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The advertisement call of *Megophrys jingdongensis* Fei and Ye, 1983 and a new record from Lai Chau Province, Northeast Vietnam

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Megophrys frogs Kuhl and van Hasselt, 1822, are morphologically conserved but male advertisement call data, which may aid the identification of species in the field, have not routinely been included in *Megophrys* species descriptions. *Megophrys jingdongensis* Fei and Ye, 1983 is a relatively widely distributed species of *Megophrys* and is included within the subgenus *Panophrys* Rao and Yang, 1997. *Megophrys jingdongensis* has been reported from China (Jingdong, Luchun, Pingbian, Shuangbai, Wenshan, Xingping, Xishuangbanna and Yuanyang counties in Yunnan Province and Tianlin County in northwestern Guangxi Province: Fei and Ye, 2016; Chen et al., 2016), and Lao Cai and Ha Giang provinces in northern Vietnam (Ohler et al., 2000; Chen et al., 2016; Nguyen et al., 2016). This species is known to occur in subtropical evergreen forests between 1000–2400 m a.s.l. (Fei et al., 2004) and is assessed as Least Concern on the IUCN Red List of Threatened Species (Fei et al., 2004).

We encountered *M. jingdongensis* at night during field work in the Hoang Lien Range in June 2012, June 2016 and September 2017. *Megophrys jingdongensis*

was observed at two sites in disturbed broad leaf forest between 1685–2153 m a.s.l. in Lao Cai Province and two sites in disturbed broad leaf forest Lai Chau Province between 1887–1923 m a.s.l. (see Table 1). This is the first record of *M. jingdongensis* in Lai Chau Province, and this collection site is 372 km southeast of the approximate type locality. This new locality is not a significant range extension, it has been collected previously from adjoining Sa Pa District in Lao Cai Province although precise collection site details were not reported (Fei et al., 2004; Chen et al., 2016).

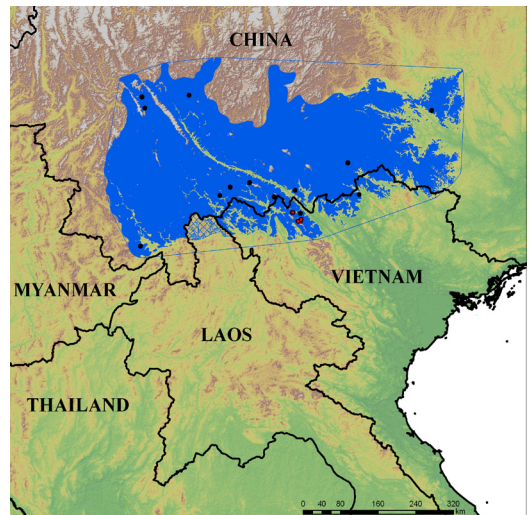


Figure 1. A distribution map of *Megophrys jingdongensis* in northern Vietnam, northern Laos and southern China. Red points represent the collection sites in this study and black points represent those reported in the literature (see text for details). Solid blue area represents presumed range and cross hatched blue area denotes areas where this species may be possibly extant. Blue outline denotes EOO.

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Specimens were euthanized using a 20% solution of benzocaine applied to the ventral surface of the frog. Tissue samples (liver) for molecular analyses were extracted prior to formalin fixation. Specimens were deposited at the Australian Museum, Sydney (AMS), and the Hoang Lien National Park headquarters (HLNP).

The specimens were identified morphologically as *M. jingdongensis* on the basis of the following characters: (1) a large adult size, SVL > 50.0 mm; (2) a distinct tympanum; (3) the presence of vomerine teeth and ridges; (4) the presence of maxillary teeth; (5) the presence of nuptial pads on breeding males; (6) the presence of a small palpebral tubercle; and (7) the absence of a light longitudinal marking on the upper lip (Fei and Ye, 1983; Fei *et al.*, 2012; Li *et al.*, 2014). Species identity was confirmed through molecular analysis (See Tapley *et al.*, 2017 for molecular methods). The newly obtained sequences (Table 1) differed from specimens collected approximately 40 km from the type locality (GenBank accession number KX811877; Chen *et al.*, 2016) in Zhenyuan County, Yunnan Province, China by 0.38% uncorrected *p*-distance at the 16S rDNA fragment examined (~530bp). This is congruent with intraspecific variation reported in frogs (e.g. Fouquet *et al.*, 2007). Uncorrected *p*-distance between sequences from specimens collected as part of this study

ranged from 0.00–0.18%, the newly obtained sequences were deposited in GenBank (see Table 1 for accession numbers).

An updated species range map was created in ArcMap 10.2.2 (Fig. 1) incorporating sites where we encountered the species and published records (Fei *et al.*, 2012; Fei and Ye, 2016; Chen *et al.*, 2016; Nguyen *et al.*, 2016). The elevation range within which *M. jingdongensis* is likely to occur was estimated by adding a buffer of 50 m to the lowest and highest known elevation records of the species (Tapley *et al.*, 2017). Areas of habitat were deemed suitable and included in maps if they are within the species' estimated elevation range; are covered with forest; and, are not separated from known localities by any continuous stretch of unsuitable habitat with a distance equal to or above 1 km. Extent of Occurrence (EOO) was measured using the IUCN EOO Calculator tool v1.2 and was calculated as 237981.6 km². The species would likely still qualify as Least Concern (IUCN, 2012) in accordance with the IUCN Red List of Threatened Species categories in view of its wide distribution, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category. The data herein are presented for easy assimilation into a Red List assessment for the species by the regional IUCN amphibian Red List authority. We observed ongoing

Table 1. Collection localities of *Megophrys jingdongensis*.

Specimen number	Latitude	Longitude	Elevation (m asl)	District	Province	Collection date	Genbank accession
AMS R185925 Field tag JJLR 02700	22.3483 °N	103.7302 °E	1887	Tam Duong	Lai Chau	15.06.12	MN876205
AMS R185926 Field tag JJLR 02701	22.3483 °N	103.7302 °E	1887	Tam Duong	Lai Chau	15.06.12	MN871430
AMS R185927 Field tag JJLR 02702	22.3483 °N	103.7302 °E	1887	Tam Duong	Lai Chau	15.06.12	N/A
AMS R185924 Field tag JJLR03823	22.3479 °N	103.7722 °E	1923	Tam Duong	Lai Chau	24.06.16	MN871425
AMS R185928 Field tag JJLR 02760	22.3821 °N	103.7875 °E	1708	Sa Pa	Lao Cai	18.06.12	MN876206
AMS R185929 Field tag JJLR 02761	22.3821 °N	103.7875 °E	1708	Sa Pa	Lao Cai	18.06.12	N/A
HLNP2016062200007 Field tag JJLR03811	22.3821 °N	103.7870 °E	1722	Sa Pa	Lao Cai	22.06.16	MN871429
Unvouchered	22.3865 °N	103.7834 °E	1685	Sa Pa	Lao Cai	23.06.16	N/A
HLNP20180908200008 Field tag JJLR03849	22.5095 °N	103.6219 °E	2153	Bat Xat	Lao Cai	08.09.17	MN871424
AMS R185930 Field tag BX001	22.5099 °N	103.6311 °E	2153	Bat Xat	Lao Cai	08.09.17	MN871433



Figure 2. *Megophrys jingdongensis* (male) AMS R185924 in situ.

disturbance to the species' habitat due to grazing of domestic livestock and forest clearance for agriculture as well as fuel wood collection for a nearby tourist camp at the site in Bat Xat District (see Tapley et al., 2018a for details). The presence of *M. jingdongensis* in Laos requires confirmation.

One specimen (specimen AMS R185924; Fig. 2.) was recorded vocalising on the 24th of June 2016. The call description is based on the calls of this specimen (Table 2; Fig. 3.). The specimen was perched on a

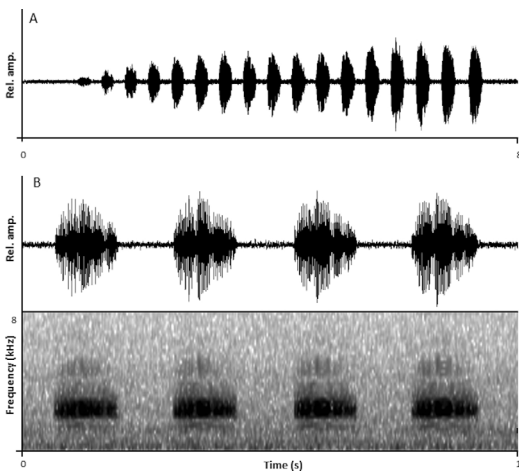


Figure 3. Advertisement call of *Megophrys jingdongensis* JJLR03823. (A) 8 s waveform of relative amplitude (Rel. amp.) over time for one call group, (B) 1 s waveform and spectrogram of Rel. amp. and frequency for four calls. Recorded at an ambient air temperature of 18.5 °C.

rocky overhang 1.8 m above and 1.5 m from a 3 m wide cascading stream at the base of Love Waterfall (thác Tình Yêu) in disturbed evergreen forest on Mount Fansipan, Hoang Lien National Park, Tam Duong District, Lai Chau Province, Vietnam. Advertisement calls were recorded with an Edirol R-09HR WAVE/MP3 Recorder (96 kHz sampling rate and 24-bit encoding) with a Røde NTG-2 condenser shotgun microphone. Calls were recorded at a distance of approximately 0.2 m and ambient temperatures at the calling site were taken immediately after recordings using a Kestrel 3500 hand-held weather meter. Calls were analysed with Raven Pro 1.5© software (<http://www.birds.cornell.edu/raven>). Audiospectrograms in figures were calculated with fast-Fourier transform (FFT) of 512 points, 50% overlap using Hanning windows. We follow Duellman (1970) in the definition of call units and pulses, except that single call is defined as a vocalisation produced during one expiration (Brown and Richards 2008). We used the definitions of Cocroft and Ryan (1995), when measuring the temporal and spectral parameters of calls with the exception of fundamental frequency, where the definition of Duellman (1970) was used. For each call recording, we measured the call duration (ms), intercall interval (ms), number of calls per call group, call repetition rate (calls/s), whether a call was pulsed, and dominant frequency (kHz). Comparative advertisement call characters for *Megophrys* species were taken from references, with advertisement calls known for 12 of the 41 known species of *Megophrys* (subgenus *Panophrys*; Jiang et al., 2002; Li et al., 2014; Wang et al., 2014; Tapley et al., 2017; Tapley et al., 2018b; Wang et al., 2019). Inconsistencies in published call descriptions precluded direct comparison with the calls of all congeners with published call descriptions.

Advertisement calls were recorded at 18.5 °C ambient temperature. Calls were an average of 132.7 ms (117.0–147.0 ms) in duration. The average dominant frequency of calls was 2.4 kHz (2.4–2.6 kHz). Calls were repeated at 3.94 calls per second, and the intercall interval ranged from 102–127 ms. The number of calls within the single call group was 17. The call group began with 4 calls at relatively low amplitude, the amplitude increasing with each of these four calls, after which amplitude remained relatively constant (Fig. 3B). Amplitude was modulated in individual calls, peaking towards the middle (Fig 3B.). The advertisement call of *M. jingdongensis* differs from those of its 11 congeners (subgenus *Panophrys*) for which calls have been described (see Table 2 for comparisons).

Table 2. Bioacoustic comparison between the male advertisement call of *Megophrys jingdongensis* and other species in the subgenus for which calls have been described. The mean value and ranges are presented. * indicates that only mean values were provided in the referred publication. Grey shading represents non-overlapping call parameters. Grey shading indicates that only mean values were provided in the referred publication. Grey shading represents non-overlapping call parameters.

Species	Number of call groups analysed	Number of calls analysed	Number of calls per call group	Call duration (ms)	Number of pulses per call	Inter-call interval (ms)	Call repetition rate (calls/s)	Dominant frequency (kHz)	Temperature °C	Reference
<i>M. jingdongensis</i>	1	17	17	132.7 (117.0–147.0)	Indistinct pulsation	113.0 (102.0–127.0)	3.9	2.5 (2.4–2.6)	18.5	This study
<i>M. acuta</i>	4	33	8.3 (7.0–9.0)	136.0 (106.0–172.0)	Not reported	425.0 (335.0–747.0)	2.0 (1.9–2.0)	Not reported	26.9	Li <i>et al.</i> , 2014
<i>M. boettgeri</i>	3	76	25.3*	54.0*	Not reported	215.0*	5.0*	Not reported	15.0–18.0	Wang <i>et al.</i> , 2014
<i>M. cheni</i>	9	88	9.2*	143.0*	Not reported	Not reported	Not reported	Not reported	15.0–18.0	Wang <i>et al.</i> , 2014
<i>M. fiansipanensis</i>	20	60	22.8 (10–41.0)	42.0 (34.0–49.0)	11.6 (10.0–14.0)	204.4 (180.0–290.0)	3.9 (3.8–4.0)	3.8 (3.6–4.7)	15.3–18.3	Tapley <i>et al.</i> , 2018b
<i>M. hoanglienensis</i>	5	20	17.0 (11.0–21.0)	102.9 (96.0–108.0)	18.7 (12.0–22.0)	274.2 (178.0–565.0)	2.6	3.0 (2.8–3.0)	18.5	Tapley <i>et al.</i> , 2018b
<i>M. jinggangensis</i>	25	420	26.1*	77.0*	Not reported	127.0*	5.7*	Not reported	15.0–18.0	Wang <i>et al.</i> , 2014
<i>M. huangshanensis</i>	3	42	35.0*	86.0*	Not reported	165.0*	4.1*	Not reported	15.0–18.0	Wang <i>et al.</i> , 2014
<i>M. kuatunensis</i>	4	45	11.3*	208.0*	Not reported	970.0*	1.0*	Not reported	15.0–18.0	Wang <i>et al.</i> , 2014
<i>M. lini</i>	18	164	10.2*	106.0*	Not reported	Not reported	Not reported	Not reported	15.0–18.0	Wang <i>et al.</i> , 2014
<i>M. minor</i>	Not reported	13	8.0–16.0	90.8 (75.0–110.0)	Not reported	253.0 (213.0–363.0)	4.0*	3.5 (3.4–3.5)	14	Jiang <i>et al.</i> , 2002
<i>M. rubrimera</i>	11	60	38.7 (16.0–51.0)	73.3 (62.0–85.0)	23.1 (19.0–25.0) or indistinct pulsation	221.3 (190.0–261.0)	3.3 (3.1–3.4)	3.3 (3.2–3.4)	21.0–22.9	Tapley <i>et al.</i> , 2017
<i>M. shunhuangensis</i>	Not reported	40	Not reported	109.6 (97–129)	17.2 (14–20)	287.2 (210–359)	approx. 2.0	3.79–3.88	16.4	Wang <i>et al.</i> , 2019

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