



The tadpoles of five *Megophrys* Horned frogs (Amphibia: Megophryidae) from the Hoang Lien Range, Vietnam

BENJAMIN TAPLEY^{1,*}, LUAN THANH NGUYEN², TIMOTHY CUTAJAR³, CHUNG THANH NGUYEN⁴, CHRISTOPHER PORTWAY³, HAO VAN LUONG⁴ & JODI J. L. ROWLEY^{3,5}

¹Zoological Society of London, Regent's Park, London, NW1 4RY, United Kingdom.

✉ ben.tapley@zsl.org; <https://orcid.org/0000-0002-9787-3793>

²Asian Turtle Program - Indo-Myanmar Conservation, CT1 Bac Ha C14 Building, To Huu Road, Ha Noi, Vietnam.

✉ nguyenluanbio@gmail.com; <https://orcid.org/0000-0002-4663-125X>

³Australian Museum Research Institute, Australian Museum, 1 William St, Sydney, NSW, 2010, Australia.

✉ timothy.cutajar@australian.museum; <https://orcid.org/0000-0001-7841-9205>

⁴Hoang Lien National Park, Sa Pa, Lao Cai, Vietnam. ✉ chung.crco@gmail.com; <https://orcid.org/0000-0001-8533-9721>

⁵Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052, Australia. ✉ Jodi.Rowley@austmus.gov.au; <https://orcid.org/0000-0002-2011-9143>

*Corresponding author. ✉ ben.tapley@zsl.org

Abstract

Frogs in the genus *Megophrys* are an Asian radiation of stream-breeding frogs. The tadpoles of many *Megophrys* species are undescribed; those that are described are often dubiously allocated to species by association with post metamorphic specimens at collection sites and without supportive molecular data. We provide detailed descriptions of the larvae of five species of *Megophrys* from the Hoang Lien Range in northwest Vietnam: *Megophrys fansipanensis*, *M. gigantea*, *M. hoanglienensis*, *M. jingdongensis* and *M. maosonensis*. Tadpoles from different subgenera differ from each other via a combination of patternation in life, oral disc shape and tail morphology but given the small sample size, and limited number of species it is unlikely that these differences can be applied more widely to delineate subgenera. Morphological differences between tadpoles from species within the subgenus *Panophrys* were insufficient to clearly delineate all species. The ability to identify tadpoles is likely to advance our understanding of the frog fauna in mainland Southeast Asia.

Key words: *Atympanophrys*, Megophryinae, *Megophrys fansipanensis*, *Megophrys jingdongensis*, *Megophrys hoanglienensis*, *Megophrys gigantea*, *Megophrys maosonensis*, *Panophrys*, Southeast Asia, larvae, *Xenophrys*

Tóm tắt tiếng Việt

Cho đến nay, nòng nọc của nhiều loài trong giống *Megophrys* vẫn chưa được mô tả; trong khi đó có nhiều nghi vấn về sự chính xác trong việc định loại đối với nòng nọc những loài đã được mô tả do việc định danh các loài trên được tiến hành chủ yếu bằng cách liên kết với các cá thể trưởng thành thu thập tại địa điểm thu mẫu mà không có sự hỗ trợ bởi dữ liệu phân tử. Trong bài báo này chúng tôi mô tả nòng nọc của năm loài cóc sừng từ dãy núi Hoàng Liên Sơn, bao gồm *Megophrys fansipanensis*, *Megophrys gigantea*, *Megophrys hoanglienensis*, *Megophrys jingdongensis*, và *Megophrys maosonensis* đồng thời so sánh chúng với nhau và với nòng nọc của loài *Megophrys rubrimera*, một loài có cùng khu vực phân bố. Đồng thời, tầm quan trọng của việc khảo sát nòng nọc và tìm hiểu hệ sinh thái của chúng để nghiên cứu đánh giá nhanh đa dạng sinh học và các hoạt động bảo tồn cũng được bàn luận.

Introduction

The megophryid frog genus *Megophrys* Kuhl and Van Hasselt is comprised of 107 described species (Li *et al.* 2020; Liu *et al.* 2020; Lyu *et al.* 2020; Mahony *et al.* 2020; Nguyen *et al.* 2020; Wang *et al.* 2020; Xu *et al.* 2020) within seven subgenera (Mahony *et al.* 2017). The genus occurs from southern and central China, the eastern and southern

Himalayas, mainland Southeast Asia, the islands of the Sunda shelf and parts of the Philippines (Mahony *et al.* 2017).

Species in the genus *Megophrys* are stream-breeding and their tadpoles exhibit conservative morphology (Grosjean 2003). The morphology of *Megophrys* tadpoles has not always been described in sufficient detail to allow for the tadpoles of different species to be distinguished morphologically (e.g., Fei & Ye 2016). This is particularly the case as all known *Megophrys* tadpoles have umbelliform oral discs and lack labial tooth rows (Altig & McDiarmid 1999a; Poyarkov *et al.* 2017; Tapley *et al.* 2017; Li *et al.* 2018); the latter of which are often relied upon when delineating species (Dubois & Ohler 1998). In addition, some *Megophrys* tadpoles have been assigned to a species merely because they are found in sympatry with the adult frogs present at a particular site at the time of collection (Leong & Chou 1998; Fei *et al.* 2009; Wang *et al.* 2012). This is problematic as several *Megophrys* species may be present at a site (e.g., Li *et al.* 2014; Wang *et al.* 2014; Chen *et al.* 2017; Tapley *et al.* 2018a,b). Molecular data is often not used to confirm species identity; of the 107 described *Megophrys* species, there are only ten tadpole descriptions where specific identity has been confirmed with molecular data, morphology has been described and measurements have been provided for individual tadpoles of specified developmental stage; *M. lekaguli* Stuart, Chuaynkern, Chandard and Inger; *M. dringi* Inger, Steubing and Tan, tadpole described by Oberhammer *et al.* (2014); *M. intermedia* (Smith), tadpole described by Tapley *et al.* (In Press); *M. jiangi* Liu, Li, Wei, Xu, Cheng, Wang and Wu; *M. lini* (Wang, Zhao, Yang, Zhou, Chen, and Liu); *M. popei* (Zhao, Yang, Chen, Chen, and Wang); *M. monticola* (Günther), tadpole described by Deuti *et al.* (2017); Che and Mahony; *M. rubrimera* Tapley, Cutajar, Mahony, Nguyen, Dau, Nguyen, Luong and Rowley; *M. leishanensis* Li, Xu, Liu, Jiang, Wei, and Wang. Furthermore, tadpole descriptions are often published with ratios of character measurements for a series (e.g., Stuart *et al.* 2006; Wang *et al.* 2012), or with very brief information on morphology and with very few measurements of characters (e.g., Wang *et al.* 2014; Zhao *et al.* 2014) rather than precise measurements for each developmental stage. This makes robust interspecific comparison using published tadpole descriptions challenging.

Vietnam contains some of the highest known species richness in the genus *Megophrys*, with 22 species representing five subgenera known from the country: *Atympanophrys* Liu; *Brachytarsophrys* Boulenger; *Ophryophryne* Boulenger; *Panophrys* Rao and Yang; and *Xenophrys* Günther (Orlov *et al.* 2002; Ohler 2003; Le *et al.* 2006; Nguyen *et al.* 2009; Chen *et al.* 2017; Mahony *et al.* 2017; Poyarkov *et al.* 2017; Tapley *et al.* 2018a; Luong *et al.* 2019; Nguyen *et al.* 2020). Most *Megophrys* are from the north of the country and the Hoang Lien Range in northern Vietnam is known to have nine species (Ohler *et al.* 2000; Tapley *et al.* 2018a; Luong *et al.* 2019), many of which have been described in the last decade; *M. fansipanensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong, and Rowley; *M. gigantea* (Liu, Hu and Yang), *M. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong, and Rowley; *M. jingdongensis* Fei and Ye; *M. maosonensis* Bourret; *M. microstoma* (Boulenger), *M. pachyproctus* Huang; *M. palpebralespinosa* Bourret, and *M. rubrimera*.

During recent field work in the Hoang Lien Range, northwest Vietnam, we encountered *Megophrys* tadpoles that appeared morphologically distinct from one another. Tadpoles were assigned to species using molecular data. We provide the first tadpole description for two species and provide detailed redescriptions for a further three.

Materials and methods

Specimens were collected by day and by night in forest stream habitats in the Hoang Lien Range, Sa Pa and Bat Xat districts, Lao Cai Province (Vietnam), and Tam Duong District, Lai Chau Province (Vietnam) during December 2017, March, June and September 2018. Geographic coordinates were obtained using a Garmin GPSMAP 60CSx GPS receiver (Garmin Ltd., Kansas, USA) and recorded in datum World Geodetic System 1984 (or WGS 84). Specimens were photographed in life before being humanely euthanised in food grade plastic bags containing a 20% solution of benzocaine and stream water. Tissue samples (tail clips) for molecular analyses were extracted from freshly euthanised specimens and stored in EDTA/DMSO or ethanol prior to fixation of specimens with 10% formalin and storage in 70% ethanol. Specimens were deposited at the Vietnam National Museum of Nature, Hanoi (VNMN) and Hoang Lien National Park headquarters. Comparative material was examined at the Australian Museum, Sydney; *Megophrys* (*Panophrys*) *rubrimera* (N=1): 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).

Molecular data: The identity of the tadpoles was confirmed by analysing the tissue collected from each tadpole (GenBank accession numbers provided in Table 1). Total genomic DNA was extracted with DNeasy tissue extraction kits (Qiagen), and we used the primers 16SAR and 16SBR of Palumbi *et al.* (1991) to amplify 550 base pairs of the 16S rRNA gene. PCR amplification was carried out in 24 µl reactions with 1000 ng of genomic DNA, 1 x 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 batch. Thermocycling was performed on an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 94 °C (2 min), two 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, Ohio, 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, Michigan, USA), aligned with the Clustal option in MEGA 7 (Kumar *et al.* 2016), and refined by eye. The new sequences were then checked on BLAST (NCBI) to verify their approximate identity. Uncorrected *p*-distance (with partial deletion of gaps and missing data) was calculated between the new sequences and sequences from *Megophrys* species known from the Hoang Lien Range using MEGA 7.

Misidentifications of sequences on GenBank are common for morphologically conservative frogs such as *Megophrys*. We used the ruler function on Google Earth to measure the distance between the collection site of sequenced specimens and their type locality as this measurement may indicate the reliability of the sequence data for species.

Morphological data: To measure the tadpoles, we used ImageJ 1.49 (Schneider *et al.* 2012) on photographs of preserved specimens that were taken next to a scale. For *M. maosonensis*, measurements of the head, body and tail base were taken from preserved specimens but we had to rely on scaled images in life to measure aspects of the tail as the tip had been removed for molecular analyses. We also used photographs taken of tadpoles in life to describe mouthpart shape, the arrangement of submarginal papillae, oral disc width in life and colouration. Staging follows Gosner (1960) and terminology for external morphology is that of Altig & McDiarmid (1999b). Measurements and morphometric abbreviations follow Oberhammer *et al.* (2014) and 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); total length (TTL); tail length = TTL–BL (TAL); 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); and upper fin height at point of maximal tail height (UFH).

We compare the newly collected tadpole specimens from the Hoang Lien Range with one another and with *M. rubrimera*, the only sympatric species with a detailed tadpole description.

Results

Megophrys (Atympanophrys) gigantea

Figs. 1, 2A, 3A, and 4

Molecular data: 16S sequences were generated from tissue samples of two tadpoles. Uncorrected *p*-distance between the tadpoles in this study and sequences from *M. gigantea* specimens collected approximately 390 km to the northwest in the proximity of the type locality; Mount Wuliang, Yunnan Province, China (GenBank accession numbers; MH406775; MH406776 & KX811898) were 0.2%.

Collection site: The following tadpole description is based on 5 specimens VNMN010898, VNMN010899, VNMN010900, HLNP 20180320 00009 & HLNP 20180320 00010) at Stage 35. Specimens were found actively feeding near the bank in a large pool in a 2 m wide stream in heavily disturbed evergreen forest on Mount Pu Ta

Leng, Bat Xat Nature Reserve, Bat Xat District, Lao Cai Province, Vietnam (22.4322°N 103.6297°E, 1912 m asl; Figs. 1 and 2A), collected at 15:00 h on 20 March 2018 by Luan Nguyen, Christopher Portway, Chung Nguyen and Benjamin Tapley.

Morphology: The body is longitudinally oval shaped and dorsally compressed; the nares are oval and are closer to the eyes than to the snout, the rims of the nares are serrated, raised from the body wall and open anterolaterally; the internarial distance is less than the interorbital distance; the eyes are positioned dorsolaterally, the pupils are round; the spiracle is sinistral and the spiracular tube protrudes from nearly two thirds of the way along the body wall just anterior to the maximal trunk width, the spiracular tube opens laterally; the mean tail length is 66.6% (63.2–68.4%, $n=5$) of the total length; the dorsal tail fin inserts behind the body-tail junction, the dorsal fin is low, particularly at the proximal half of the tail length; the mean basal tail width is 42.1% (37.2–45.1%, $n=5$) of the maximal trunk width, the tail tip is broadly rounded; the oral disc is subterminal and antero-dorsal, the lateral corners fold medially and turn upward when not fully extended (and in preservative), the mean width of the umbelliform oral disc makes up 92.4% (82.4–96.7%, $n=5$) of the maximal width of the trunk; in life the maximal BW is 62.6% the maximal width of the fully extended ODW ($n=1$); the lower lip is triangular with basal lobes when the mouthparts are fully expanded in life, we define this as hastate shaped (Fig. 4B), marginal papillae are absent; six rows of submarginal papillae are present on the upper lip when they are counted medially at the maximal width of the oral disc; these are regularly positioned; those present medially at the maximal width of the oral disc are rounded, but those in the innermost two rows become increasingly oblong shaped towards the edges of the disc; five rows of rounded submarginal papillae are present on the lower lip when they are counted medially at the maximal width of the oral disc; these are regularly positioned, longitudinally oblong shaped on the three inner rows and they become smaller and circular in shape on the outermost row; the lower lip is deeper than the upper lip; labial teeth are absent; the upper jaw sheath is serrated and has a deep medial notch; the lower jaw sheath is serrated and lacks a medial notch. See Table 1 for measurements.

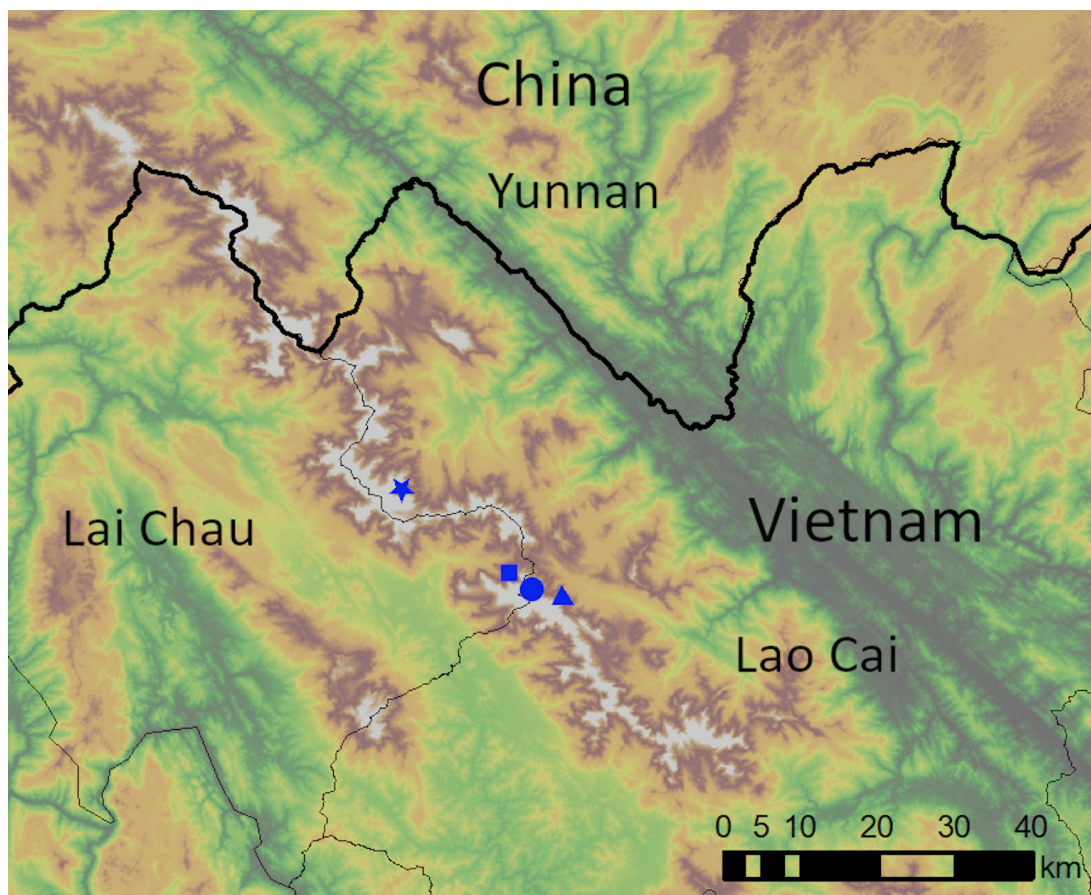


FIGURE 1. Survey sites in the Hoang Lien Range. Blue star, *Megophrys gigantea* and *Megophrys jingdongensis* tadpole collection site; blue square, *Megophrys hoanglienensis* tadpole collection site; blue circle, *Megophrys fansipanensis* tadpole collection site; blue triangle *Megophrys maosonensis* tadpole collection site. Pale grey areas indicative of higher elevation, dark green indicative of lowest elevation.

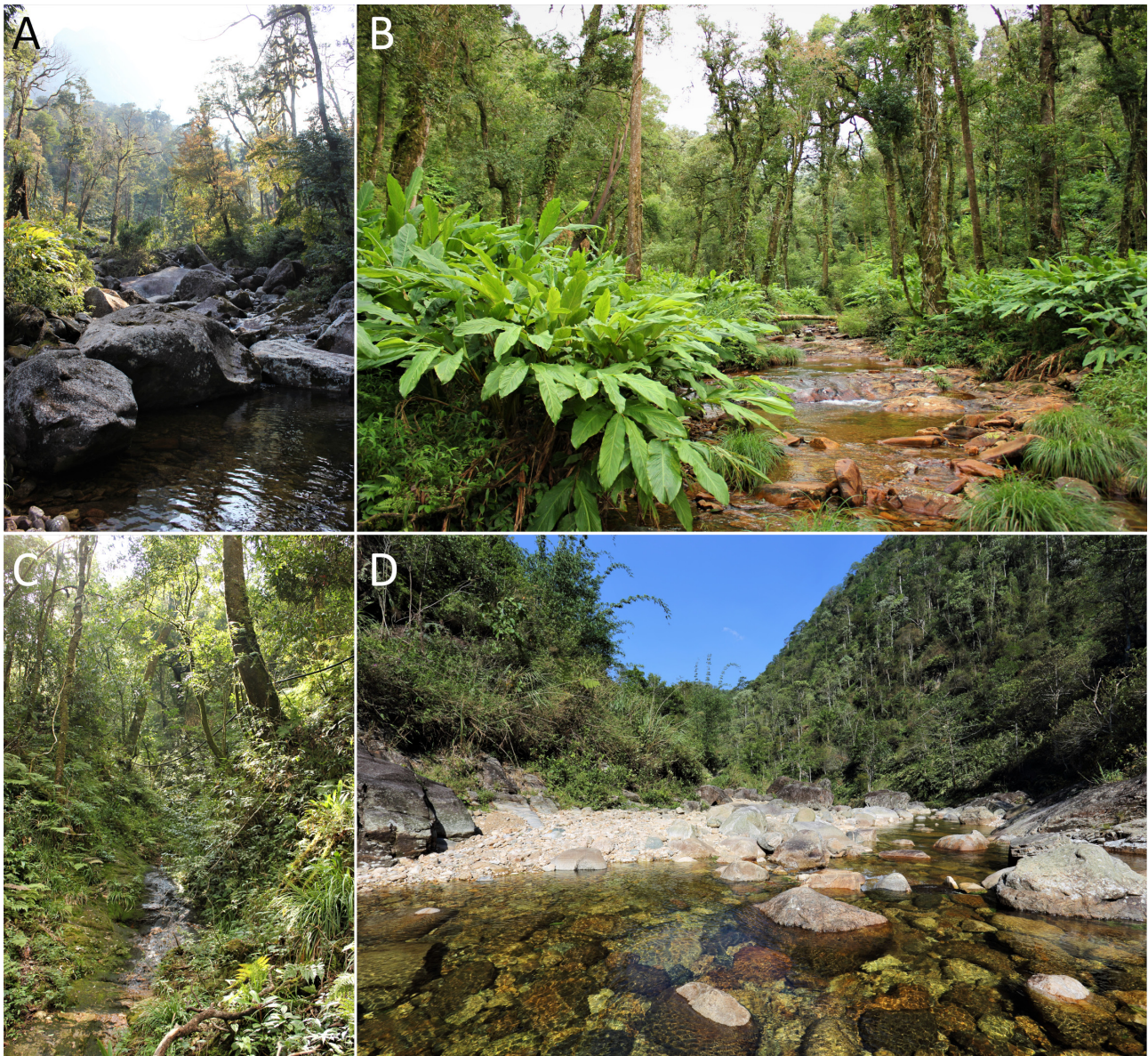


FIGURE 2. Collection sites in the Hoang Lien Range: (A) *Megophrys gigantea* and *Megophrys jingdongensis* tadpole collection site, Bat Xat District; (B) *Megophrys hoanglienensis* tadpole collection site, Tam Duong District; (C) *Megophrys fansipanensis* tadpole collection site, Sa Pa District, and (D) *Megophrys maosonensis* tadpole collection site, Sa Pa District.

Colour in life: (Fig. 4) Dorsally, the head and body are light brown with pale neuromasts; the area surrounding the nares is dark brown; the oral disc is a translucent tan brown with dark brown submarginal papillae; the dorsal and ventral fins are opaque, pale yellowish brown without speckles; the lower fin is more yellow in colour than the upper fin and also without speckles; the ventral and lateral sides of the body are a translucent dark grey and speckled with white; the underside of the oral disc is speckled with white; the gills and coils of the gut are visible in the ventral view through the transparent ventral skin; the sclera of the eye is black with green-gold flecks; the pupil is black and rounded; the iris is orange and speckled with black dots; the outer margin of the upper jaw sheath is dark brown.

Colour in preservative: (Fig. 3A) Body brown; the gills and coils of the gut are visible in the ventral view through the transparent ventral skin; the oral disc is translucent grey brown with darker brown submarginal papillae; the dorsal and ventral fins are an opaque, pale grey brown; the venter is grey; the iris is dark grey.

Variation: VNMN010899 has a shorter tail than the other *M. gigantea* specimens collected from the Hoang Lien Range, this is probably due to a historic tail injury and subsequent regeneration (Figs. 3A and 4A). The tadpole of *M. gigantea* at Stage 34 was described previously by Fei *et al.* (2009) although it is not clear if species identity was supported with molecular data. The description is broadly congruent with the Stage 35 specimens collected in

this study but there are some notable differences. At stage 34 the TTL is reported as 39.0 mm (Fei *et al.* 2009); this is considerably smaller than the mean TTL of 50.7 mm in this study. Fei *et al.* (2009) describe the body and tail muscle as having grey patterns (also depicted in the associated line drawing). The specimens we collected in the Hoang Lien Range lacked grey patterns on the body and on the tail muscle.

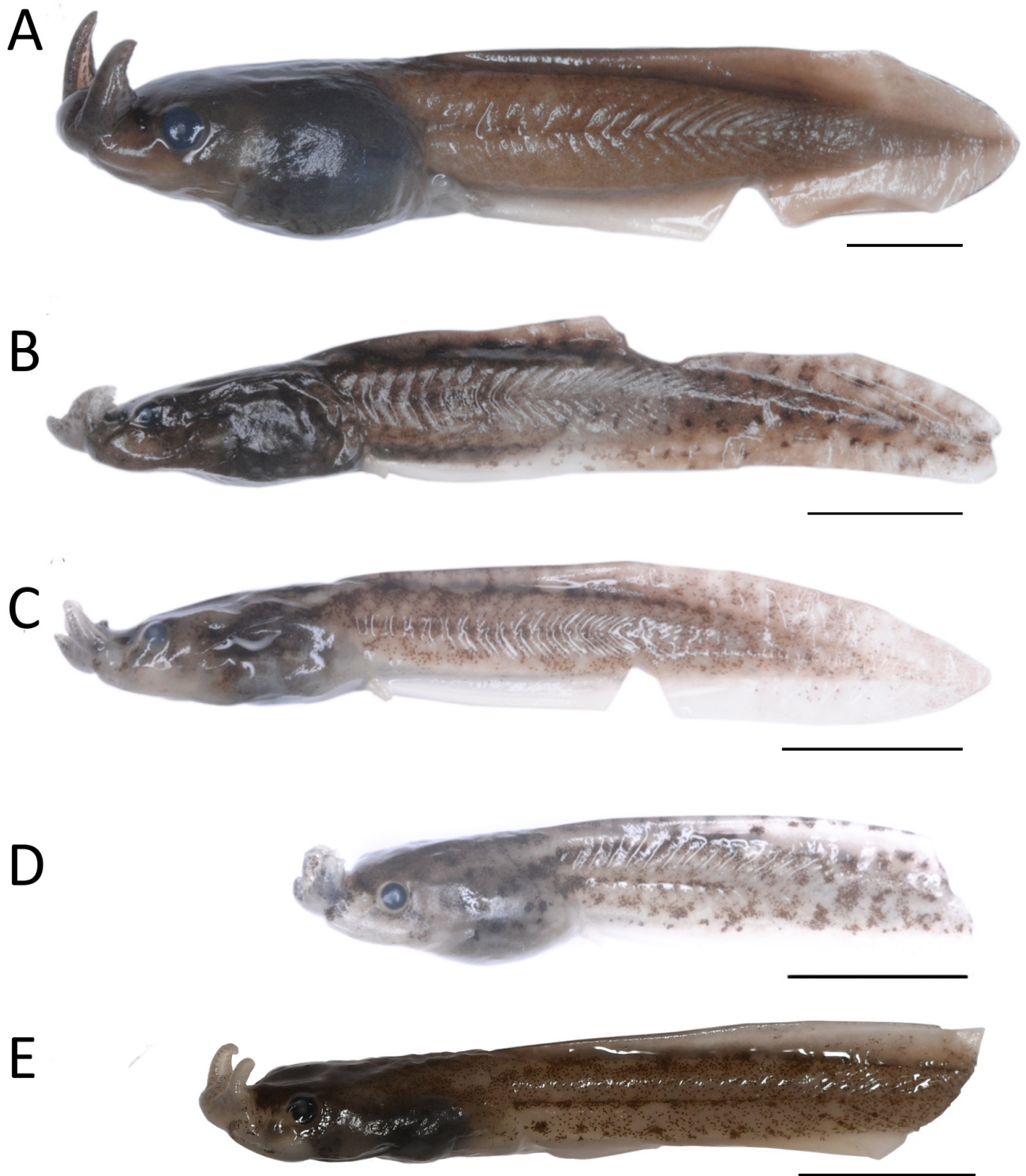


FIGURE 3. Tadpoles of five *Megophrys* species from the Hoang Lien Range in preservative: (A) *Megophrys gigantea* (VNMN010899); (B) *Megophrys fansipanensis* (HLNP 20171230 00011); (C) *Megophrys jingdongensis* (VNMN010902); (D) *Megophrys hoanglienensis* (VNMN010903) and (E) *Megophrys maosonensis* (VNMN010904). Scale bar is 0.5 cm.

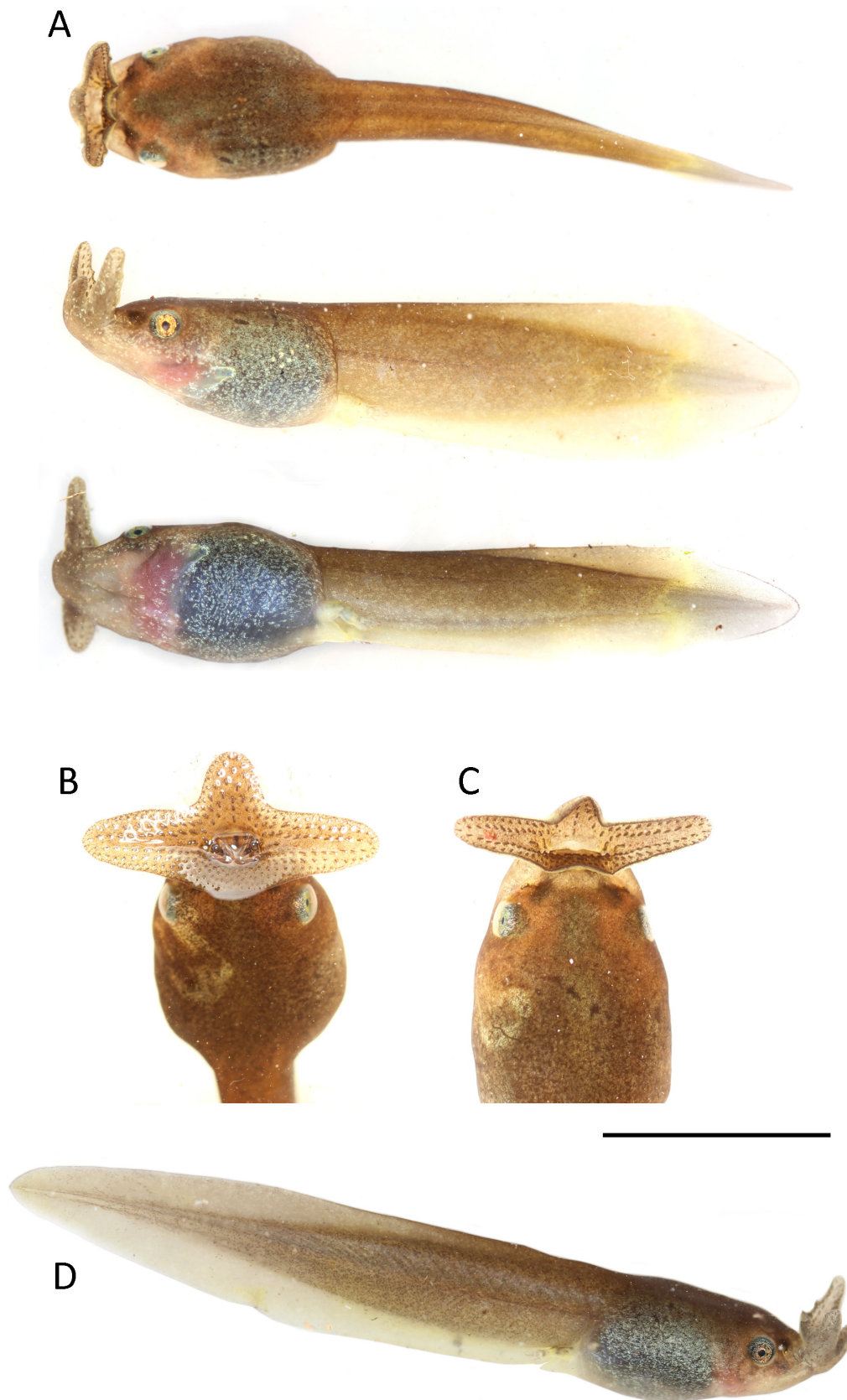


FIGURE 4. *Megophrys gigantea* tadpole VNMN010899. (A) Dorsal, lateral, ventrolateral views (15 mm scale), and (B) detail of the oral disc of a live tadpole (15 mm scale). (C) Oral disc of the euthanised specimen (15 mm scale). (D) Lateral view of unvouchered specimen to demonstrate intraspecific variation (not to scale).

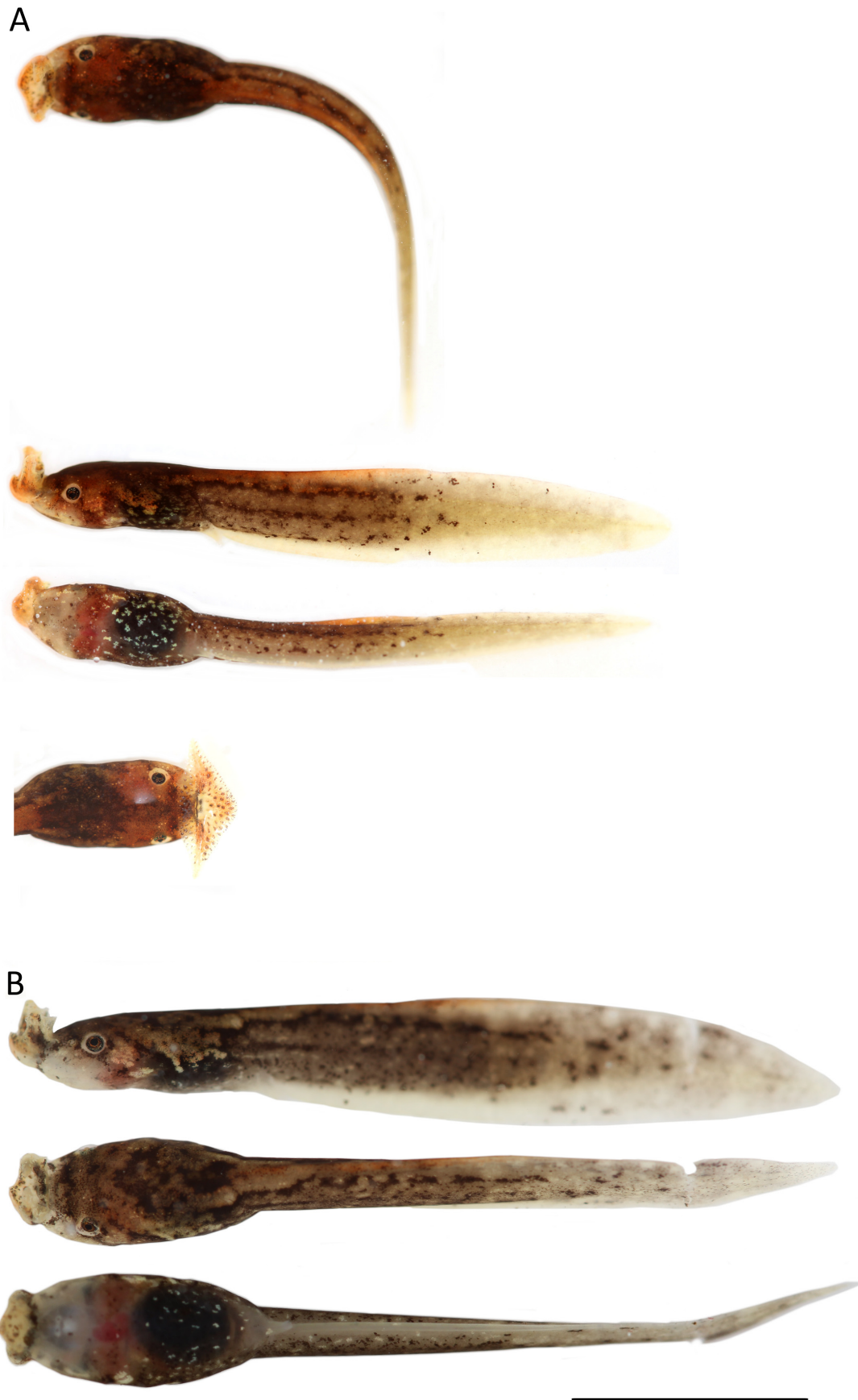


FIGURE 5. *Megophrys fansipanensis* tadpoles. (A) Dorsal, lateral, ventrolateral views, and detail of the oral disc in a live tadpole VNMN010901. (B) Lateral, dorsal and ventral views of the live tadpole HLNP 20171230 00011. 10 mm scale.

TABLE 1. Comparisons between tadpoles of *Megophrys* species from the Hoang Lien Range. Measurements are presented as mean (range). All measurements in preservative unless stated otherwise. * Denotes measurement obtained from only one specimen. ^ Denotes mean value used to calculate ratios.

Subgenus	<i>Ayampanophrys</i>			<i>Panophrys</i>			<i>Xenophrys</i>			
	<i>M. gigantea</i>	<i>M. fiansipanensis</i>	<i>M. jingdongensis</i>	<i>M. hoanglienensis</i>	<i>M. rubrimera</i>	<i>M. maosonensis</i>	GenBank	Fei <i>et al.</i> 2009	Tapley <i>et al.</i> 2017	Fei <i>et al.</i> 2009
Species	<i>M. gigantea</i>	<i>M. fiansipanensis</i>	<i>M. jingdongensis</i>	<i>M. hoanglienensis</i>	<i>M. rubrimera</i>	<i>M. maosonensis</i>				
GenBank	MT412122, MT413142	MT413143	MT413144	MT413146		MT412390				
N	5	2	1	1	1	2				10
Gosner Stage	35	25	25	26	37	25				37–39
BL	16.9 (15.7–18.0)	9.1 (7.4–10.8)	8.9	7.1	10.5	8.8 (8.1–9.5)				14.1 (12.5–15)
BH	7.9 (7.6–8.3)	3.3 (2.7–3.9)	3.4	3.7		3.3 (2.8–3.7)				
BS	6.3 (4.2–7.5)	3.3 (2.9–3.6)	3.6	3.0		4.2 (3.6–4.7)				
BW	8.9 (8.5–9.2)	4.7 (3.5–5.8)	4.1	4.6	5.4	4.1 (–)				7.5 (7–8)
ED	1.6 (1.3–1.8)	0.7 (0.5–0.8)	0.6	0.7		0.9 (0.8–1.0)				
ES	5.5 (5.2–6.0)	3.6 (2.8–4.4)	3.2	1.9		2.4 (2.2–2.5)				
IND	4.6 (4.2–5.1)	2.6 (1.8–3.3)	1.9	2.1		2.4 (2.1–2.6)				
IOD	6.8 (6.1–7.3)	3.6 (2.5–4.6)	2.7	3.2		3.3 (3.0–3.5)				
NE	1.6 (1.4–1.7)	0.9 (0.8–1.0)	0.8	0.4		1.0 (0.9–1.0)				
SN	4.0 (3.6–4.2)	2.7 (1.9–3.4)	2.5	1.0		1.3 (1.1–1.4)				
SS	10.8 (9.8–13.8)	5.0 (4.6–5.3)	5.3	4.3	4.5	5.0 (4.9–5.1)				
ODW	8.2 (7.5–8.9)	3.6 (2.9–4.3)	2.6	2.2		2.7 (2.6–2.8)				
LFH	2.9 (2.4–3.3)	1.3 (0.9–1.6)	1.2	1.1		1.4 (–)				
MTH	8.7 (8.2–9.0)	4.8 (3.7–5.9)	4.4	4.7	5.9	5.6 (5.5–5.6)				8.1 (7.4–9)
TAL	33.8 (26.9–36.9)	21.7 (19.1–24.3)	19.0	19.4	22.8	27.4 (26.3–28.5)				34.8 (29–38.5)
TTL	50.7 (42.6–54.9)	30.8 (26.5–35.0)	27.9	26.5	33.3	35.5 (34.4–36.6)				49.0
TMH	4.9 (4.4–5.4)	2.7 (1.9–3.4)	2.8	3.0	3.1	3.6 (3.5–3.7)				5.5 (5–6)
TMW	3.8 (3.2–4.1)	2.5 (1.7–3.2)	2	2.0	3.4	2.3 (2.1–2.5)				4.6 (3.6–5.4)
UFH	2.1 (1.6–2.6)	1.25 (0.9–1.6)	1	0.9		1.8 (1.7–1.9)				
ODW in life	14.7*	5.4*	5.1*	5.8*		5.6*				
BW/ODW in life	62.6*	63.0*	80.4*	79.3*		73.2*				
TMW/BW	42.1 (37.2–45.1)	51.9 (48.6–55.2)	55.2	43.5	60.0	56.1 (51.2–61)				61.3^
Mouthpart shape	hastate	bi-triangular	bi-triangular	bi-triangular	bi-triangular	bi-triangular				
Pattern on tail	absent	present	present	present	present	present				
Tail tip	broadly rounded	broadly rounded	rounded	pointed	rounded	narrowly rounded				
			pointed, rounded in text							

Megophrys (Panophrys) fansipanensis

Figs. 3B and 5

Molecular data: 16S sequences were generated from tissue samples collected from two tadpoles (VNMN010901 & HLNP 20171230 00011) and uncorrected *p*-distance between these sequences and that of the holotype of *Megophrys fansipanensis* also collected on Mount Fansipan, Sa Pa District, Lao Cai Province, Vietnam (GenBank accession number MH514886) was 0.0%.

Collection site: The following tadpole description is based on two specimens (VNMN010901 & HLNP 20171230 00011) at Stage 35. Specimens were collected from a small pool in a 1 m wide low-gradient stream in disturbed evergreen forest on Mount Fansipan, Hoang Lien National Park (HLNP), Sa Pa District, Lao Cai Province, Vietnam (22.3281°N 103.7817°E, 2242 m asl; Figs. 1 and 2C), collected on 30 December 2017 by Luan Nguyen and Chung Nguyen.

Morphology: The body is elongated and slender; the nares are oval and their rims raised from the body wall, the nares are closer to the eyes than to the snout; the internarial distance is less than the interorbital distance; the eyes are positioned dorsolaterally, the pupils are round; the spiracle is sinistral and the spiracular tube protrudes from midway along the body wall just anterior to the maximal trunk width and opens laterally; the tail length ranges from 69.1–72.1% (*n*=2) of the total length; the dorsal tail fin inserts behind the body-tail junction, the dorsal fin is low, particularly towards the proximal half of the tail length; the basal tail width ranges from 53.1–60.3%, (*n*=2) of the maximal trunk width; the tail tip is pointed; the oral disc is subterminal and antero-dorsal; the lateral corners fold medially and turn upward when not fully extended (and in preservative), the width of the umbelliform oral disc makes up 74.1–82.9%, (*n*=2) of the maximal width of the trunk; in life the maximal BW is 64.8% (*n*=1) the maximal width of the fully extended ODW; the lower lip is bi-triangular shaped when fully expanded in live specimens; marginal papillae are absent; five rows of submarginal papillae are present on the upper lip when they are counted medially at the maximal width of the oral disc; these are regularly positioned and rounded, and become substantially smaller in the outermost row; five rows of rounded submarginal papillae are present on the lower lip when they are counted medially at the maximal width of the oral disc; these are regularly positioned, longitudinally oblong shaped at the edge of the disc and become increasingly rounded towards the point at which the maximal width of the disc is at its greatest, the submarginal papillae become smaller and more longitudinally elongated in shape on the outermost row; the lower lip is deeper than the upper lip; labial teeth are absent; the upper jaw sheath is serrated and has a medial notch; the lower jaw sheath lacks a medial notch. See Table 1 for measurements.

Colour in life: (Fig. 5) Dorsally, the head and body are brown with dark brown speckles which become increasingly dense towards the posterior half of the body; there are obvious pale neuromasts; the oral disc is a translucent beige brown with dark brown submarginal papillae; the dorsal apex of the tail has a light brown saddle, the margins of which are bordered by a dark brown band on the lateral surfaces of the tail, this saddle extends from the body and terminates half way along the length of the tail; at the point where the tail meets the body there is a brown stripe that extends from the body-tail junction and runs along the midline of the lateral tail surface, this stripe becomes increasingly broken and fades into brown blotches half way along the tail; the dorsal and ventral tail fins are opaque, pale yellowish brown with a few dark brown speckles; the venter is a translucent grey brown and speckled with metallic blue and flecked with dark brown; the gills and coils of the gut are visible in the ventral view through the transparent ventral skin; the sclera of the eye is broad and black with green-gold flecks; the pupil is black and rounded; the iris is orange and speckled with black dots; the outer margin of the upper jaw sheath is dark brown.

Colour in preservative: (Fig. 3B) The body is brown with darker speckles; the oral disc is a translucent grey brown with dark brown submarginal papillae; the dorsal and ventral fins are opaque, pale grey brown with darker speckles; the venter is speckled grey and brown.

Variation: HLNP 20171230 00011 is larger than VNMN010901 (Fig. 5) The ventral surface of the body of VNMN010901 is more densely speckled with metallic blue. TMW/BW is greater in HLNP 20171230 00011.

Megophrys (Panophrys) jingdongensis

Figs. 3C and 6

Molecular data: A 16S sequence was generated from a tissue sample obtained from the single tadpole

(VNMN010902). Uncorrected *p*-distance between the tadpole in this study and a *M. jingdongensis* specimen collected approximately 390 km to the northwest in the proximity of the type locality; Wenlong, Jingdong County, Yunnan Province, China (GenBank accession number KX811874) was 0.1%.



FIGURE 6. *Megophrys jingdongensis* tadpole VNMN010902 in life. Dorsal, lateral, and ventral views. 10 mm scale.

Collection site: The following tadpole description is based on a single specimen (VNMN010902) at Stage 25. The specimen was found near the bank in a large, 2 m wide pool in heavily disturbed evergreen forest on Mount Pu Ta Leng, Bat Xat Nature Reserve, Bat Xat District, Lao Cai Province, Vietnam (22.4322°N 103.6297°E, 1912 m asl; Figs. 1 and 2A), at 15:00 h on 20 March 2018 by Luan Nguyen, Christopher Portway, Chung Nguyen and Benjamin Tapley.

Morphology: The body is elongated and slender; the nares are oval and are closer to the eyes than to the snout, the rims of the nares are serrated and raised from the body wall, the internarial distance is less than the interorbital distance; the eyes are positioned dorsolaterally, the pupils are round; the spiracle is sinistral and the spiracular tube protrudes from midway along the body wall and anterior to maximal trunk width and opens laterally; the tail length is 68.1% of the total length; the dorsal tail fin inserts behind the body-tail junction, the dorsal fin is low, particularly towards the proximal half of the tail length; the basal tail width is 48.8% of the maximal trunk width, the tail tip is rounded; the oral disc is subterminal and antero-dorsal; the lateral corners fold medially and turn upward when not fully extended (and in preservative), the width of the umbelliform oral disc makes up 63.4% of the maximal width of the trunk; in life the maximal BW is 80.4% ($n=1$) the maximal width of the fully extended ODW; the lower lip is bi-triangular shaped; marginal papillae are absent; regularly positioned, round and oblong shaped submarginal papillae are present on both the upper and lower lip; the lower lip is deeper than the upper lip; labial teeth are absent; the upper jaw sheath is serrated and has a medial notch; the lower jaw sheath lacks a medial notch. See Table 1 for measurements.

Colour in life: (Fig. 6) Dorsally, the head and body are dark brown with a series of cream blotches bordered by orange flecks, diffuse beige flecks cover the dorsal and lateral surfaces of the body; neuromasts are indistinct; the oral disc is a translucent beige brown and the submarginal papillae are dark brown; the dorsal apex of the tail has four cream saddles, the margins of which are bordered with dark brown, the saddles extend from the body and terminate half way along the length of the tail; at the point where the tail meets the body, there is a brown stripe that extends from the body-tail junction and runs along the midline of the lateral tail surface, this stripe becomes increasingly broken and fades into brown blotches half way along the length of the tail, the dorsal and ventral tail fins are opaque, the dorsal fin has many dark brown speckles along its length; the ventral fin has few, very dispersed dark brown speckles; the vent is grey brown and speckled with metallic blue; the sclera of the eye is black; the pupil is black and round; the iris is gold, speckled with black dots; the gills and coils of the gut are visible in the ventral view through the transparent ventral skin.

Colour in preservative: (Fig. 3C) The body is brown with lighter speckles and blotches; the oral disc is a translucent grey brown with dark brown submarginal papillae; the dorsal and ventral fins are opaque, pale grey brown with darker speckles; the venter is speckled grey and brown.

Variation: The tadpole of *M. jingdongensis* at Stage 37 was described previously by Fei *et al.* (2009) although it is unclear if species identity was supported with molecular data. The description is broadly congruent with the specimen collected in this study but there are some notable differences. In the text of Fei *et al.* (2009) the tail tip is described as blunt, but the line drawing of the tadpole shows a specimen with a pointed tail tip. Fei *et al.* (2009) describe the body of *M. jingdongensis* as Stage 37 as unmarked, this is incongruent with our observation of a single specimen at Stage 25 where the body is heavily marked both in life and in preservative.

Megophrys (Panophrys) hoanglienensis

Figs. 3D and 7

Molecular data: A 16S sequence was generated from tissue samples collected from one tadpole (VNMN010903) and uncorrected *p*-distance between this sequence and that of the holotype of *M. hoanglienensis* also collected on Mount Fansipan, Sa Pa District, Lao Cai Province, Vietnam (Genbank accession number MH514889) was 0.0%.

Collection site: The following tadpole description is based on a single specimen (VNMN010903) at Stage 26. The specimen was found feeding in a 5 m wide stream in disturbed evergreen forest on Mount Fansipan, Hoang Lien National Park, Tam Duong District, Lai Chau Province, Vietnam (22.3483°N 103.7700°E, 1901 m asl; Figs. 1 and 2B), on 10 September 2018 by Luan Nguyen, Chung Nguyen and Luong Hoang.

Morphology: The body is longitudinally oval shaped and dorsally compressed; the nares are oval and are closer to the eyes than to the snout, the rims of the nares are serrated and raised from the body wall, the internarial distance is less than the interorbital distance; the eyes are positioned dorsolaterally, the pupils are round; the spiracle is sinistral and the spiracular tube protrudes from midway along the body wall and anterior to maximal trunk width and opens laterally; the tail length is 73.2% of the total length; the dorsal tail fin inserts behind the body-tail junction, the dorsal fin is low, particularly towards the anterior half of the tail length; the basal tail width is 43.5%, of the maximal trunk width, the tail tip is pointed; the oral disc is subterminal and antero-dorsal; the lateral corners fold medially and turn upward when not fully extended (and in preservative), the mean width of the umbelliform oral disc makes up 47.8% of the maximal width of the trunk, in life the width of the umbelliform oral disc exceeds the maximal width of the trunk; in life the maximal BW is 79.3% ($n=1$) the maximal width of the fully extended ODW; the lower lip is bi-triangular shaped; marginal papillae are absent, the edges of the lips are smooth; four rows of submarginal papillae are present on the upper lip when they are counted medially at the maximal width of the oral disc; these are longitudinally oblong shaped at the edge of the disc and become increasingly rounded towards the maximal width of the disc, and become substantially smaller in the outermost row; five rows of longitudinally oblong submarginal papillae are present on the lower lip when they are counted medially at the maximal width of the oral disc; these are regularly positioned and become smaller on the outermost row; the lower lip is deeper than the upper lip; labial teeth are absent; the upper jaw sheath is serrated and has a medial notch; the lower jaw sheath lacks a medial notch. See Table 1 for measurements.

Colour in life: (Fig. 7) Dorsally, the head and body are dark brown, a darker brown irregular shaped bar runs dorsally from nare to nare, a large reddish brown blotch is present posterior to each of the eyes, the area between

these reddish brown blotches and mid dorsum is reticulated with blackish brown; the upper lateral surface of the body is beige with darker brown reticulations and light grey flecks, the skin of the lower lateral surface of the body is also flecked with grey over slightly translucent skin, the colour of the organ structures is visible beneath; the neuromasts are distinct; the oral disc is translucent beige brown, the colouration becoming darker towards the outer margins of the disc, the submarginal papillae are dark brown; the dorsal apex of the tail has light brown saddle, the margins of which are bordered by a dark brown band on the lateral surfaces of the tail, this saddle extends from the body and terminates closer to body than to tail tip; at the point where the tail meets the body there is a brown stripe that extends from the body-tail junction and runs along the midline of the lateral tail surface, this stripe becomes increasingly broken and fades into brown blotches half way along the length of the tail; the dorsal and ventral tail fins are opaque, the upper tail fin is a pale yellowish brown with many dark brown speckles, the lower tail fin is largely translucent with many dark brown speckles; the venter is translucent, speckled with metallic grey blue flecks; the sclera of the eye is broad and black with green-gold flecks; the pupil is black and round; the iris is orange and speckled with black dots.

Colour in preservative: (Fig. 3D) The body is brown with darker speckles; the oral disc is a translucent grey brown with dark brown submarginal papillae; the dorsal and ventral fins are opaque, upper fin pale grey brown with darker speckles; the venter is speckled grey and brown.



FIGURE 7. *Megophrys hoanglienensis* tadpole VNMN010903 in life. Dorsal, lateral, and ventrolateral views. 5 mm scale.

Megophrys (Xenophrys) maosonensis

Figs. 3E and 8

Molecular data: A 16S sequence was generated from a tissue sample obtained from the single tadpole (VNMN010904). Uncorrected *p*-distance between the tadpole in this study and a *M. maosonensis* specimen also collected in the rough proximity of one of the type specimens; Sa Pa District, Lao Cai Province, Vietnam (GenBank accession number KX811786) was 0.0%.

Collection site: The following tadpole description is based on two specimens at Gosner stage 25 (VNMN010904 and HLNP 2018090900015). The specimens were found feeding in a 8 m wide stream in disturbed evergreen forest on Mount Fansipan, Hoang Lien National Park, Cat Cat river, Sa Pa District, Lao Cai Province, Vietnam (22.3214°N 103.8264°E, 1244 m asl; Figs. 1 and 2D), on 9 September 2018 by Luan Nguyen, Chung Nguyen and Luong Hoang.



FIGURE 8. *Megophrys maosonensis* tadpole VNMN010904 in life. Dorsal, lateral, and ventral views. 5 mm scale.

Morphology: The body is elongated and slender; the nares are oval and are approximately equal in distance from the eyes and the snout, the rims of the nares are serrated and raised from the body wall, the internarial distance is less than the interorbital distance; the eyes are positioned dorsolaterally, the pupils are round; the spiracle is sinistral and the spiracular tube protrudes from one third of the way along the body, anterior to maximal trunk width and opens laterally; the tail length is 76.5–77.9% of the total length; the dorsal tail fin inserts behind the body-tail junction, the dorsal fin is low, particularly towards the proximal half of the tail length; the basal tail width is 51.2–61.0%, of the maximal trunk width; the tail tip is narrowly rounded; the oral disc is subterminal and antero-dorsal; the lateral corners fold medially and turn upward when not fully extended (and in preservative), the mean width of the umbelliform oral disc makes up 63.4–68.3% of the maximal width of the trunk; in life, the maximal BW is 73.2% ($n=1$) the maximal width of the fully extended ODW and the width of the umbelliform oral disc exceeds the maximal width of the trunk; the lower lip is bi-triangular shaped; marginal papillae are absent; five rows of submarginal papillae are present on the upper lip when they are counted medially at the maximal width of the oral disc; these are longitudinally oblong shaped and become substantially smaller in the outermost row; four rows of longitudinally oblong submarginal papillae are present on the lower lip when they are counted medially at the maximal width of the oral disc; these are regularly positioned and become smaller on the outermost row the lower lip is deeper than the upper lip; labial teeth are absent; the upper jaw sheath is serrated and has a medial notch; the lower jaw sheath lacks a medial notch. See Table 1 for measurements.

Colour in life: (Fig. 8) Dorsally, the head and body are brown with dark brown speckles which become increasingly dense towards the posterior half of the body; a dark brown bar runs from each nare to the anterior edge of the

eye; there are obvious pale neuromasts; the oral disc is translucent orange brown with darker brown submarginal papillae; the dorsal apex of the tail muscle on the anterior half of the tail is orange brown and forms a saddle which is speckled with dark brown, the lower margins of the orange brown saddle fade to cream bordered by a dark brown band which runs half way along the tail length on the lateral surface; at the point where the tail meets the body there is an area of dense dark brown flecks which form a thin line which extends from the body-tail junction and runs along the midline of the lateral tail surface; the dorsal and ventral tail fins are opaque, beige brown with a few dark brown speckles; the ventral surface is a translucent cream with and speckled with metallic grey blue; the lower lip is bordered by a “V” shaped area of dark stippling when viewed from the ventral surface; the sclera of the eye is broad and black with green-gold flecks; the pupil is black and round; the iris orange and speckled with black dots.

Colour in preservative: (Fig. 3E) The body is grey with darker speckles; the oral disc is a translucent grey with dark brown submarginal papillae; the dorsal and ventral fins are opaque, pale grey with few visible darker speckles; the ventral surface is dark grey.

Variation: The tadpole of *M. maosonensis* at Stages 37–39 was described previously by Fei *et al.* (2009; see Table 1 for measurements) as *M. major* Boulenger; a species now only definitively known from northeast India (Mahony *et al.* 2018), although species identity was not apparently supported with molecular data in Fei *et al.* (2009). The line drawing depicts a tadpole with a very pointed tail tip, the specimens from the Hoang Lien Range have a narrowly rounded tail tip (Fig. 8). Robust comparison cannot be made with the description of Fei *et al.* (2009) as the tadpoles were of a much later stage (37–39) than those collected in the Hoang Lien Range (Stage 25).

Discussion

Amphibians in Southeast Asia are under increasing threat as they are poorly studied, and their habitats are being lost rapidly (Rowley *et al.* 2010). Most species of *Megophrys* are forest-dependent, and a number are threatened by habitat loss and degradation (Wu & Yang 2004; IUCN 2016; Tapley *et al.* 2017, 2018a,b; Mahony *et al.* 2018). In addition, frogs in the genus are poorly known, with undiagnosed species diversity (Chen *et al.* 2017; Liu *et al.* 2018), a high rate of species discovery and a lack of basic biological data for the species that are known. The description of *Megophrys* tadpoles from the Hoang Lien Range helps address a knowledge deficit and will aid further research.

Detailed descriptions of tadpoles are important when undertaking rapid biodiversity inventories as these descriptions may aid in the detection of species. Tadpoles are often relatively easy to locate in the field as they usually occur within a defined area of aquatic habitat and they may be present, and indeed abundant, for several months (Grosjean *et al.* 2015) or even years (Morrison & Hero 2003). In contrast, post metamorphic amphibians often disperse from breeding sites and individuals, particularly of arboreal species, may be difficult to find outside of the breeding season. In the case of *Megophrys*, we have failed to detect post metamorphic individuals during field surveys in the Hoang Lien Range in December 2017 and March 2018, yet *Megophrys* tadpoles have been observed at this time (L. Nguyen unpublished data). Furthermore, the presence of *M. gigantea* at our study sites was first confirmed by the presence of their large active tadpoles which were much more conspicuous than the adult frogs outside of the breeding season. In the Hoang Lien Range, more than one *Megophrys* species may often be found in syntopy; and we found the tadpoles of both *M. gigantea* and *M. jingdongensis* at one site at the same time and did not observe the adults of either species. A thorough understanding of both the microhabitat for both larvae and post metamorphic amphibians is essential for informing amphibian conservation strategies, such as which habitat to protect and optimal periods in which to monitor populations of a species. This is of particular importance as *M. gigantea* is assessed as Vulnerable by the IUCN (Wu & Yang 2004) and both *M. fansipanensis* and *M. hoanglienensis* likely qualify for listing as Endangered (Tapley *et al.* 2018a).

Tadpole characteristics may also assist with diagnosing evolutionary relationships in cases where adults are highly morphologically conserved. There have been proposals to raise the seven current subgenera to genera within the current genus *Megophrys sensu lato* (Delorme *et al.* 2006; Frost *et al.* 2006; Li & Wang 2008; Chen *et al.* 2017; Li *et al.* 2020). Whilst there are some reported differences between the tadpoles of some of the proposed genera (e.g., between the subgenus *Brachytarsophrys* and other Megophryinae; Li *et al.* 2011), there are no currently known reliable morphological characters in either tadpoles (Grosjean 2003) or post metamorphic individuals that can be used to define all of the proposed genera (see review in Mahony *et al.* 2017). Our sample size was too small to draw any well supported conclusions on differences between subgenera. There were interspecific differences in BW/ODW in

life as well as tail tip shape (Table 1) which could be useful when assigning tadpoles to a species within the Hoang Lien Range. Only *M. gigantea* has a broadly rounded tail and only *M. maosonensis* has a narrowly rounded tail. In our study, both *M. fansipanensis* and *M. hoanglienensis* have pointed tails and the former differs from the latter by having a smaller BW/ODW (63% in *M. fansipanensis* versus 79.3% in *M. hoanglienensis*). *Megophrys jingdongensis* and *M. rubrimera* are syntopic with one another in the Hoang Lien Range (Tapley *et al.* 2018b); only one tadpole specimen of both *M. jingdongensis* and *M. rubrimera* was examined and these specimens differed in their stage of development. There were no obvious interspecific differences between the larvae of *M. jingdongensis* and *M. rubrimera*; additional specimens would be required for the elucidation of any interspecific differences. The hastate shape mouthparts of tadpoles in the subgenus *Atympanophrys* is only clearly visible in life, in preservative the mouthparts become folded and the shape is not clearly apparent. Li *et al.* (2011) used fixed specimens in their study of megophryid tadpole types (including *Atympanophrys*) and this feature may not have been obvious in preserved specimens. Further research should focus on whether this character is found in other species in the subgenus and whether this larval character can be considered diagnostic for the subgenus.

Acknowledgements

We are grateful to the staff at Hoang Lien National Park for their assistance and collaboration. In particular we would like to thank Director Nguyen Quang Vinh for continued support and partnership. We thank Mr Nguyen Huu Hanh, Vice Chief of Lao Cai FPD for his support in arranging the office's work at Ba Xat. The Vietnamese Ministry of Agriculture and Rural Development and staff at Hoang Lien National Park kindly facilitated surveys and issued permissions (Permit numbers 1343/TCCL-BTTN, 3762/UBND-NC, 4198/UBND-NLN and 3762/UBND-NC). Ethical approval was granted by the Zoological Society of London's ethics committee (project ZFP1) and the Australian Museum Animal Ethics Committee (#15-07). We would also like to extend our thanks to the People's Committee of Lao Cai Province for supporting this programme of research. This work was supported by Ocean Park Conservation Foundation Hong Kong (B. Tapley, L. Nguyen and J. Rowley) and a ZSL EDGE Fellowship (L. Nguyen). We are grateful to Daniel Kane for permitting us to use his image of a collection site. We are grateful to an anonymous reviewer and Florencia Vera Candioti for their comments which helped refine this paper.

References

- Altig, R. & McDiarmid, R.W. (1999a) Diversity: familial and generic characterization. *In*: McDiarmid, R.W. & Altig, R. (Eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, Chicago, pp. 24–51.
- Altig, R. & McDiarmid, R.W. (1999b) Body plan: development and morphology. *In*: McDiarmid, R.W. & Altig, R. (Eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, Chicago, pp. 295–337.
- Chen, J.M., Zhou, W.W., Poyarkov Junior, N.A., Stuart, B.L., Brown, R.M., Lathrop, A., Wang, Y.Y., Yuan, Z.Y., Jiang, K., Hou, M., Chen, H.M., Suwannapoom, C., Ngoc Nguyen, S., Van Duong, T., Papenfuss, T.J., Murphy, R.W., Zhang, Y.P. & Che, J. (2017) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus *Megophrys sensu lato* (Anura: Megophryidae). *Molecular Phylogenetics and Evolution*, 106, 28–43.
- Delorme, M., Dubois, A., Grosjean, S. & Ohler, A. (2006) Une nouvelle ergotaxinomie des Megophryidae (Amphibia, Anoures). *Alytes*, 24, 6–21.
- Deuti, K., Grosjean, S., Nicolas, V., Vasudevan, K.V. & Ohler, A. (2017) Nomenclatural puzzle in early *Xenophrys* nomina (Anura, Megophryidae) solved with description of two new species from India (Darjeeling hills and Sikkim). *Alytes*, 34, 20–48.
- Dubois, A. & Ohler, A. (1998) A new species of *Leptobrachium* (*Vibrissaphora*) from northern Vietnam, with a review of the taxonomy of the genus *Leptobrachium* (Pelobatidae, Megophryinae). *Dumerilia*, 4, 1–32.
- Fei, L., Hu, S.Q., Ye, C.Y. & Huang, Y.Z. (2009) *Fauna Sinica. Amphibia. Volume 2. Anura*. Chinese Academy of Science, Science, Beijing, XIII + 957 pp.
- Fei, L. & Ye, C.Y. (2016) *Amphibians of China, Volume I*. Science Press, Beijing, 1040 pp.
- Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F., De Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C. & Raxworthy, C.J. (2006) The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297, 1–291.
[https://doi.org/10.1206/0003-0090\(2006\)297\[0001:TATOL\]2.0.CO;2](https://doi.org/10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2)
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.

- Grosjean, S. (2003) A redescription of the external and buccopharyngeal morphology of the tadpole of *Ophryophryne microstoma* Boulenger, 1903 (Megophryidae). *Alytes*, 21, 45–58.
- Grosjean, S., Ohler, A., Chuaynkern, Y., Cruaud, C. & Hassanin, A. (2015) Improving biodiversity assessment of anuran amphibians using DNA barcoding of tadpoles. Case studies from Southeast Asia. *Comptes Rendus Biologies*, 338, 351–361. <https://doi.org/10.1016/j.crv.2015.03.015>
- IUCN (2016) *Megophrys damrei*. *The IUCN Red List of Threatened Species* 2016: e.T48101780A48101799. <https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T48101780A48101799.en>
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
- Le, D.T., Pham, A.V., Nguyen, S.H.L., Ziegler, T. & Nguyen, T.Q. (2006) First records of *Megophrys daweimontis* Rao and Yang, 1997 and *Amolops vitreus* (Bain, Stuart and Orlov, 2006) (Anura: Megophryidae, Ranidae) from Vietnam. *Asian Herpetological Research*, 6, 66–72.
- Leong, T.M. & Chou, L.M. (1998). Larval identity of the montane horned frog, *Megophrys longipes* (Boulenger) (Amphibia: Anura: Megophryidae). *Raffles Bulletin of Zoology*, 46, 471–476.
- Li, C. & Wang, Y.Z. (2008) Taxonomic review of *Megophrys* and *Xenophrys*, and a proposal for Chinese species (Megophryidae, Anura). *Acta Zootaxonomica Sinica*, 33, 104–106.
- Li, C., Guo, X.G. & Wang, Y.Z. (2011) Tadpole types of Chinese megophryid frogs (Anura: Megophryidae) and implications for larval evolution. *Current Zoology*, 57, 93–100. <https://doi.org/10.1093/czoolo/57.1.93>
- Li, S., Xu, N., Liu, J., Jiang, J.P., Wei, G. & Wang, B. (2019 “2018”) A new species of the Asian toad genus *Megophrys* sensu lato (Amphibia: Anura: Megophryidae) from Guizhou Province, China. *Asian Herpetological Research*, 9, 224–239.
- Li, Y.L., Jin, M.J., Zhao, J., Liu, Z.Y., Wang, Y.Y. & Pang, H. (2014) Description of two new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Heishiding Nature Reserve, Fengkai, Guangdong, China, based on molecular and morphological data. *Zootaxa*, 3795, 449–471. <http://dx.doi.org/10.11646/zootaxa.3795.4.5>
- Li, Y., Zhang, D.D., Lyu, Z.T., Wang, J., Li, Y.L., Liu, Z.Y., Chen, H.H., Rao, D.Q., Jin, Z.F., Zhang, C.Y. & Wang, Y.Y. (2020) Review of the genus *Brachytarsophrys* (Anura: Megophryidae), with revalidation of *Brachytarsophrys platyparietus* and description of a new species from China. *Zoological Research*, 41, 105–122.
- Liu, J., Li, S., Wei, G., Xu, N., Cheng, Y., Wang, B. & Wu, J. (2020) A new species of the Asian toad genus *Megophrys* sensu lato (Anura: Megophryidae) from Guizhou Province, China. *Asian Herpetological Research*, 11, 1–18.
- Liu, Z., Chen, G., Zhu, T., Zeng, Z., Lyu, Z., Wang, J., Messenger, K., Greenberg, A.J., Guo, Z., Yang, Z. & Shi, S. (2018) Prevalence of cryptic species in morphologically uniform taxa—fast speciation and evolutionary radiation in Asian frogs. *Molecular Phylogenetics and Evolution*, 127, 723–731.
- Luong, A.M., Pham, A.V., Nguyen, T.T. & Nguyen, T.Q. (2019) First record of *Megophrys gigantea* Liu, Hu & Yang, 1960 (Anura: Megophryidae) from Vietnam. *Russian Journal of Herpetology*, 26, 201–204.
- Lyu, Z.T., Li, Y.Q., Zeng, Z.C., Zhao, J., Liu, Z.Y., Guo, G.X. & Wang Y.Y. (2020) Four new species of Asian horned toads (Anura, Megophryidae, *Megophrys*) from southern China. *ZooKeys*, 942, 105–140. <https://doi.org/10.3897/zookeys.942.47983>.
- Mahony, S., Foley, N., Biju, S.D. & Teeling, E. (2017) Evolutionary history of the Asian Horned Frogs (Megophryinae): integrative approaches to timetree dating in the absence of a fossil record. *Molecular Biology and Evolution*, 34, 744–771.
- Mahony, S., Kamei, R.G., Teeling, E.C. & Biju, S.D. (2018) Cryptic diversity within the *Megophrys major* species group (Amphibia: Megophryidae) of the Asian Horned Frogs: Phylogenetic perspectives and a taxonomic revision of South Asian taxa, with descriptions of four new species. *Zootaxa*, 4523, 1–96.
- Mahony, S., Kamei, R.G., Teeling, E.C. & Biju, S.D. (2020) Taxonomic review of the Asian Horned Frogs (Amphibia: *Megophrys* Kuhl & Van Hasselt) of Northeast India and Bangladesh previously misidentified as *M. parva* (Boulenger), with descriptions of three new species. *Journal of Natural History*, in press.
- Morrison, C. & Hero, J.M. (2003) Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology*, 72, 270–279. <https://doi.org/10.1046/j.1365-2656.2003.00696.x>
- Nguyen, S.V., Ho, T.C. & Nguyen, T.Q. (2009) *Herpetofauna of Vietnam*, Edition Chimaira, Frankfurt am Main, 768 pp.
- Nguyen, T.Q., Nguyen, T.T., Luong, A.M. & Ziegler, T. (2020) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from Vietnam. *Zootaxa* 4722, 401–422.
- Oberhammer, E., Barten, C., Schweizer, M., Das, I., Haas, A. & Hertwig, S.T. (2014) Description of the tadpoles of three rare species of megophryid frogs (Amphibia: Anura: Megophryidae) from Gunung Mulu, Sarawak, Malaysia. *Zootaxa*, 3835, 59–79. <http://dx.doi.org/10.11646/zootaxa.3835.1.3>
- Ohler, A. (2003) Revision of the genus *Ophryophryne* Boulenger, 1903 (Megophryidae) with description of two new species. *Alytes* 21, 23–44.
- Ohler, A., Marquis, O., Swan, S. & Grosjean, S.T. (2000) Amphibian biodiversity of Hoang Lien Nature Reserve (Lao Cai Province, northern Vietnam) with description of two new species. *Herpetozoa*, 13, 71–87.
- Orlov, N.L., Murphy, R.W., Ananjeva, N.B., Ryabov, S.A. & Ho, C.T. (2002) Herpetofauna of Vietnam, a checklist. Part 1.

- Amphibia. *Russian Journal of Herpetology*, 9, 81–104.
- Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) The simple fool's guide to PCR. Department of Zoology, University of Hawaii, Honolulu, 47 pp.
- Poyarkov Jr, N.A., Duong, T.V., Orlov, N.A., Gogoleva, S.S., Vassilieva, A.B., Nguyen, L.T., Nguyen, V.D.H., Nguyen, S.G., Che, G. & Mahony, S. (2017) Molecular, morphological and acoustic assessment of the genus *Ophryophryne* (Anura, Megophryidae) from Langbian Plateau, southern Vietnam, with description of a new species. *Zookeys*, 62, 49–120. <https://doi.org/10.3897/zookeys.62.10624>
- Rowley, J.J.L., Brown, R., Bain, R., Kusriani, M., Inger, R., Stuart, B., Wogan, G., Thy, N., Chan-ard, T., Trung, C.T., Diesmos, A., Iskandar, D.T., Lau, M., Minh, L.T., Makchai, S., Nguyen, T.Q., Phimmachak, S. (2010) Impending conservation crisis for Southeast Asian amphibians. *Biology Letters*, 6, 336–338.
- Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis, *Nature Methods*, 9, 671–675.
- Stuart, B.L., Chuaynkern, Y., Chan-ard, T. & Inger, R.F. (2006) Three new species of frogs and a new tadpole from eastern Thailand. *Fieldiana Zoology*, 111, 1–19. [https://doi.org/10.3158/0015-0754\(2006\)187\[1:TNSOFA\]2.0.CO;2](https://doi.org/10.3158/0015-0754(2006)187[1:TNSOFA]2.0.CO;2)
- Tapley, B., Cutajar, T., Mahony, S., Nguyen, T.C., Dau, V.Q., Nguyen, T.T., Luong, V.H. & Rowley, J.J.L. (2017) The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. *Zootaxa*, 4344, 465–492. <https://doi.org/10.11646/zootaxa.4344.3.3>
- Tapley, B., Cutajar, T., Mahony, S., Nguyen, C.T., Dau, V.Q., Luong, A.M., Le, D.T., Nguyen, T.T., Nguyen, T.Q., Portway, C., Luong, H.V. and Rowley, J.J., (2018a). Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. *Zootaxa*, 4508, 301–333. <https://doi.org/10.11646/zootaxa.4508.3.1>
- Tapley, B., Cutajar, T., Nguyen, L.T., Nguyen, C.T., Harding, L., Portway, C., Luong, H.V., Rowley, J.J.L. (2018b) A new locality and elevation extension for *Megophrys rubrimera* in Bat Xat Nature Reserve, Lao Cai Province, northern Vietnam. *Herpetology Notes*, 11, 865–868
- Tapley, B., Nguyen, L.T. & Le, M.V. (2020) A description of the tadpole of *Megophrys* “*Brachytarsophrys*” *intermedia* (Smith, 1921), *Zootaxa*, 4845 (1), 26–34. <https://doi.org/10.11646/zootaxa.4845.1.2>
- Wang, B., Wu, Y.-Q., Peng, J J.-W., Shi, S.C., Lu, N.-N. & Wu, J. (2020) A new *Megophrys* Kuhl & Van Hasselt (Amphibia, Megophryidae) from southeastern China. *ZooKeys*, 904, 35–62.
- Wang, Y.Y., Zhang, T.-d., Zhao, J., Sung, Y.-h., Yang, J.H., Pang, H. & Zhang, Z. (2012) Description of a new species of the genus *Xenophrys* Günther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. *Zootaxa*, 3546, 53–67.
- Wang, Y., Zhao, J., Yang, J., Zhou, Z., Chen, G. & Liu, Y. (2014) Morphology, molecular genetics, and bioacoustics support two new sympatric *Xenophrys* toads (Amphibia: Anura: Megophryidae) in Southeast China. *PLoS ONE*, 9(4), e93075.
- Wu, G. & Yang, D. (2004) *Megophrys giganticus*. The IUCN Red List of Threatened Species 2004: e.T57637A11667811. <http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T57637A11667811.en>
- Xu, N., Li, S.Z., Liu, J., Wei, G. & Wang, B. (2020) A new species of the horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. *ZooKeys*, 943, 119–144. <https://doi.org/10.3897/zookeys.943.50343>
- Zhao, J., Yang, J., Chen, G., Chen, C. & Wang, Y. (2014) Description of a new species of the genus *Brachytarsophrys* Tian and Hu, 1983 (Amphibia: Anura: Megophryidae) from Southern China based on molecular and morphological data. *Asian Herpetological Research*, 5, 150–160. <https://doi.org/10.3724/SP.J.1245.2014.00150>