

The advertisement call of the Perak spadefoot toad *Megophrys aceras* from Langkawi Island, Kedah, Peninsular Malaysia

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Megophrys aceras Boulenger 1903 (Anura: Megophryidae), is distributed in Peninsular Thailand to the northern and the western part of Peninsular Malaysia, at an altitude of up to 1500 m (Chan-ard, 2003; Grismer et al., 2006; Thong-aree et al., 2011; Sumarli et al., 2015). Frogs of the genus *Megophrys* produce a loud advertisement call to attract females during the mating season. We follow Mahony et al. (2017) in the treatment of Megophryinae as a single genus, *Megophrys*, with a subgenus-level classification due to the lack of reliable morphological characters that can be used to define all previously proposed genera. There are nine *Megophrys* species in Malaysia (Frost, 2021), and call descriptions have been published for *Megophrys nasuta* (Malkmus et al., 2002), *M. kobayashii* (Malkmus et al., 2002), and *Megophrys baluensis* from Sabah (Malkmus & Riede, 1996; Malkmus et al., 2002).

The advertisement calls of frogs are species-specific (Pavan, 2008; Köhler et al., 2017) and it is well known that there is a strong relationship between anuran phylogenetic relatedness and the acoustic similarity of their advertisement calls (Blair, 1958; Gingras et al., 2013). Consequently, information on advertisement calls can help to identify cryptic species in the field (Inger et al., 2017) and is a valuable tool for taxonomy, systematics and biodiversity research. Furthermore, frogs from the genus of *Megophrys* contain an undiagnosed diversity of morphologically cryptic species and this information is essential especially in species delimitation. However, call descriptions for many species within this genus are still lacking. Hence this study describes the characteristics of the advertisement call of *M. aceras* from Langkawi Island, Kedah, Peninsular Malaysia.

Acoustic Encounter Survey (AES) was used to detect the presence of *M. aceras*. The advertisement calls of *M. aceras* were recorded as uncompressed/raw .wav files using a rechargeable portable digital voice recorder (8GB, OEM, Rechargeable Dictaphone MP3 player, AS331C_V1000) with attached mini microphone. The advertisement calls were recorded within 20 cm of the frog and analysed using Adobe Audition CC 11.0.0.199 software (Dehling, 2010). The figures of spectrograms and oscillograms were exported using Raven Lite 2.0 audio editing software.

The calling individual was photographed in-situ using a digital camera (Nikon COOLPIX L840) and then collected for further identification and measurement. This species can be identified by its truncated snout, distinct tympanum (three



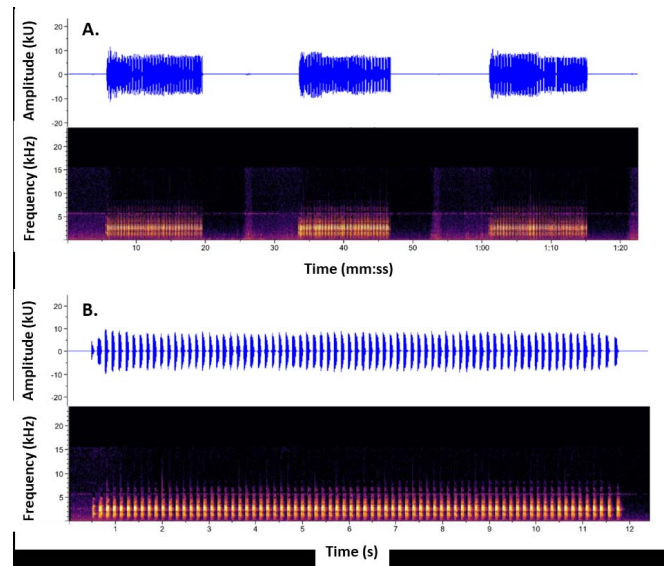
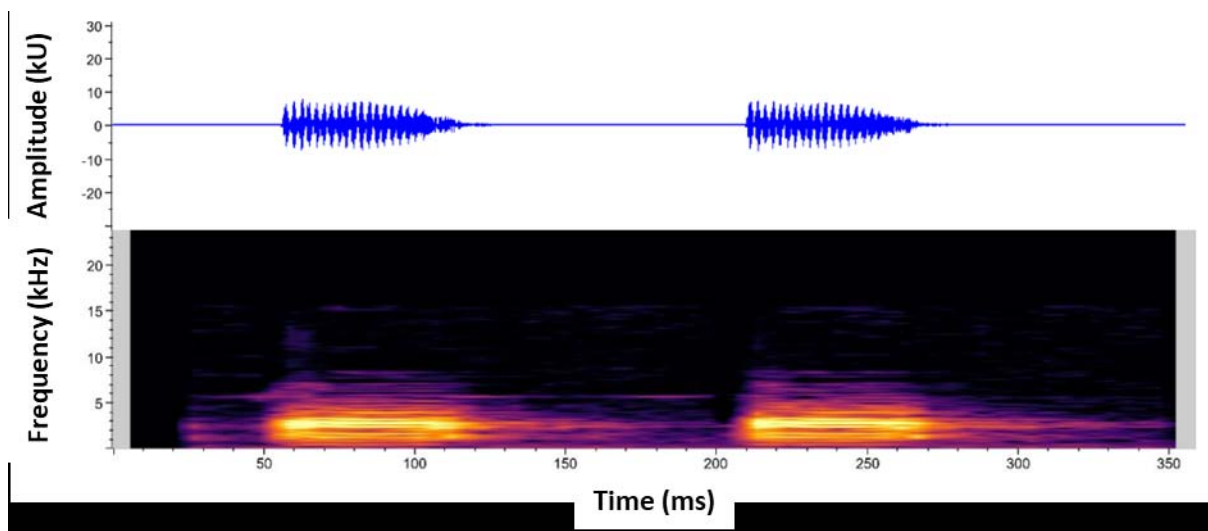
Figure 1. Male *Megophrys aceras* (20USM/LA/MA03) from which acoustic recordings were made, in-situ at the calling site

quarter diameter of the eye), a short dermal projection on the eyelids, a large triangular mark between the eyes, slightly swollen fingertips, and short limbs with dark crossbars (Smith, 1930). The frog was preserved as a voucher specimen (20USM/LA/MA03) in 70 % ethanol and deposited at School of Pharmaceutical Sciences, Universiti Sains Malaysia, for future reference. This frog was euthanised by injecting absolute ethanol straight into the frog's heart using a sterilised needle and syringe for immediate death. This procedure was approved by the USM institutional Animal Care and Use Committee, USM/IACUC/2019/(121)(1039).

The advertisement call of the male *M. aceras*, was recorded at 20:40 h on the 2nd November 2020 in Gunung Raya Forest Reserve (6.39098, 99.79596; 323 m a.s.l.), Langkawi Island, Kedah, Peninsular Malaysia. The air temperature and relative humidity of the sampling site were 28° C and 83 %, respectively (obtained from the AccuWeather application, android version). The specimen was measured using a digital Vernier calliper (Fisher scientific), and was weighed using an analytical balance (Ohaus). The morphometric measurements (after Watters et al., 2006) were tibia length (the length of the flexed knee's outer surface to the heel/tibiotarsal inflexion) - 26 mm; snout-vent length (the distance from the snout tip to posterior margin of the vent) - 55 mm; head width (the

Table 1. List of the call parameters measured in this study and observed values for *Megophrys aceras*

Parameter	Description	Parameter range (mean \pm sd)
Call group	A single group of calls produced during a single expiration	2.24 – 20.94 s (11.13 \pm 3.83, N = 51)
Call groups per minute	Number of the call groups per minute	2 – 4
Call group interval	Time interval between each call group	5.43 – 56.46 s (13.81 \pm 7.83, N = 50)
Call number	Numbers of calls per call group	31 – 111 calls (72.74 \pm 25.08, N = 51)
Call duration	The time between the beginning and end of a single call	74 – 94 ms (64.04 \pm 4.63, N = 228)
Inter-call interval	The time interval between each call	73 – 112 ms (86.50 \pm 7.12, N = 226)
Pulse number	Number of pulses contained in a single call	13 – 24 pulses (21.92 \pm 24, N = 228)
Pulse duration	Interval time between each peak of each pulse	2 – 3 ms (2.27 \pm 3, N = 228)
Dominant frequency	Dominant occurrence of call frequency	3.75 kHz

**Figure 2.** Oscillogram and spectrogram representation of a *Megophrys aceras* advertisement call - **A.** Three call groups, **B.** One call group**Figure 3.** Oscillogram and spectrogram representation of a *Megophrys aceras* advertisement call (2 calls)

widest portion of the head or angle of the jaws) - 25 mm, and weight - 27 g.

The calling frog was recorded while sitting in the water of a sandy spring-water creek, beside the road at a forest edge (Fig. 1). The call parameters analysed are listed in Table 1 following Duellman (1970), Cocroft & Ryan (1995), Brown & Richards (2008), and Tapley et al. (2017). A total of 51 call groups were recorded and analysed, with 2 – 4 calls per minute. The call group of *M. aceras* has a repetitive “EARK” sound. The oscillogram and spectrogram of the advertisement call are illustrated in Figures 2 and 3. The values for the call parameters are shown in Table 1. This call has been deposited in FonoZoo (recording number F_SOUND_CODE 12963)

In South-east Asia and north-east India, there are 61 *Megophrys* species (Mahony et al., 2020; Frost, 2021).

However, the advertisement calls of only sixteen species of five subgenera have been analysed and described from this region (Table 2). Whereas, only a call description has been reported for a frog in the subgenus *Xenophrys* (*M. oropedian*) (Mahony et al., 2013) (Table 2). A constant range limit of dominant frequency is notable in each subgenus. For example, frogs from the subgenus *Xenophrys*, have a dominant frequency limit of above 3 kHz. Frogs from the subgenus *Brachytarsophrys*, have the lowest dominant frequency, followed by *Pelobatrachus*, *Xenophrys*, *Panophrys*, and *Ophryophryne*. This order may reflect body size since the calls of larger frogs (e.g. *Brachytarsophrys* and *Pelobatrachus*) are expected to be at a lower dominant frequency. The sequence of these subgenera is aligned with the maximum likelihood tree by Mahony et al. (2017). Subgenus *Brachytarsophrys* diverges the earliest compared

Table 2. The advertisement calls of congeneric species of *Megophrys* from South-east Asia and north-east India

Subgenus	Species	Ambient temp. (°C)	Dominant frequency (kHz)	Call duration (ms)	Inter-call interval (ms)	References
<i>Xenophrys</i>	<i>M. aceras</i>	28.0	3.75	64.04±4.63 (74–94)	84.50 ±7.12(73–112)	This study
<i>Xenophrys</i>	<i>M. oropedion</i>	18.0	3.10–3.25	44.6±7.33 (30–55)	-	Mahony et al. (2013)
<i>Pelobatrachus</i>	<i>M. baluensis</i>	17–19	2.8	90–100	550–700	Malkmus & Riede (1996); Malkmus et al. (2002)
<i>Pelobatrachus</i>	<i>M. nasuta</i>	Not reported	2.0–2.5	160–170	150–160	Malkmus et al. (2002)
<i>Pelobatrachus</i>	<i>M. kalimantanensis</i>	20.7	2.1±0.15 (1.8–2.3)	137.9±16.18 (116.2–211.5)	185.8±23.53 (141.8–233.8)	Munir et al. (2019)
	Type 1	20.7	2.04±0.1 (1.9–2.2)	536.9±195.08 (268.7–885.6)	434.7±75.93 (323.7–535.1)	
	Type 2					
	<i>M. kobayashii</i>	21.0	-	163 (135–188)	150 (134–182)	Malkmus et al. 2002
<i>Panophrys</i>	<i>M. fansipanensis</i>	15.3–18.3	3.6–4.7	42.0 (34.0–49.0)	204.4 (180–290)	Tapley et al. (2018)
<i>Panophrys</i>	<i>M. hoanglienensis</i>	18.5	3.0 (2.8–3.0)	102.9 (96.0–108.0)	274.2 (178–565)	Tapley et al. (2018)
<i>Panophrys</i>	<i>M. jingdongensis</i>	18.5	2.4 (2.4–2.6)	132.7 (117.0–147.0)	113.0 (102.0–127.0)	Cutajar et al. (2020)
<i>Panophrys</i>	<i>M. minor</i>	14	3.5 (3.4–3.5)	90.8 (75.0–110.0)	253.0 (213.0–363.0)	Jiang et al. (2002)
<i>Panophrys</i>	<i>M. rubrimeria</i>	21.0–22.9	3.2 (3.2–3.4)	74.2 (56–83)	207.0 (190–235)	Tapley et al. (2017)
<i>Brachytarsophrys</i>	<i>M. feae</i>	13.0	1.38	340.0–474.0	293–482	Wogan et al. (2004)
<i>Brachytarsophrys</i>	<i>M. intermedia</i>	21.4	0.56–0.82	99.0–240.0	267–611	Tran (2013)
<i>Ophryophryne</i>	<i>M. elfina</i>	11.3–17.5	4.03–4.92	73 ± 0.23 (25–112)	207 ± 2.06 (96–942)	Poyarkov et al. (2017)
<i>Ophryophryne</i>	<i>M. gerti</i>	11.3–17.5	4.45–5.10	104 ± 0.56 (75–152)	421.54 ± 4.17 (275–813)	Poyarkov et al. (2017)
<i>Ophryophryne</i>	<i>M. synoria</i>	11.3–17.5	3.60–3.89	62 ± 0.46 (37–85)	143 ± 3.32 (56–528)	Poyarkov et al. (2017)

to other subgenera (Mahony et al., 2017). Based on this comparison we suggest that the dominant frequency of calls between each subgenus to be a phenotypic character expressing phylogenetic time divergence. Further research on the calls of *Megophrys* species is required to determine whether call parameters can be used to delimited *Megophrys* subgenera.

As frogs are ectotherms, the temporal character of call duration and inter-call interval are dependent on temperature (Platz 1989; Gerhardt & Bee, 2006). Call duration increases with temperature (Gerhardt, 1978; Gerhardt & Huber, 2002) while inter-call interval decreases (Gerhardt, 1978; Gerhardt & Huber, 2002). Call duration and inter-call are distinct in some species within the same subgenus (Table 2) but this may be due to differences in ; ambient temperature at the time of recording (Licht, 1969; Pough et al., 1983; Cree, 1989; Navas, 1996). This may account for the higher call duration of *M. aceras* compared to *M. oropedion* (Table 2).

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