OGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 36: 123–128 (2023)
DOI 10.3897/herpetozoa.36.e101646

Description of the advertisement call of *Boulenophrys nanlingensis* (Anura, Megophryidae), with a case of individual identification using its dorsum pattern

Tianyu Qian[1], Guoxing Deng[2], Yonghui Li[2], Daode Yang[1]

1 *Institute of Wildlife Conservation, Central South University of Forestry and Technology, Changsha 410004, China*
2 *Administration Bureau of Hunan Mangshan National Nature Reserve, Chenzhou 423000, Hunan, China*

https://zoobank.org/12A6681B-C4A3-47E7-92CB-3CF1D7F4DCE8

Corresponding author: Daode Yang (csfuyydd@126.com)

Academic editor: Eva Ringler ♦ **Received** 8 February 2023 ♦ **Accepted** 25 April 2023 ♦ **Published** 4 May 2023

Abstract

We describe the advertisement call of the Nanling horned frog, *Boulenophrys nanlingensis* for the first time, based on recordings obtained from four individuals. One of these individuals, which was identified using its dorsum pattern, was recorded twice after nine months. Distinct shifts in the temporal parameters and call rate were observed from calls of the re-captured individual, which was suspected to be related to temperature and social context. However, due to the limited sample size, further research is needed to confirm these findings. We highlight the potential of mark-recapture method using dorsum pattern for studying and monitoring the Nanling horned frog and other megophyinid frogs.

Key Words

bioacoustics, frogs, mark-recapture, Megophryinae, southern China

Introduction

The genus *Boulenophrys* Fei, Ye & Jiang, 2016 is the largest branch of the Asian horned frog subfamily Megophryinae Bonaparte, 1950, comprising 65 species found in southern China and the Indochina peninsula (Frost 2023). During their breeding season, male *Boulenophrys* frogs emit repeated, monosyllabic calls from stream banks and often form small chorus groups (Pope 1931; Liu 1950; Wang et al. 2014). These calls are species-specific, with clear differences particularly evident amongst sympatric species (Liu et al. 2018; Cutajar et al. 2020). As a result, call parameters have become a diagnostic character in recent taxonomic studies for identifying *Boulenophrys* species (Tapley et al. 2017, 2018, 2020).

Boulenophrys nanlingensis (Lyu, Wang, Liu & Wang, 2019) is distributed throughout Nanling Mountains in southern China. According to Wang et al. (2019), the breeding season of *B. nanlingensis* was from August to December. Notably, this period contains seasonal changes with the air temperature decreasing during this period in southern China. Although the advertisement call of *B. nanlingensis* was not described, a recently published guide, "A Field Guide to the Amphibians of Eastern China" (Ding et al. 2022), has illustrated its spectrogram and oscillogram and provided an audio file for field identification. Thus, we could easily identify this species in the field from its distribution area.

Methods

Field observation

On 18 November 2021, during a night survey conducted in Mangshan (also known as Mt. Mang), Yizhang County, Hunan Province, China (24.945°N, 112.938°E, ca. 1220 m elev.), we observed a group of at least five male *B. nanlingensis* calling in chorus on rocky areas along the bank of a mountain stream. The stream was approximately 5 m wide and several individuals of *Leptobrachium liui* could also be heard calling nearby. We were able to locate four individual frogs of *B. nanlingensis*, three of which were hiding under a crevice with their feet submerged in shallow water, while one remained hidden under fallen leaves. Each individual was positioned at least 1 m apart from the others. We recorded their advertisement calls individually between 20:30 and 22:00 h and captured and photographed two of them next to an improvised scale bar (the shotgun microphone). Both were released immediately after photographing.

After nearly nine months, when we revisited this site during a night survey on 13 August 2022, a single calling male *B. nanlingensis* was located under a crevice about 5 m from the rocky areas which we visited in November 2021. After recording its advertisement call between 20:00 and 20:30 h, we captured the frog and held it in captivity for a few days before releasing it back to the collection site on 18 August 2022. During this period, we took measurements and photographs of the frog. Upon comparing photos taken during both surveys, we confirmed that this frog belongs to one of the photographed individuals we had encountered in November 2021 (see Results).

Data collection

During our initial survey, the calls were recorded by using a Zoom F6 digital sound recorder with a Boya BM6060L shotgun microphone, held approximately 0.2–1 m from each frog. Two recordings from four individuals (vocally marked as No.1–4 in the recordings) were made at a sample rate of 192 kHz and a resolution of 24-bit. The ambient air temperature was recorded as 12.3 °C by using a digital thermometer (0.1 °C, AZ Instrument 8918). For the second survey, we used a Zoom F3 digital sound recorder with a Sennheiser ME66/K6 shotgun microphone held approximately 0.5 m from the frog. A single recording was made at 192 kHz sampling rate and 32-bit float resolution. The air ambient temperature was recorded as 19.7 °C.

The snout-vent length (SVL) of the re-captured individual was measured by using a digital caliper (0.01 mm, to the nearest 0.1 mm). We also estimated the SVL of the two photographed frogs in November 2021 by measuring the columns on the shotgun microphone.

Acoustic analysis

All recordings obtained from the field were resampled to 44.1 kHz and 16-bit by using Adobe Audition 2023 and were then analysed with Raven Pro v.1.6.4 (K. Lisa Yang Center for Conservation Bioacoustics 2023). Spectrogram measurements were taken as follows: Hann window, DFT = 512 samples, overlap = 50%, Hop Size = 256 samples. We used "call-centred" terminology as summarised by Köhler et al. (2017), in which the fundamental unit was defined as a "call" and the continuous units were defined as a "call group". The following acoustic parameters were measured: call duration (ms); call interval (ms); number of calls per call group; call repetition rate (calls/s), measured by counting the total number of calls (*k*) within a call group and dividing *k*-1 by the duration between the onset of the first call and the onset of the last call of the call group (modified from Bee et al. (2013)); number of pulses per call; dominant frequency (Hz), measured using the function "Peak Frequency" in Raven Pro. We also reported the frequency bandwidth (Hz) by measuring frequencies 5% and 95%. The spectrogram and oscillogram figures were generated using Seewave v.2.2.0 (Sueur et al. 2008) and TuneR 1.4.2 (Ligges et al. 2013) packages in R programme 4.2.2 (R Core Team 2021) with a "Hanning" window size of 256 samples and an overlap of 50%.

Results

The photos from the re-captured individual that were taken in both surveys are shown in Fig. 1. Multiple colour patterns and morphological characters (enlarged tubercles) indicate that the two photos belong to a same frog.

Measurements of acoustic parameters of the four individuals are shown in Table 1, the recaptured individual being marked as "No. 3". The advertisement call of

Table 1. Call parameters of *Boulenophrys nanlingensis*. *N* = number of call groups/calls analysed, NM = not measured.

Individuals	No. 1 (*N* = 18/138)	No. 2 (*N* = 15/171)	No. 4 (*N* = 4/35)	No. 3 (*N* = 10/116)	No. 3 (*N* = 7/135)
Recording date	18 Nov 2021	18 Nov 2021	18 Nov 2021	18 Nov 2021	13 Aug 2022
Air temperature (°C)	12.3	12.3	12.3	12.3	19.7
SVL (mm)	NM	NM	ca. 34	ca. 33	37.3
Condition	Chorus	Chorus	Chorus	Chorus	Solo
Call duration (ms)	169.3±18.3(73.5–196.8)	196.9±21.2(74.0–268.0)	199.2±26.7 (94.7–298.6)	176.9±21.6 (96.4–248.6)	101.6±8.1 (60.2–113.6)
Call interval (ms)	584.5±206.9 (295.9–1313.1)	513.9±150.3 (185.1–1030.2)	526.3±138.0 (376.1–819.0)	548.6±165.2 (321.9–1129.5)	195.0±34.8 (137.8–348.3)
Dominant frequency (Hz)	3448±237 (2412–4307)	3366±91 (3187–3704)	3411±92 (3359–3618)	3285±86 (3101–3445)	3276±96 (3187–3618)
Frequency 5% (Hz)	3008±213 (2067–3273)	3123±106 (2153–3187)	3102±186 (2326–3187)	2978±275 (2067–3187)	2712±282 (1378–3015)
Frequency 95% (Hz)	4284±145 (3445–4479)	3969±123 (3618–4393)	4243±79 (4048–4393)	3968±232 (3618–4737)	4179±100 (3790–4393)
No. of pulses per call	21.7±1.7 (14–28), *N*=133	22.5±1.9 (15–26), *N*=166	23.1±1.3 (20–26), *N*=32	20.5±1.7 (15–25), *N*=116	22.6±1.9 (13–28), *N*=124
No. of calls per call group	7.7±1.9 (4–11)	11.4±6.4 (5–32)	8.8±4.3 (5–15)	11.6±5.2 (4–18)	19.3±5.2 (14–29)
Call repetition rate (calls/s)	1.4±0.2 (1.0–1.8)	1.4±0.2 (1.0–1.6)	1.3±0.2 (1.2–1.7)	1.4±0.1 (1.1–1.6)	3.4±0.1 (3.3–3.6)

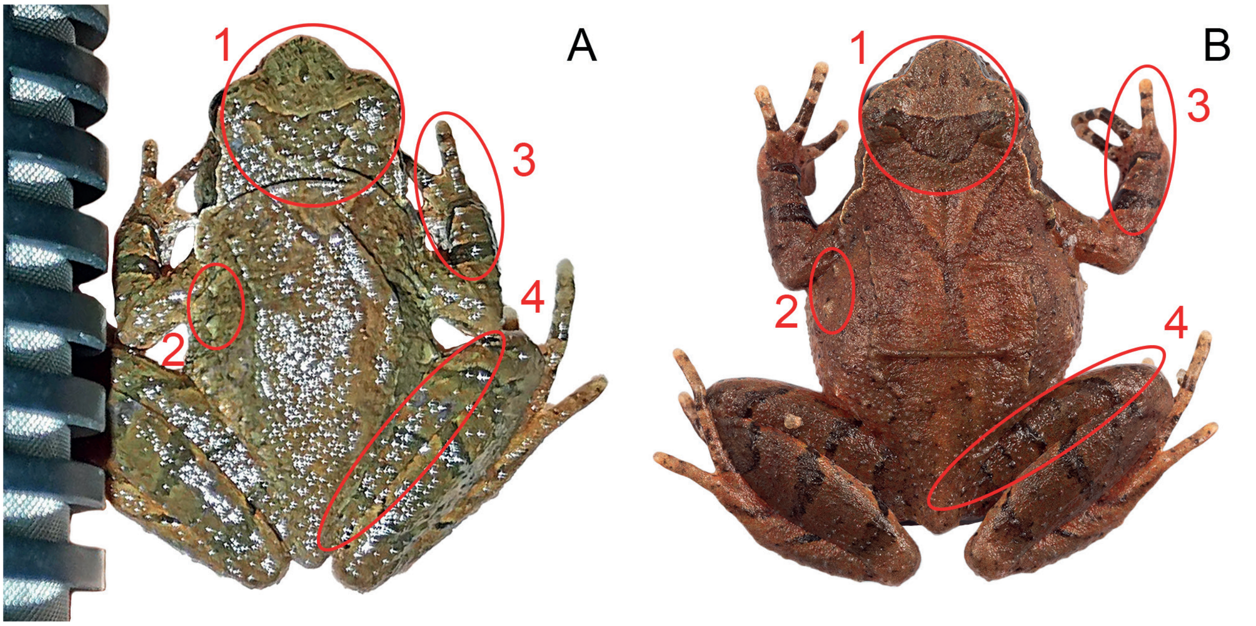

Figure 1. Comparison of the photos taken in November 2021 (**A**) and August 2022 (**B**) from the re-captured individual of *B. nanlingensis*. Red circles showing characters used for individual identification: (1) triangle pattern between upper eyelids, (2) prominent tubercles on upper left flank, (3) black bands on lower right arm and right fourth finger and (4) five black bands on right outer thigh. Images not to scale.

B. nanlingensis is a group of repeated pulsative calls. Call amplitude was consistent within each call group, except for the first 1–2 calls which had a lower amplitude. Within each call, the first pulse begins with a moderate amplitude, followed by a distinct interval. The second pulse experiences a sudden increased amplitude modulation, which then gradually increases to reach its peak amplitude at approximately 1/3 of the way through the call. The amplitude then gradually decreases to the end of the call.

The mean value of call duration amongst individuals recorded in November 2021 varied from 169.3 ms to 199.2 ms and the mean value of call interval varied from 513.9 to 584.5 ms. However, these values obtained from the re-captured individual recorded in August 2022 were considerably shorter (101.6 ms and 195.0 ms, respectively). As a result, this individual exhibited a much higher mean call repetition rate of 3.4 calls/s, compared to the calls recorded in November 2021 (1.3–1.4 calls/s). Fig. 2 demonstrates the differences in calls recorded from the re-captured individual "No. 3" between different seasons.

Discussion

According to Qian et al. (2023), *Boulenophrys nanlingensis* is in sympatry with *B. shimentaina* and *B. ombrophila* in Mangshan, while the dominant frequency of the advertisement call of *B. nanlingensis* (2.3–4.1 kHz) is lower than that of *B. shimentaina* (vs. 4.7–5.2 kHz; Lyu et al. (2020)), but overlapped with that of *B. ombrophila* (3.5–3.6 kHz; Messenger et al. (2019)). However, during our surveys in 2021 and 2022, we consistently observed *B. ombrophila* ceased its calling activities by mid-June. Thus, since *B. nanlingensis* breeds from August to December, it is unlikely that these two species with overlapping dominant frequencies will be present at the same time of the year.

Compared to the published calls of the other species in *Boulenophrys*, the advertisement call of *B. nanlingensis* differs considerably, reinforcing the specific identity of this taxon. For example, the call duration of *B. nanlingensis* (60.2–298.6 ms) is longer than that of *B. fansipanensis* (34.0–49.0 ms; Tapley et al. (2018)), *B. frigida* (43.0–50.0 ms; Tapley et al. (2021)) and *B. boettgeri* (54.0 ms, mean value; Wang et al. (2014)). The number of pulses per call of *B. nanlingensis* (13–28) is larger than that of *B. frigida* (10–11; Tapley et al. (2021)). The call repetition rate of *B. nanlingensis* (1.0–3.6 calls/s) is lower than that of *B. boettgeri* (5.0 calls/s, mean value; Wang et al. (2014)), *B. huangshanensis* (4.1 calls/s, mean value; Wang et al. (2014)), *B. jinggangensis* (5.7 calls/s, mean value; Wang et al. (2014)) and *B. minor* (4.0 calls/s, mean value; Jiang et al. (2002)).

Temperature has been reported to affect temporal parameters and call rates in most anurans (reviewed in Gerhardt (1994)). In this study, we observed distinct shifts in temporal parameter (i.e. call duration and call interval) and call rate from the recaptured individual, whose calls were recorded twice during different seasons. A similar result was reported from another megophryinid frog *Ophryophryne elfina*, whose calls were recorded at different temperatures (11.3 °C and 17.5 °C, Poyarkov et al. (2017)). However, the social context was also related to call rate shift (Capshaw et al. 2020). During our first encounter, the frogs were calling in a chorus with several conspecific males. We have noticed that these individuals would adjust their call rhythm by lengthening the call interval to avoid overlapping when other males attempted

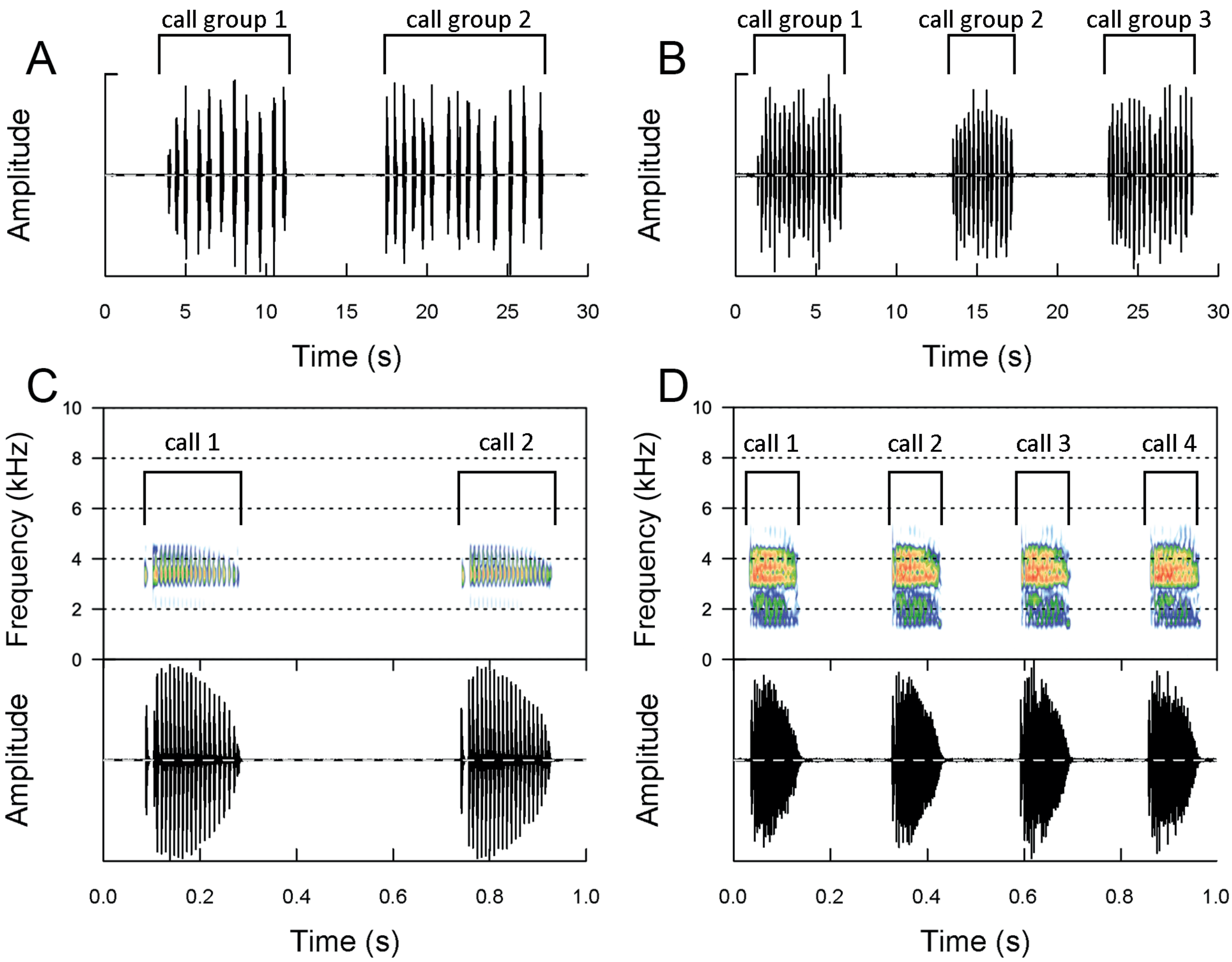

Figure 2. Comparison of the advertisement call of a recaptured individual of *B. nanlingensis* recorded from November 2021 (**A, C**) and August 2022 (**B, D**). 30 s oscillograms showing two call groups recorded in November (**A**) and three call groups recorded in August (**B**). 1 s oscillograms and corresponding spectrograms showing two calls recorded in November (**C**) and four calls recorded in August (**D**).

to join the chorus. However, during the second encounter, the frog was calling alone.

Several megophryinid frogs are known to be exhibit loyalty to their breeding habitats or calling sites. For instance, Liu (1950) observed that *B. omeimontis* "*This frog has the special habit of appearing on the same stone near the margin of the water every night*", while Mulkmus et al. (2002) reported that *Pelobatrachus baluensis* frogs "*live in very stationary calling communities*" and *P. kobayashii* frogs "*live in permanent colonies*", with five colonies of *P. baluensis* traced by Mulkmus et al. (2002) from same spots over five years. In our study, we observed a potentially similar pattern of loyalty to breeding habitats in the recaptured individual of *B. nanlingensis*, which did not move its calling site more than 5 m over a nine-month period.

The mark-recapture method has been long and widely used in amphibians in demographic, home range, behaviour and other aspects of studies (e.g. Martof (1953); Zweifel (1968); Nelson and Graves (2004); Pettitt et al. (2013)). Toe-clipping was previously the most frequently used method to mark an individual, but was reported to be harmful to individuals or influence their behaviour (reviewed in Wells (2007)). To avoid such unexpected consequences, non-invasive methods, such as individual recognition, based on colour patterns, were proposed (Bradfield 2004; Kenyon et al. 2009; Zheng et al. 2011; Caorsi et al. 2012; Morrison et al. 2016; Patel and Das 2020). Although only one individual from *B. nanlingensis* was tested in this study, we have found that its dorsal pattern and prominent skin tubercles did not show obvious changes over a nine-month period. We propose that this method could potentially be a useful non-invasive tool for studying or monitoring Nanling horned frogs and other megophryinid species which having distinct dorsum patterns (as well as ventral patterns, which were not recorded in this study) that varied between individuals.

Acknowledgements

We thank Eva Ringler and two anonymous reviewers for their constructive comments on the previous version of this manuscript. We thank Wenbao Zheng and Jun Chen for their assistance in the fieldwork. TQ thanks the Cornell Lab of Ornithology for providing licence support for Raven Pro software. This work was supported by the National Natural Science Foundation of China (Grant No. 31472021), the Project for Wildlife Conservation and Management of the National Forestry and Grassland Administration of China (Grant No. 2022-HN-001) and the Wildlife Conservation Project of Hunan Province (Grant No. HNYB2022-001).

References

Bradfield KS (2004) Photographic identification of individual Archey's frogs, *Leiopelma archeyi*, from natural markings. Doc science internal series 191: 1–36.

Bee MA, Suyesh R, Biju SD (2013) The vocal repertoire of *Pseudophilautus kani*, a shrub frog (Anura: Rhacophoridae) from the Western Ghats of India. Bioacoustics 22(1): 67–85. https://doi.org/10.1080/09524622.2012.712750

Caorsi VZ, Santos RR, Grant T (2012) Clip or snap? An evaluation of toe-clipping and photo-identification methods for identifying individual southern red-bellied Toads, *Melanophryniscus cambaraensis*. South American Journal of Herpetology 7(2): 79–85. https://doi.org/10.2994/057.007.0210

Capshaw G, Foss-Grant AP, Hartmann K, Sehuanes JF, Moss CF (2020) Timing of the advertisement call of the common tink frog (*Diasporus diastema*) shifts with the acoustic behaviour of local conspecifics. Bioacoustics 29(1): 79–96. https://doi.org/10.1080/09524622.2018.1555715

Cutajar T, Rowley JJL, Nguyen LT, Nguyen CT, Portway C, Harding L, Luong HV, Tapley B (2020) The advertisement call of *Megophrys jingdongensis* Fei and Ye, 1983 and a new record from Lai Chau Province, Northeast Vietnam. Herpetology notes 13: 139–143.

Ding GH, Hu HL, Chen JY (2022) A field guide to the amphibians of Eastern China. Beijing: China Agricultural Science and Technology Press, 169 pp.

Gerhardt HC (1994) The evolution of vocalization in frogs and toads. Annual Review of Ecology and Systematics 25: 293–324. https://doi.org/10.1146/annurev.es.25.110194.001453

Frost DR (2023) Amphibian Species of the World: an Online Reference. Version 6.1. Electronic Database. American Museum of Natural History, New York. https://amphibiansoftheworld.amnh.org/index.php [accessed: 23 Mar 2023]

Jiang JP, Xie F, Fei L, Ye CY, Zheng MQ (2002) Mating calls of six forms of pelobatid in Wawu Mountain National Forest Park, Sichuan, China (Anura: Pelobatidae). Zoological Research 23(1): 89–94.

K. Lisa Yang Center for Conservation Bioacoustics (2023) Raven Pro: interactive sound analysis software (Version 1.6.4). The Cornell Lab of Ornithology, Ithaca. https://ravensoundsoftware.com/

Kenyon N, Phillott AD, Alford RA (2009) Evaluation of the photographic identification method (PIM) as a tool to identify adult *Litoria genimaculata* (Anura: Hylidae). Herpetological Conservation and Biology 4(3): 403–410.

Ligges U, Krey S, Mersmann O, Schnackenberg S (2013) Tuner: Analysis of music. http://r-forge.r-project.org/projects/tuner [Accessed 20 Jan 2023]

Liu CC (1950) Amphibians of western China. Fieldiana: Zoology Memoirs 2: 1–397. [pls. 1–10.] https://doi.org/10.5962/bhl.part.4737

Liu ZY, Chen GL, Zhu TQ, Zeng ZC, Lyu ZT, Wang J, Messenger K, Greenberg AJ, Guo ZX, Yang ZH, Shi SH, Wang YY (2018) Prevalence of cryptic species in morphologically uniform taxa – Fast speciation and evolutionary radiation in Asian frogs. Molecular Phylogenetics and Evolution 127: 723–731. https://doi.org/10.1016/j.ympev.2018.06.020

Lyu ZT, Li YQ, Zeng ZC, Zhao J, Liu ZY, Guo GX, Wang YY (2020) Four new species of Asian horned toads (Anura, Megophryidae, *Megophrys*) from southern China. Zookeys 942: 105–140. https://doi.org/10.3897/zookeys.942.47983

Martof B (1953) Home range and movements of the green frog, *Rana clamitans*. Ecology 34(3): 529–543. https://doi.org/10.2307/1929725

Morrison TA, Keinath D, Estes-Zumpf W, Crall JP, Stewart CV (2016) Individual identification of the endangered Wyoming toad *Anaxyrus baxteri* and implications for monitoring species recovery. Journal of Herpetology 50(1): 44–49. https://doi.org/10.1670/14-155

Malkmus R, Manthey U, Vogel G, Hoffmann P, Kosuch J (2002) Amphibians & Reptiles of Mount Kinabalu (North Borneo). Fuldaer Verlagsanstalt, Fulda, 424 pp.

Messenger KR, Dahn HA, Liang Y, Xie P, Wang Y, Lu C (2019) A new species of the genus *Megophrys* Gunther, 1864 (Amphibia: Anura: Megophryidae) from Mount Wuyi, China. Zootaxa 4554(2): 561–583. https://doi.org/10.11646/zootaxa.4554.2.9

Nelson GL, Graves BM (2004) Anuran population monitoring: comparison of the north American amphibian monitoring program's calling index with mark-recapture estimates for *Rana clamitans*. Journal of Herpetology 38(3): 355–359. https://doi.org/10.1670/22-04A

Patel NG, Das A (2020) Shot the spots: A reliable field method for individual identification of *Amolops formosus* (Anura, Ranidae). Herpetozoa 33:7–15. https://doi.org/10.3897/herpetozoa.33.e47279

Pettitt BA, Bourne GR, Bee MA (2013) Advertisement call variation in the Golden Rocket frog (*Anomaloglossus beebei*): evidence for individual distinctiveness. Ethology 199: 244–256. https://doi.org/10.1111/eth.12058

Pope CH (1931) Notes on amphibians from Fukien, Hainan, and other parts of China. Bulletin of the American Museum of Natural History 61(8): 397–611.

Poyarkov Jr NA, Duong TV, Orlov NL, Gogoleva SS, Vassilieva AB, Nguyen LT, Nguyen VDH, Nguyen SN, Che J, Mahony S (2017) Molecular, morphological and acoustic assessment of the genus *Ophryophryne* (Anura, Megophryidae) from Langbian Plateau, southern Vietnam, with description of a new species. ZooKeys 672: 49–120. https://doi.org/10.3897/zookeys.672.10624

Qian T, Li Y, Chen J, Li P, Yang D (2023) Tadpoles of four sympatric megophryinid frogs (Anura, Megophryidae, Megophryinae) from Mangshan in southern China. Zookeys 1139: 1–32. https://doi.org/10.3897/zookeys.1139.81641

R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Sueur J, Aubin T, Simonis C (2008) Seewave, a free modular tool for sound analysis and synthesis. Bioacoustics 18(2): 213–226. https://doi.org/10.1080/09524622.2008.9753600

Tapley B, Cutajar T, Mahony S, Nguyen CT, Dau VQ, Nguyen TT, Luong HV, Rowley JJL (2017) The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. Zootaxa 4344(3): 465–492. https://doi.org/10.11646/zootaxa.4344.3.3

Tapley B, Cutajar T, Mahony S, Nguyen CT, Dau VQ, Luong AM, Le DT, Nguyen TT, Nguyen TQ, Portway C, Luong HV, Rowley JJL (2018) Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. Zootaxa 4508(3): 301–333. https://doi.org/10.11646/zootaxa.4508.3.1

Tapley B, Cutajar T, Nguyen LT, Portway C, Mahony S, Nguyen CT, Harding L, Luong HV, Rowley JJL (2021) A new potentially Endangered species of *Megophrys* (Amphibia: Megophryidae) from Mount Ky Quan San, north-west Vietnam. Journal of Natural History 54(39–40): 2543–2575. https://doi.org/10.1080/00222933.2020.1856952

Wang J, Lyu ZT, Liu ZY, Liao CK, Zeng ZC, Zhao J, Li YL, Wang YY (2019) Description of six new species of the subgenus *Panophrys* within the genus *Megophrys* (Anura, Megophryidae) from southeastern China based on molecular and morphological data. ZooKeys 851: 113–164. https://doi.org/10.3897/zookeys.851.29107

Wang YY, Zhao J, Yang JH, Zhou ZX, Chen GL, Liu Y (2014) Morphology, molecular genetics, and bioacoustics support two new sympatric *Xenophrys* toads (Amphibia: Anura: Megophryidae) in southeast China. PLoS ONE 9(4): e93075. https://doi.org/10.1371/journal.pone.0093075

Wells KD (2007) The Ecology and Behavior of Amphibians. The University of Chicago Press, Chicago and London, 1148 pp.

Ziegler L, Arim M, Bozinovic F (2016) Intraspecific scaling in frog calls: the interplay of temperature, body size and metabolic condition. Oecologia 181: 673–681. https://doi.org/10.1007/s00442-015-3499-8

Zheng YC, Rao DQ, Murphy RW, Zeng XM (2011) Reproductive behavior and underwater calls in the Emei mustache toad, *Leptobrachium boringii*. Asian Herpetological Research 2(4): 199–215. https://doi.org/10.3724/SP.J.1245.2011.00199

Zweifel RG (1968) Effects of temperature, body size, and hybridization on mating calls of toads, *Bufo a. americanus* and *Bufo woodhousii fowleri*. Copeia 1968(2): 269–285. https://doi.org/10.2307/1441753