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Female reciprocal calling of pearly tree frog (*Nyctixalus margaritifer* Boulenger, 1882) in West Java, Indonesia

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Less than 1% of female anurans vocalize (Gerhardt & Bee 2007; Köhler *et al.* 2017). In some species, females emit calls as a response to male calls. This behaviour has been referred to as a reciprocal call (Schlaepfer & Figeroa-Sandí 1998). As most female frogs respond to male calls only when they are at the peak of reproduction (Bush *et al.* 1996), experimental playback becomes a beneficial method for revealing their acoustic signal (Capranica 1965). Nevertheless, the study of female calls often receives less attention from researchers because the behaviour is challenging to observe (Willaert *et al.* 2016). However, a reference collection is needed for taxonomy purposes to provide clear guidelines for species identification or delimitation (Köhler *et al.* 2017).

Nyctixalus margaritifer, also known as the pearly tree frog, was described based on a male specimen purchased from the East Indies (Boulenger 1882). The Royal Museum, Brussels, lost the specimen between 1882 and 1884. Nonetheless, in 1884, V. Huegel 1884 discovered a male specimen from Willis Mountains (Java), which was established as this species' neotype (Dubois 1981). *Nyctixalus margaritifer* is endemic and disjunct in Java, Indonesia, in undisturbed highland forests above 700 m elevation (Iskandar 1998). In 1998, Iskandar discovered female species specimens in Situ Gunung in Mount Gede Pangrango West Java (Iskandar 1998). Later, in 2019, Rumanta found five specimens at Telaga Warna in Mount Gede, West Java (Rumanta *et al.* 2019). All previous research, however, did not include descriptions or recordings of the species' acoustic signals. Per the previous studies, this paper focuses on species found in West Java. Using the playback experiment method, we have described and documented the previously unknown call of the female *N. margaritifer*.

This study was conducted from August 2022 to July 2023 at Telaga Warna Lake in Bogor and at Situ Gunung Lake in Mount Gede Pangrango National Park, Sukabumi, West Java. In both locations, we conducted a visual search for the frogs with flashlights three nights per week (from 7 p.m. to 4 a.m. Western Indonesia Time) in clear weather. Studies suggest that female characteristics were determined based on indicators such as snout-vent length (SVL) (females of *N. margaritifer* are 42.6–45 mm in size whereas males are between 30 mm and 33 mm), the presence of egg masses in the abdomen, the absence of a nuptial pad on the first finger, and linea masculina on the abdomen (Inger 1966; Iskandar 1998; Priambodo *et al.* 2021). A YW-201 digital humidity and temperature sensor was used to measure the air temperature (Celsius) and humidity (%) of the areas as close as possible to the calling sites of the frogs. We also used a mobile phone to document the marble patterns found on the frogs' abdomens to distinguish between them. The detailed marble patterns are presented in the supplementary Figure.

For the playback experiment performed in field conditions, acoustic stimuli were applied using a one-minute recording of a male *N. margaritifer* call from captivity in the USA. The call was arranged in note groups consisting of several notes between 10 and 12 notes. Each note group is separated from the other by silent intervals, which have a longer duration

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than the inter-note interval. The notes within the groups had unpulsed and sparse harmonic spectrum characteristics with varied harmonic bands (Fig. 1e).

The recording was created by contributing artist Michael Ready and made available by the Indonesian National Research and Innovation Agency's (BRIN) Research Centre for Biosystematics and Evolution (Audio S1). We considered the possibility that the female frogs would not emit any calls due to the stress of being observed and measured at the start of our experiment. To prevent this and to ensure the frogs emit clear communication signals, the acoustic stimuli were activated 30 minutes after we made our measurements, using a small portable speaker raised ± 70 cm above the ground and placed 5-7 m from the female frogs. Our sound recordings were made using a Zoom H1N handy recorder placed ±1 m from the females, with the resulting audio saved as WAV files in 48000 Hertz (Hz) and 16 bits.

The calls were digitalised 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 & Hamidy 2017). The oscillogram and spectrogram were used to measure several quantitative parameters in response to acoustic stimuli: the duration of the responding female calls (in seconds), call rate (call per minute), call duration (in seconds), inter-call intervals (in seconds), and dominant frequency in Hz (Köhler *et al.* 2017).

We also measured the dominant frequency modulation values as a parameter, with values < 1/-1 Hz/ms categorised as 'not frequency modulated' and > 1/-1 Hz/ms categorised as 'frequency modulated' (Emmrich *et al.* 2020). We then calculated the Modulation Index (MI) using the algorithm described by Castellano & Rosso (2006). The MI ranged from 0, indicating no modulation, to 1, indicating complete modulation with intervals of silence. The call analysis was made using the Raven Pro software version 16.4 (Centre for Conservation Bioacoustics 2019). To visualise the oscillogram and spectrogram, we used the Seewave package version 2.2.3 (Sueur *et al.* 2008). The data was presented as a mean ± SD (minimum–maximum).

The study resulted in four recordings, three of which were obtained from Telaga Warna Lake (6°42'5.29"S, 106°59'50.01"E; 6°42'3.26"S, 106°59'50.41"E; 6°41'56.10"S, 106°59'50.01"E) and one from Situ Gunung Lake in Mount Gede Pangrango (6°50'1.11"S, 106°55'29.58"E) (Fig. 1a, Audio S2, Video S4). The female specimens were found perched on the leaves of shrubs/bushes 30 cm to 80 cm above the ground. The air temperature and humidity were recorded at 19.65 ± 1.63 (17.8–21.5 degrees Celsius) and 87.8 ± 3.19 (84%–92%).

Based on the results, the female frogs vocally responded to the acoustic stimuli with a response duration of 2.1 ± 1.5 (0.09–8.0 s) and 13–20 calls (n = 62 calls) in 04:46–06:16 minutes. The calls rate averaged 2.84 ± 0.23 (2.7–3.12 calls/min), with a duration of 0.21 ± 0.07 (0.06–0.31 s) and inter-calls interval of 19.96 ± 7.3 (11.11–43.56 s). The females exhibited several distinct types of calls that were visible in spectrogram patterns. The first type featured nearly flat, unpulsed notes with 50% showing modulation (n = 29) as depicted in Fig. 1b. The response duration was 2.6 ± 1.6 (0.94–7.68 s) to acoustic stimuli, while the call duration, dominant frequency modulation, and the mean modulation index were 0.23 ± 0.04 (0.11–0.30 s), 1.39 ± 0.44 (1.12–1.9 Hz/ms, n = 3), and 1.26 ± 0.21 (1–1.6, n = 16), respectively.

The second type of calls consisted of unpulsed notes, with sparse-harmonic notes and an energy sweep-up towards the end (n = 23, Fig. 1c). The calls exhibited a response duration of 1.8 ± 1.5 (0.09–8.00 s) to acoustic stimuli. Meanwhile, the calls duration, dominant frequency modulation, and the mean modulation index were 0.15 ± 0.06 (0.06–0.26 s), 4.57 ± 2.89 (1.08–11.16 Hz/ms), and 1.13 ± 0.19 (1–1.5, n = 6), respectively. The third type of call comprises unpulsed notes with a sparse harmonic and an energy sweep–down at the end (n = 10, Fig. 1d). The calls exhibited a response duration of 1.4 ± 1.2 (0.16–4.37 s) to acoustic stimuli. Meanwhile, the call duration, dominant frequency modulation, and mean modulation index were 0.23 ± 0.03 (0.19–0.27 s), 1.91 ± 0.31 (1.39–2.32 Hz/ms), and 1.2 (n = 1), respectively. Detailed acoustic parameters are presented in supplementary Table S3.

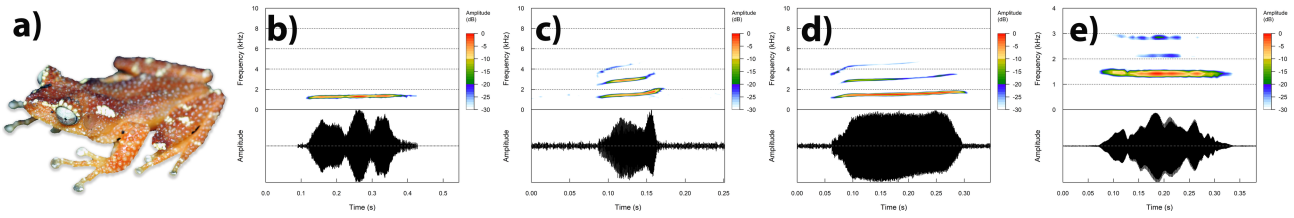

FIGURE 1. A female *N. margaritifer* (a). The first type of call (b), the second type of call (c), the third type of call (d), and the note type structure of a male call (e).

Based on the results of the playback experiment, the female frogs recognized and responded to the conspecific acoustic stimuli we provided. We recorded 28 response calls emitted after the call of note groups consisting of 10 notes with a response duration of 1.7 ± 0.8 (0.09–4.44 s) and 34 calls emitted after note groups consisting of 12 notes with a response duration of 2.43 ± 1.9 (0.56–8 s). Regarding the number of call responses, these results prove that the female *N. margaritifer* can emit calls and be attracted to long and constant male calls, as commonly observed in other anurans (Faggioni *et al.* 2017).

The female calls had unpulsed characteristics consisting of numerous harmonics within a sparse spectrum (Fig. 1b–d), which is similar to the note type in call of a male *N. margaritifer* recorded by Michael Ready (Fig. 1e) and male *N. pictus* (Matsui 1996). A total of 36 calls exhibited a dominant frequency modulation of 3.57 ± 2.67 (1.1–11.16 Hz/ms), while 25 calls had an index modulation of 1.22 ± 0.21 (1.0–1.6). The results indicate that the female frogs consistently maintained their calls with amplitude modulation, dominant frequency modulation, or both. Nevertheless, the limitations of this study were that we only obtained recordings from four females. Therefore, future studies with representative samples are needed to explore the acoustical signal and vocalization in the female *N. margaritifer*.

Audio recordings and supplementary material are available at: https://figshare.com/s/d60b43d28b06ceed1cf0

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References

Boulenger, G.A. (1882) V.—Description of a new genus and species of frogs of the family *Ranidae*. *Annals and Magazine of Natural History*, Series 5, 10 (55), 35. https://doi.org/10.1080/00222938209459662

Bush, S.L., Dyson, M.L. & Halliday, T.R. (1996) Selective phonotaxis by males in the Majorcan midwife toad. Proceedings of the Royal Society of London. *Series B: Biological Sciences*, 263 (1372), 913–917. https://doi.org/10.1098/rspb.1996.0135

Capranica, R.R. (1965) n.k. *In*: *The evoked vocal response of the bullfrog: A study of communication by sound. Research Monograph 33.* MIT Press, Cambridge, Massachusetts, pp. 29–54.

Castellano, S. & Rosso, A. (2006) Variation in call temporal properties and female preferences in *Hyla intermedia*. *Behaviour*, 143 (4), 405–424. https://doi.org/10.1163/156853906776240650

Centre For Conservation Bioacoustics. (2019) Raven Pro: Interactive sound analysis software. Version 1.6. 1. Computer software. The Cornell Lab of Ornithology. https://ravensoundsoftware.com/ (accessed 5 January 2024)

Dubois, A. (1981) List of nominal genera and subgenera De *Ranoidea* (Anura amphibians) of the world, With Identification of their species-types. Nomenclatural consequences. Pubblicazioni Del Centro Di Studio Per La Faunistica Ed Ecologia Tropicali Del C.N.R. CCXVI. *Monitore Zoologico Italiano*, Supplemento, 15 (1), 225–284. https://doi.org/10.1080/03749444.1981.10736637

Emmrich, M., Vences, M., Ernst, R., Köhler, J., Barej, M.F., Glaw, F., Jansen, M. & Rödel, M.O. (2020) A guild classification system proposed for anuran advertisement calls. *Zoosystematics and Evolution*, 96 (2), 515–525. https://doi.org/10.3897/zse.96.38770

Faggioni, G., Souza, F., Uetanabaro, M., Landgref-Filho, P., Furman, J. & Prado, C. (2017) Reproductive biology of the nest building vizcacheras frog *Leptodactylus bufonius* (Amphibia. Anura. *Leptodactylidae*). Including a description of unusual courtship behaviour. *Herpetological Journal*, 27 (1), 73–80.

Gerhardt, H.C. & Bee, M.A. (2007) Recognition and localisation of acoustic signals. *In*: Narins, P.M., Feng, A.S., Fay, R.R. & Popper, A.N. (Eds.), hearing and sound communication in amphibians. Springer, New York, pp. 113–146. https://doi.org/10.1007/978-0-387-47796-1_5

Inger, R.F. (1966) The Systematics and Zoogeography of The Amphibia of Borneo. *Fieldiana Zoology*, 52, 349–352. https://doi.org/10.5962/bhl.title.3147

Iskandar, D.T. (1998) The Amphibians of Java and Bali. Bogor, Indonesia: Research and Development Centre for Biology—LIPI and GEF Biodiviersity Collections Project, pp. 83–85.

Köhler, J., Jansen, M., Rodríguez, A., Kok, P.J.R., Toledo, L.F., Emmrich, M., Glaw, F., Haddad, C.F.B., Rödel, M.O. & Vences, M. (2017) The Use of Bioacoustics in Anuran Taxonomy: Theory. Terminology. Methods And Recommendations for Best

Practice. *Zootaxa*, 4251 (1), 1–124.
https://doi.org/10.11646/zootaxa.4251.1.1
Kurniati, H. & Hamidy, A. (2017) Advertisement Calls Variation on *Hylarana nicobariensis* (Stoliczka, 1870) of Five Different Populations in Indonesia (Anura: Ranidae). *Indonesian Journal of Biology*, 12 (2), 165–173. [unknown pagination]
Matsui, M. (1996) Call Characteristics and Systematic Relationships of a Malayan Treefrog *Nyctixalus pictus* (Anura, *Rhacophoridae*). *Herpetological Journal*, 6 (2), 62–64.
Priambodo, B., Firmansyah, R., Pranata, D.C., Aninnas, A.N., Susanto, M.A.D., Aji, F.D.N., Widodo, T.W., Gunawan, G., Permana, D., Adiba, F.Y., Ristanto, Y., Erfanda, M.P. & Kadafi, A.M. (2021) Rediscovery of pearly tree frog, *Nyctixalus margaritifer* Boulenger, 1882 (Amphibia: *Rhacophoridae*) from Mt. Wilis after 135 years. *Turkish Journal of Zoology*, 45 (4), 329–334.
https://doi.org/10.3906/zoo-2104-22
Rumanta, M., Kunda, R.M. & Iryani, K. (2019) Diversity and Species composition of anura in telaga warna nature conservation, west java, Indonesia. *International Journal of Zoology Studies*, 4 (5), 35–40.
Schlaepfer, M.A. & Figeroa-Sandí, R. (1998) Female Reciprocal Calling in a Costa Rican Leaf-Litter Frog, *Eleutherodactylus podiciferus*. *Copeia*, 1998 (4), 1076–1080.
https://doi.org/10.2307/1447362
Sueur, J., Aubin, T. & Simonis, C. (2008) Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18, 213–226.
https://doi.org/10.1080/09524622.2008.9753600
Willaert, B., Suyesh, R., Garg, S., Giri, V.B., Bee, M.A. & Biju, S.D. (2016) A unique mating strategy without physical contact during fertilization in Bombay Night Frogs (*Nyctibatrachus humayuni*) with the description of a new form of amplexus and female call. *PeerJ*, 4, e2117.
https://doi.org/10.7717/peerj.2117

Supplementary Materials

The following supporting information are available online from: https://figshare.com/s/d60b43d28b06ceed1cf0

Supplementary Audio S1. Acoustic stimuli of male *N. margaritifer*.
Supplementary Audio S2. Recording files of female *N. margaritifer* in the wild.
Supplementary Table S3. Table of call distribution and acoustic parameters of females *N. margaritifer*.
Supplementary Video S4. Female call response to acoustic stimuli.

The following supporting information can be downloaded at the DOI landing page of this paper: Supplementary Figure S5. Oscilogram,spectrogram acoustic stimuli and marble patterns on the frogs' abdomens.