# A reference collection of the acoustic signals of male *Nyctixalus margaritifer* Boulenger, 1882 (Anura: Rhacophoridae)

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**Abstract.** *Nyctixalus margaritifer* was initially documented in 1882 in the East Indies. However, the first and only acoustic signal within the genus *Nyctixalus* to be recorded and classified was from *Nyctixalus pictus*. As part of an effort to establish a reference collection, this study aims to investigate the acoustic characteristics exhibited by *N. margaritifer*. The findings revealed that male calls were organised in note groups, which were arranged in different series. Additionally, the notes showed characteristics of an unpulsed, sparse spectrum and consisted of numerous harmonics. This fundamental note type had fast/shorter calls and was present in every note group. In certain conditions, male frogs emitted notes that were longer and slower compared to the fundamental note type. These notes were found to be the first (introductory) or last in a group of notes. In some species, the first (introductory) note of a call is commonly associated with chorus territorialisation, social behaviour, and enhanced information transmission. However, the significance of the last note in calls/note groups remains unknown. The results also indicate that male frogs consistently maintained their notes either with modulated or non-modulated dominant frequency, or both. Nevertheless, further research is essential to uncover the mechanisms and anomalies within the acoustic signals of *N. margaritifer*, particularly for taxonomy purposes.

Key words. Anura, bioacoustics, introductory note, Nyctixalus, Rhacophoridae

#### INTRODUCTION

In most anuran species, acoustic signals are essential to mediate intraspecific or interspecific communication (Wells, 2007; Köhler et al., 2017). In addition to molecular studies, acoustic signals provide clear guidelines for the delimitation and identification of species for taxonomic and phylogenetic studies (Köhler et al., 2017), uncovering the relationships between the taxa and description of cryptic species (Padial et al., 2008; Rodriguez et al., 2017; Hamidy et al., 2018; Köhler et al., 2021; Ong & Shahrudin, 2022; Oswald et al., 2023). Nevertheless, descriptions of the calls of several species remain unreported or do not discuss intraspecific variation (Sukumaran et al., 2010; Forti et al., 2015). Therefore, to improve comparisons between species, a quantitative description of the characteristics of a particular anuran's

© National University of Singapore ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print) advertisement and other types of calls is needed (Márquez & Eekhout, 2006; Batista et al., 2015).

*Nyctixalus margaritifer* was first documented in the East Indies in 1882 (Boulenger, 1882). The presence of generic characters that were similar to the genus *Philautus*, particularly *Ixalus pictus* Peters, 1871 (Smith, 1931; Inger, 1966) and *Hazelia spinosa* Taylor, 1920 (Taylor, 1962; Liem, 1970), prompted a reassessment of various genera that resulted in the loss of the name '*Nyctixalus*'. The name '*Nyctixalus*' was later re-established (Dubois, 1981) and the genus currently consists of three species, namely *N. margaritifer*, *N. pictus*, and *N. spinosus* (Frost, 2022).

The efforts of earlier researchers in clarifying the *Nyctixalus* genus have been explicitly directed towards establishing a robust and reliable classification framework. Nevertheless, the acoustic features within this genus have been documented solely for *N. pictus* in Sarawak, Malaysia, which serves as the species' type locality (Matsui, 1996). Regrettably, the acoustic attributes of *N. margaritifer* have not been previously delineated or documented in scholarly literature. Henceforth, to establish a comprehensive reference collection, our study delves into the nuanced acoustic attributes exhibited by this species within its natural habitat.

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#### MATERIAL AND METHODS

This research was conducted between 13 December 2022 and 25 September 2023 in Telaga Warna, Bogor, West Java (6°42′6.12″S, 106°59′50.34″E), and Mount Slamet, Central Java (7°16″30.84″S, 109°12′27.91″E). Visual Encounter Surveys (VES) were used to search for frogs in the area three nights per week, using flashlights and in clear weather (from 7 p.m. to 1 a.m. West/Central Indonesia Time). A Zoom H1N handy recorder was used to record resulting acoustic calls. The device was placed as close as possible to the specimens to capture the clearest possible sounds. The recordings were saved as WAV files in 48,000 Hertz (Hz) and 16 bits.

After recording, we measured the specimens' snout-vent length (SVL) with a digital calliper ( $\pm 0.1$  millimetres), then released them into their natural habitat. Air temperature (degrees Celsius) and humidity (%) were measured with a digital temperature and humidity meter (YW-201) placed  $\pm 1$  m from specimens as we were recording. The calls were digitised and normalised to reach -1 decibel (db) below the maximum limit of WAV files. Temporal and frequency information was measured from an audio spectrogram with a 512-point fast Fourier transform (FFT), with a 50% overlap and applying the Hanning window (Kurniati, 2011; Pettitt et al., 2012).

We measured call duration (in seconds), periods of silence between calls (in seconds), note rates (notes per seconds), note duration (in seconds), inter-note intervals (in seconds), note frequency (Hz), dominant frequency (Hz), peak harmonic (Hz), and dominant frequency modulation (Hz/ ms) (Köhler et al., 2017). Dominant frequency modulation values were obtained by subtracting the end frequency from the start frequency and dividing this value by call duration (in minutes). Values of < 1/-1 Hz/ms indicate no frequency modulation and values of > 1/-1 Hz/ms indicate frequency modulation (Emmrich et al., 2020).

Subsequently, calls were identified and analysed using the Raven Pro version 1.6 software (Centre for Conservation Bioacoustics, 2019). To visualise the oscillogram and spectrogram, we used RStudio version 2022.12.0+353 with the Seewave package version 2.2.3 (Sueur et al., 2008; RStudio Team, 2020). The data obtained was presented as a mean  $\pm$  SD minimum–maximum, and the hygiene protocols for handling frogs in the field followed the procedures of Murray et al. (2011).

#### RESULTS

We obtained three recordings of male *Nyctixalus margaritifer* calls. The first recording was from a male at Telaga Warna, West Java, just 2 m from another male. The second male was  $\pm$  3 m away from a female frog. The third recording was captured from a male in Mt. Slamet, Central Java. It was positioned 4–6 m from two females. During our observation, we discovered that males were perched on leaves/branches in

areas with dense herbaceous plants. The SVLs ranged from 38.5 mm to 40.1 mm, while the temperature and humidity were recorded at 18.25–19.1°C and 80%–84%, respectively.

In the first recording, a total of 23 calls were analysed. Among these calls, 15 were arranged in note groups consisting of two to eight notes, and only eight calls had nine to 12 notes (Fig 1A, S2). The male producing these calls showed a note rate of 0.52 notes/sec over 05:00 minutes. Note groups had an average duration of  $7.93\pm3.36$  (3.3-14.63 sec) with intervals of  $4.55\pm3.69$  (1.3-16.78 sec). Each had an average duration of  $0.17\pm0.07$  (0.07-0.5 sec), with intervals of  $1.17\pm0.62$  (0.72-4.12 sec). Among the 155 notes, 48 had a dominant frequency modulation ranging from 1.2 /-0.9 to -0.3 Hz/ms.

From the second recording of a different male, we observed 21 calls. Among these calls, 16 were arranged in note groups consisting of nine to 10 notes, while only five had two, three, and eight (n = 3) notes (Fig 1B, S3). Calls emitted from this male had a note rate of 1.04 notes/sec over 02:46 min, with an average duration of  $5.03\pm1.12$  (1.72-6.91 sec) and periods of silence between note groups measured at  $2.46\pm0.9$  (10.2-4.12 sec). The notes had a duration of  $0.35\pm0.06$  (0.17-0.77 sec) with intervals of  $0.29\pm0.21$  (0.13-1.8 sec) between notes. Among the 175 notes in the groups, only one note had a dominant frequency modulation of -0.3 Hz/ms.

Despite the geographical separation of male frogs in Central Java, it was observed that the 23 emitted note groups consisted of several repeated notes (nine to 13), with only two calls comprising two and five notes (Fig. 1C, S4). This note group had an average duration of  $10.05\pm2.51$  (1.70-13.41 sec), with periods of silence measured at  $16.82\pm3.32$  (8.15-25.27 sec). Furthermore, the notes in the group had a duration of  $0.13\pm0.05$  (0.03-0.31 sec), with intervals of  $0.86\pm0.2$  (0.59-1.81 sec) between notes, at a rate of 0.38 notes/sec over 10:48 min. Among the 248 notes, 31 had a dominant frequency modulation ranging from 1.2 - 1.8/0.9 - 0.5 Hz/ms. A detailed acoustic parameter is provided in Table 1 and Supplementary Table S1.

### A spectral analysis of male calls

According to our observations, the notes were characterised by an unpulsed, sparse spectrum and consisted of numerous harmonics (n = 573), as presented in Fig. 2B. This fundamental note type had fast/shorter calls and was present in every note group. Additionally, the note duration was  $0.21\pm0.11$ (0.03-0.44 sec) with a dominant frequency of  $1287.22\pm80.11$ (1125-1500 Hz). The peak frequencies of the first and second harmonics were  $1246.24\pm50.62$  (1031.25-1406.25 Hz) and  $2609.83\pm120.97$  (2156.25-2718.75 Hz).

In certain conditions, when a male was near a female or to other males, the male frogs in West Java emitted notes which had similar characteristics with the fundamental note type but sounded longer and slower. These notes were found to be the first (introductory, n = 2) or last (n = 3) in a group of notes (Fig. 2C, S5). The notes had an average duration of 0.56±0.12 (0.50–0.77 sec) and a dominant frequency of 1312.5 Hz. The peak frequencies of the first and second

Table 1. Acoustic parameters of the male Nyctixalus margaritifer.

Acoustic parameters	Telaga Warna First male	Telaga Warna Second male	Mt. Slamet First male
	Mean±SD (Min–Max)	Mean±SD (Min–Max)	Mean±SD (Min–Max)
Note duration (s)	0.17±0.07	0.35±0.06	0.13±0.05
	(0.07–0.50)	(0.17–0.77)	(0.03–0.31)
Inter-note interval (s)	1.17±0.62	0.29±0.21	0.86±0.20
	(0.72–4.12)	(0.13–1.80)	(0.59–1.81)
Note frequency (Hz)	223.92±36.42	406.70±74.08	297.65±63.20
	(130.75–326.88)	(215.78–619.49)	(89.67–463.53)
Dominant frequency (Hz)	1263.51±46.98	1379.46±45.90	1216.67±28.69
	(1218.75–1312.50)	(1312.50–1500.00)	(1125.00-1312.50)
Peak first harmonic (Hz)	1218.15±25.05	1315.18±18.61	1215.73±23.67
	(1125.00-1312.50)	(1218.75–1406.25)	(1031.25–1312.50)
Peak second harmonic (Hz)		2655.51±52.71 (2531.25–2718.75)	2394.43±109.59 (2156.25–531.25)



Fig. 1. Various note groups emitted by males of *Nyctixalus margaritifer*. A–B, note groups consisting of seven to eight notes from males from Telaga Warna, West Java. C) Note group of 12 notes emitted from a male from Mt. Slamet, Central Java.



Fig. 2. A, male *Nyctixalus margaritifer*. B–C, general structure of notes within a note group. B, one of the basic/fundamental note types. C, one of the longer notes.

harmonics were  $1275\pm51.35$  (1218.75-1312.5 Hz) and  $2562.5\pm54.13$  (2531.25-2625 Hz), respectively. Most of these types are unmodulated (<1/-1 Hz/ms). Recordings of the longer and slower notes are provided in Supplementary Audio S5.

#### DISCUSSION

This research showed that male calls were organised in note groups, which were arranged in different series. However, calls emitted by males that were in close proximity to other males were not as resonant. Most of the note groups also had fewer repeated notes, between two and eight notes (n = 15 note groups), but the spacing between the series of notes was irregular. When a male was in close proximity to a female, a significant increase was observed in the number of notes, between 9 and 13, with shorter intervals between notes. In the call recordings of all male samples from Telaga Warna, we observed that some note groups had longer first (introductory) and last notes. In some male anurans, longer first (introductory) notes have been associated with chorus territorialisation (Duellman & Fouquette, 1968), social behaviour (Schwartz, 2001), and enhanced information transmission (Fang et al., 2019).

Increasing vocalisation or the number of notes is an effective competitive strategy that enhances the probability of signal recognition and detection, reception among competing males, or mate-attraction ability (Rand & Ryan, 1981; Wells & Taigen, 1989; Shen et al., 2008; Fang et al., 2019). It is common for female anurans to be attracted to long, loud, and constant male calls (Wilczynski et al., 1995; Howard & Young, 1998; Welch et al., 1998).

Nevertheless, emitted note groups with higher notes were easily detected by predators (Fang et al., 2019). In some species, calls with modulations provide mis-vocalisation or poor localisation cues to allow conspecific communication without indicating the location of frogs to predators (Narins et al., 2003; Hsieh & Saberi, 2009). Modulation also leads to more efficient sound transmission as a competitive strategy for intrasexual and territorial interactions (Schwartz et al., 2002; Ellinger & Hodl, 2003; Bosch & De La Riva, 2004; Foratto et al., 2021), preventing frequency interference from other frogs (Drewry & Rand, 1983).

In conclusion, this research presented data on the acoustic characteristics of male N. margaritifer. The results indicate that males emit calls organised into diverse note groups, likely serving communicative purposes within their natural habitat, especially for interacting with conspecifics, notably females. The note groups generally consisted of unpulsed notes with sparse-spectrum and numerous harmonics. In certain conditions, male frogs emitted notes that sounded longer and slower compared to fundamental note type. These notes were found to be the first (introductory) or last in a group of notes. The results also indicate that male frogs consistently maintained their notes either with modulated or non-modulated dominant frequency, or both. However, definitively identifying the advertising call in this species remains inconclusive due to our limited sample size. Further research is essential to uncover the mechanisms and anomalies within the acoustic signals of N. margaritifer, particularly for taxonomic purposes.

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## SUPPLEMENTARY DATA

The following data are available online:

Supplementary Table S1 (https://figshare.com/s/c21cd520ddf4e1ba981a)

Supplementary Audio S2. Acoustic calls of male number 1 from Telaga Warna, West Java (https://figshare.com/ s/9e62b9d4454536f6f248).

Supplementary Audio S3. Acoustic calls of male number 2 from Telaga Warna, West Java (https://figshare.com/s/ e200c095830951ad04dc).

Supplementary Audio S4. Acoustic calls of male number 3 from Mt. Slamet, Central Java (https://figshare.com/s/ d9a97d5ee3160ce22000).

Supplementary Audio S5. First and last notes from acoustic calls with a long duration.