THE ADVERTISEMENT CALL OF THE INDOCHINESE BROWN BULLFROG, *Kaloula indochinensis* CHAN, BLACKBURN, MURPHY, STUART, EMMETT, HO, AND BROWN, 2013 (ANURA: MICROHYLIDAE) FROM GIA LAI PROVINCE, VIETNAM

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SUMMARY

Call descriptions may facilitate studies on behavioural ecology and support taxonomists in their efforts to delimit species. The Indochinese Brown Bullfrog (*Kaloula indochinensis*) was described in 2013 and occurs in evergreen forest in Indochina. Whilst the tadpole of the species was recently described, little else is known about the biology and ecology of the species including the call characters. In this paper, we describe the male advertisement calls of the Indochinese Brown Bullfrog, *Kaloula indochinensis* from evergreen forest in Gia Lai province, in the Central Highlands of Vietnam for the first time. This species has a slow and low-pitched call that is similar to the sounds of a trumpet to the human ear. The call description of *Kaloula indochinensis* is based on recordings from two males. This species has a single call type consisting of a single note per call with a dominant frequency of 0.34 - 0.43 kHz (calls recorded at an air temperature of 20.5° C. The call duration ranged from 194 - 250 milliseconds and the average intercall interval ranged from 481 - 1627 milliseconds. The male advertisement calls of *Kaloula indochinensis* is compared with the male advertisement calls for the six congeneric species with published male advertisement call descriptions.

Keywords: Amphibians, bioacoustics, Indochina, Tay Nguyen Plateau.

1. INTRODUCTION

The genus Kaloula Gray, 1831 is currently comprised of 19 species and are commonly known as Asian Narrowmouth Toads (Frost, 2022). Members of this genus occur from Korea and Northern China to the Lesser Sundas and the Philippines, Bangladesh, and India (Frost, 2022). Although call descriptions may facilitate studies on behavioural ecology and support taxonomists in their efforts to delimit species (Köhler et al., 2017), calls have been described for just six of the 19 species in the genus (Diesmos et al., 2002; Heyer 1971; Lalremsanga et al., 2018). Parameters of advertisement calls have been used as lines of evidence to delimit species of Kaloula (Diesmos et al., 2002). The Indochinese Brown Bullfrog Kaloula indochinensis was described in 2013 and occurs in evergreen forest

in Indochina (Vietnam, Laos, and Cambodia) (Chan *et al.*, 2013; Frost, 2022). After nearly 10 years since scientific description, it remains very poorly known. Whilst the tadpole of the species was recently described (Vassilieva 2021), little else is known about the biology and ecology of the species.

During recent fieldwork in Gia Lai province, in the highlands of central Vietnam, we recorded the calls of *K. indochinensis* in a small pool near the Ho Chi Minh Road after heavy rain. After confirming the species identification based on morphology of the observed specimens, we describe the male advertisement call of this species for the first time and provide a comparison with the male advertisement call of six congeneric species.

2. RESEARCH METHODOLOGY

The field survey was conducted at night in Kon Von II village, Dak Roong commune, K'Bang district, Gia Lai province, Vietnam on 29 July 2016 (geographic coordinates 14.54334°N, 108.41396°E, ca. 1200 m a.s.l.). Geographic coordinates and elevation were obtained using a Garmin GPSMAP 64CSx GPS receiver (Garmin Ltd., Kansas, USA) and recorded in datum World Geodetic System 1984.

Specimens were photographed in life before being euthanised using a 20% solution of benzocaine applied to the ventral surface of the frog; prior to fixation in 5% formalin with subsequent storage in 70% ethanol. Sex was determined by direct observation of calling in the field. Specimens were subsequently deposited in the Institute of Tropical Biology Collection of Zoology (ITBCZ), Ho Chi Minh City, Vietnam under accession number ITBCZ 3607 (an adult male) and ITBCZ 3608 (an adult male). The identity of species was confirmed by morphology following the description of Chan *et al.* (2013).

Male advertisement calls (described as calls hereafter) were recorded with a TASCAM DR-05 Linear PCM Recorder (44.1 kHz sampling rate and 16-bit encoding) with a Neewer[©] 14.37" Shotgun Microphone. Calls were recorded at a distance of approximately 0.4-0.5 m from the calling males; a Kestrel 3500 hand-held weather meter (Kestrel, Minneapolis, USA) was used to take ambient temperatures and humidity at the calling site immediately after the recording.

The software Raven Pro 1.4[©] (http://www.birds.cornell.edu/raven) was used to analyse the recorded calls. Audio-spectrograms in figures were calculated with fast-Fourier transform (FFT) of 512 points, 50% overlap and 188 Hz grid-spacing, using Hanning window. The terminology of call analysis and description using a call-centered

approach (defining uninterrupted units as call whenever they are separated by long silent intervals) follows Köhler *et al.* (2017). We examined oscillograms (waveforms) and audiospectrograms of vocalisations and measured the call duration (millisecond - ms), intercall interval duration (ms), call repetition rate (calls/s and calls/min), number of notes per call (notes/call), harmonic frequency (kHz) and dominant frequency (kHz).

We compare the call of Kaloula indochinensis with six species with published descriptions: К. baleata (Müller) call (Malkmus et al., 2002), K. conjuncta (Peters) (Diesmos et al., 2002), K. kalingensis Taylor (Diesmos et al., 2002), K. picta (Duméril and Bibron) (Diesmos et al., 2002), K. pulchra Gray (Heyer 1971; Lalremsanga et al., 2018), and K. walteri Diesmos, Brown, and Alcala (Diesmos et al., 2002).

A short call of this species is also published in a Vietnamese website about Amphibian of Vietnam (vnherps.com) for public reference at the link:

https://www.vnherps.com/anura/microhylid ae/microhylinae/kaloula/kaloula-indochinensis.

3. RESULTS

Species identification: the morphology of the collected specimens (collected 23 km in a straight line from the type locality, measured by the ruler function on Google Earth) matches the species description of *K. indochinensis* in Chan *et al.* (2013): snout–vent length (SVL) 52.4–52.7 mm in two males; finger tips expanded into large, transversely expanded discs, third finger disc width 3.3–3.4 mm in males; the majority of individuals with two subarticular tubercles on fourth toe; inner metatarsal tubercle elongate, slightly raised, shorter than first toe; dorsal black or brown with orange-yellow patch on either side of the neck posterior to eyes; large, bright, orangeyellow axillary and inguinal patch (Figure 1). Calling males of *K. indochinensis* have a large and single subgular vocal sac.

The call description is based on the recordings of two calling individuals at an air temperature of 20.5°C. The calls of *K. indochinensis* are slow and low-pitched and is similar to the sound of a trumpet to the human ear. Table 2 shows details of call parameters among examined individuals of the species. *Kaloula indochinensis* has a male advertisement call with an average duration of

215.6 ms (194–250 ms, N = 40). Calls were repeated at a rate of approximately 0.76–1.25 calls per second (60.1 [45.9–75.2] calls per min) and had an average intercall interval of 789.3 ms (481–1627 ms, N = 40). The fundamental frequency was not evident, and the average dominant frequency was 0.34–0.43 kHz. The 2nd, 3rd, and 4th harmonics were detected at 0.80, 1.20, and 1.37 kHz, respectively in both individuals (Table 1, Figure 2). Amplitude modulation was present, with calls tending to decline in intensity with each successive call.



Figure 1. Male Kaloula indochinensis in life. A. ITBCZ 3607 (SVL 52.4 mm) and B. ITBCZ 3608 (SVL 52.7 mm) (Photos by Luan Nguyen)

Parameter values are given as means (and ranges)						
Specimen	ITBCZ 3607	ITBCZ 3608	Summary			
Number of calls measured	20	20	40			
Call duration (ms)	214.0 (200-227)	217.1 (194–250)	215.6 (194-250)			
Intercall interval (ms)	656.3 (550-766)	922.3 (481–1627)	789.3 (481–1627)			
Call repetition rate (call/s)	1.1 (1.00–1.25)	0.87 (0.76-0.98)	1.0 (0.76-1.25)			
Note/call	1	1	1			
Dominant frequency (kHz) (1 st	0.42(0.42, 0.42)	0.24(0.24, 0.24)	0.38 (0.34-0.43)			
harmonic	0.45 (0.45–0.45)	0.34 (0.34–0.34)				
2 nd harmonic (kHz)	0.80	0.80	0.80			
3 rd harmonic (kHz)	1.20	1.20	1.20			
4 th harmonic (kHz)	1.37	1.37	1.37			
Temperature, °C	20.5	20.5	20.5			





Figure 2. The call of *Kaloula indochinensis* from Gia Lai province (A, B) 20 s waveform of relative amplitude (Rel. amp.) and corresponding spectrogram over time for ITBCZ 3607 and ITBCZ 3608 respectively; (C, D). 1.5 s waveform and corresponding spectrogram of two calls of ITBCZ 3607 (obtained from the last two calls in A); and (E, F). 1.5 s waveform and corresponding power spectrum of ITBCZ 3608 (obtained from the last two calls in B)

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Table 2. Comparison of male advertisement calls for all Kaloula with known calls

Species	Kaloula indochinensis	Kaloula baleata	Kaloula conjuncta	Kaloula kalingensis	Kaloula picta	<i>Kaloula pulchra</i> (from Thailand)	<i>Kaloula pulchra</i> (from India)	Kaloula walteri
Number of individuals	2	NR	1	1	1	1	1	1
Number of calls measured	40	NR	NR	8	22	NR	10	5
Call duration (ms)	215.6 (194–250)	280–300	950.0 (300–1800)	155–200	290	560-600	527.8 (450.8-620.3)	650.8 (200–900)
Intercall interval (ms)	789.3 (481–1627)	NR	NR	NR	NR	NR	2876–3902	1275.0 (600–1800)
Call repetition rate (call/s)	1.0 (0.76–1.25)	NR	NR	NR	1.2	NR	0.23 (0.21–0.25)	2.2
Call repetition rate (call/minute)	60.1 (45.9–75.2)	NR	1–2 calls/min	1–3 calls/min	NR	NR	NR	NR
Notes/call	1	NR	1	1	1	1	1	1
Pulse/call	1	Described as pulsed	1	1	32–37	18–21	36–45	44–198
Dominant frequency (kHz)	0.38 (0.34–0.43)	1–3	2.3–2.4	1.8–2.2	2.2 (1.7–2.8)	0.25	1.26	2.2
Temperature (°C)	20.5	23	25	25–27	25	NA	NA	25
Reference	This study	Malkmus et al. (2002)	Diesmos <i>et al.</i> (2002)	Diesmos <i>et al.</i> (2002)	Diesmos <i>et al.</i> (2002)	Heyer (1971)	Lalremsanga <i>et al.</i> (2018)	Diesmos <i>et al.</i> (2002)

**NR* = *Not Reported*

4. DISCUSSION

Six *Kaloula* species have published call descriptions, the call of all known species including *Kaloula indochinensis* have a single note per call type. See Table two for a comparison of the male advertisement calls for *K. indochinensis* and six congeneric species

The male advertisement call of Kaloula indochinensis differs to the calls of K. baleata by having shorter call duration (mean 215.6, 194-250 ms vs. 280-300 ms) and a lower dominant frequency (0.34-0.43 kHz vs. 1.0-3.0 kHz); from K. coniuncta by having shorter call duration (mean 215.6, 194-250 ms vs. mean 950.0, 300-1800 ms), a greater call repetition rate (45.9–75.2 calls per min vs. 1–2 calls per min), and a lower dominant frequency (0.34–0.43 kHz vs. 2.3–2.4 kHz); from K. kalingensis by having a greater call repetition rate (45.9–75.2 calls per min vs. 1-3 calls/min), and a lower dominant frequency (0.34–0.43 kHz vs. 1.8–2.2 kHz); from K. picta by having a shorter call duration (mean 215.6, 194-250 ms vs. 290 ms), a single pulsed-note vs. 32-37 pulses per call; and a lower dominant frequency (0.38 kHz vs. 1.7-2.8 kHz); from K. pulchra (populations from Thailand and India) by having a shorter call duration (mean 215.6, 194-250 ms vs. 450-620 ms); a longer intercall interval (mean 789.3, 481–1627 vs. 2876–3902 ms); a greater call repletion rate (0.76-1.2 5 calls/s vs. 0.21-0.25 calls/s); and having a single pulsed-note vs. 18–45 pulses per call; and from *K. alteri* by having a shorter intercall interval call (mean 789.3, 481-1627 vs. mean 1275.0, 600-1800 ms), fewer calls per second (0.76-1.25 vs. 2.2 call/s), and a lower dominant frequency (0.34-0.43 vs. 2.2 kHz).

In Southern Vietnam, *Kaloula indochinensis* is sympatric with *K. pulchra* in some lowland areas (e.g., Phu Yen province, our data). This call description will facilitate rapid identification and differentiation between the two species in the field. This description will support further studies on the reproductive biology of the species.

5. CONCLUSION

In this study, we recorded and describe the calls of the Indochinese Brown Bullfrog Kaloula indochinensis from Gia Lai province, central Vietnam based on the calls of two individuals. The call of Kaloula indochinensis has a single call type consisting of a single note per call. The call duration ranged from 194-250 milliseconds and the average intercall interval ranged from 481-1627 milliseconds. The calls have a dominant frequency of 0.34-0.43 kHz (calls recorded at an air temperature of 20.5°C). To the human ear, this species has a slow and low-pitched call that is similar to the sounds of a trumpet. The comparison of male calls of Kaloula indochinensis with the male calls for the six congeneric species with published male call descriptions has also been provided. The call description will not only support further studies on the reproductive biology of the species but are also a useful; public reference.

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TIẾNG KÊU THÔNG BÁO CỦA LOÀI ỄNH ƯƠNG ĐÔNG DƯỜNG, Kaloula indochinensis CHAN, BLACKBURN, MURPHY, STUART, EMMETT, HO, AND BROWN, 2013 (BỘ KHÔNG ĐUÔI: HỌ NHÁI BẦU) TẠI TỈNH GIA LAI, MIỀN TRUNG VIỆT NAM

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TÓM TẮT

Mô tả tiếng kêu của loài có thể tạo điều kiện thuận lợi cho các nghiên cứu về sinh thái học hành vi và hỗ trợ các nhà phân loại học trong nỗ lực phân loại các loài. Loài ễnh ương đông dương, *Kaloula indochinensis* được mô tả năm 2013 và ghi nhận tại các nước Việt Nam, Lào và Camphuchia. Ở Việt Nam, loài này phân bố từ Phú Yên vào tới miền Nam (Đồng Nai). Trong bài báo này, chúng tôi lần đầu tiên mô tả tiếng kêu thông báo của loài Ēnh ương Đông Dương, *Kaloula indochinensis* tại tỉnh Gia Lai, Tây Nguyên, miền Trung Việt Nam dựa trên tiếng kêu của hai cá thể đực. Tiếng kêu thông báo của loài *Kaloula indochinensis* nhanh và trầm, nghe như tiếng thổi kèn đối với tai người. Tiếng kêu có dạng đơn nốt, mỗi tiếng kêu có 1 xung với tần số trội dao động trong khoảng 0,34 – 0,43 kHz (ghi âm ở nhiệt độ 20,5°C). Thời gian của mỗi tiếng kêu dao động trong khoảng 194 – 250 mili giây, trong khi thời gian giữa các tiếng kêu lớn hơn, từ 481 – 1627 mili giây. Hai cá thể đực được ghi âm kêu gần nhau ở trên mặt vũng nước tạm sau mưa dưới tán rừng thường xanh. Chúng tôi cũng cung cấp thông tin so sánh đặc điểm tiếng kêu thông báo của loài *Kaloula indochinensis* với năm loài khác trong giống *Kaloula* có tiếng kêu thông báo đã được mô tả trước đó.

Từ khóa: Âm học, âm sinh học, lưỡng cư, Tây Nguyên.

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