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## The Vietnamese population of *Megophrys kuantunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China

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### Abstract

The Asian frog genus *Megophrys* is a diverse group of morphologically conserved, forest-dwelling frogs. The genus harbours highly localised species diversification and new species continue to be described on a regular basis. We examined the taxonomic status of a population of *Megophrys* frogs from the Hoang Lien Range in northern Vietnam and southern China previously identified as *M. kuantunensis* (subgenus *Panophrys*). Preliminary phylogenetic analyses using a fragment of 16S rDNA places the species in question within the *Megophrys* (subgenus *Panophrys*) species group, a primarily Chinese radiation within the genus. On the basis of morphological, molecular and bioacoustic data, we conclude that this population does not represent *M. kuantunensis*, or any known species in the genus. We herein describe this species of *Megophrys* as new. Known only from Sa Pa District, Lao Cai Province in Vietnam and Jinping County, Yunnan Province in China, the new species is likely to be threatened by ongoing deforestation in the region. We provide an updated species description of *M. kuantunensis* based on type specimens, and suggest that *M. kuantunensis* is likely to be restricted to eastern China.

**Key words:** Bioacoustics, cryptic species, Hoang Lien, *Megophrys rubrimera* sp. nov., Southeast Asia, tadpole

### Tóm tắt tiếng Việt

Giống ếch châu Á *Megophrys* là nhóm có thành phần loài đa dạng và các loài mới tiếp tục được mô tả gần đây. Trong nghiên cứu này chúng tôi xem xét tình trạng phân loại của một loài thuộc giống *Megophrys* từ dãy núi Hoàng Liên Sơn ở phía Bắc Việt Nam và phía Nam Trung Quốc trước đây được xác định là *M. kuantunensis* (phân giống *Panophrys*). Các phân tích phát sinh loài bằng cách sử dụng một đoạn của gen 16S rDNA xác định các loài đang được đề cập đến trong giống *Megophrys* (phân giống *Panophrys*) là một nhánh chủ yếu của giống này từ Trung Quốc. Trên cơ sở dữ liệu hình thái, phân tử và âm sinh học, chúng tôi cho rằng quần thể này không thuộc loài *M. kuantunensis*, hoặc bất kỳ loài nào được biết đến trong giống. Chúng tôi mô tả loài mới thuộc giống *Megophrys*, hiện biết ở huyện Sa Pa, tỉnh Lào Cai, Việt Nam và huyện Kim Bình, tỉnh Vân Nam, Trung Quốc, loài mới này có thể bị đe dọa bởi nạn phá rừng đang diễn ra ở khu vực sống của chúng. Chúng tôi cũng cung cấp bản mô tả lại loài *M. kuantunensis* dựa trên mẫu vật và cho rằng *M. kuantunensis* có thể chỉ phân bố hạn chế ở phía Đông Trung Quốc.

## Introduction

Frogs of the genus *Megophrys* Kuhl & Van Hasselt, 1822 are morphologically conservative terrestrial frogs that inhabit hill streams across southern and central China, the eastern and southern Himalayas and south into Peninsular Malaysia, islands of the Sunda shelf in Indonesia and parts of the Philippines (Mahony *et al.* 2017). The genus harbours cryptic diversity and also highly localised species diversification due to the rugged mountain terrain and the limited dispersal ability of these frogs (Li *et al.* 2014; Mahony 2011; Mahony *et al.* 2011, 2013, 2017; Wang *et al.* 2012, 2014; Chen *et al.* 2017). Species delineation in the genus has been hampered by the fact that *Megophrys* are morphologically conserved and because detailed information, specifically call and molecular data, has not routinely been included in species descriptions.

The higher systematics of *Megophrys sensu lato* had been debated for some time and there has been several proposals to split the genus on the basis of morphological and limited molecular data (Delorme *et al.* 2006; Frost *et al.* 2006; Li & Wang 2008; Chen *et al.* 2017). As there is no clear morphological differentiation for some of the proposed genera (e.g., *Megophrys*, *Xenophrys*) we follow Mahony *et al.* (2017) in the treatment of the Megophryinae as a single genus, *Megophrys*, with previously described genus-level groups treated as subgeneric-level taxa. By implication, the genus *Megophrys* is currently comprised of 71 species, 19 of which have been reported from Vietnam (Ohler 2003; Le *et al.* 2006; Nguyen *et al.* 2009; Orlov *et al.* 2002, 2015; Chen *et al.* 2017; Mahony *et al.* 2017); *M. brachykolos* Inger & Romer, 1961; *M. daweimontis* Rao & Yang, 1997; *M. elfina* Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony *et al.*, 2017; Poyarkov *et al.* 2017; *M. feae* Boulenger, 1887; *M. gerti* Ohler, 2003; *M. hansii* Ohler, 2003; *M. intermedia* Smith, 1921; *M. jingdongensis* Fei & Ye, 1983; *M. kouii* Mahony, Foley, Biju & Teeling, 2017; *M. kuatunensis* Pope, 1929; *M. latidactyla* Orlov, Poyarkov & Nguyen, 2015; *M. major* Boulenger, 1908; *M. maosonensis* Bourret, 1937; *M. microstoma* Boulenger, 1903; *M. minor* Stejneger, 1926; *M. pachyproctus* Huang, 1981; *M. palpebralespinosa* Bourret, 1937; *M. parva* Boulenger, 1893 and *M. synoria* Stuart, Sok & Neang, 2006.

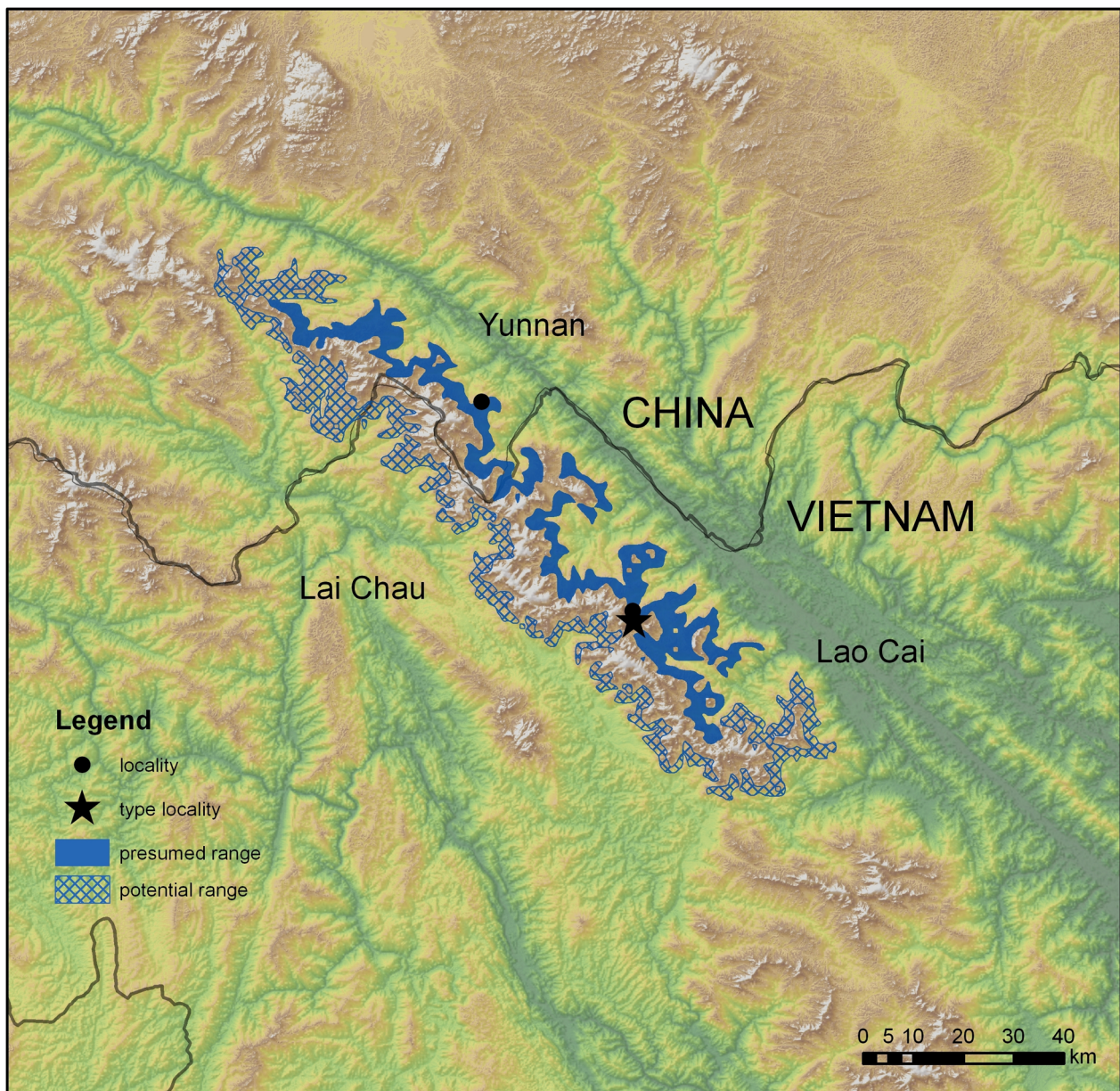
During recent fieldwork in the Hoang Lien Range and adjacent Sa Pa District, in northern Vietnam, we encountered a *Megophrys* species that superficially resembled *M. kuatunensis*, but is geographically distant from confirmed populations of this species in eastern China. By providing an updated species description for *M. kuatunensis* through re-examination of the holotype and partial paratype series, we conclude that the Vietnam species differs from *M. kuatunensis*. Furthermore, we demonstrate that this species differs from all other described congeners in terms of morphology, bioacoustics and molecular sequences, and describe the species herein as new.

## Materials and methods

Specimens were collected at night in forest stream habitats in the Hoang Lien Range (Fig. 1), Sa Pa District, Lao Cai Province, Vietnam during June of 2012 and 2016. Geographic coordinates were obtained using a Garmin GPSMAP 60CSx GPS receiver and recorded in datum WGS 1984. Salifert test kits were used to measure pH and alkalinity of the water body; water samples were taken in the mid-section of the stream at 10 cm depth and tests were carried out *in situ* following the manufacturer's guidelines. Tissue samples for molecular analyses were extracted from freshly euthanased specimens (liver from post-metamorphic frogs, and tail muscle from tadpoles) prior to formalin fixation. Specimens were deposited at the Vietnam National Museum of Nature, Hanoi (VNMN), the Australian Museum, Sydney (AMS), and at the Hoang Lien National Park headquarters. Comparative material has been examined from the Natural History Museum, London (NHMUK; specimen acronym BMNH), Field Museum of Natural History, Chicago (FMNH, formerly CNHM), and American Museum of Natural History, New York (AMNH).

**Molecular data:** Genomic DNA was extracted from EDTA/DMSO or ethanol preserved tissues using DNeasy® Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany), using the protocols for purification of genomic DNA from animal tissues. We amplified a ~550 bp section of 16S (mtDNA) using the primers (5'-3') 16Sar CGCCTGTTTATCAAAAACAT and 16Sbr CCGGTCTGAAGTCTCAGATCACGT (Palumbi 1991). PCR amplification was carried out in 24 µl reactions with 1000 ng of genomic DNA, 1 × Reaction Buffer (Bioline My Taq Red Reagent Buffer), 2 pmol of corresponding primers and Bioline My Taq Red DNA polymerase (0.5 units). Negative controls were included in each PCR. Thermocycling was performed on an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 94 °C (2 min), 2 cycles of 94 °C (20 s) denaturation, 52 °C (40 s) annealing and 72 °C (60 s) extension, followed by 33 cycles of 94 °C (20 s) denaturation, 50 °C (40 s) annealing and 72 °C (50 s) extension, followed by a final extension step at 72 °C (5 min).

All PCR products were purified using Exo-Sap-IT (USB Corporation, OH, USA), and sequenced in both directions at Macrogen (Seoul, Korea). Sequences were edited and checked for quality through reference of chromatograms using Sequencher v. 4.10 (Gene Codes, Ann Arbor, MI). Preliminary molecular barcoding analyses including sequences from four specimens (three adult males and a single tadpole) placed the new species in the genus *Megophrys* (subgenus *Panophrys*), so available homologous sequences on GenBank belonging to 22 of the 25 known species of this subgenus, and representatives of all other subgenera were included in the alignment. Phylogenetic analyses performed also placed the species in *Panophrys* but the sequence data was insufficient to resolve a meaningful phylogenetic tree, as observed by other studies using short mitochondrial sequence datasets (see Mahony *et al.* 2017 for examples). Uncorrected pairwise sequence divergence (with pairwise deletion of gaps and missing data) was calculated using MEGA 7. The specific identity of the tadpole (AMS R177680) was confirmed by a 100% sequence match with adults from the site. Distance from the site of sequence collection and the type locality of each species was estimated using the ruler function on Google Earth. Locality information and GenBank accession numbers for all sequences included in the analyses can be found in Table 1.



**FIGURE 1.** Distribution of *Megophrys rubrimera* sp. nov. in the Hoang Lien Range, northern Vietnam and Ailao Mountain Range in Yunnan Province, China.



**TABLE 1.** List of specimens and GenBank accession numbers for all DNA sequences included in the analysis.

Species	Locality	Distance from type locality	Voucher No.	GenBank Accession No. (16S)	Source
<i>Megophrys acuta</i>	China, Guangdong, Heishiding	0km - type locality	SYS a001957	KJ579118	Chen <i>et al.</i> 2017
<i>Megophrys baolongensis</i>	China, Chongqing, Baolong	0km - type locality	KIZ019216	KX811813	Chen <i>et al.</i> 2017
<i>Megophrys binchaunensis</i>	China, Yunnan, Jizu Shan	0km - type locality	KIZ019441	KX811849	Chen <i>et al.</i> 2017
<i>Megophrys binlingensis</i>	China, Sichuan, Wawu Shan	With 30 km	KIZ025807	KX811852	Chen <i>et al.</i> 2017
<i>Megophrys boettgeri</i>	China, Jiangxi, Tongbashan	~ type locality	SYS a001700	KJ560382	Wang <i>et al.</i> 2014
<i>Megophrys brachykolos</i>	China, Hong Kong	~30 km	SYS a002258	KJ560403	Wang <i>et al.</i> 2014
<i>Megophrys carinense</i>	China, Guangxi, Dayao Shan	~ 1000km	Tissue ID: YPX20455	KX811811	Chen <i>et al.</i> 2017
<i>Megophrys cheni</i>	China, Hunan, Taoyuandong	0km - type locality	SYS a002142	KJ560398	Wang <i>et al.</i> 2014
<i>Megophrys daweimontis</i>	China, Yunnan, Dawei Shan	0km - type locality	KIZ048997	KX811867	Chen <i>et al.</i> 2017
<i>Megophrys dringi</i>	Malaysia, Sarawak, Gunung Mulu	0km - type locality	ZMH A09364	KJ831314	Oberhummer <i>et al.</i> 2014
<i>Megophrys huangshanensis</i>	China, Anhui, Huang Shan	0km - type locality	KIZ022002	KX811816	Chen <i>et al.</i> 2017
<i>Megophrys jingdongensis</i>	China, Yunnan, Wenlong	~ type locality	KIZ011821	KX811874	Chen <i>et al.</i> 2017
<i>Megophrys jinggangensis</i>	China, Hunan, Taoyuandong	~40km	SYS a001860	KJ560400	Wang <i>et al.</i> 2014
<i>Megophrys kuatunensis</i>	China, Fujian, Wuyishan	0km - type locality	SYS a001592	KJ560378	Wang <i>et al.</i> 2014
<i>Megophrys lini</i>	China, Hunan, Taoyuandong	0km - type locality	SYS a002128	KJ560416	Wang <i>et al.</i> 2014
<i>Megophrys microstoma</i>	China, Yunnan, Xiaoqiaogou	within 100 km	KIZ048799	KX811914	Chen <i>et al.</i> 2017
<i>Megophrys minor</i>	China, Sichuan, Qingcheng Shan	0km - type locality	KIZ019391	KX811896	Chen <i>et al.</i> 2017
<i>Megophrys montana</i>	Indonesia, Java, Sukabumi	within 250km	LSUMZ 81916	KX811927	Chen <i>et al.</i> 2017
<i>Megophrys monticola</i>	India, West Bengal, Sukhiapokhri-Manegthanjan road	within 150km	SDBDU 2011.1047	KY022312	Mahony <i>et al.</i> 2017
<i>Megophrys nasuta</i>	Malaysia, Sarawak, Gunung Mulu	~1750km	ZMH A13127	KJ831311	Chen <i>et al.</i> 2017
<i>Megophrys obesa</i>	China, Guangdong, Heishiding Nature Reserve	0km - type locality	SYS a001956	KJ579117	Li <i>et al.</i> 2014
<i>Megophrys omeimontis</i>	China, Sichuan, Emei Shan	0km - type locality	KIZ025765	KX811884	Chen <i>et al.</i> 2017
<i>Megophrys palpebralespinosa</i>	Vietnam, Thanh Hoa, Pu Hu Nature Reserve	within 200km	KIZ011603	KX811888	Chen <i>et al.</i> 2017
<i>Megophrys parva</i>	Vietnam, Lao Cai, Sa Pa	~ 1200 km	MNHN:199 9.5694	JN848362	Ohler <i>et al.</i> 2011

.....continued on the next page

**TABLE 1.** (Continued)

Species	Locality	Distance from type locality	Voucher #	GenBank Accession No. (16S)	Source
<i>Megophrys rubrimera</i> <b>sp. nov.</b>	Vietnam, Lao Cai, Sa Pa	0km - type locality	AMS R177676	MF536419	This study
<i>Megophrys rubrimera</i> <b>sp. nov.</b>	Vietnam, Lao Cai, Sa Pa	0km - type locality	VNMN 2017.002	MF536420	This study
<i>Megophrys rubrimera</i> <b>sp. nov.</b>	Vietnam, Lao Cai, Sa Pa	0km - type locality	VNMN 2017.003	MF536421	This study
<i>Megophrys shapingensis</i>	China, Sichuan, Wawushan	~ 50 km	No voucher	KX811905	Chen <i>et al.</i> 2017
<i>Megophrys</i> sp. 3	China, Yunnan, Maandi	Not applicable	KIZ020425	KX811871	Chen <i>et al.</i> 2017
<i>Megophrys spinata</i>	China, Guizhou, Fanjing Mountain	0km - type locality	ZYC644	AY526205	Zheng <i>et al.</i> 2004
<i>Megophrys tuberogramulata</i>	China, Hunan, Badagongshan Nature Reserve	within 50km	Tissue ID: YPX10987	KX811823	Chen <i>et al.</i> 2017
<i>Megophrys wuliangshanensis</i>	China, Yunnan, Huangcaoling	within 250 km	KIZ046812	KX811881	Chen <i>et al.</i> 2017
<i>Megophrys wushanensis</i>	China, Sichuan, Guangwu Shan	within 380 km	KIZ045469	KX811838	Chen <i>et al.</i> 2017
<i>Pelodytes punctatus</i>	France, Argeles-sur-Mer	~ 650 km	Pdp-2/AE08	DQ642114	Veith <i>et al.</i> 2006

**Morphological data:** For post metamorphic specimens we recorded morphological data (to the nearest 0.1 mm) with digital callipers from specimens fixed in 10% formalin and then stored in 70% ethanol. Measurements and morphometric abbreviations follow Mahony *et al.* (2011). Morphometric abbreviations are as follows: Snout to vent length (SVL); head width, measured at the posterior angle of the jaws (HW); head length, measured from the rear of the mandible to the tip of the snout (HL); snout length, measured from the tip of the snout to the anterior orbital border of the eye (SL); distance from the nostril to the tip of the snout (SN); distance from the front of the eye to the nostril (EN); eye length, i.e. the horizontal distance between the bony orbital borders of the eye (EL); inter-upper eyelid width, i.e., the shortest distance between the upper eyelids (IUE); maximum upper eyelid width (UEW); internarial distance (IN); internal front of eyes, i.e., the shortest distance between the anterior orbital borders of the eyes (IFE); internal back of eyes, i.e., the shortest distance between the posterior orbital borders of the eyes (IBE); maximum tympanum diameter (TYD); distance from the anterior border of the tympanum to the posterior orbital border (TYE); forearm length, measured from the elbow to the wrist (FAL); hand length, measured from the wrist to the tip of the third finger (HAL); first finger length, measured from the base of the second finger to the tip of the first finger (FIL); second finger length, measured from the base of the first finger to the tip of the second (FIIL); third finger length, measured from the base of the second finger to the tip of the third (FIIL); fourth finger length, measured from the base of the third finger to the tip of the fourth (FIVL); thigh length, measured from the cloaca to the knee when the thigh is held at a right angle to the body (TL); shank length, measured from the knee to the tibio-tarsal articulation (SHL); foot length, measured from the base of the inner metatarsal tubercle to the tip of the fourth toe (FOL); length of the inner metatarsal tubercle (IMT). Sex was determined by the presence of nuptial pads and gonadal inspection. Body mass was recorded in life (to the nearest 0.1 g), using Pesola scales. To measure the single tadpole of the new species, we used ImageJ 1.49 (Schneider *et al.* 2012) from photographs of the preserved specimen. Staging follows Gosner (1960), and tadpole terminology is that of Altig and McDiarmid (1999). Measurements and morphometric abbreviations follow Oberhammer *et al.* (2014). Morphometric abbreviations are as follows: body length from snout to the point where the axis of the tail myotomes touches the body wall (BL); maximal body height at trunk (BH); body end to centre of spiracles (BS); maximal body width (BW); eye diameter (ED); eye snout distance (ES); internarial distance measured from the centre of each nare (IND); interorbital distance measured from centre to centre (IOD); lower fin height at point of maximal tail height (LFH); maximal tail height, including fins (MTH); distance from centre of nare to centre of eye (NE); oral disc width (ODW); distance from centre of the nare to the snout (SN);

distance from snout to centre of spiracle (SS); tail length = TTL–BL (TAL); total length (TTL); maximal tail muscle height at body–tail junction where the ventral line of the musculature meets the contour of the trunk (TMH); maximal tail muscle width (TMW); upper fin height at point of maximal tail height (UFH).

We compare the new species with all congeners reported from mainland Southeast Asia (excluding the southern peninsula south of the Isthmus of Kra) and bordering provinces of southern China: *M. acuta* Wang, Li & Jin, 2014; *M. auralensis* Ohler, Swan & Daltry, 2002; *M. binchuanensis* Ye & Fei, 1995; *M. boettgeri* Boulenger, 1899; *M. brachykolos*; *M. carinense* Boulenger, 1889; *M. damrei* Mahony, 2011; *M. daweimontis* Rao & Yang, 1997; *M. elfina*; *M. feae*; *M. gerti*; *M. glandulosa* Fei, Ye & Huang, 1990; *M. hanshi*; *M. intermedia*; *M. jingdongensis*; *M. kouii*; *M. kuatunensis*; *M. latidactyla*; *M. lekaguli* Stuart, Chuaynkern, Chan-ard & Inger, 2006; *M. major*; *M. maosonensis*; *M. microstoma*; *M. minor*; *M. omeimontis* Liu, 1950; *M. pachyproctus*; *M. palpebrapespinoza*; *M. parva*; *M. spinata* Liu & Hu, 1973; *M. synoria*; *M. takensis*; Mahony, 2011 and *M. wuliangshanensis* Ye & Fei, 1995. Specimens of congeners were examined from museum collections when available and supplemented with additional morphological data from the following literature: Boulenger 1899; Bourret 1937; Fei *et al.* 2009; Fei *et al.* 2012; Huang *et al.* 1981; Li *et al.* 2014; Mahony 2011; Neang *et al.* 2013; Ohler *et al.* 2002; Ohler 2003; Orlov *et al.* 2015; Poyarkov *et al.* 2017; Rao & Yang 1997; Stuart *et al.* 2006a, 2006b. Due to the degree of undiagnosed diversity within the genus, where available, we relied on the examination of topotypic material and/or original species descriptions.

**Acoustic data:** Advertisement calls of three individuals 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© v.1.5 software (<http://www.birds.cornell.edu/raven>). Audiospectrograms provided in figures were calculated with fast-Fourier transform (FFT) of 512 points, 50% overlap using Hanning windows. The units of a call group and pulse were defined according to Duellman (1970), and we define a single call as a vocalisation produced during a single expiration (Brown & Richards 2008). Temporal and spectral parameters of calls were measured using the definitions of Cocroft and Ryan (1995). For each call recording, we measured the call duration (ms), intercall interval (ms), number of calls per call group, call repetition rate (calls/s), number of pulses per call, and dominant frequency (kHz). Comparative advertisement call characters for *Megophrys* species were taken from references, with advertisement calls known for 7 of the 25 known species of *Megophrys* (subgenus *Panophrys*) (Jiang *et al.* 2001; Ziegler *et al.* 2002; Li *et al.* 2014; Wang *et al.* 2014; Xiong *et al.* 2015).

**Species distribution mapping:** Species range maps were created in ArcMap 10.2.2. Each species' range was generated using data from map layers for local topography, vegetation communities, and land use. We used the ESRI World Topographic basemap layer, WorldClim Altitude raster (Hijmans *et al.* 2005), and IUCN Elevation raster to visualise topography and the ESRI World Imagery basemap layer to determine local land cover.

The elevation range within which each species is likely to occur was estimated by adding a buffer to the lowest and highest known elevation records of the species. The range of each buffer was determined by the distance between observations and the species' perceived likelihood of dispersal between known localities. Conservative buffers were augmented if they excluded vast areas between recorded points on the assumption that localities must be at least partially connected by habitable topography for dispersal and colonisation to have occurred. For both species, a buffer of 50 m was sufficient to link recorded localities.

Areas of habitat were deemed suitable and included in maps as inferred or potential range if they: are within the species' estimated elevation range; are covered with forest; and, for the new species are not separated from known localities by any continuous stretch of unsuitable habitat with a distance equal to or above 1 km.

Area of occupancy (AOO) was measured using the Calculate Geometry tool in ArcMap, and extent of occurrence (EOO) was measured using the IUCN EOO Calculator tool v1.2.

## Results

**Molecular data:** The sequences from the newly collected *Megophrys* species were most similar to “*Megophrys* sp. 3” (*sensu* Chen *et al.* 2017), differing by only 0.8% uncorrected pairwise divergence at the 16S rDNA fragment examined. Uncorrected

**TABLE 2.** Uncorrected 16S pairwise distances among *Megophrys rubrimera* sp. nov. and all species for which homologous sequences were available on GenBank belonging to the subgenus *Megophrys* (*Panophrys*) and representatives of all subgenera.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	<i>M. acuta</i> KJ579118																
2	<i>M. baolongensis</i> KX811813	0.073															
3	<i>M. binchuanensis</i> KX811849	0.081	0.044														
4	<i>M. binlingensis</i> KX811852	0.086	0.036	0.025													
5	<i>M. boettgeri</i> KJ560382	0.070	0.027	0.040	0.036												
6	<i>M. brachykolos</i> KJ560403	0.081	0.046	0.053	0.063	0.047											
7	<i>M. carinense</i> KX811811	0.136	0.084	0.078	0.078	0.082	0.087										
8	<i>M. cheni</i> KJ560398	0.078	0.027	0.038	0.040	0.027	0.044	0.078									
9	<i>M. daweimontis</i> KX811867	0.070	0.040	0.028	0.027	0.034	0.063	0.076	0.036								
10	<i>M. dringi</i> KJ831314	0.149	0.101	0.105	0.103	0.097	0.107	0.089	0.105	0.103							
11	<i>M. rubrimera</i> sp. nov. VNNM 2017.002	<b>0.097</b>	<b>0.042</b>	<b>0.025</b>	<b>0.025</b>	<b>0.028</b>	<b>0.059</b>	<b>0.072</b>	<b>0.038</b>	<b>0.023</b>	<b>0.097</b>						
12	<i>M. rubrimera</i> sp. nov. VNNM 2017.003	<b>0.097</b>	<b>0.042</b>	<b>0.025</b>	<b>0.025</b>	<b>0.028</b>	<b>0.059</b>	<b>0.072</b>	<b>0.038</b>	<b>0.023</b>	<b>0.097</b>	<b>0.000</b>					
13	<i>M. rubrimera</i> sp. nov. AMS R177676	<b>0.097</b>	<b>0.042</b>	<b>0.025</b>	<b>0.025</b>	<b>0.028</b>	<b>0.059</b>	<b>0.072</b>	<b>0.038</b>	<b>0.023</b>	<b>0.097</b>	<b>0.000</b>	<b>0.000</b>				
14	<i>M. huangshanensis</i> KX811816	0.070	0.030	0.044	0.040	0.008	0.051	0.089	0.030	0.036	<b>0.036</b>	<b>0.036</b>	<b>0.036</b>				
15	<i>M. jingdongensis</i> KX811874	0.086	0.042	0.030	0.025	0.042	0.066	0.068	0.040	0.025	<b>0.023</b>	<b>0.023</b>	<b>0.023</b>	0.046			
16	<i>M. jinggangensis</i> KJ560400	0.073	0.038	0.046	0.044	0.036	0.048	0.080	0.029	0.044	<b>0.046</b>	<b>0.046</b>	<b>0.046</b>	0.044	0.044		
17	<i>M. kuatunensis</i> KJ560378	0.081	0.039	0.054	0.054	0.034	0.049	0.104	0.027	0.052	<b>0.054</b>	<b>0.054</b>	<b>0.054</b>	0.039	0.062	0.047	
18	<i>M. lini</i> KJ560416	0.070	0.047	0.049	0.051	0.034	0.040	0.084	0.030	0.044	<b>0.053</b>	<b>0.053</b>	<b>0.053</b>	0.105	0.106	0.105	0.039
19	<i>M. microstoma</i> KX811914	0.154	0.105	0.110	0.101	0.099	0.110	0.131	0.103	0.105	<b>0.106</b>	<b>0.106</b>	<b>0.106</b>	0.105	0.106	0.105	0.119
20	<i>M. minor</i> KX811896	0.098	0.059	0.053	0.053	0.044	0.063	0.082	0.053	0.049	<b>0.049</b>	<b>0.049</b>	<b>0.049</b>	0.049	0.057	0.059	0.067
21	<i>M. montana</i> KX811927	0.174	0.118	0.122	0.115	0.118	0.128	0.122	0.122	0.113	<b>0.116</b>	<b>0.116</b>	<b>0.116</b>	0.124	0.115	0.119	0.159
22	<i>M. monticola</i> KY022312	0.149	0.104	0.116	0.106	0.108	0.121	0.121	0.113	0.102	<b>0.113</b>	<b>0.113</b>	<b>0.113</b>	0.113	0.116	0.119	0.118
23	<i>M. nasuta</i> KJ831311	0.150	0.131	0.131	0.131	0.133	0.133	0.130	0.131	0.127	<b>0.135</b>	<b>0.135</b>	<b>0.135</b>	0.131	0.129	0.132	0.156
24	<i>M. obesa</i> KJ579117	0.081	0.070	0.076	0.081	0.049	0.076	0.117	0.035	0.062	<b>0.070</b>	<b>0.070</b>	<b>0.070</b>	0.059	0.073	0.062	0.049
25	<i>M. omeimontis</i> KX811884	0.087	0.042	0.025	0.023	0.036	0.051	0.067	0.034	0.023	<b>0.021</b>	<b>0.021</b>	<b>0.021</b>	0.040	0.021	0.040	0.047
26	<i>M. palpebralespinosa</i> KX811888	0.084	0.049	0.036	0.040	0.049	0.064	0.078	0.051	0.040	<b>0.044</b>	<b>0.044</b>	<b>0.044</b>	0.049	0.044	0.053	0.071
27	<i>M. parva</i> JN848362	0.089	0.061	0.050	0.048	0.048	0.070	0.085	0.057	0.041	<b>0.033</b>	<b>0.033</b>	<b>0.033</b>	0.057	0.033	0.057	0.076
28	<i>M. shapingensis</i> KX811905	0.114	0.070	0.066	0.066	0.074	0.085	0.063	0.072	0.061	<b>0.072</b>	<b>0.072</b>	<b>0.072</b>	0.080	0.065	0.068	0.096
29	<i>M. spinata</i> AY526205	0.089	0.047	0.029	0.012	0.047	0.079	0.091	0.039	0.034	<b>0.034</b>	<b>0.034</b>	<b>0.034</b>	0.052	0.032	0.054	0.057
30	<i>M. tuberoanulatus</i> KX811823	0.070	0.015	0.034	0.027	0.015	0.044	0.076	0.019	0.032	<b>0.030</b>	<b>0.030</b>	<b>0.030</b>	0.019	0.032	0.029	0.030
31	<i>M. wuliangshanensis</i> KX811881	0.089	0.044	0.032	0.034	0.040	0.061	0.086	0.042	0.027	<b>0.027</b>	<b>0.027</b>	<b>0.027</b>	0.044	0.028	0.049	0.052
32	<i>M. wushanensis</i> KX811838	0.081	0.021	0.040	0.038	0.027	0.048	0.080	0.030	0.036	<b>0.038</b>	<b>0.038</b>	<b>0.038</b>	0.030	0.044	0.036	0.040
33	<i>Megophrys</i> sp. 3 KX811871	0.097	0.046	0.030	0.030	0.032	0.059	0.074	0.042	0.028	<b>0.008</b>	<b>0.008</b>	<b>0.008</b>	0.036	0.027	0.049	0.059
34	<i>Peldoyres punctatus</i> DQ642114	0.214	0.160	0.149	0.155	0.158	0.166	0.155	0.166	0.149	<b>0.151</b>	<b>0.151</b>	<b>0.151</b>	0.162	0.162	0.164	0.200

.....continued on the next page



TABLE 2. (Continued)

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	<i>M. acuta</i> KJ579118																
2	<i>M. baolongensis</i> KX811813																
3	<i>M. binchuanensis</i> KX811849																
4	<i>M. binlingensis</i> KX811852																
5	<i>M. boettgeri</i> KJ560382																
6	<i>M. brachykalos</i> KJ560403																
7	<i>M. carinense</i> KX811811																
8	<i>M. cheni</i> KJ560398																
9	<i>M. daweimontis</i> KX811867																
10	<i>M. dringi</i> KJ831314																
11	<i>M. rubrimera</i> <b>sp. nov.</b> VNNM 2017.002																
12	<i>M. rubrimera</i> <b>sp. nov.</b> VNNM 2017.003																
13	<i>M. rubrimera</i> <b>sp. nov.</b> AMS R177676																
14	<i>M. huangshanensis</i> KX811816																
15	<i>M. jingdongensis</i> KX811874																
16	<i>M. jinggangensis</i> KJ560400																
17	<i>M. kuatunensis</i> KJ560378																
18	<i>M. lini</i> KJ560416																
19	<i>M. microstoma</i> KX811914	0.106															
20	<i>M. minor</i> KX811896	0.061	0.105														
21	<i>M. montana</i> KX811927	0.120	0.153	0.122													
22	<i>M. monticola</i> KY022312	0.117	0.148	0.125	0.132												
23	<i>M. nasuta</i> KJ831311	0.124	0.156	0.130	0.152	0.153											
24	<i>M. obesa</i> KJ579117	0.049	0.122	0.081	0.172	0.135	0.172										
25	<i>M. omeimontis</i> KX811884	0.040	0.099	0.051	0.113	0.108	0.130	0.065									
26	<i>M. palpebralespinosa</i> KX811888	0.047	0.101	0.059	0.118	0.115	0.122	0.089	0.036								
27	<i>M. parva</i> JN848362	0.057	0.102	0.061	0.123	0.128	0.149	0.076	0.039	0.057							
28	<i>M. shapingensis</i> KX811905	0.074	0.114	0.078	0.115	0.104	0.129	0.105	0.061	0.065	0.080						
29	<i>M. spinata</i> AY526205	0.057	0.113	0.071	0.139	0.125	0.149	0.073	0.029	0.057	0.060	0.081					
30	<i>M. tuberoanulata</i> KX811823	0.038	0.099	0.048	0.120	0.106	0.129	0.054	0.034	0.046	0.050	0.068	0.034				
31	<i>M. wuliangshanensis</i> KX811881	0.053	0.108	0.055	0.118	0.108	0.137	0.065	0.028	0.046	0.070	0.044	0.034				
32	<i>M. wushanensis</i> KX811838	0.046	0.105	0.059	0.126	0.113	0.132	0.062	0.034	0.049	0.070	0.049	0.015	0.038			
33	<i>Megophrys</i> sp. 3. KX811871	0.053	0.110	0.055	0.116	0.119	0.135	0.076	0.023	0.044	0.037	0.080	0.039	0.034	0.042		
34	<i>Peldoytes punctatus</i> DQ642114	0.168	0.180	0.159	0.165	0.172	0.180	0.216	0.153	0.149	0.176	0.151	0.199	0.158	0.157	0.145	

sequence divergences between the newly collected specimens and all other homologous sequences available on GenBank were  $\geq 2.1\%$ . An uncorrected sequence divergence of  $\geq 2.1\%$  is comparable with genetic distances between other morphologically diagnosable species in the genus (Wang *et al.* 2014; Chen *et al.* 2017), and indeed Chen *et al.* (2017) used 2% divergence as the threshold *p*-distance for discriminating 19 putative new species. An uncorrected sequence divergence of 0.8%, as observed here between *Megophrys* sp. 3 and the newly collected species is broadly congruent with intraspecific variation reported elsewhere within the subgenus (Li *et al.* 2014). Sequences from the newly collected *Megophrys* specimens were most closely related to species within the subgenus *Panophrys* (Table 2), supporting the position of the species in the subgenus.

***Megophrys (Panophrys) rubrimera* sp. nov.**

Figs. 2, 3, 4, 5 & 6; Table 3.

—*Megophrys kuatunensis* Orlov *et al.* 2000:10 (partim: *Megophrys* cf. *kuatunensis* “Mount Fan Si Pan, Hoang Lien Range (Sa Pa District, Lao Cai Province)”; Orlov *et al.* 2002:83 (partim: “northern Vietnam”); Nguyen *et al.* 2009:88 (partim: Sa Pa District, Lao Cai Province, Vietnam).

**Holotype:** VNMN 2017.002 adult male calling beside a 1 m wide rocky stream (stream bed 5–6 m wide) in heavily disturbed evergreen forest, Sa Pa District, Lao Cai Province, Vietnam (22.38205°N, 103.78745°E, 1708 m asl; Fig. 1). Collected at 22:15 h on 18 June 2012 by Jodi J. L. Rowley, Dau Quang Vinh, Pham Van Sang, Tran Van Tu, Hang A Su, Hoang A Di and Dinh Van Xuan.

**Paratypes:** Two male specimens (VNMN 2017.003, AMS R177676) collected from a 3.5 m wide rocky stream in slightly disturbed evergreen forest, Sa Pa District, Lao Cai Province, Vietnam (22.39829°N, 103.78545°E, 1400 m asl; Fig. 1) on 17 June 2012 by Jodi J. L. Rowley, Dau Quang Vinh, Pham Van Sang, Tran Van Tu, Hang A Su, Hoang A Di and Dinh Van Xuan. One adult male specimen (AMS R177675) collected from disturbed habitat; a 2 m wide rocky stream in slightly disturbed evergreen forest, Sa Pa District, Lao Cai Province, Vietnam (22.38208°N, 103.78699°E, 1722 m asl) between 22:00 and 22:30 h on 22 June 2016 by Jodi J. L. Rowley, Benjamin Tapley and Nguyen Thanh Chung. Three adult male specimens (AMS R177677, AMS R177678 and AMS R177679) collected from disturbed habitat; a steep north-easterly facing road side bank with seepages and a small stream, with evergreen forest nearby, Sa Pa District, Lao Cai Province, Vietnam (22.3809°N, 103.78798°E, 1714 m asl; Figs 1 & 7 C–E) between 22:00 and 22:30 h on 23 June 2016 by Jodi J. L. Rowley, Benjamin Tapley and Nguyen Thanh Chung.

**Referred specimens:** One adult male specimen (field tag JJLR03813 - HNLP2016062200001) collected from disturbed habitat; a 2 m wide rocky stream in slightly disturbed evergreen forest, Sa Pa District, Lao Cai Province, Vietnam (22.38208°N, 103.78699°E, 1722 m asl) between 22:00 and 22:30 h on 22 June 2016 by Jodi J. L. Rowley, Benjamin Tapley and Nguyen Thanh Chung. Specimen deposited at Hoang Lien National Park Headquarters, Vietnam. Tadpole specimen (AMS R177680) collected from a pool in a 2 m wide rocky stream in slightly disturbed evergreen forest, Sa Pa District, Lao Cai Province, Vietnam (22.38208°N, 103.78699°E, 1722 m asl) between 22:00 and 22:30 h on 22 June 2016 by Jodi J. L. Rowley, Benjamin Tapley and Nguyen Thanh Chung. The pH of the water body was 7.7, alkalinity 0.1 ppm and the water temperature was 17.6 °C.

**Etymology:** The specific name “*rubrimera*”, an invariable noun in apposition, derived from the Latin word *ruber* (prefix *rubri-*) meaning red, and the Latinised version of the Greek noun *mera*, meaning thigh, in reference to the bright red-orange colouration of the groin, and the inner and outer thighs of the new species.

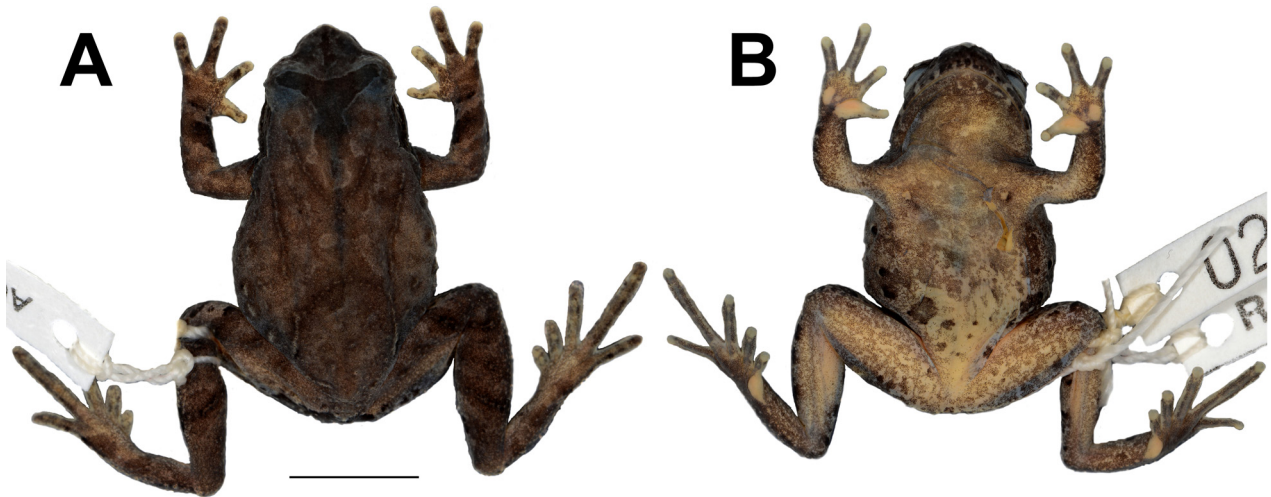
**Suggested vernacular name:** Red-thighed horned frog (English), Cóc sùng đùi đỏ (Vietnamese)

**Diagnosis:** Assigned to the genus *Megophrys* on the basis of tadpole morphology; tadpoles have dorsally orientated umbrelliform oral discs (Dubois & Ohler 1998; Li *et al.* 2011), which is diagnostic for the genus within Megophryidae; and to the subgenus *Panophrys* on the basis of molecular data.

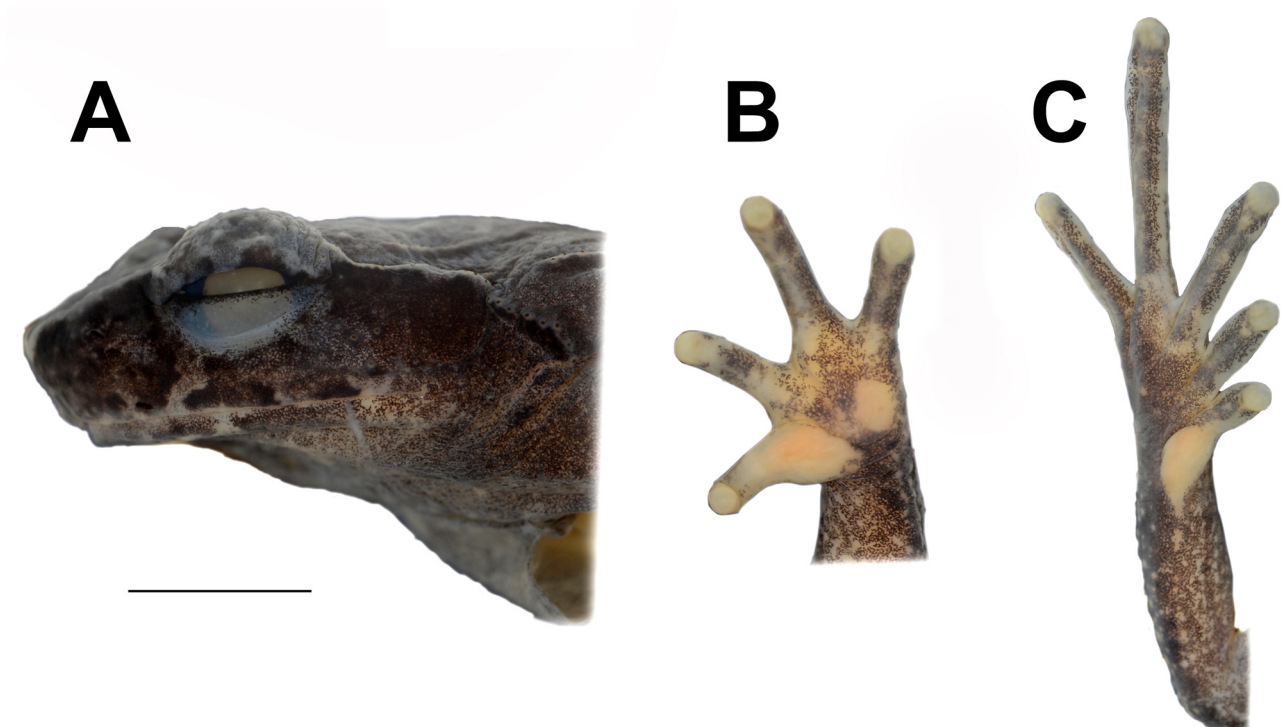
*Megophrys rubrimera* sp. nov. differs from its congeners by a combination of the following morphological characters (based on eight adult males): (1) small size (SVL 26.7–30.5 mm); (2) very small palpebral horn on upper eyelid; (3) toes lacking interdigital webbing but possessing narrow lateral fringes; (4) tympanum diameter : eye diameter 58.0–76.0 mm; (5) shank length: snout vent length 48.0–56.0%; (6) groin, inner surface of thighs and outer surface of shanks red-orange; (7) absence of subarticular tubercles on fingers and toes; (8) red-orange inner

metatarsal tubercle; (9) head width greater than head length; (10) head width : snout vent length 38.0–42.0%; (11) weakly defined vomerine ridges with teeth; and (12) an advertisement call with a dominant frequency of 3.23 kHz.

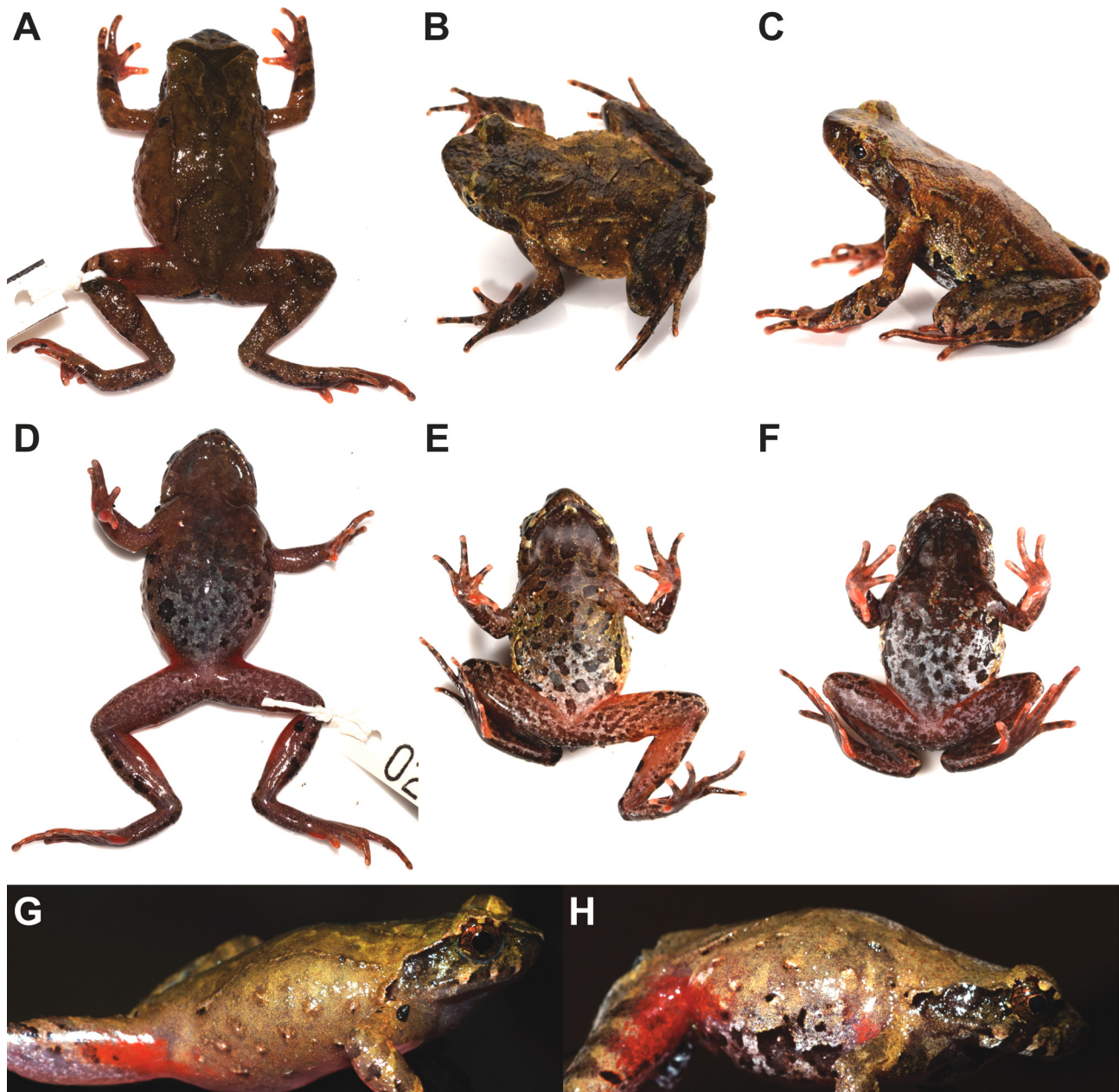
**Description of holotype:** Sexually mature male (Figs. 2 & 3). Head small, wider than long; the snout rounded in dorsal view and obtusely protruding in lateral view, rostral appendage absent (Figs. 3A & 4G); loreal region vertical and concave; canthus rostralis angular; dorsal region of the snout is slightly concave; eye diameter nearly twice the maximum tympanum diameter and subequal to snout length; eye-tympanum distance is smaller than the maximum tympanum diameter; tympanum round and orientated vertically. Pupil in life oval, vertically orientated when dilated; nostril orientated laterally, closer to eye than snout; internarial distance exceeds eyelid width, and subequal to narrowest point between upper eyelids; weakly defined vomerine ridges present, oblique and barely separated anteriorly with small vomerine teeth; maxillary teeth present. Tongue moderately large and not clearly notched posteriorly.



**FIGURE 2.** *Megophrys rubrimera* sp. nov. in preservative (holotype VNMN 2017.002). (A) Dorsal view, and (B) ventral view. Scale bar 10.0 mm.



**FIGURE 3.** *Megophrys rubrimera* sp. nov. in preservative (holotype VNMN 2017.002). (A) lateral view of head, (B) palmar surface of left hand, and (C) plantar surface of right foot. Scale bar 5.0 mm.



**FIGURE 4.** *Megophrys rubrimera* sp. nov. in life. (A) Dorsal view of holotype VNMN 2017.002, (B) dorsolateral view of paratype AMS R177679, (C) lateral view of referred specimen HNL2016062200001, (D) ventral view of holotype VNMN 2017.002, (E) ventral view of paratype AMS R177679, (F) ventral view of referred specimen HNL2016062200001, and (G) lateral view showing groin coloration of holotype VNMN 2017.002 and (H) lateral view showing groin coloration of paratype AMS R177676.

Forelimbs short and stocky, forearm length shorter than hand length; fingers short and wide without lateral fringes (Fig. 3B), finger length formula  $I < II < IV < III$ ; interdigital webbing absent, subarticular tubercles absent, palmar tubercles absent; supernumerary tubercles absent, but skin raised on articulations of Fingers III, IV; thenar tubercle absent; finger tips slightly expanded and flattened to oval pads; terminal grooves absent. Hind limbs relatively short and stocky; thigh longer than shank, and longer than foot; toes relatively short and wide, lateral fringes present (Fig. 3C); toe tips very slightly dilated, terminal grooves absent; toes not webbed; outer metatarsal tubercle, subarticular and supernumerary tubercles absent; inner metatarsal tubercle prominent.

Skin of dorsal surfaces of body, limbs, and dorsal and lateral surfaces of head weakly granular; tympanum granular with its borders slightly raised; very small pointed tubercle present on outer edge of upper eyelid; supratympanic fold narrows as it passes above the tympanum, terminating above axilla, supratympanic fold with

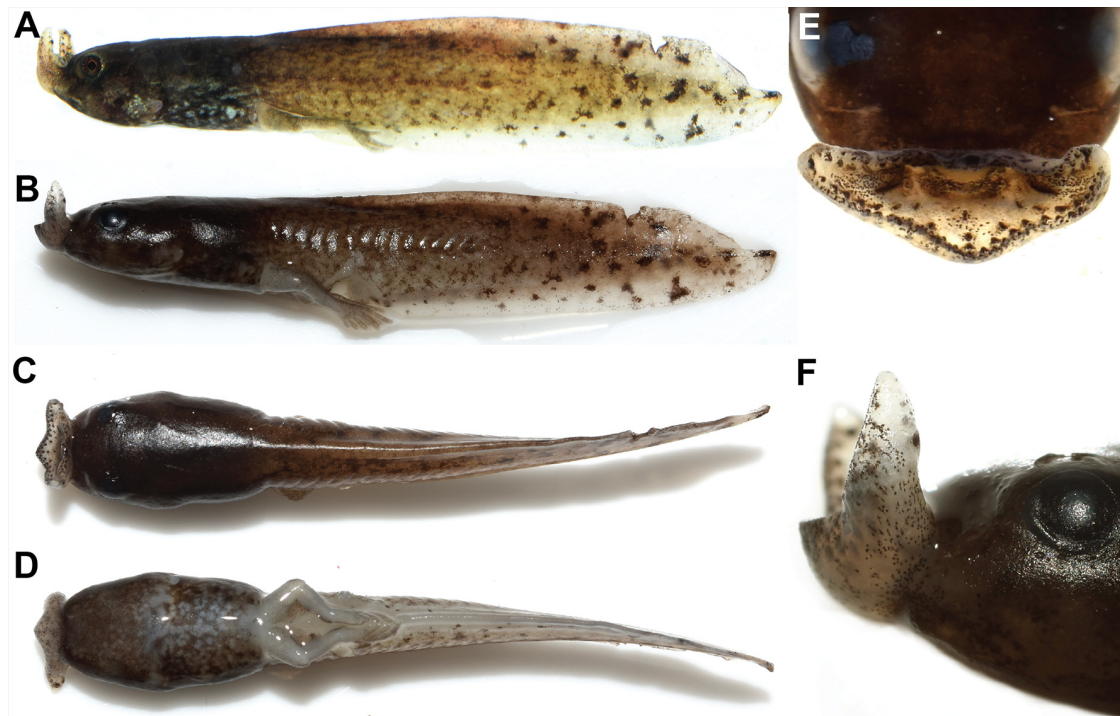


many dark asperities along its crest (Fig. 4A); flanks with small scattered tubercles, some of which terminate in darkened asperities; thin dorsolateral fold extending from behind supratympanic fold to approximately three-quarters distance to groin; a weak, “V”-shaped parietoscapular ridge present, its two sides extending posteriorly from above tympanum and meeting medially beyond level of axilla; a second inverted “U”-shaped ridge present on mid-dorsum which joins laterally with dorsolateral folds (Figs. 2A & 4A); ridges with some dark asperities; small tubercles tipped with dark asperities on dorsal surface of shanks, and arranged into distinct transverse rows on the thighs and forearms. Large distinct tubercles present in the inguinal region; gular region, chest and ventral surfaces of limbs smooth; pectoral glands obvious, small, slightly raised, positioned on level with axilla (Figs. 2B & Fig. 4D); femoral glands small, slightly raised, one positioned equidistant from knee and cloaca on the posterior surface of each thigh.

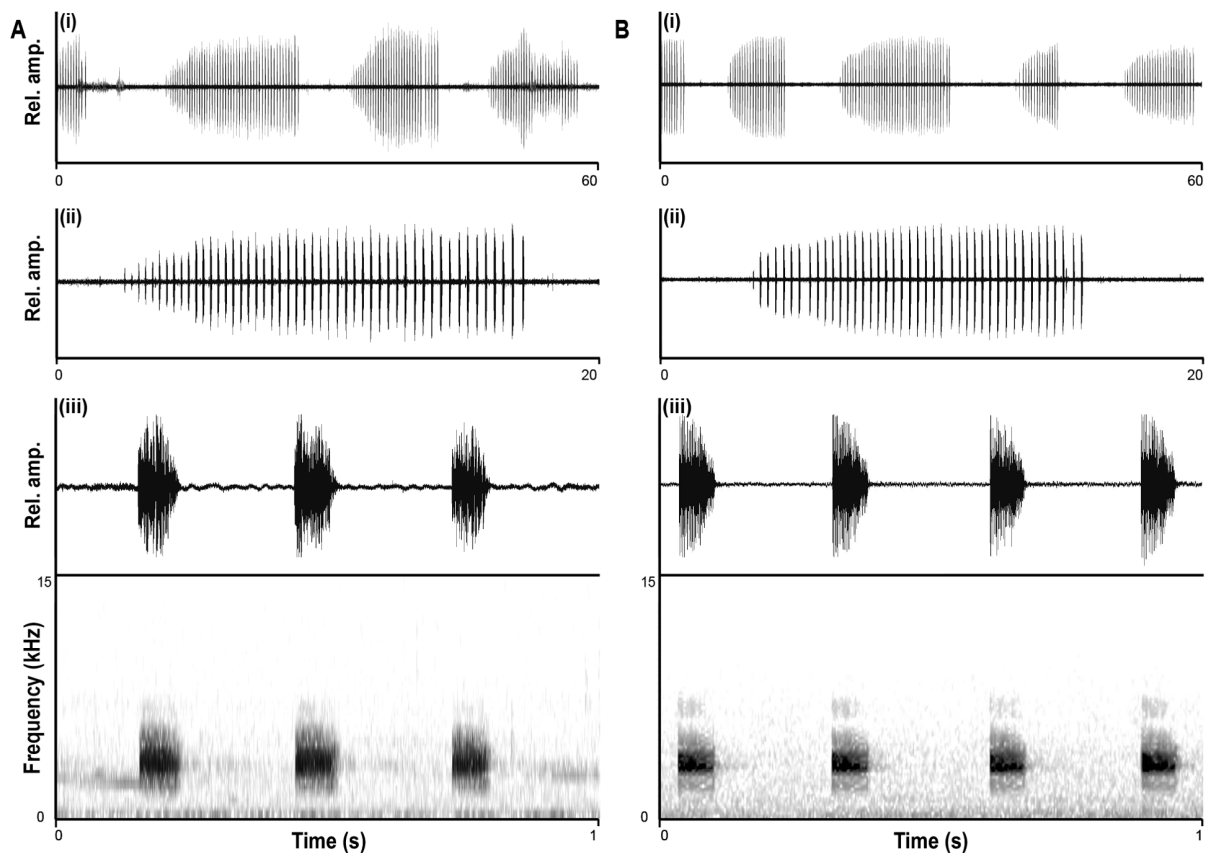
**Colour of holotype in life** (Figs. 4A–B, D & F): Dorsally light tan; darker “Y”-shaped marking on dorsum between the eyes, border of marking beige; vertical dark brown bar below eyes; lateral surface of the eyelid dark with a single medial light bar; tympanum dark brown; hind and forelimbs possess dark tan bars, darker on the forelimbs than the hind limbs; tubercles on flanks encircled by darker tan; groin red-orange; gular and pectoral region grey-brown; abdomen light grey with dark grey blotches and white speckling; ventral surface of thighs pinkish grey with darker blotches; inner thighs and outer surface of shanks red-orange; underside of Fingers III, IV orange; thenar tubercles red-orange; inner metatarsal tubercle on feet red-orange; iris metallic brown.

**TABLE 3.** Measurements (mm) of *Megophrys rubrimera* sp. nov. All specimens are male. Abbreviations defined in Materials and methods section. \*Holotype, \*\*paratypes, ^referred specimen.

	VNMN 2017.002*	VNMN 2017.003**	AMS R177676**	AMS R177675**	HNL 2016062200001^	AMS R177677**	AMS R177678**	AMS R177679**
SVL	29.8	27.9	28.7	30.5	30.2	26.7	27.4	30.4
HW	11.2	11.1	11.7	11.6	11.6	11.1	10.4	12.2
HL	9.4	10.1	11.6	11.2	11.2	10.6	9.8	11.1
IFE	3.5	3.8	4.2	4.0	4.1	3.9	3.7	4.1
IBE	3.8	3.6	4.0	4.4	3.7	3.5	3.7	4.0
EL	3.3	3.6	3.4	3.5	3.5	3.2	3.3	3.2
TYD	2.0	2.1	2.0	2.3	2.2	2.1	2.5	2.2
TYE	1.9	2.4	2.0	2.1	1.8	2.2	1.5	1.9
SL	3.9	3.8	4.4	4.1	4.0	3.6	2.8	3.9
EN	1.6	1.9	1.5	1.9	1.9	1.9	1.9	1.9
SN	2.0	2.2	2.7	1.9	1.9	1.8	1.5	1.7
IUE	4.0	3.7	4.0	3.3	3.4	3.2	3.3	3.2
IN	4.0	3.3	4.3	3.3	3.5	3.0	2.6	3.1
UEW	3.1	3.1	3.9	3.7	3.5	3.1	3.1	3.3
FAL	6.5	6.4	7.5	6.6	7.1	6.3	6.3	7.3
HAL	7.5	7.8	9.2	8.5	8.8	8.4	7.8	8.1
FIL	2.4	3.3	3.6	3.5	3.8	3.0	3.1	3.8
FIIL	2.8	3.5	4.4	3.5	4.2	2.9	3.4	3.5
FIIL	4.1	4.8	5.6	4.9	5.1	5.1	4.7	5.7
FIVL	2.9	3.1	3.3	3.6	3.4	3.3	3.4	3.6
SHL	14.4	14.8	16.1	15.6	15.5	14.9	13.6	15.9
TL	14.6	14.9	15.6	15.9	15.8	14.8	14.7	15.9
FOL	13.5	12.4	15.4	13.9	13.3	12.9	14.1	14.7
IMT	2.7	2.4	2.1	2.6	2.5	2.4	2.4	2.41
Mass in life (g)	2.4	2.0	2.3	2.5	2.5	2.3	2.3	2.6



**FIGURE 5.** *Megophrys rubrimera* sp. nov. tadpole AMS R177680. (A) Lateral view in life, (B) lateral view in preservative, (C) dorsal view in preservative, (D) ventral view of in preservative, (E) dorsal view of mouthparts in preservative, and (F) lateral view of mouthparts in preservative.



**FIGURE 6.** Advertisement call of *Megophrys rubrimera* sp. nov.: 60 s waveform of relative amplitude (Rel. amp.) over time for several call groups of AMS R177677 (Ai) and unvouchered individual (b) (Bi); 20 s waveform of Rel. amp. over time for one call group of AMS R177677 (Aii) and unvouchered individual (b) (Bii); 1 s waveform and spectrogram of Rel. amp. and frequency for three and four calls of AMS R177677 (Aiii) and unvouchered individual (b) (Biii), respectively.

**Colour of holotype in preservative:** (Figs. 2 & 3): Majority of dorsal and lateral surfaces of the head, body, forelimbs and hind limbs brown; darker brown “Y”-shaped marking between eyes; darker brown “X”-shaped marking over opposing “V”-shaped and “U”-shaped ridges; dorsolateral folds and flank tubercles bordered by darker margin; front of snout and lateral canthus rostralis dark brown; narrow vertical dark brown bar below eyes and dark brown blotch covering tympanum; upper jaw with darker blotches; two dark brown blotches on the dorsal surface of forearms; dorsal surface of Fingers II, III and IV with dark brown blotches. Gular region, chest and anterior part of abdomen primarily creamy-grey, with grey brown speckling on gular region; abdomen blotched with dark brown; ventral surfaces of thighs with grey brown mottling, and shanks with grey brown speckling; ventral surfaces of feet grey brown; area surrounding vent with dark brown blotches; forelimbs ventrally mottled and blotched with light and dark brown; extending to the ventral surface of hands; inner metatarsal tubercles unmarked and beige, tips of fingers unmarked and beige; tips of toes only lightly speckled with grey brown, if at all; lateral fringe on toes, beige, unmarked; pectoral and femoral glands beige.

**Measurements (in mm):** Holotype. SVL 29.8, HW 11.2, HL 9.4, SL 3.9, SN 2.0, EN 1.6, EL 3.3, IUE 4.0, UEW 3.1, IFE 3.5, IBE 3.8, TYD 2.0, TYE 1.9, FAL 6.5, HAL 7.5, FIL 2.4, FIIL 2.8, FIIL 4.1, FIVL 2.9, TL 14.6, SHL 14.4, FOL 13.5, IMT 2.7, weight in life 2.4 g.

**Variation:** Measurements of the type series are shown in Table 3. Paratypes and referred specimens generally agree with the holotype morphologically, but with the following exceptions: in VNMN 2017.003, AMS R177676 the middorsal “U”-shaped ridge (on the holotype) is replaced by an inverted “V”-shaped ridge. AMS R177676 with distinct dark grey blotches on ventral surface of thighs, this is lacking in VNMN 2017.003 and the holotype specimen. In life, AMS R177676 has a vivid red-orange flash on the axillary region (Fig. 4G). Gular region of VNMN 2017.002 and AMS R177678 lack dark blotches, present in all other specimens. Ventral surface of thighs (on AMS R177679 and AMS R177676) are covered in dark blotches, which are not present on any of the other specimens. Tongue weakly notched in one specimen (HNL2016062200001).

**Secondary sexual characters:** Females of *Megophrys rubrimera* sp. nov. are currently unknown. Two male specimens (AMS R177678 and AMS R177679) possessed raised oval nuptial pads covered in microspinules on the base of Finger II; protruding fleshy projection (secondary sexual characteristic of some male *Megophrys* e.g. *M. caudoprocta*, *M. koui* and *M. pachyproctus*) above the cloaca absent from all specimens. After fixation it was very difficult to open the mouths of the specimens without damaging them due to the small head size. It was therefore not possible to determine the position, presence or absence of internal vocal slits which are a secondary sexual characteristic used as a diagnostic character to the species level (e.g. Mahony *et al.* 2013).

**Tadpole:** (Fig. 5): The following tadpole description is based on a single specimen (AMS R177680) at Stage 37, the tadpole was confirmed as that of *M. rubrimera* sp. nov. by molecular analysis. Body elongated and slender; nares oval and are closer to the eye than to the snout; internarial distance is subequal to the interorbital distance; eyes positioned dorsolaterally, the pupils are round; the spiracle is sinistral and the spiracular tube protrudes from midway along the body wall just posterior to maximal trunk width and opens laterally; the tail makes up 68% of the total body length; the dorsal tail fin is low, particularly at the proximal half of the tail length; the basal tail width is 60% of the maximal trunk width; the oral disk is subterminal and antero-dorsal; the width of the umbelliform oral disc makes up ~80% of the maximal width of the trunk; the upper and lower lips each have deep medial emarginations; submarginal papillae are present on both sides between the oral orifice and the emarginations of the upper and lower lip, concentrated in a row around the margins; keratodonts are absent.

**Colour in life:** Dorsally, the head and body are brown with darker speckles; obvious pale neuromasts; the oral disk is translucent yellowish brown with dark brown submarginal papillae; the dorsal and ventral fins opaque, pale yellowish brown with speckles; the venter is speckled white and brown; iris orange, speckled with black dots.

**Colour in preservative:** Body brown with darker speckles; oral disc translucent grey brown with dark brown submarginal papillae; the dorsal and ventral fins opaque, pale grey brown with darker speckles; the venter is speckled grey and brown.

**Tadpole body measurements (in mm):** BL 10.5, BH 4.4, BS 4.3, ES 1.7, IND 1.7, IOD 4.1, BW 5.4, ED 1.2, LFH 1.3, MTH 5.9, NE 1.9, ODW 4.5, SN 0.6, SS 5.7, TAL 22.8, TTL 33.3, TMH 3.1, TMW 5.4, UFH 1.3).

**Advertisement call:** Call descriptions are based on the calls of one paratype (AMS R177677) and two unvouchered individuals. Advertisement calls were recorded at 21.0–22.9 °C ambient temperature. Calls were an average of 73.3 ms (62–85 ms) in duration (Table 4; Fig. 6). Calls of unvouchered individual (b) contained an average of 23.05 pulses (19–25), whereas the calls of AMS R177677 and another unvouchered individual (a) were

not distinctly pulsed. The average dominant frequency of calls was 3.3 kHz (3.2–3.4 kHz). Calls were repeated at a rate of approximately 3.25 (3.05–3.37) calls per second, and had an average intercall interval of 221.3 ms (190–261 ms). The number of calls within each call group ranged from 16–51, with an average of 38.7. Most call groups began at a relatively low amplitude, increasing with each call up to approximately a quarter to a third of the duration, after which amplitude remained relatively constant (Fig. 6A i–ii & B i–ii). Individual calls either began with a medium relative amplitude and peaked near the middle before declining towards the end (Fig. 6A iii), or peaked at the beginning, steadily declining throughout (Fig. 6B iii).

**TABLE 4.** Measurements of advertisement call parameters for adult *Megophrys rubrimera* **sp. nov.** Parameter values are given as means (and ranges).

	AMS R177677	Unvouchered individual (a)	Unvouchered individual (b)
Number of call groups measured	3	3	5
Total number of calls analysed	20	20	20
Call duration (ms)	74.2 (56–83)	79.6 (72–85)	66.0 (62–70)
Intercall interval (ms)	207.0 (190–235)	232.8 (217–261)	225.6 (208–324)
Call repetition rate (calls/s)	3.3	3.1	3.4
Calls/call group	39.3 (34–50)	45.6 (42–51)	31.2 (16–47)
Pulses/call	Not distinctly pulsed	Not distinctly pulsed	23.05 (19–25)
Dominant frequency (kHz)	3.2 (3.2–3.4)	3.4 (3.4–3.4)	3.2 (3.2–3.4)
Temperature (°C)	22.9	21.0	22.1

**Natural history:** All specimens of *Megophrys rubrimera* **sp. nov.** were found in disturbed evergreen secondary forest. Males were calling during June 2012 and 2016, and tadpoles (Stage 37) were collected in June 2016, suggesting a prolonged breeding season or that the species has a long larval period. Males were observed calling on stream-side vegetation (Fig. 7A–B) and a steep north-eastern facing roadside bank with seepages and a small stream (Fig. 7C–E). Females were not observed.

**Distribution and conservation status:** This species is known from between 1400 m asl and 1722 m asl at two localities, 2 km apart in Sa Pa District, Lao Cai Province, Vietnam and 50 km north-west at Maandi, Jinping County, Ailao Mountain Range, Yunnan Province, China (Fig. 1; Chen *et al.* 2017). The species' area of occupancy (AOO) and extent of occurrence (EOO) are currently predicted to be 385 km<sup>2</sup> and 2298 km<sup>2</sup>, respectively. The Vietnamese and Chinese portions of this species' range comprise two threat-defined locations; land use likely differs between the two countries, and habitat loss probably affects the species independently in each. Ongoing disturbance to the species' habitat due to forest clearance for agriculture has been observed in Sa Pa District. We recommend that *Megophrys rubrimera* **sp. nov.** is listed as Endangered in accordance with the IUCN Red List of Threatened Species categories and criteria B1ab(iii).

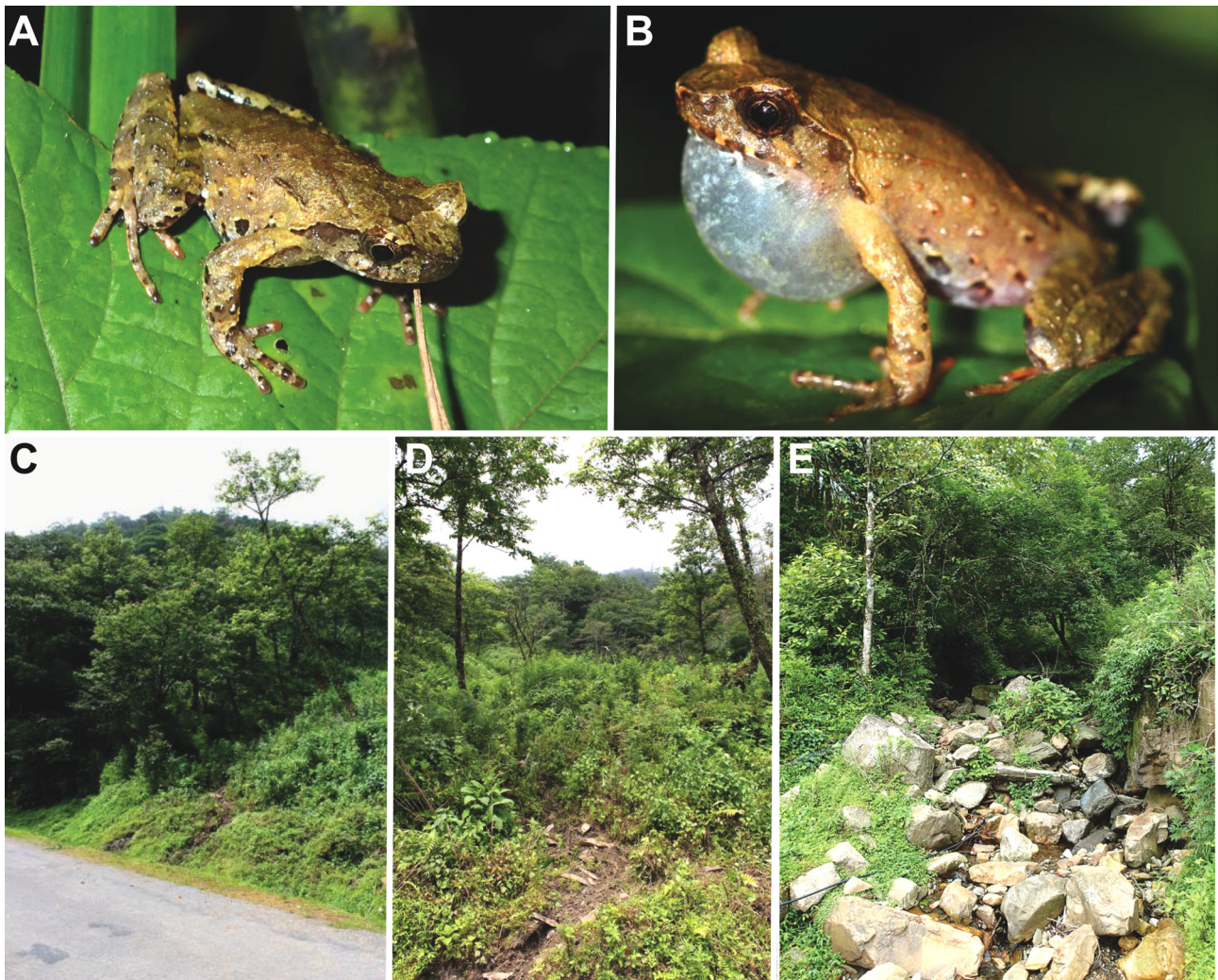
**Comparisons:** *Megophrys (Panophrys) rubrimera* **sp. nov.** can be distinguished from all other congeners found in mainland southeast Asia, north of the Isthmus of Kra and neighbouring provinces of China on the basis of morphology, and from all congeners for which comparable data is available on the basis of molecular and acoustic data. Comparisons with each subgenus are discussed separately below.

Subgenus *Brachytarsophrys*: *Megophrys rubrimera* **sp. nov.** can be distinguished from the species in the subgenus *Brachytarsophrys* (*M. carinense*, *M. feae* and *M. intermedia*) in mainland southeast Asia and neighbouring provinces of China by the absence of a transverse fold at the base of the head (versus presence), and having a smaller adult male size, SVL 26.7–30.5 mm (versus SVL > 79.1 mm; examined material).

Subgenus *Ophryophryne*: *Megophrys rubrimera* **sp. nov.** can be distinguished from *M. elfina* by the presence of lateral fringes on the toes (versus absence; Poyarkov *et al.* 2017) and by the presence of vomerine teeth (versus absence Poyarkov *et al.* 2017); from *M. gerti* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 31.7–42.2 mm; Poyarkov *et al.* 2017; material examined); from *M. hanshi* by having a smaller adult male size, SVL 33.4–43.1 mm; Ohler 2003; Poyarkov *et al.* 2017; material examined); from *M. microstoma* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 34.3–44.4 mm; Poyarkov *et al.* 2017; material examined); from *M. kouli* by the absence of a protruding fleshy projection above the cloaca in sexually mature males (secondary sexual



characteristic of sexually mature *M. kouii*); from *M. synoria* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 38.2–53.7 mm; Stuart *et al.* 2006b; Poyarkov *et al.* 2017; material examined) and further from all species in the subgenus *Ophryophryne* due to the presence (versus absence) of maxillary teeth.



**FIGURE 7.** *Megophrys rubrimera* sp. nov. *in situ*, and habitat at collection localities. (A) Adult male paratype AMS R177675 and (B) unvouchered calling male. (C–E) Habitat at type locality in Sa Pa District, Lao Cai Province, Vietnam.

Subgenus *Xenophrys*: *Megophrys rubrimera* sp. nov. differs from *M. auralensis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 60.1–76.7 mm; Ohler *et al.* 2002; Neang *et al.* 2013), and a lack of interdigital toe webbing (versus rudimentary webbing); from *M. damrei* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 47.5–57.1 mm; Mahony 2011; Neang *et al.* 2013; material examined), a lack of interdigital toe webbing (versus rudimentary), and the presence of lateral fringes on the toes (versus absence); from *M. glandulosa* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 76.7–81.6 mm; Fei *et al.* 2009; material examined), and a lack of interdigital webbing between the toes (versus basal webbing); from *M. leakaguli* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 55.6–68.1 mm; Stuart *et al.* 2006a; material examined), a lack of webbing between the toes (versus rudimentary webbing), and the presence of lateral fringes on the toes (versus absence); from *M. major* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 72.4–79.4 mm; material examined), a lack of toe webbing (versus at least one quarter webbed), and the absence of a light-coloured upper lip stripe (versus presence); from *M. maosonensis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 58.0–76.0 mm; Bourret 1937), the absence of toe webbing (versus toes up to one quarter webbed), and the absence of a light-coloured upper lip stripe (versus presence); from *M. pachyproctus* by having a larger tympanum to eye ratio, TYM : EYE 58.0–76.0% (versus 29.0%; Huang *et al.* 1981), and the absence of a protruding projection posterior to cloaca on male specimens (versus presence); from *M. parva* by having a smaller

adult male size, SVL 26.7–30.5 mm (versus 33.9–36.0 mm; material examined), a relatively larger tympanum to eye ratio, TYM : EYE 58.0–76.0% (versus 43.6–55.0%; material examined), and the presence of lateral fringes on the toes (versus absence); from *M. takensis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 47.3–53.0 mm; Mahony 2011), a lack of toe webbing (versus rudimentary webbing), and the presence of lateral fringes on the toes (versus absence)

Subgenus *Panophrys*: Phylogenetic analysis places *Megophrys rubrimera* **sp. nov.** in the subgenus *Panophrys*. *Megophrys rubrimera* **sp. nov.** is most similar, in terms of morphology, to *M. kuatumensis* with whom it has been confused with in the past, but differs from this species by typically having relatively longer shanks, SHL : SVL 48.0–56.0% (versus 42.7–48.6%; examined material), possessing narrow lateral fringes on the toes (versus absent), the presence of vomerine teeth (versus absence), and advertisement call (see bioacoustic comparison). It could also be mistaken for other small sized (minimum SVL < 35.0 mm) species within the subgenus *Panophrys* but differs from *M. acuta* by the presence (versus absence) of vomerine teeth, and absence (versus presence) of subarticular tubercles on the base of each finger; from *M. binchuanensis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 32.0–36.0 mm; Li *et al.* 2014) and the presence of vomerine teeth (versus absence); from *M. boettgeri* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 34.5–38.3 mm; Boulenger 1899; material examined), a lack of toe webbing (versus rudimentary webbing), and advertisement call (see bioacoustic comparison); from *M. brachykolos* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 33.9–40.5 mm; material examined), possessing narrow lateral fringes on the toes (versus absent), and the presence of vomerine teeth (versus absence); from *M. daweimontis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 34.0–37.0 mm; Rao & Yang 1997), the presence of lateral fringes on the toes (versus absence), and Finger I being shorter than the Finger II (versus Finger I being longer than Finger II); from *M. minor* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 32.2–40.5 mm; Li *et al.* 2014; examined material), a lack of toe webbing (versus rudimentary webbing), the presence of vomerine teeth (versus absence), and advertisement call (see bioacoustic comparison); from *M. wuliangshanensis* by the presence of vomerine teeth (versus absence), and the presence of lateral fringes on the toes (versus absence). *Megophrys rubrimera* **sp. nov.** differs from larger bodied (SVL > 35.0 mm) species within the subgenus *Panophrys*, all of which possess interdigital webbing to some degree. *Megophrys rubrimera* **sp. nov.** differs from *M. jingdongensis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 53.0–57.0 mm; Fei *et al.* 2012; Li *et al.* 2014), and a lack of interdigital webbing (versus at least one quarter webbed); from *M. latidactyla* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 38.9 mm; Orlov *et al.* 2015), possessing narrow lateral fringes on the toes (versus wide), and the absence of interdigital webbing (versus at least one quarter webbed); from *M. omeimontis* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 56.0–60.9 mm; Li *et al.* 2014; examined material), and a lack of toe webbing (versus rudimentary webbing); from *M. palpebralespinosa* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 36.2–39.1 mm; Li *et al.* 2014; examined material), the presence of vomerine teeth (versus absence), and a lack of interdigital webbing between the toes (versus approximately half webbed); from *M. spinata* by having a smaller adult male size, SVL 26.7–30.5 mm (versus 47.2–54.4 mm; Li *et al.* 2014), the presence of vomerine teeth (versus absence), the lack of interdigital webbing between toes (versus at least one quarter webbed), and the absence of large keratinised spines on nuptial pads of sexually mature males (versus presence).

**Bioacoustic comparison:** The male advertisement call of *Megophrys rubrimera* **sp. nov.** differs from those of its seven congeners (subgenus *Panophrys*) for which calls have been described. The male advertisement call of *Megophrys rubrimera* **sp. nov.** differs from *M. acuta* by having a call duration of 62–85 ms (versus 106–172 ms), 16–51 calls per call group (versus 7–9), an intercall interval of 190–261 ms (versus 335–747 ms), a call repetition rate of 3.05–3.37 calls/s (versus 1.87–2.04 calls/s), and relatively high amplitude variation within calls (versus relatively constant amplitude within calls) at 21.0–22.9 °C (versus 26.9 °C); from *M. boettgeri* by having an average call duration of 73.3 (62–85) ms (versus average 54 ms), an average call repetition rate of 3.25 (3.05–3.37) calls/s (versus an average of 4.99 calls/s) at 21.0–22.9 °C (versus 15.0–18.0 °C); from *M. jinggangensis* by having an average call repetition rate of 3.25 (3.05–3.37) calls/s (versus average 5.70) at 21.0–22.9 °C (versus 15.0–18.0 °C); from *M. huangshanensis* by having an average call duration of 73.3 (62–85) ms (versus average 86.0 ms), an average intercall interval of 221.3 (190–261) ms (versus average 165.0 ms), and an average call repetition rate of 3.25 (3.05–3.37) calls/s (versus average 4.10) at 21.0–22.9 °C (versus 15.0–18.0 °C); from *M. kuatumensis* by having an average call duration of 73.3 (62–85) ms (versus average 208.0 ms), an average intercall interval of 221.3 (190–261) ms (versus average 970.0 ms), an average call repetition rate of 3.25 (3.05–3.37) calls/s (versus

average 0.97), and an average of 38.7 (16–51) calls per call group (versus average 11.3) at 21.0–22.9 °C (versus 15.0–18.0 °C); from *M. lini* by having an average call duration of 73.3 (62–85) ms (versus average 106 ms), and an average of 38.7 (16–51) calls per call group (versus average 10.2) at 21.0–22.9 °C (versus 15.0–18.0 °C); from *M. minor* by having a dominant frequency of 3.2–3.4 kHz (versus 3.4–3.5 kHz), a call duration of 62–85 ms (versus 75–110 ms), an intercall interval of 190–261 ms (versus 213–363 ms), an average call repetition rate of 3.25 (3.05–3.37) calls/s (versus average 4), and an average of 38.7 (16–51) calls per call group (versus 8–16) at 21.0–22.9 °C (versus 14.0 °C).

### ***Megophrys (Panophrys) kuatunensis* Pope, 1929**

Figs. 8 & 9; Table 5.

*Megalophrys kuatunensis* Pope 1929:1 (partim: see Remarks section).

—*Megophrys kuatunensis* Gee & Boring 1929:20.

—*Megophrys (Megophrys) kuatunensis* Dubois 1980:472.

—*Panophrys kuatunensis* Rao and Yang 1997:98.

—*Xenophrys kuatunensis* Delorme *et al.* 2006:17.

—*Megophrys (Panophrys) kuatunensis* Mahony *et al.* 2017:755.

**Holotype:** Adult male (AMNH 30126), type locality: “Kuatun [Village], Chungan Hsien, northwest Fukien Province, China, 5500–6000 feet.” (=Guadun [*ca.* 27°40'N, 117°40'E, *ca.* 1675–1830 m asl], Wuyishan County, Nanping Prefecture, Fujian Province), collected by Clifford H. Pope, April–September 1926 (Pope 1929, 1931).

**Paratypes:** AMNH 30123–30124; AMNH 30239–30258, FMNH 24406 (formerly AMNH 30230), FMNH 24408 (formerly AMNH 30232), FMNH 24411–24413 (formerly AMNH 30235–30237), BMNH 1961.956 (formerly AMNH 30238, then FMNH 24414), BMNH 1985.1294 (formerly AMNH 30234, then FMNH 24410), BMNH 1985.1295 (formerly AMNH 30231, then FMNH 24407), MCZ 28297 (formerly AMNH 30233, then FMNH 24409).

**Examined specimens:** Full morphological datasets were taken for five adult males, holotype and paratypes (AMNH 30126, 30240–30241, 30243–30244), and four adult female paratypes (AMNH 30242, BMNH 1961.956, BMNH 1985.1295, FMNH 24411). SVL measurements were taken for three additional adult male paratypes (FMNH 24406, FMNH 24412–24413).

**Holotype description:** (Figs. 8 & 9; Table 5 for measurements): Sexually mature adult male. Head moderately small, width subequal to length; snout rounded in dorsal view, obtusely protruding in lateral view, without rostral appendage (Fig. 8); loreal region vertical and weakly concave; canthus rostralis angular; dorsal region of snout slightly concave; eye diameter greater than three times as long as maximum tympanum diameter, and longer than snout; eye-tympanum distance subequal to maximum tympanum diameter; tympanum circular, its upper edge not concealed by supratympanic ridge (Fig. 8); nostril orientated laterally, closer to eye than snout; internarial distance greater than eyelid width, and subequal to narrowest point between upper eyelids; pineal ocellus not visible externally; vomerine ridges and vomerine teeth absent; tongue moderately large, weakly notched posteriorly, with no medial lingual process.

Forelimbs moderately short and thin, forearm moderately enlarged relative to upper forelimb, and shorter than hand; fingers short and narrow without lateral fringes (Fig. 9B), finger length formula  $IV < I < II < III$ ; interdigital webbing and supernumerary tubercles absent; subarticular tubercles on Fingers I and II only; thenar and outer palmar tubercles weak; finger tips rounded, narrower than adjoining end of distal phalanges; pads on finger tips and terminal grooves absent. Hindlimbs relatively short and thin, shanks overlap when thighs are held at right angle to body; thigh shorter than shank and foot; toes long and rounded without lateral fringes (Fig. 9), relative toe lengths  $I < II < V < III < IV$ ; toe tips rounded, narrower than adjoining end of distal phalanges, with weakly discernible pads; terminal grooves absent; webbing, outer metatarsal tubercle, subarticular and supernumerary tubercles absent; inner metatarsal tubercle weak; ridge of callous tissue on ventral surface of digits absent.

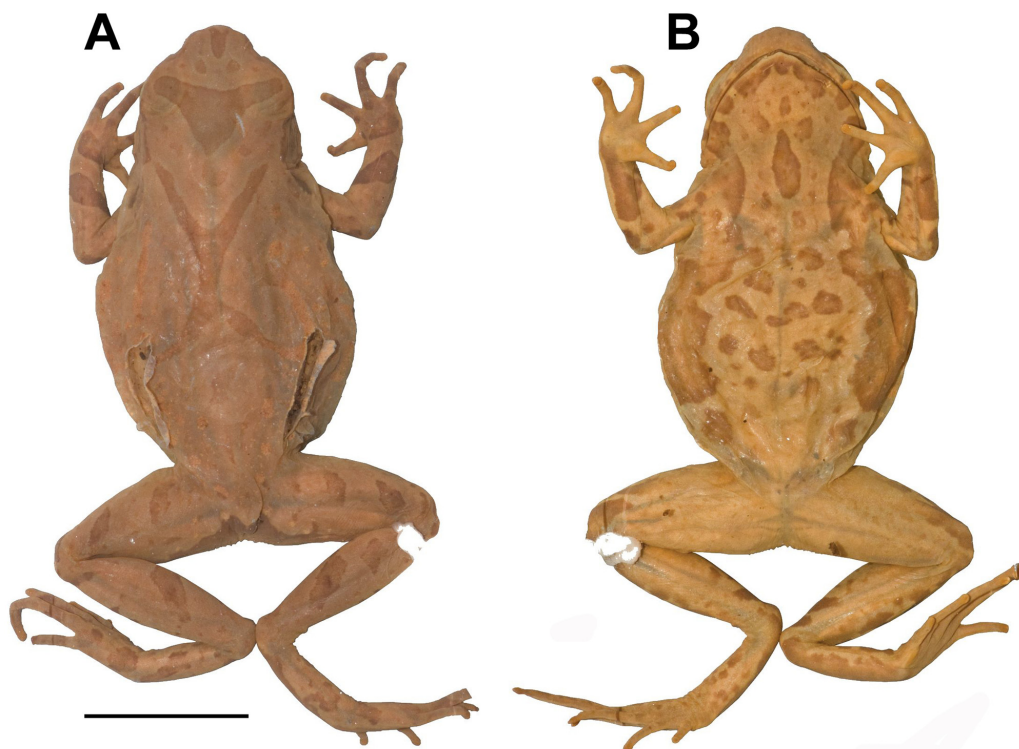
Skin of dorsal, lateral and ventral surfaces of head, body and limbs primarily smooth; tympanum smooth with borders not raised; outer edge of upper eyelid with a short weak ridge; supratympanic fold narrow anteriorly, thick and glandular posteriorly, extending from orbit and sloping down along upper portion of tympanum, where it curves abruptly down, terminating above forearm insertion (Fig. 8A); flanks with distinct patches of glandular skin which may have represented tubercles when the specimen was freshly preserved; dorsolateral folds and other



dorsal ridges indistinct (Fig. 8A); pectoral glands tiny, flat, positioned on level slightly posterior to axilla (Fig. 8B); femoral gland small, slightly raised, positioned subequally distant from knees and cloaca on posterior surface of each thigh; dermal asperities absent on all surfaces.

**Colouration in preservative:** (Figs. 8 & 9): Dorsal and lateral surfaces of head, body, forelimbs and hindlimbs primarily mid-brown; solid dark brown triangular marking on dorsal surface of head between eyes; a dark brown “X”-shaped marking on dorsum, with scattered dark brown spots and blotches on dorsum around “X”-shaped marking and on dorsal surface of snout; front of snout, lateral canthus rostralis and lower half of supratympanic folds dark brown; wide vertical dark brown bar below eyes; dark brown blotch covering tympanum present; a short longitudinal dark brown stripe on central dorsum of snout; two dark brown crossbars on dorsal surface of forearms; dorsal surface of outer three fingers with dark brown transverse blotches; dorsal surface of hindlimbs with large dark brown transverse crossbars, two on thighs, one on shank, and one on tarsus, with dorsal surfaces of feet spotted. Ventral surfaces of head, body and limbs primarily light brown, with dark brown blotches along outer margin of ventral mandibles, gular region, chest and abdomen; a longitudinal dark brown blotch positioned medially, extending from the posterior gular region onto chest; a dark brown stripe extends laterally from the rear of the mandible, over the pectoral region and ventral proximal surface of forelimbs to approximately 70% distance to groin on both sides; ventral surfaces of thighs and shanks without markings; ventral surfaces of tarsus and feet dark brown; area surrounding vent and posterior surfaces of thighs dark brown; ventral surfaces of hands plain grey-brown and forearms ventrally with large dark brown blotch.

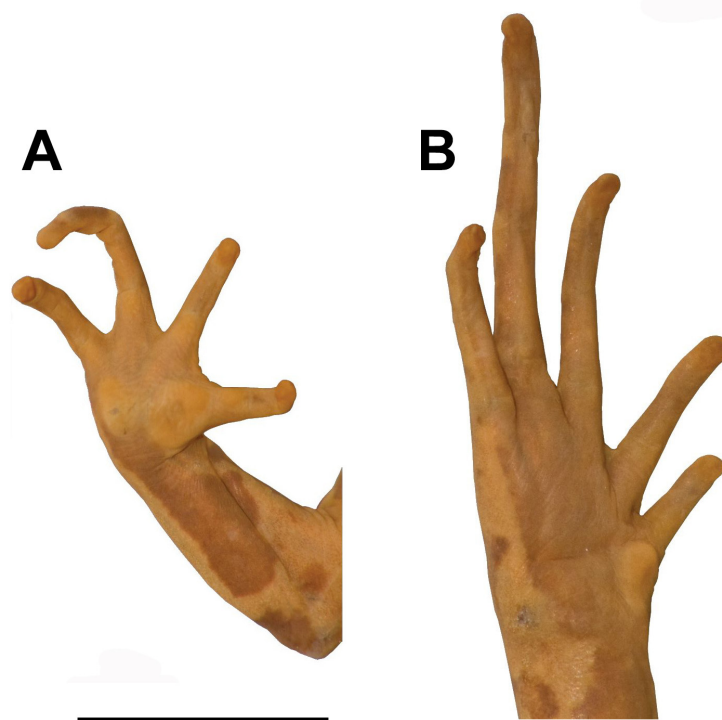
**Colouration in life:** Not recorded.



**FIGURE 8.** Adult male *Megophrys kuantunensis* holotype, AMNH 30126. (A) Dorsal view, and (B) ventral view. Scale bar 10.0 mm.

**Variation:** Refer to Table 5 for morphometric variation for five adult males and four adult females. Adult male SVL for eight paratypes ranges from 28.3 to 31.4 mm. Finger length formula varied slightly,  $IV \leq I < II < III$ . Outer palmar, thenar and outer metatarsal tubercles are more distinct on all examined paratypes. Dorsal and lateral surfaces of the head and body of examined paratypes with scattered granules and small tubercles, indicating that the smoothness of the holotype is most likely an artifact of preservation condition. Subarticular tubercles are not distinct on the base of fingers on FMNH 24411, but appear to be present on the base of all fingers on BMNH 1985.1295. No visible notch on the posterior edge of the tongue of AMNH 30242, but this may be an anomaly of preservation. Dorsolateral folds are indistinct on the holotype and some paratypes (AMNH 30240, AMNH 30242,

FMNH 24411), weakly developed and on anterior third to half of trunk length on both sides on AMNH 30241 and AMNH 30243, anterior half of trunk length on the left side only on BMNH 1985.1295, narrow and moderately to strongly developed on the anterior three quarters of the trunk length on AMNH 30244 and BMNH 1961.956, or represented by a weak row of tubercles on FMNH 24406. Supratympanic folds do not obscure the tympanum rim on all specimens except AMNH 30244 and BMNH 1961.956, on which the upper margins are covered. AMNH 30240, AMNH 30241 and BMNH 1985.1295 have outer upper eyelids slightly thickened medially, and BMNH 1961.956 has a short raised transverse fold medially, extending to the outer edge of the upper eyelids, but no specimens have distinctly pointed tubercle/s on the upper eyelids. Pectoral glands are visible on all examined paratypes, small, and situated approximately level with the axilla. Femoral glands vary from comparatively small to moderately large in size. Dorsal ridges vary from indistinct on the holotype and paratype FMNH 24411, to moderately well developed (i.e., on AMNH 30240, AMNH 30243, BMNH 1985.1295), with the following configurations observed on examined specimens: parietoscapular ridge only (“>”), parietoscapular-middorsal ridge (“>-”), complete parietoscapular-sacral ridge (“>-<”), or separate parietoscapular and sacral ridges (“> <”). The holotype is the only male with no dermal asperities. AMNH 30240 has small white spinular dermal asperities sparsely distributed on tops of tubercles and on the parietoscapular fold on the anterior dorsum, becoming moderately dense posteriorly; on AMNH 30241, asperities are visible from approximately mid trunk length, increasingly in density posteriorly; on AMNH 30242 and AMNH 30243, asperities are sparse on the tympanic region and on posterior lateral surfaces of head and anterior lateral dorsum, sparse on anterior central dorsum but from mid dorsum increases in density posteriorly. Asperities are absent from all remaining surfaces on these specimens. FMNH 204411 is the only female with dermal asperities with just a few scattered on the posterior dorsum. Colouration in preservation of examined paratypes differed from holotype based on the following characters: triangular marking between the eyes had a light central spot on AMNH 30243 and AMNH 30244; dark brown dorsolateral stripe is present on AMNH 30244; AMNH 30240, AMNH 30241 and AMNH 30243 have two to three transverse crossbars on shanks; palmar, thenar and inner metatarsal tubercles distinctly lighter than surrounding surfaces on paratypes; dark brown blotches on gular region, chest and abdomen vary in intensity and density from almost absent on AMNH 30240 to mottled on AMNH 30241; pectoral and femoral glands noticeably lighter than surrounding surfaces on all examined paratypes; ventral surfaces of thighs and shanks faintly mottled on AMNH 30240, AMNH 30241 and AMNH 30244.



**FIGURE 9.** Adult male *Megophrys kuatunensis* holotype, AMNH 30126. (A) Palmar surface of right hand, and (B) plantar surface of right foot. Scale bar 5.0 mm.

**TABLE 5.** Measurements (mm) for *Megophrys kuatunensis* partial type series. Abbreviations defined in Materials and methods section. \*Holotype, M-male, F-female.

	*AMNH A 30126	AMNH A 30240	AMNH A 30241	AMNH A 30243	AMNH A 30244	AMNH A 30242	BMNH 1961.956	BMNH 1985.1295	FMNH 24411
Sex	M	M	M	M	M	F	F	F	F
SVL	31.4	29.3	28.8	29.4	29.3	26.6	31.1	30.2	37.3
HW	10.3	9.5	9.8	10.0	9.7	9.1	10.3	10.3	11.7
HL	10.2	10.2	10.1	10.6	10.1	9.4	10.3	10.3	11.6
IFE	5.5	5.2	5.2	5.5	4.9	5.2	5.7	4.8	6.0
IBE	8.9	8.3	8.2	7.9	8.3	7.8	8.1	7.8	9.1
EL	4.1	4.3	3.9	3.7	3.7	3.5	3.6	3.9	4.2
TYD	1.5	1.4	ind	1.7	1.4	1.4	1.6	1.7	1.6
TYE	1.4	1.8	ind	1.7	1.5	1.5	1.4	1.7	2.3
SL	3.7	3.7	3.6	3.7	3.5	3.4	4.0	3.4	4.1
EN	1.7	1.7	1.8	1.8	1.9	1.7	2.1	1.4	1.9
SN	2.2	2.0	2.1	2.1	1.9	1.9	2.3	2.2	2.3
IUE	3.5	2.7	3.3	3.1	3.1	2.9	2.8	2.8	3.1
IN	3.5	3.0	3.5	3.5	2.8	3.5	3.7	3.3	3.8
UEW	3.1	2.9	3.1	2.9	2.7	2.6	3.1	2.6	3.7
FAL	6.3	5.6	6.4	6.5	6.1	5.4	8.1	7.5	7.6
HAL	7.3	6.8	7.3	7.4	7.5	6.3	8.4	7.6	9.3
FIL	3.1	2.4	2.8	2.8	3.2	2.2	3.2	3.1	3.3
FIIL	3.4	2.6	3.1	3.3	3.3	2.7	3.4	3.2	3.3
FIIL	4.8	4.2	4.5	4.8	4.3	4.3	5.8	5.0	6.0
FIVL	2.8	2.4	2.7	3.1	2.8	2.2	3.0	3.3	3.7
SHL	13.4	13.1	13.8	13.9	13.3	12.5	15.1	14.6	17.1
TL	11.5	11.8	12.3	13.1	12.7	11.1	13.3	14.2	15.8
TFOL	20.1	18.2	19.1	19.4	17.8	16.7	22.4	21.1	25.2
FOL	12.9	11.7	11.8	12.8	11.2	10.8	14.7	12.9	15.4
IMT	1.5	1.5	1.8	1.8	1.6	1.7	1.8	1.4	2.2

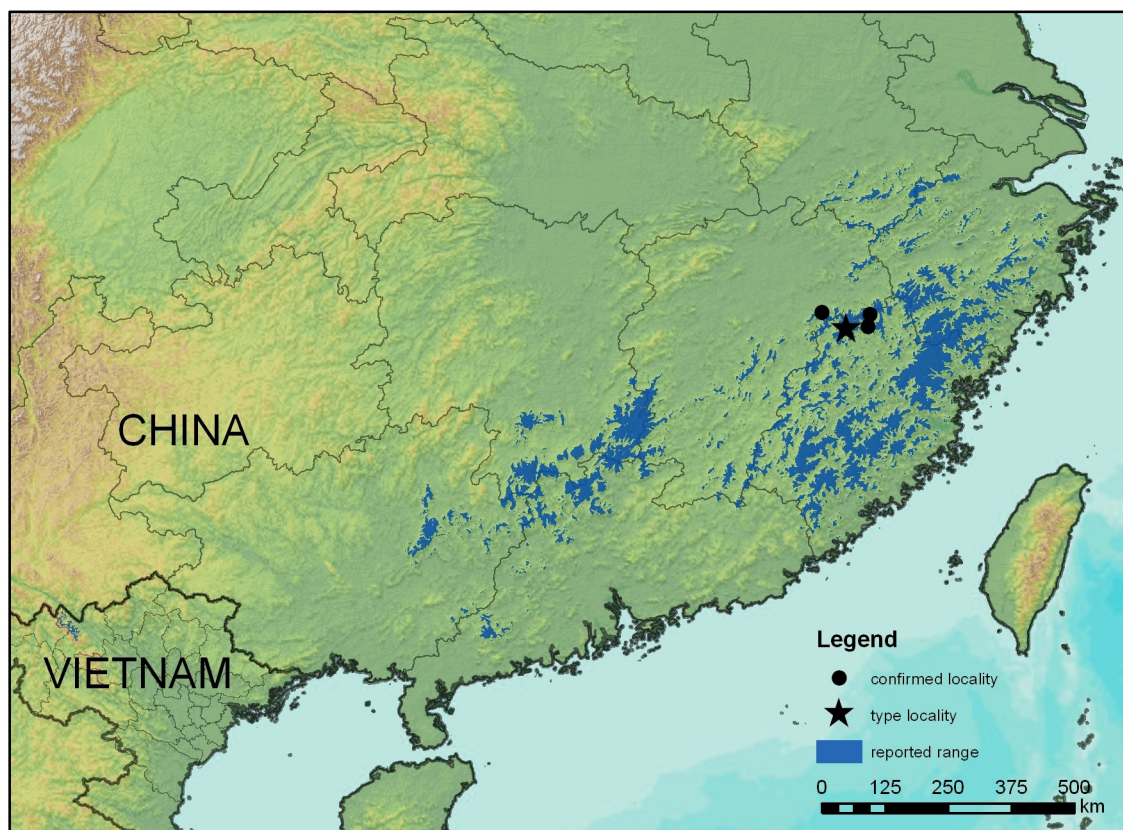
**Secondary sexual characters:** Males: weakly raised nuptial pads present, appearing smooth and translucent, or covered with brown microspinules, covering most of the dorsal surface of Finger I, narrowing distally and extending to the base of the distal phalange; nuptial pad absent on Finger II; vocal sac indistinct in preservation; internal vocal slits present near the rear of the mandible; forearms slightly to moderately enlarged relative to the upper forelimbs. Females: mature ova without pigmented poles; nuptial pads, internal vocal slits and enlarged forearms absent.

**Distribution** (Fig. 10): To our knowledge, the presence of *M. kuatunensis* has only been positively confirmed with molecular data in Fujian and Jiangxi provinces (Li *et al.* 2014; Wang *et al.* 2014; Chen *et al.* 2017). The IUCN Red List assessment for this species indicates a much wider distribution (Huiqing *et al.* 2004) including locations in Zhejiang, Hunan and Guangxi provinces. Geological barriers exist between these locations that may limit the dispersal of *Megophrys*. The identity of populations assigned to *M. kuatunensis* in Zhejiang, Hunan and Guangxi provinces should be confirmed with molecular data.

**Remarks:** Pope (1929) provided a relatively detailed description of the holotype of his new species, *M. kuatunensis*, including a brief comparison with *M. boettgeri* and *M. minor*. He later expanded on this description providing a figure of the holotype, a detailed variation section, and morphological and biological comparison with the sympatric *M. boettgeri* (Pope 1931). The type series of *Megophrys kuatunensis* was based on the holotype and 31 paratypes (Pope 1929). Pope (1931:445) included an additional specimen as a paratype, AMNH 30324, stating



that “An additional specimen has been found since the appearance of the original description which listed only 31 paratypes”. This action does not validate AMNH 30324 as a paratype, and thus this specimen is not available for lectotype designation in the future should the holotype become lost/destroyed (International Commission of Zoological Nomenclature 1999). Within the section titled “Notes on paratypes”, Pope (1929) includes a statement that “Tadpoles were secured”, neither designating a museum number, nor counting them among the 31 specimens referred to directly as paratypes, indicating that Pope did not intend to regard these tadpoles as paratypes. Pope (1931) further comments that of the two series of tadpoles (AMNH 30606 and AMNH 30645) collected from the type locality, he would only tentatively assign the former to *M. kuatunensis*. These tadpoles should not be considered to be paratypes of *M. kuatunensis*. Marx (1958) lists three paratypes in the CNHM (FMNH 24408, 24412, 24413), omitting two paratypes FMNH 24406 (formerly AMNH 30230) and FMNH 24411 (formerly AMNH 30235) from his annotated catalogue of type specimens in the collection. This omission was not amended in supplementary catalogue of FMNH types (Marx 1976), however, we confirm that both of these specimens were present in the collection during a recent visit (S. Mahony, pers. com.).



**FIGURE 10.** Distribution of *Megophrys kuatunensis* after Huiqing *et al.* 2004. Blue polygons represent the elevational range of the species; 430–1950 m asl within the distributional range reported by the IUCN. Star denotes type locality and black circles denote the locations where *M. kuatunensis* have been confirmed with molecular data.

In their description of a new species, *M. brachykolos*, Inger and Romer (1961) examined part of the type series of *M. kuatunensis* and were the first to note that the paratype series of *M. kuatunensis* is heterogeneous in species composition. They identified a *M. kuatunensis* female paratype FMNH (as CNHM) 24408 as *M. brachykolos* on the basis of several measurements and characters that fell within the variation range of the female *M. brachykolos* types. They designated this specimen as a paratype of their new species (Inger and Romer 1961; Marx 1976). Our examination of approximately half of the type series of *M. kuatunensis* (including FMNH 24408) revealed two additional paratypes (AMNH 30239 and BMNH 1985.1294 [formerly AMNH 30234, then FMNH 24410]) that are morphologically conspecific with FMNH 24408, and not *M. kuatunensis sensu stricto*. Though we partially agree with Inger and Romer (1961) in so far as these specimens share superficial similarities with *M. brachykolos sensu stricto* (and its sister taxon *M. acuta*), we suggest that their identification should be considered tentative pending

further study of this population due to geographic distance from the type locality of *M. brachykolos*, and the recognition of extensive cryptic diversity in *Megophrys* (Chen *et al.* 2017; Mahony *et al.* 2017).

## Discussion

With the description of *Megophrys rubrimera* **nov. sp.** there are now 72 species in the genus *Megophrys*, 19 of which are reported from Vietnam (two species within the subgenus *Brachytarsophrys*; six species within the subgenus *Ophryophryne*; eight species within the subgenus *Panophrys* and three species within the subgenus *Xenophrys*). *Megophrys kuatunensis* was first formally reported from Vietnam in 2002 (Orlov *et al.* 2002), prior to this, it had been tentatively identified as *Megophrys* cf. *kuatunensis* and recorded from elevations of 1400–2000 m asl on Mount Fansipan, Hoang Lien National Park (see photographs in Orlov *et al.* 2000). We suspect all reports of *Megophrys kuatunensis* in Lao Cai Province refer to *Megophrys rubrimera* **nov. sp.** To our knowledge, the presence of *Megophrys kuatunensis* has never been confirmed from Vietnam and this species should be considered endemic to China. The suspected ranges of *Megophrys rubrimera* **nov. sp.** and *M. kuatunensis* are at least 750 km apart (Huiqing *et al.* 2004). Moreover the presence of *M. kuatunensis* has only been confirmed with molecular data from sites 1780 km north-east of the predicted range of *Megophrys rubrimera* **nov. sp.** (Wang *et al.* 2014). There are no published reports of specimens referable to *Megophrys rubrimera* **nov. sp.** being collected elsewhere in Vietnam although 16S data from specimens (*M. sp.* 3) collected from Maandi, Jinping County, Yunnan Province, China (Chen *et al.* 2017) confirm that *Megophrys rubrimera* **nov. sp.** occurs 50 km to the north-west of the type locality.

Lao Cai Province is amongst the most well-surveyed regions in Vietnam in terms of amphibians (Ohler *et al.* 2000), with approximately 80 species recorded to date (Ohler *et al.* 2000; Bain & Stuart 2006; Bain *et al.* 2007; Nguyen *et al.* 2009; Orlov *et al.* 2011; Poyarkov *et al.* 2015; Matsui *et al.* 2017). Despite this, new amphibian species continue to be discovered in the Province, with four species of frog described in the last four years alone (Nguyen *et al.* 2013; Orlov *et al.* 2013; Rowley *et al.* 2013; Matsui *et al.* 2017).

Ten species of *Megophrys* are now known from Sa Pa District in Lao Cai Province; *M. daweimontis*, *M. feae*, *M. jingdongensis*, *M. kouii*, *M. maosonsensis*, *M. major*, *M. minor*, *M. pachyproctus*, *M. parva*, *M. palpebralespinosa* and *Megophrys rubrimera* **nov. sp.** (Bourret 1937; Ohler *et al.* 2000; Nguyen *et al.* 2009; Chen *et al.* 2017). Preliminary molecular analysis suggests that *Megophrys major* is a complex of species (Vassileva *et al.* 2016; Chen *et al.* 2017; Mahony *et al.* 2017), and these authors removed *M. maosonsensis* from the synonymy of this species. Further taxonomic work is required in order to ascertain whether or not *M. major* occurs in Vietnam. There are likely more undescribed species of *Megophrys* in Lao Cai Province; Chen *et al.* (2017) found putative evidence for many undescribed *Megophrys* species, including two other undescribed species they refer to as *M. sp.* 1 and *M. sp.* 5 from Sa Pa District, Lao Cai Province.

*Megophrys rubrimera* **nov. sp.** is likely Endangered according to the IUCN Red List of Threatened Species criteria (IUCN 2012). Future research should focus on determining the distribution and elevational range of the species. *Megophrys rubrimera* **nov. sp.** may be present on the western slopes of the Hoang Lien Range and forest patches in this region should be surveyed as a priority.

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## APPENDIX I.

Comparative material examined:

*Megophrys (Brachytarsophrys) carinense* ( $N=6$ ): BMNH 1947.2.24.88 paralectotype, “Karin Hills”, Myanmar; BMNH 1947.2.24.89 paralectotype, “Mount Carin, 900–1,200 m, Karin Hills”, Myanmar; BMNH 1947.2.24.90–91 paralectotype, “Yado, Karin Hills”, Myanmar; BMNH 1921.4.1.316, Me Yum, Me Wang Forest, 1,000–1,500 ft, N. Thailand; CAS 243791, along gas pipeline, ‘Khotama Camp’ ( $14^{\circ}43'56.7''N$   $98^{\circ}15'01.7''E$ , 240 ft), Yephyu Township, Dawei District, Tanintaryi, Myanmar.

*Megophrys (Brachytarsophrys) feae* ( $N=10$ ): BMNH 89.3.25.60–61 topotypes, “Kakhyen hills”, Myanmar; BMNH 1936.7.4.17, “Nam Ti Valley, Upper Burma”, Myanmar; BMNH 1974.8.09, “4,800’, Pangnamdin, N. Burma”, Myanmar; BMNH 1940.6.2.11–12, BMNH 1940.6.2.14–15, “Ahke, Pangnamdim, Triangle Upper Burma”, Myanmar; BMNH 1940.6.2.13, “Mahtun, Pangnamdim, Triangle Upper Burma”, Myanmar; BMNH 1908.9.18.3, “Mogok, Upper Burma”, Myanmar.

*Megophrys (Brachytarsophrys) cf. feae* ( $N=4$ ): AMNH 168659, forest and streams above Nam Tha River (1,300–1,400 m:  $21^{\circ}54'56''N$   $104^{\circ}21'39''E$ ), Nam Tha Commune, Van Ban District, Lao Cai Province, Vietnam; FMNH 254038–254040, Tam Dao, Vinh-Phu Province, Vietnam.

*Megophrys (Brachytarsophrys) intermedia* ( $N=18$ ): BMNH 1947.2.25.21–31, BMNH 1972.1496–1498, syntypes, Dalat, Langbian Plateau, Vietnam; FMNH 258093–258096, Xe Sap National Biodiversity Conservation Area (1,200–1,280 m), Kaleum District, Xe Kong Province, Lao P.D.R.

*Megophrys (Ophryophryne) gerti* ( $N=4$ ): BMNH 1921.4.1.324 holotype, “Camly, Lang Bian Plateau”, Vietnam; BMNH 1921.4.1.323 paratype, “Dran, Lang Bian Plateau”, Vietnam; BMNH 1972.1524 paratype, “Pak Maat”, Laos; AMNH 163668, near camp 1 (980 m,  $15^{\circ}11'41''N$   $108^{\circ}02'25''E$ ), Tra Don Commune, Tra My District, Quang Nam Province, Vietnam.

*Megophrys (Ophryophryne) hansii* ( $N=18$ ): FMNH 252879, 252880, 252882, 252884 holotype and paratypes, 60 km to the northwest from Kannack, Annam mountains (1,000–1,200 m), Kon Cha Rang village, Kannack town, An Khe District, Gia-Lai Province, Vietnam; FMNH 252873 paratype, 20 km to the northwest from Kannack (700–750 m,  $14^{\circ}20'N$   $108^{\circ}36'E$ ), Buon Luoi Village, Kannack town, Annam mountains, An Khe District, Gia-Lai Province, Vietnam; AMNH 163680, camp 1: (930 m,  $15^{\circ}11'41''N$   $108^{\circ}02'25''E$ ), Tra Don Commune, Tra My District, Quang Nam Province, Vietnam; AMNH 169284, Vu Vu stream ( $16^{\circ}14'30''N$   $107^{\circ}29'23''E$ ), far stream off of A Ma Road, Tram Tra Ve (Forestry station of Huong Giang State Forestry Enterprise), Huong Nguyen Commune, A Luoi District, Thua Thien Hue Province, Vietnam; AMNH 169286, Khe Dau Station (109 m,  $16^{\circ}17'58''N$   $107^{\circ}33'18''E$ ), Binh Thanh Commune, Huong Thuy District, Thua Thien Hue Province, Vietnam; AMNH 161353, Rao An Region (200 m,  $18^{\circ}21'53''N$   $105^{\circ}13'13''E$ ), Huong Son Reserve, Huong Son District, Ha Tinh Province, Vietnam; FMNH 258009–258010, FMNH 258046–258051, Xe Sap National Biodiversity Conservation Area (near  $16^{\circ}04'10''N$   $106^{\circ}58'45''E$ , 1,100–1,280 m), Kaleum District, Xe Kong Province, Lao P.D.R.; FMNH 258008, along Houay Alung stream ( $16^{\circ}00'32''N$   $106^{\circ}55'31''E$ , 920–1,000 m), Xe Sap National Biodiversity Conservation Area, Kaleum District, Xe Kong Province, Lao P.D.R.

*Megophrys (Ophryophryne) microstoma* ( $N=6$ ): BMNH 1947.2.22.50–53 lectotype and paralectotypes, Mau Son Mts, Tonkin, 3,000–4,000 ft., Vietnam; AMNH 168682, tributary to Khe Pan River (ca. 331 m,  $21^{\circ}56'39''N$   $104^{\circ}24'12''E$ ), Nam Tha Commune, Van Ban District, Lao Cai Province, Vietnam; BMNH 1974.2334, “N.E. Thailand”.

*Megophrys (Ophryophryne) cf. poilani* ( $N=1$ ): AMNH 169287, stream 1-directly across road from Range Station to the northwest, watersource for station (ca. 653 m,  $16^{\circ}04'28''N$   $107^{\circ}29'14''E$ ), Ho Chi Minh Highway marker kilometer 398+700, A Pat Forestry Protection Department Range Station, A Roang Commune, A Luoi District, Thua Thien Hue Province, Vietnam.

*Megophrys (Ophryophryne) synoria* ( $N=2$ ): FMNH 262778–262779 holotype and paratype, Samling Logging Concession, O Chung Chry stream (500 m, near  $12^{\circ}17'30''N$   $107^{\circ}03'06''E$ ), O'Rang District, Mondolkiri Province, Cambodia.

*Megophrys (Panophrys) binlingensis* ( $N=6$ ): FMNH 232868, 232873–232877 topotypes, 9 km W Bin Ling, Wa Shan camp, Hongya Xian, Sichuan, China.

*Megophrys (Panophrys) boettgeri* ( $N=7$ ): BMNH 1947.2.25.4, BMNH [18]99.4.24.87–91 syntypes, “Kuatun, a village about 270 miles from Foochow, in the mountains at the north-west of the Province of Fokien [=Fujian], at an altitude of 3,000 to 4,000 feet or more”, China; BMNH 1985.1293 topotype, “Ch'ungan hsien, Fukien, China”.

*Megophrys (Panophrys) brachykolos* ( $N=20$ ): FMNH 69063–69065, FMNH 109977, FMNH 109979, FMNH 109981, FMNH 109982, FMNH 109985 holotype and paratypes, The Peak, Hong Kong Island, China; BMNH 1983.322, BMNH 1951.1.6.38–39 topotypes, The Peak District, Hong Kong Island, China; BMNH 1983.321, “Near Tai Tam Intermediate Reservoir, Hong Kong Island”, China; BMNH 1951.1.6.40, “Hong Kong”, China; FMNH 256939, trail from Ngong Ping to Shek Pik Reservoir, 2 km S Ngong Ping (ca. 200 m), Lantau, Hong Kong, China; FMNH 256940, Ma Po Ping (ca. 3 m), Lantau, Hong Kong,



China; AMNH A 30239, FMNH 24408, BMNH 1985.1294 paratypes of *Megophrys kuatunensis*, Ch'ungan Hsien, Fukien, China.

*Megophrys (Panophrys) kuatunensis* ( $N=12$ ): AMNH A 30126, AMNH A 30240–30244, FMNH 24406, FMNH 24411–24413, BMNH 1961.956, BMNH 1985.1295 holotype and paratypes, Ch'ungan Hsien, Kuatun village, Fukien, China;

*Megophrys (Panophrys) cf. minor* ( $N=6$ ): FMNH 167973, “Pan-lung-shan, Szechwan, China”; FMNH 49523, “Mt. Omei, Szechwan, China”; FMNH 213944–213945, BMNH 1979.441, Doi Inthanon, 1,500 m, Chiangmai, Thailand; BMNH 1934.10.2.10, “Lohit Valley, Sadiya Frontier Valley, Assam”.

*Megophrys (Panophrys) omeimontis* ( $N=7$ ): FMNH 49406–49407, FMNH 170696 syntypes, “Mount Omei, Szechwan, altitude 3,600 feet”, China; FMNH 232869–232872, 9 km west of Bin Ling, Wa Shan camp, Hongya Xian, Sichuan, China.

*Megophrys (Panophrys) palpebralespinosa* ( $N=4$ ): FMNH 258098–258100, near Nam Ou River (600 m), Phou Dendin National Biodiversity Conservation Area, Phongsaly District, Phongsaly Province, Lao P.D.R.; AMNH 163858, scouting expedition camp (1,020 m, 22°54'15"N 105°13'38"E), Khau Ria Village, Du Gia Commune, Yen Minh District, Ha Giang Province, Vietnam.

*Megophrys (Xenophrys) auralensis* ( $N=1$ ): FMNH 267763, Knorgl Louk - Louk Mountain-top (1,200 m, 1314799 N, 0355440 E), Pursat Province, Cambodia.

*Megophrys (Xenophrys) damrei* ( $N=2$ ) BMNH 1972.1522–1523 holotype and paratype, Bokor Plateau (1,000 m), Elephant Mountains, Kampot Province, Cambodia.

*Megophrys (Xenophrys) cf. glandulosa* ( $N=3$ ): CAS 221395, on road between Ahtonga and Babaw, (27°17'24.3"N 97°51'52.4"E), Machanbaw Township, Putao District, Kachin State Province, Myanmar; CAS 221442, Aureinga Camp (27°17'36.4"N 97°51'50.0"E), Naung Mon Township, Putao District, Kachin State Province, Myanmar; CAS 221484, rd between Ahtonga and Babaw (27°15'27.2"N 97°50'32.4"E), Machanbaw Township, Putao District, Kachin State Province, Myanmar.

*Megophrys (Xenophrys) lekaguli* ( $N=4$ ): FMNH 213946 holotype, Khao Soi Daow watershed (ca. 600–700 m), Chantaburi Province, Thailand; FMNH 191456–191457 paratypes, Pong Nam Ron, Khao Soi Dao Tai (1,000 m), Chantaburi Province, Thailand; BMNH 1979.440 topotype, Khao Soi Daow W.S, Chanthaburi, Thailand.

*Megophrys (Xenophrys) major* ( $N=5$ ): BMNH 1947.2.4.93, BMNH 1947.2.4.95, BMNH 1947.2.4.98, BMNH 1947.2.4.99, BMNH 1947.2.25.1 syntypes, “Darjeeling”, West Bengal, India

*Megophrys (Xenophrys) parva* ( $N=6$ ): BMNH 1947.2.25.9–12 paralectotypes of *Leptobrachium parvum*, “District of Karin Bia-po”, Myanmar; BMNH 1921.4.1.317–318, “Pa Meang, N. Siam”.

*Megophrys (Xenophrys) takensis* ( $N=7$ ): BMNH 1972.1516–1520 holotype and paratypes, “2,500', Ban Pa Che, NW of Tak, Thailand”; BMNH 1972.1521 topotype, “2,500', Ban Pa Che, NW of Tak, Thailand”; FMNH 261711, Khlong Lan National Park (305 m, 16°07'47.4"N 99°16'54.0"E), Kampaeng, Thailand.