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Call characters of *Zhangixalus* spp. frogs from Southeast Asia, with a new call profile for *Zhangixalus faristsalhadii* from Central Java, Indonesia (Anuran: Rhacophoridae)

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The genus *Zhangixalus* was formerly included the genus *Rhacophorus* Kuhl & Van Hasselt 1822 (Kuhl & Hasselt 1822; O'Connell *et al*. 2018) and was recognized as an independent genus (Jiang *et al*. 2019). The genus currently contains 46 species distributed across northeastern India, Nepal, Bhutan, continental and eastern Asia, and Sundaland (Jiang *et al*. 2019; Frost 2024; Gonggoli *et al*. 2024). Acoustic studies have only been performed on some members of this genus in China (Matsui & Wu 1994; Fang *et al*. 2018), Vietnam (Nguyen *et al*. 2020), Laos (Brakels *et al*. 2023), and Taiwan (Cheng *et al*. 2022). In recent years, a number of new species have been identified and descried based on molecular and morphometric studies (O'Connell *et al*. 2018; Brakels *et al*. 2023; Gonggoli *et al*. 2024), However, no bioacoustics information is available for the *Zhangixalus* populations from Sundaland. Therefore, we aim to characterize the call features of the Sundaland *Zhangixalus*, particularly in connection to the newly described *Zhangixalus faritsalhadii* from central Java, Indonesia (Gonggoli *et al*. 2024) which might be of value in taxonomy for the understanding of speciation and signal evolution (Kohler *et al*. 2017).

Calls of male of *Zhangixalus faritsalhadii* were recorded and collected at Mt. Slamet, Kalipagu, Ketenger Village, Baturaden District, Banyumas Regency, Central Java Province, Indonesia on 25 and 26 January 2025 which is the type locality of the species. The advertisement calls of a single individual were recorded approximately 1–2 metres from the subject with an A-Zoom H1N handy recorder as WAV files with a sampling rate of 44.1 kHz and 16 bits. The snout–vent length (SVL) of the male was 37.6 mm, and air temperature recorded near the calling male was 19.5–20.2 °C.

For comparison, we analysed recordings of three related species: *Zhangixalus dulitensis* in Ulu Temburong National Park, Brunei, recorded by Prof. Dr. Ulmar Grafe (Accessible at https://soundcloud.com/frogvoicesofborneo/51-rhacophorus-dulitensis); *Zhangixalus achantharrhena* at Mt. Singgalang, Balingka, West Sumatra, Indonesia, recorded by late Dr. Misbahul Munir that were subsequently deposited in the collections of the Museum of Zoologicum Bogoriense (MZB), Directorate of Scientific Collection Management, National Research and Innovation Agency, Cibinong, West Java, Indonesia; and *Zhangixalus prominanus,* made by David Boyle at Telekom Loop, Fraser's Hill, Pahang Malaysia (Accessible at www.xeno-canto.org/921945, file type mp3).

We measured note durations (in seconds), inter-note intervals (in seconds), note rates (notes per second), pulse durations (in seconds), inter-pulse intervals (in seconds), pulse minimum and maximum, dominant frequency, peak harmonics, and frequency dominant modulations (in Hertz/millisecond) (Köhler *et al*. 2017). We measured only notes, pulses, inter-note intervals, and inter-pulse durations for *Z. prominanus* because the audio files were in mp3 format. The spectral and temporal parameters were analysed using the Raven Pro version 1.6 software (Centre for Conservation Bioacoustics 2019) and visualised using RStudio version 2022.12.0+353 with the Seewave package version 2.2.3 (Sueur *et al*. 2008; RStudio Team 2020). Spectrograms were obtained using the Blackman window function at 3db Filter

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Bandwidth of 158 Hz; grid spacing of 21.5 Hz; overlap 90% (Köhler *et al*. 2021). The data was presented as a mean ± SD (minimum–maximum).

In this study, we observed a male *Z. faritsalhadii* that emitted complex, distinct calls containing different note types composed of one to five pulses (pulse group) (Fig. 1B, Supplementary Audio S1). These calls were dominated by notes composed of two pulses (n = 38) and three pulses (n = 24) of 93 notes. The calls had a duration of 0.1 ± 0.04 seconds, inter-note interval of 5.6 ± 3.5 seconds, and inter-pulse intervals of 0.01 ± 0.01 seconds. The calls were emitted at a rate of 0.16 ± 0.05 (n = 93 notes) notes per second with 0.43 ± 0.01 (n = 222 pulses) pulses per second. Each pulse formed up to the fifth harmonic band ranging from 1498.8 to 8035.7 Hz. However, only 44 pulses were modulated at a rate of 2,4 ± 3,4 Hz/ms.

The male *Z. prominanus* also emitted complex, distinct calls containing different note types of 6 to 17 pulses per note (Fig. 1E). The calls were dominated by notes consisting of 10 pulses (n = 103). The call durations ranged from 0.1 to 0.4 sec (n = 198) with an inter-note interval of 3.6 ± 2.2 seconds, emitted at a rate of 0.26 notes per second and at a rate of 2.78 pulses per second. The pulses duration ranges from 0,002 – 0,04 sec (n = 2069) and most inter-pulse intervals between first with second pulses lasted 0,03 ± 0,002 (0,007 – 0,03 sec).

The calls of the male *Z. dulitensis* with uniform pulsed notes consisted of three pulses (n = 17) with 44 pulses modulated at 37.4 ± 29.9 Hz/ms (Fig. 1D). The calls lasted 0.11 ± 0,01seconds with an inter-note interval of 1.8 ± 0.3 seconds and an inter-pulse interval of 0.01 ± 0.002 seconds, emitted at a rate of 0.57 notes per second with 1.70 pulses per second. Each pulse formed up to four harmonic bands ranging from 1609.5 to 6524 Hz. Meanwhile, the calls of the male *Z. achantharrhena* consisted of three pulses per note (n = 21) with 56 pulses modulated at 16,6 ± 8,9 Hz/ms emitted at a rate of 0.12 notes per second with 0.36 pulses per second (Fig. 1C). These calls lasted around 0,3 ± 0,01 seconds with an inter-note interval of 8,5 ± 7,8 seconds, an inter-pulses interval of 0,03 ± 0,01 seconds and one to eight spare harmonic bands raging 1312.5–10857.4 Hz.

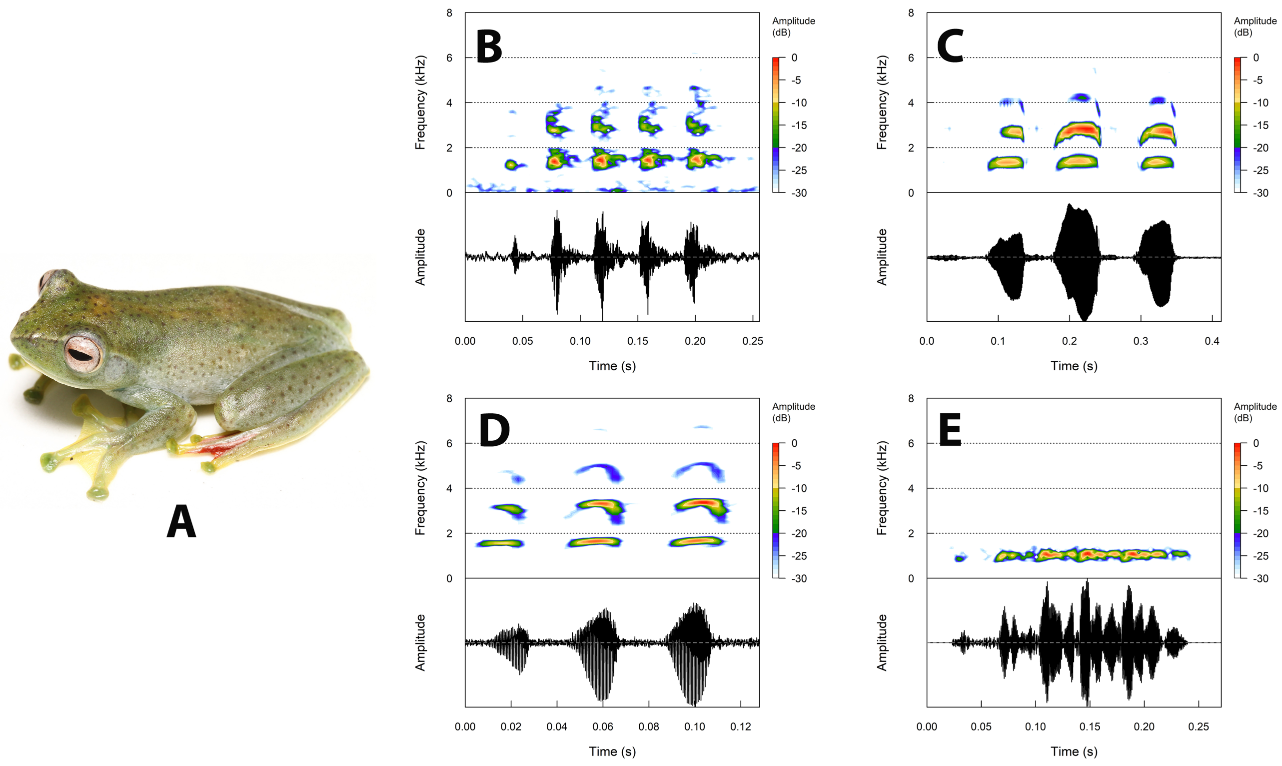

FIGURE 1. A male *Zhangixalus faritsalhadii* (A). Spectrograms (top) and oscillograms (bottom) of various pulses group emitted by males *Zhangixalus* spp.: B) *Zhangixalus faritsalhadii*. C) *Zhangixalus achantharrhena*. D) *Zhangixalus dulitensis*. E) *Zhangixalus prominanus*. Photograph by Misbahul Munir (*Z. faritsalhadii*).

Based on the pulse patterns of *Z. faritsalhadii*, we observed a unique pattern across the one-pulse notes and multi-pulse groups in which the last pulses were longer in duration 0.05 ± 0.02 (n = 79) than the previous pulses 0.03 ± 0.01 (n = 143). This pattern was also found in the dominant frequencies and harmonics of two pulses per note. We found that the final pulses had a dominant frequency (1580.9 ± 58.3 Hz) and harmonics that were (1577,6–8096.5 Hz) higher than the first pulse (1551.5 ± 62.9 Hz; 1545.9–7967.3 Hz). However, in calls with three-pulse notes, the last pulses had a dominant frequency (1492.9 ± 103.3 Hz) and harmonics (1500.2–6425.5 Hz) that were lower than the second pulses (1509.1 ± 108.3 Hz; 1489.4–6498.2 Hz) but much better than the first pulses (1423.9 ± 145.3 Hz; 1428.4–6268.6 Hz). This pattern also resembled four pulses per note, where the last pulses had a dominant frequency (1500.7 ± 123.8 Hz) and harmonics (1497.4–4837.8 Hz) lower than the third pulses (1557 ± 75.3 Hz; 1510.6–4745.9 Hz) but higher than the first (1338.4 ± 117.9 Hz; 1358.3–4737.3 Hz) and second pulses (1469.2 ± 127.4 Hz; 1437.8–4737.3 Hz). There is only one call that has five pulses per note pattern, further research requires to determine whether it is a common call behaviour of the species.

The *Z. dulitensis* exhibited durations in the first to third pulses (0.03 ± 0.003; n = 51). However, the dominant frequency (3336,4 ± 14,2 Hz) and harmonics (1641.6–6642.3) of the third pulses are more significant than the first (1549,1 ± 14,2 Hz; 1550.4–6063.8 Hz) and second pulses (2800,6 ± 774,6 Hz; 1636.5–6541 Hz). Among the *Z. achantharrhena,* the first and third pulses were shorter (0.07 ± 0.01 sec; n = 42) than the second pulses (0,08 ± 0,01 sec; n = 21). Nevertheless, the dominant frequency (2431,9± 586,6 Hz) and harmonic (1303.6–10788.5 Hz) of the third pulses are lower than the second (2678,6 ± 309,8 Hz; 1330.4–10916.7 Hz) but mostly higher than the first pulses (1638,4 ± 586,7 Hz; 1312.5–10687.5 Hz). Detailed acoustic parameters are presented in supplementary Table S2 and S3.

The divergence in the vocal behaviour among *Zhangixalus* spp. in Sundaland can result from geographic variations, environmental selection, sexual selection, and isolation (Kohler *et al.* 2017). In this research, we identified their advertisement calls are of different structure and distinguishable to the human ear and have narrow quantitative differences. However, due to lack of comparative calls *Z. prominanus* from Malaysia further research is required.

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Supplementary Materials. The following data are available online: https://figshare.com/s/74a5729ce9f0c709059f

Supplementary Audio S1. Acoustic male *Z. faritsalhadii*

Supplementary Table S2. Table of acoustic parameters of *Zhangixalus* spp.

Supplementary Table S3. Raw data of acoustics parameters of *Zhangixalus* spp.