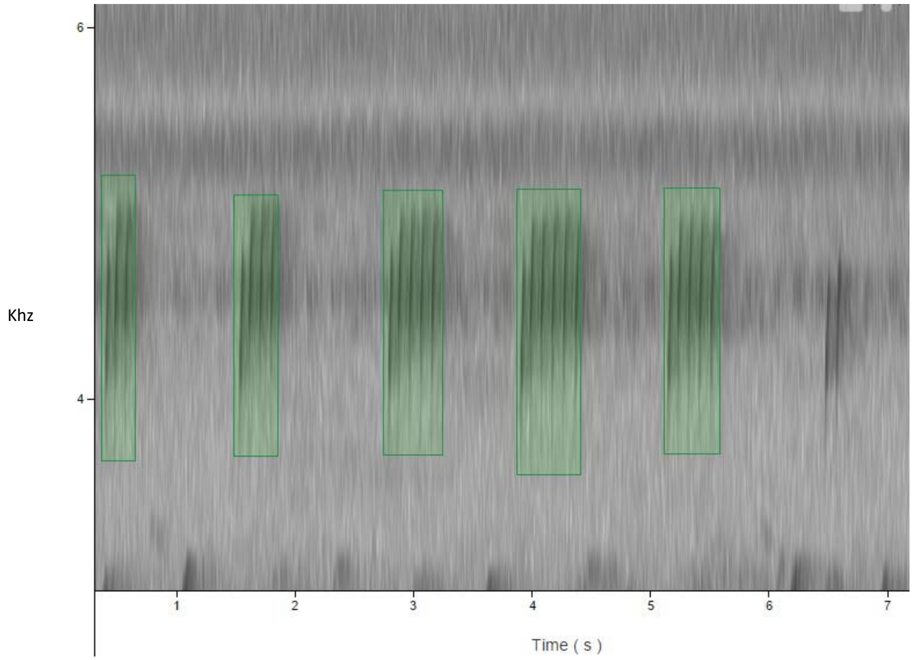

Figure 5. Sound spectrogram from *Eleutherodactylus brittoni*. Note patterns are denoted by the green squares (**, ***, ****, *****, ******, *****, repeat). Asterisk (*) represent pulses. Sound spectrogram generated by ARBIMON II.

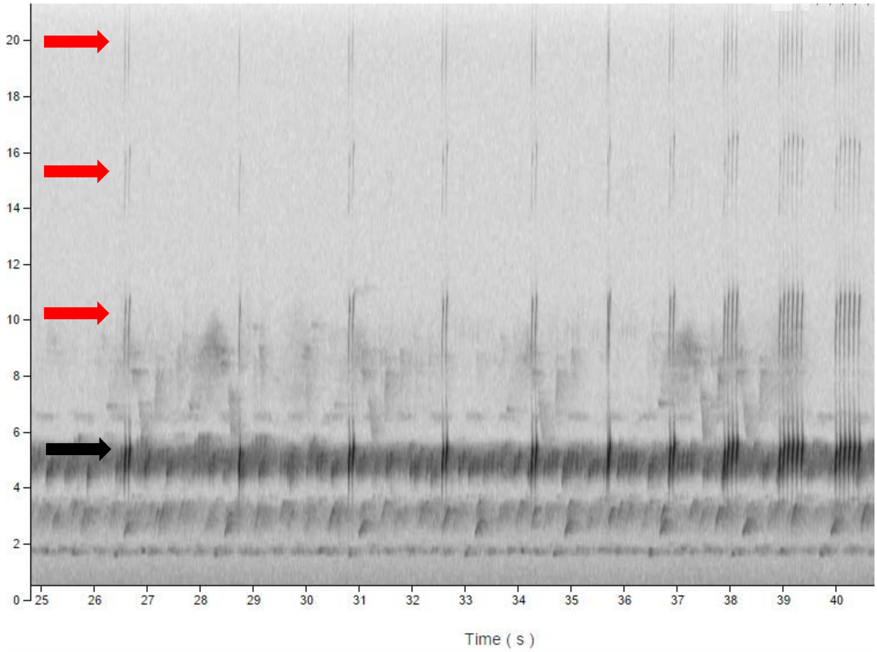

Figure 6. Harmonics (Red) shown in ARBIMON II from *Eleutherodactylus brittoni* ranging from 3.3kHz to 22.0 kHz.

Furthermore, males generate harmonics extending above 22.0kHz, well in the ultrasound realm (Figure 6). It is not clear what is the functional significance of these ultrahigh vocalizations. But some species use them to avoid masking by background noise (Feng et al. 2006), and some species, such as *Hylarana taipehensis* (Van Denburgh, 1909), formerly known as *Rana taipehensis*, seem to use high intensity calls also, to cope with noisy environments (Sun and Narins 2005).

More research on the vocalization complexities of *Eleutherodactylus* may shed light into these unanswered vocalization mysteries. For now, however, when we hear (or detect) the sounds of tropical frogs, we could consider that they may not just be vocalizing a melody; they could also be generating harmonics. These data are part of an ongoing research in which these parameters are being scrutinized to further understand how these organisms communicate. By studying their auditory papillae and larynx we can better understand how these sounds are produced and how they are received by their conspecific.

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