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Description of the Advertisement and Aggressive Calls of the Enigmatic Trinidad Thin-Toed Frog *Leptodactylus nesiotus*

***Amaël Borzée*[1,2,*], *Hoa Quynh Nguyen*[3,4,*], *Michael J. Jowers*[5,**]**

[1] *Laboratory of Animal Behaviour and Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing, People's Republic of China.*
[2] *Division of EcoScience, Ewha Womans University, Seoul, 03760, Republic of Korea.*
[3] *Interdisciplinary Program of EcoCreative, Department of Life Science, Ewha Womans University, Seoul, 03760, Republic of Korea.*
[4] *Centre for Research and Development of Membrane Technology, Institute of Environmental Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam.*
[5] *Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrario De Vairão, 4485-661, Vairão, Portugal.*
[*] *These authors contributed equally.*
[**] *Corresponding author. Email: michaeljowers@hotmail.com*

Abstract. Research on elusive amphibians is challenging, resulting in a lack of basic information on their natural history. This is the case of *Leptodactylus nesiotus,* a rare and elusive frog found in a small swamp in the southwestern peninsula of the island of Trinidad (West Indies). Here, we provide additional call descriptions for data collected in the field (Trinidad) and laboratory in 2004, including descriptions of both advertisement and aggressive calls. The notes in the advertisement calls were divided in two types of pulses, both lasting on average less than 0.04 s and with average peak frequencies less than 16,000 Hz. The aggressive calls also displayed two types of pulses lasting 0.06 s maximum on average and with highest average peak frequency of 1,612 Hz. The only significant difference between these two call types relates to the temporal structures, but a larger dataset is needed to confirm the pattern. There were no temporal significant differences between the calls described here and the ones described in earlier literature, although we provide a more detailed call description.

Keywords. Call characteristics; *Leptodactylus nesiotus;* Trinidad; West Indies.

INTRODUCTION

Threats to amphibian species are multiple (Stuart et al., 2004; Beebee and Griffiths, 2005; Wake, 2012), and these species are under a comparatively higher threat level than other groups of animals (Wake and Vredenburg, 2008; Pimm et al., 2014). Warnings against the loss of knowledge before their discovery are multiple (Brower, 2008; Scheffers et al., 2012), and hundreds of amphibians are in serious decline or already extinct (Honegger, 1981; Wake and Vredenburg, 2008). The Trinidad thin-toed frog (*Leptodactylus nesiotus* Read, 1981) is an interesting case. Until recently this species had an extremely reduced distribution, with its original population being found only in a small swamp within the Icacos Peninsula, in the island of Trinidad (Heyer, 1998). Thus, this population is highly susceptible to habitat alteration such as drought, mostly resulting from human activities, which would most likely cause an important population decline or even the extinction of the species locally. Recent sightings (and identification through capture of some individuals) have shown that *L. nesiotus* has expanded throughout some areas of southern and central Trinidad. However, the limited knowledge on its ecology and natural history makes it a threatened species (Hardy, 2004), and the data deficiency makes it unlikely to predict future areas of expansion or declines for its conservation management.

Species are described using a combination of characters such as morphology, phylogenetic relationships, osteology, and call properties (Vitt and Caldwell, 2013). Call properties are important as they allow the identification of species in the field when other cues such as morphology or genetics are not immediately accessible (Duellman and Trueb, 1986; Wells, 2010). However, some amphibian species have large variations in call properties (Gerhardt, 1994; Gerhardt and Huber, 2002) and extensive call repertoires (Toledo et al., 2015). Different species express several of the following types of calls: advertisement, territorial, distress, warning, and release, among others. Advertisement calls are used to attract mates for breeding, while distress, aggressive, and release calls are produced when interacting with other individuals; call repertoires varies among species (Wells, 2010).

Leptodactylus nesiotus was described in 1987 (Read, 1987), and morphological and phylogenetic relationships were subsequently assessed (Murphy, 1997; Heyer, 1994,

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1998; Ponssa, 2008; Ponssa et al., 2010; de Sá et al., 2014), including osteological (Ponssa, 2008; Ponssa et al., 2010) and external morphological characters (Heyer, 1994). Its advertisement call was described as consisting of a call duration of 0.03 s, 4–5 partial pulses, and a "broadcast frequency" (i.e., equivalent of the larger variability of the frequency range) of 1,500–2,000 Hz. The harmonic structure was noted to be ambiguous (Heyer, 1994). Here, we clearly describe the call properties of both the advertisement and aggressive calls of *L. nesiotus*.

MATERIALS AND METHODS

Species overview

Leptodactylus nesiotus is a small frog (32–33 mm; $n = 3$; Heyer, 1994) described from a single locality in Icacos Peninsula (Bonasse swamp; 10°05′05.8″N, 61°49′37.9″W; 10 m a.s.l.) on the southwestern peninsula of Trinidad Island, Trinidad and Tobago. The species is now also known from Guyana, Suriname and French Guiana (Jairam and Fouquet, 2018). It is listed as Vulnerable D2 in the IUCN red list of Endangered Species (Hardy, 2004). The species calls at night "in a burrow under a mass of fern roots in a stagnant swamp" (Hardy, 2004).

Field sampling, call analysis, and laboratory behavior

Sightings of calling males in the field are uncommon because of the elusive behavior of this species. Despite the acoustic identification of *Leptodactylus nesiotus* males ($n = 10$) during several visits to the swamp throughout the wet season of 2002 and 2003 by various researchers, all surveys prior to 2004 resulted in no sightings or captures. During May and June 2004, males could be heard calling between the thick reeds, and three *L. nesiotus* males and one female ($n = 4$) were collected at Bonasse swamp (10°05′05.8″N, 61°49′37.9″W; 10 m a.s.l.) on 3 July 2004. These individuals were taken to the laboratory and housed in tanks (0.95 cm × 35 cm × 35 cm) with a central mud islet with plentiful shrub branches, wood, a few stones, leaf litter, and water to resemble their natural habitat (Ponssa et al., 2010).

Three advertisement calls were recorded in adequate quality to be analyzed, including one in the field and two in laboratory settings. Two calls with a different structure were recorded in laboratory setting, and a single call type was recorded per individual. The calls were recorded with a directional TECT model UEM-81 super-cardioid/cardioid condenser microphone and a Sharp® MD-MT280E(S) portable minidisc recorder. Temperature recordings fluctuated at times of recording between 26–30 degrees.

We used Raven Pro 1.4 (Center for Conservation Bioacoustics. 2011) to extract acoustic properties of each call following the recommendations of Köhler et al. (2017). Background noise was filtered out at 1 kHz, and the spectrogram configuration was set to Hann window of 256-sample window size, 128-sample hop size with 50% frame overlap, and 172 Hz frequency grid spacing with 256-sample DFT size. Each call was analyzed for both temporal and spectral domains.

Two types of calls were identified and analyzed separately. One corresponded to the advertisement call described by Heyer (1994), which included the call recorded in the field and two recordings in the laboratory. The other two recordings in laboratory conditions appear to be aggressive calls as individuals were interacting with each other.

Temporal character measurements ranged from fine-scale pulses to larger-scale notes and even phrases in the aggressive calls. Specifically, we identified pulse duration (PD), inter-pulse interval (IPI), pulse period (PP) and pulse rate (PR, inverse of pulse period), note duration (ND), inter-note interval (INI), note rate (NR, inverse of note period) and inter-call interval (ICI). Spectral characters consisted of peak frequency (PF), which indicates the frequency of highest energy, and bandwidth 90% (BW90%), which refers to the frequency range from 5% to 95% of total energy. Additionally, and despite the low sample size, we tested for significant difference ($\alpha < 0.05$) between the two types of calls for all non-correlated variables though a non-parametric independent-sample Kruskal-Wallis test. The dependent variable was the type of call, and the covariates were PD, PF, number of pulses, IPI, and ICI. Variation is summarized as $\bar{x} \pm$ SD.

Comparison with previous call description

The first call description of *Leptodactylus nesiotus* was based on three individuals (Heyer, 1994: fig. 30) and re-analyzed by de Sá et al. (2014: fig. 118). We conducted this comparative analysis to assess the match between the advertisement calls we recorded and the previously described calls, thereby ensuring the reliability of our analysis. We assessed the differences between our results and earlier results through one-way ANOVAs. All analyses were conducted in SPSS v.21 (IBM Corp., 2016).

RESULTS

Generally, the advertisement call of *Leptodactylus nesiotus* was a series of notes consisting of two types of pulses, A and B (Fig. 1). These two pulses were alternatively produced during the call, although one recording was richer in pulse A than pulse B. The aggressive calls, in turn, comprised numerous pulses produced within each note (Fig. 2).

Call descriptions

Advertisement calls

The advertisement call of *Leptodactylus nesiotus* is a note with two types of pulses (Fig. 1). Pulse A lasts 0.04 ± 0.01 s with a PF of 1,555 ± 64 Hz, while pulse B lasts 0.03 ± 0.02 s and displays a PF of 1,389 ± 271 Hz. The amplitude of pulse A is however multiple factors of that of pulse B, although not in band frequency (band width BW 90% pulse A = 409 ± 152 Hz; pulse B = 1,318 ± 651 Hz). For both types of pulses combined, the average number of pulses per note was 1.31 ± 0.36, with IPI of 0.15 ± 0.16 s, PP of 0.18 ± 0.17 s, and PR of 14.88 ± 3.47 s^{-1}. Notes lasted 0.08 ± 0.05 s, with INI of 0.48 ± 0.35 s, NP of 0.56 ± 0.29 s, and NR of 3.17 ± 1.33 s^{-1}.

Agressive calls

This type of call also had two types of pulses (Fig. 2). Pulse A lasted 0.06 ± 0.01 s with a PF of 1,612 ± 26 Hz, while pulse B lasted 0.04 ± 0.01 s and displayed a PF of 1,578 ± 47 Hz. The amplitude of pulse A was lower than that of pulse B and occupied a lower band frequency as well (BW 90% pulse A = 457 ± 434 Hz; pulse B = 1244 ± 133 Hz). A note contained 3.5 ± 0.45 pulses, with IPI of 0.05 ± 0.05 s, PP of 0.11 ± 0.04 s, and PR of 10.86 ± 3.49 s^{-1}. The ND was 0.36 ± 0.14 s, with INI of 1.56 ± 1.29 s, NP of 1.96 ± 1.17 s, and NR of 1.00 ± 0.89 s^{-1}. For this type of call, we were able to measure properties of a phrase, in which phrase duration lasted 20.31 ± 12.85 s, with intervals between two consecutive phrases of 16.87 ± 2.13 s, and the number of notes in a phrase was 3.52 ± 0.45.

Table 1. Results of the Kruskal-Wallis test with call types as the dependent variable to determine the differences between the two types of calls described in this study. Significant results are indicated in bold.

	n	Z	df	P
Duration	11	1.75	1	0.186
Peak frequency	11	0.32	1	0.571
Number of pulses	7	4.5	1	**0.034**
Inter-pulse interval	7	0.5	1	0.480
Inter-call intervals	7	1.13	1	0.289

The Kruskal-Wallis analysis to determine the differences between the two types of calls showed that only the number of pulses was significantly different ($Z = 4.50$, df = 1, $P = 0.034$; Table 1). Nevertheless, the very low sample size needs to be acknowledged.

Comparison with previous call description

The one-way ANOVAs to test for variations between the call properties described here and the one described by Heyer (1994) did not display significant differences

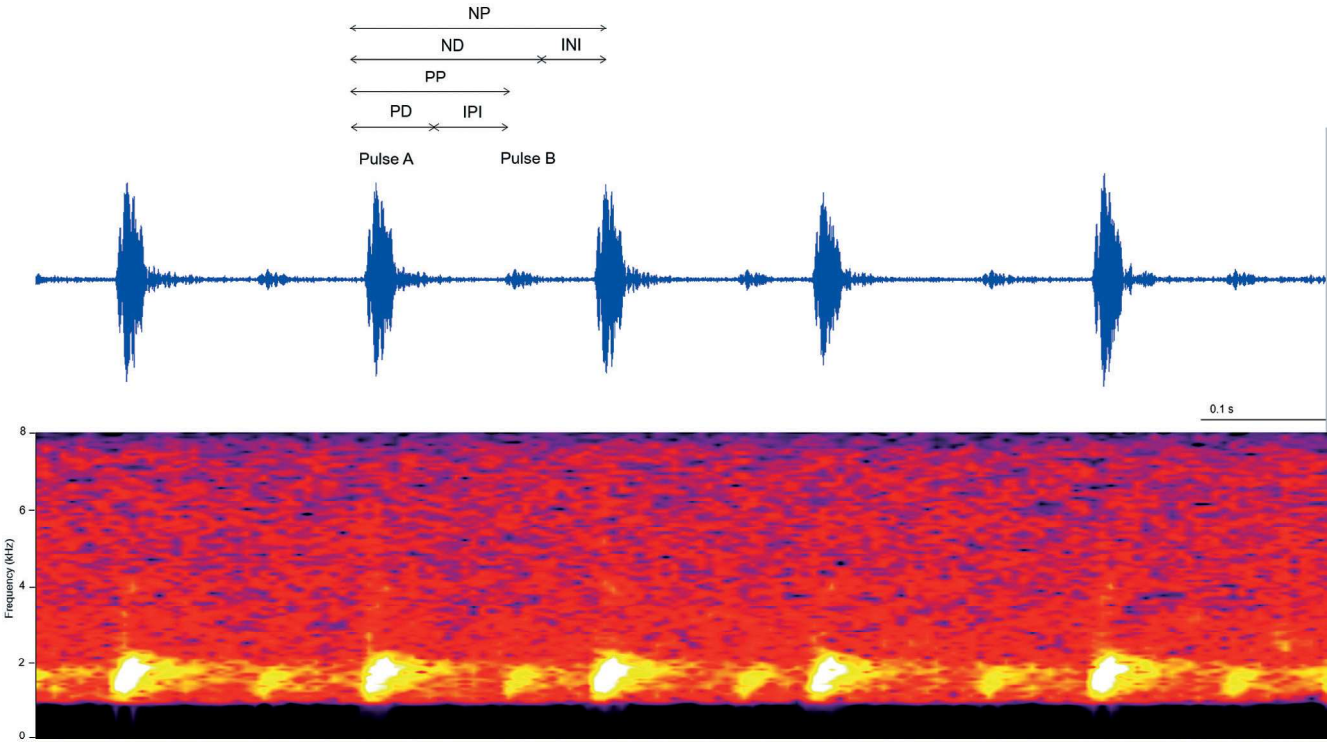

Figure 1. Oscillogram (amplitude vs. time; top) and spectrogram (frequency vs. time; bottom) of the advertisement calls produced by *Leptodactylus nesiotus*. One note is composed of two pulses: A and B. Temporal measurements include: pulse duration (PD), inter-pulse interval (IPI), pulse period (PP), note duration (ND), inter-note interval (INI), and note period (NP).

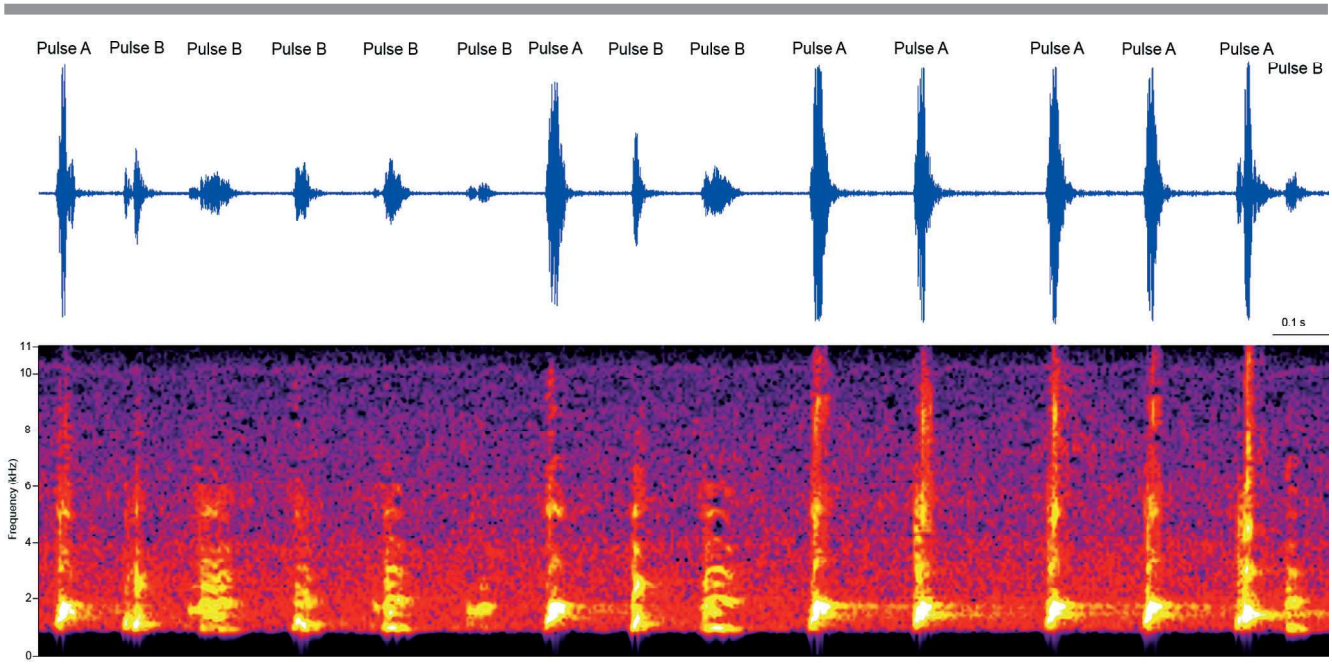

Figure 2. Oscillogram (amplitude vs. time; top) and spectrogram (frequency vs. time; bottom) of the aggressive call produced by *Leptodactylus nesiotus*. A note is also composed of two pulses: A and B.

between any of the factors. Duration (F = 0.54, df = 1, P = 0.480) and peak frequency (F = 1.73, df = 1, P = 0.213) were not different between the two studies.

DISCUSSION

Our redescription of the advertisement calls of *Leptodactylus nesiotus* was found not to be significantly different from the original calls described by Heyer (1994) in terms of peak frequency and call duration. Additionally, we describe a new vocalization for *L. nesiotus;* although the function of the call is unclear, it is likely related to male competition or aggressive calls. This call was only heard under laboratory conditions in the evenings. Distress calls have been reported in several species of *Leptodactylus* Fitzinger, 1826 (Toledo et al., 2004; Padial et al., 2006; Toledo and Haddad 2009; Dorado-Rodrigues et al., 2012; Forti et al., 2017). The genus has distinct vocalizations that are related to behavioral conditioning, such as agonistic interactions resulting in the emission of vocal croaks (encounter calls) and deep, rough sounds (territorial calls; da Silva et al., 2008).

Furthermore, species can emit several vocalizations such as growl, grunt, and trill calls (e.g., *Leptodactylus chaquensis* Cei, 1950; Heyer and Giaretta, 2009). In the field, calling males were heard in isolated islets and reported to be territorial (Ponssa et al., 2010), which may suggest that this was a male–male competition call. Report of this aggressive call in *L. nesiotus* populations might prove important for future surveys to identify possible population stress by male competition, which could be indicative of limited resource availability (e.g., swamp desiccation) or increased number of males within populations.

All three males were maintained with the female in the same tank (to increase the chances of amplexus to further understand the reproductive behavior of the species), so we cannot be certain about the sex of the individual producing the aggressive call as individuals were not seen calling from their excavated burrows (Ponssa et al., 2010). However, female vocalizations are known from a few anuran genera and are mostly related to mating behavior (Emerson and Boyd, 1999); moreover, such behavior has never been observed previously in the laboratory. Nevertheless, *Leptodactylus* females have been reported to emit a parental distress call (Sestito et al., 2016), but the amplitude seems to be very low compared to our recordings.

Interestingly, both of the types of calls described here displayed two types of pulses, which might be related to the physio-morphological characteristics of the species. Alternatively, the pattern could be the result of ecological pressures, as calls are produced to advertise the status of calling males (Wells and Schwartz, 2007).

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