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A new potentially Endangered species of *Megophrys* (Amphibia: Megophryidae) from Mount Ky Quan San, north-west Vietnam

Benjamin Tapley [a], Timothy Cutajar [b], Luan Thanh Nguyen [c,d], Christopher Portway [b], Stephen Mahony [e,f], Chung Thanh Nguyen [g], Luke Harding [h], Hao Van Luong [g] and Jodi J. L. Rowley [b,i]

aZoological Society of London, London, UK; bAustralian Museum Research Institute, Australian Museum, Sydney, Australia; cIndo-Myanmar Conservation, London, UK; dEDGE of Existence Programme, Zoological Society of London, London, UK; eSchool of Biology and Environmental Science, University College Dublin, Dublin, Ireland; fDepartment of Life Sciences, The Natural History Museum, London, UK; gHoang Lien National Park, Lao Cai, Vietnam; hWild Planet Trust, Paignton, UK; iCentre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

ABSTRACT

The genus *Megophrys* is known to harbour morphological cryptic species diversity. During field work on Vietnam's third highest peak, Mount Ky Quan San, north-west Vietnam, we collected specimens of a new species of *Megophrys* at two locations more than 2000 m above sea level (asl). Phylogenetic analyses using mitochondrial markers place the new species within the subgenus *Panophrys*, a primarily Chinese radiation within the genus *Megophrys*. We describe the species based on an all-male type series and distinguish it from all other *Megophrys* species from mainland Southeast Asia, north of the Isthmus of Kra and nearby provinces of China (Yunnan, Guangxi and Guizhou) based on morphological, molecular and bioacoustic data. The new species is inferred to form a clade along with *M. hoanglienensis* and *M. fansipanensis*; it is syntopic with the former but has a non-overlapping range with the latter. Uncorrected *p* distances for the 16S rDNA and CO1 genes between the new species and closest relatives exceed values observed between other closely related species in the *Panophrys* subgenus. The new species is most similar to *M. fansipanensis* in terms of morphology, and several call parameters also overlap with this species; however, these two species call at different times of year. This is the fourth likely range-restricted and Endangered *Megophrys* species described from the Hoang Lien Range since 2017, and this discovery further highlights the significance of the Hoang Lien Range for Vietnam's amphibian diversity.

http://zoobank.org/NomenclaturalActs/69340DD7-B21E-4DD0-836F-6741878013BB

TÓM TẮT TIẾNG VIỆT

Giống cóc sừng *Megophrys* chứa đựng sự đa dạng cao với nhiều loài tương đồng về hình thái. Trong quá trình khảo sát thực địa tại vùng đỉnh núi Ky Quan San, Tây Bắc Việt Nam, chúng tôi khám phá ra một

CONTACT Benjamin Tapley ✉ ben.tapley@zsl.org
Supplemental data for this article can be accessed here.

loài mới thuộc giống *Megophrys* ở độ cao trên 2000 m. Các phân tích về dữ liệu di truyền sử dụng các đoạn gen thuộc hệ gen ty thể cho thấy quần thể này thuộc giống phụ *Panophrys* trong giống *Megophrys*, đây là một giống chủ yếu phân bố tại Trung Quốc. Chúng tôi mô tả loài mới này dựa trên các mẫu vật đực và so sánh với các loài khác trong giống *Megophrys* ở đất liền vùng Đông Nam Á (từ phía bắc eo đất Kra, Thái Lan) và khu vực lân cận ở Trung Quốc dựa trên đặc điểm hình thái, phân tử và âm học. Loài mới được mô tả gần gũi với hai loài đặc hữu ở dãy Hoàng Liên Sơn là *Megophrys hoanglienensis* và *Megophrys fansipanensis*; loài mới có cùng khu vực phân bố với loài *M. hoanglienensis* song về hình thái và tiếng kêu thì giống với loài *M. fansipanensis* nhưng giữa chúng khác nhau về mùa sinh sản. Đây là loài thứ tư, có vùng phân bố hẹp và nguy cơ tuyệt chủng cao được mô tả từ dãy Hoàng Liên từ năm 2017 đến nay. Khám phá này cho thấy sự đa dạng cao các loài lưỡng cư và tầm quan trọng của dãy Hoàng Liên đối với khu hệ lưỡng cư ở Việt Nam.

Introduction

The Asian horned frog genus *Megophrys* Kuhl and Van Hasselt, 1822 comprises seven subgenera (Mahony et al. 2017) and 110 species, of which over 25% were described since 2017 (e.g. Li et al. 2020; Lyu et al. 2020; Mahony et al. 2020; Nguyen et al. 2020; Shi et al. 2020; Su et al. 2020; Wang et al. 2020; Xu et al. 2020). *Megophrys* are morphologically conservative stream-breeding frogs 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). The genus is known to harbour hidden species diversity within morphologically obscure species complexes; molecular analyses published by Chen et al. (2017) and Liu et al. (2018) provided evidence for more than 20 putative species of *Megophrys* from mainland Southeast Asia and China.
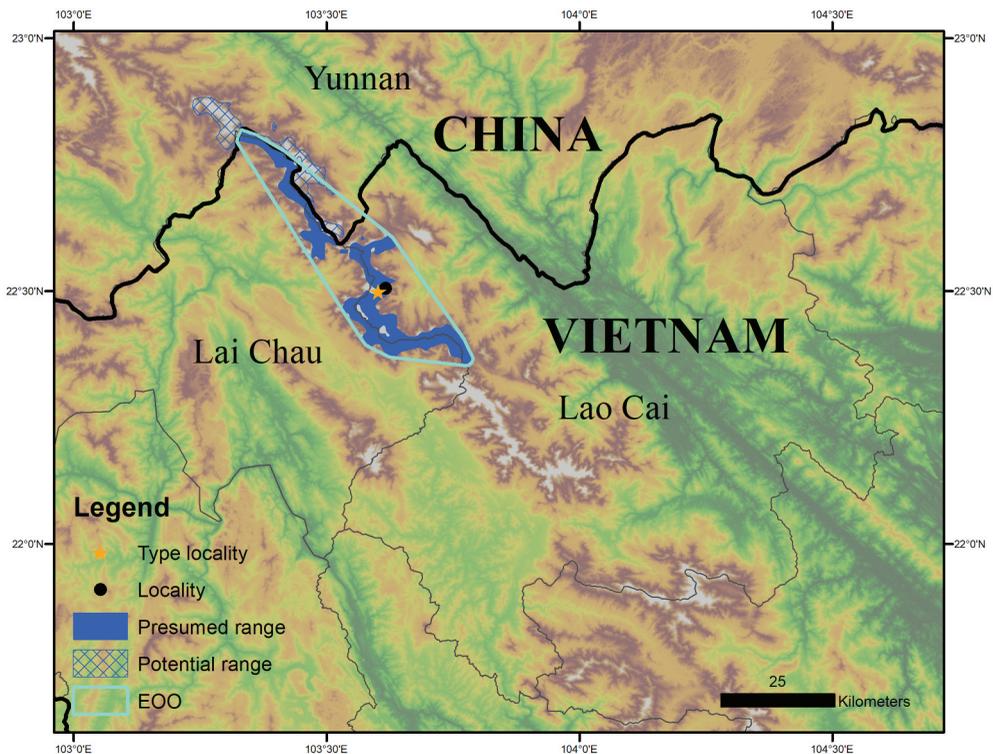
Vietnam supports the second highest number of *Megophrys* species (China has the highest), with the following 23 species reported from Vietnam to date (Orlov et al. 2002, 2015; Ohler 2003; Le et al. 2006; Nguyen et al. 2009, 2020; Chen et al. 2017; Mahony et al. 2017; Poyarkov et al. 2017; Tapley et al. 2017, 2018a; Luong et al. 2019): *M. brachykolos* Inger and Romer, 1961; *M. caobangensis* Nguyen, Pham, Nguyen, Luong and Ziegler, 2020; *M. daweimontis* Rao and Yang, 1997; *M. elfina* Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che and Mahony, 2017; *M. fansipanensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong and Rowley, 2018a; *M. feae* Boulenger, 1887; *M. gerti* (Ohler, 2003); *M. gigantica* Liu, Hu and Yang, 1960; *M. hansi* (Ohler, 2003); *M. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong and Rowley, 2018a; *M. intermedia* Smith, 1921; *M. jingdongensis* Fei and Ye, 1983 in Fei et al. (1983); *M. koui* Mahony, Foley, Biju and Teeling, 2017; *M. major* Boulenger, 1908; *M. maosonensis* Bourret, 1937; *M. microstoma* (Boulenger, 1903); *M. minor* Stejneger, 1926; *M. pachyproctus* Huang, 1981 (in Huang and Fei 1981); *M. palpebralespinosa* Bourret, 1937; *M. parva* (Boulenger, 1893); *M. poilani* (Bourret, 1937); *M. rubrimera* Tapley, Cutajar, Mahony, Nguyen, Dau, Nguyen, Luong and Rowley, 2017; and *M. synoria* (Stuart, Sok and Neang, 2006).

During field work on Mount Ky Quan San, Vietnam, we encountered a *Megophrys* species that was distinct from other congeners in mainland Southeast Asia and neighbouring provinces of China. We describe this species as new on the basis of

morphological, genetic, bioacoustic and behavioural differences, as the new species calls at a different time of year than other *Panophrys* Rao and Yang, 1997 species in the Hoang Lien Range.

Materials and methods

Specimens were collected at night in forest stream habitats on Mount Ky Quan San, Bat Xat District, Lao Cai Province, Vietnam, in September 2017 (Figure 1). Geographic coordinates were obtained using a Garmin GPSMAP 60CSx Global Positioning System receiver (Garmin Ltd., Kansas, USA) and recorded in the World Geodetic System 1984 datum. Specimens were photographed in life before being humanely euthanased using a 20% solution of benzocaine applied to the ventral surface of the frog. Tissue samples (liver) for molecular analyses were extracted from freshly euthanased specimens and stored in ethylenediaminetetraacetic acid / dimethyl sulfoxide (EDTA / DMSO) or molecular-grade ethanol prior to fixation of specimens with 10% formalin and storage in 70% ethanol. Type specimens were deposited at the Vietnam National Museum of Nature, Hanoi (VNMN) and

Figure 1. Distribution of *Megophrys frigida* sp. nov. in the Hoang Lien Range, north-west Vietnam. The solid blue area represents the presumed area where the habitat is within the species' estimated elevation range, covered with forest, and not separated from known localities by any continuous stretch of unsuitable habitat by a distance equal to or greater than 1 km. The blue cross-hatching denotes areas where this species may be extant in China (potential range). The light blue outline indicates the Extent Of Occurrence (EOO).

the Australian Museum, Sydney (AMS). A referred specimen was deposited at the Hoang Lien National Park headquarters (HLNP) as a reference for National Park scientists. Comparative material was loaned for examination from the California Academy of Science, California, USA (CAS), the American Museum of Natural History, New York, USA (AMNH) and the Field Museum of Natural History, Chicago, USA (FMNH). Specimens from the Centre for Ecological Sciences, the Indian Institute of Science, Bangalore, India (CES), the Natural History Museum, London, UK (NHMUK; specimen acronym BMNH––British Museum [Natural History]), and the Zoological Survey of India, Kolkata, West Bengal, India (ZSIC), were examined at the respective institutions (see Supplementary information I).

Molecular data

Total genomic DNA was extracted from EDTA/DMSO- or ethanol-preserved tissues using a DNeasy® Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's protocols for purification of genomic DNA from animal tissues. We amplified a ~587-bp section of 16S (mtDNA) using the primers (5′–3′) 16Sar CGCCTGTTTATCAAAAACAT and 16Sbr CCGGTCTGAACTCAGATCACGT (Palumbi et al. 1991) and a ~640-bp section of CO1 (mtDNA) using the primers (5′–3′) Chmf4 TYTCWACWAAYCAYAAAGAYATCGG and Chmr4 ACYTCRGGRTGRCCRAARAATCA (Che et al. 2012). Polymerase chain reaction (PCR) amplification was carried out in 24-μL reactions with 1000 ng of genomic DNA, 1 * Reaction Buffer (Bioline MyTaq^{TM} Red Reaction Buffer), 2 pmol of corresponding primers and Bioline MyTaq^{TM} 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) for 16S; and initial denaturation 95°C (5 min), 35 cycles of 94°C (60 s) denaturation, 46°C (60 s) annealing and 72°C (60 s) extension, followed by a final extension step at 72°C (10 min) for CO1. All PCR products were purified using ExoSap-IT^{TM} (USB Corporation, Ohio USA), and sequenced in both directions at Macrogen (Seoul, South Korea). Sequence chromatograms were edited and checked for quality using Sequencher v. 4.10 (Gene Codes, Ann Arbour, Michigan USA). The new sequences were then checked on BLAST (The National Center for Biotechnology Information) (Altschul et al. 1990) to verify their approximate identity.

Phylogenetic analysis

Results of the BLAST analyses, including sequences from the four specimens we collected from the Hoang Lien Range, showed that all of the least divergent samples were from species within the subgenus *Panophrys*. For phylogenetic analyses, 12S and 16S sequences (hereafter the 12S–16S data set) belonging to 47 of the 48 known species of this subgenus were obtained from the following papers with published sequence data (Zheng et al. 2004; Li et al. 2014; Xiang et al. 2013; Oberhummer et al. 2014; Wang et al. 2014; Chen et al. 2017; Mahony et al. 2017; Poyarkov et al. 2017; Tapley et al. 2017; Wang et al. 2017; Zhang et al. 2017; Mahony et al. 2018; Tapley et al. 2018a; Liu et al. 2018; Li et al. 2019 ["2018"]; Messenger et al. 2019; Wang et al. 2019a; Wang et al. 2019b; Wu et al. 2019; Cutajar et al.

2020; Liu et al. 2020; Lyu et al. 2020; Nguyen et al. 2020; Wang et al. 2020; Xu et al. 2020) and downloaded from GenBank (Benson et al. 2017), along with the following outgroup taxa representing all other subgenera: *Megophrys* (*Atympanophrys*) *shapingensis* Liu, 1950, *M.* (*Brachytarsophrys*) *carinense* (Boulenger, 1889), *M.* (*Ophryophryne*) *microstoma, M.* (*O.*) *synoria, M.* (*Megophrys*) *montana* Kuhl and Van Hasselt, 1822, *M.* (*Pelobatrachus*) *nasuta* (Schlegel, 1858) and *M.* (*Xenophrys*) *monticola* (Günther, 1864). Two additional taxa, *Leptobrachella oshanensis* (Liu, 1950) and *Leptobrachium boringii* (Liu, 1945), were included to root the tree, following Chen et al. (2017).

We included a second data set of 84 cytochrome c oxidase subunit I (CO1) sequences belonging to 42 of 48 known species of this subgenus available on GenBank and 10 new sequences representing the newly collected species, and *M. fansipanensis*, *M. jingdongensis, M. hoanglienensis* and *M. rubrimera* (the other *Panophrys* species that occur at elevations higher than 2000 m asl in the Hoang Lien Range; Supplementary information II) (hereafter the CO1 data set). *Leptobrachium boringii* was included to root the tree, following Chen et al. (2017).

New and previously published sequences were aligned using MAFFT (Katoh et al. 2002) on the CIPRES Science Gateway (http://www.phylo.org/sub_sections/portal; Miller et al. 2010).

Uncorrected *p* distance (with partial deletion of gaps and missing data) was calculated based on 16S and on the CO1 data set using MEGA7 (Kumar et al. 2016). As *Megophrys* are morphologically conservative, misidentifications of sequences on GenBank are common, so the distance from the collection site of sequenced specimens and the type locality of each species was estimated using the ruler function on Google Earth. This measurement may be indicative of how reliable the associated sequence data are, particularly for historically described species that do not have associated sequence data for type specimens. Locality information and GenBank accession numbers for all sequences included in the analyses can be found in Supplementary information II.

The matrilineal genealogy was inferred using two methods for the 12S–16S and CO1 data sets: Bayesian inference (BI) and maximum likelihood (ML). The optimal evolutionary models for BI analysis in both data sets were tested using MODELTEST v. 3.06 (Posada and Crandall 1998), and the best-fit model for BI was the GTR+G+I model of DNA evolution (suggested by the Akaike information criterion). ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE was used to estimate the optimal evolutionary model for ML analysis, and the best-fit model was GTR+F+I+G4 for the 12S–16S data set and TVM+F+I+G4 for the CO1 data set, chosen according to the Bayesian information criterion.

BI analysis was conducted in MRBAYES v. 3.2 for both data sets (Ronquist et al. 2012); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run for 15 million generations for the 12S–16S data set and for 5 million generations for the CO1 data set, and sampled every 1000 generations. Five independent MCMCMC runs were performed, and the first 25% of trees were discarded as burn-in. The remaining trees were combined, and a 50% majority-rule consensus tree was generated. Confidence in topology was assessed by posterior probability (BPP, Huelsenbeck et al. 2001). IQ-TREE v. 1.6.7.1 (Nguyen et al. 2015) was used to perform the ML analysis with 10,000 ultrafast bootstrap replications (Minh et al. 2013), and confidence in node topology was tested by non-parametric bootstrapping with 1000 replicates (BS; Felsenstein 1985). We considered

posterior probability and ultrafast bootstrap support values of ≥95% to indicate strong support (Felsenstein 1985; Minh et al. 2013).

Morphological data

We recorded morphological data (to the nearest 0.1 mm) with digital callipers from fixed specimens. Measurements and morphometric abbreviations follow Mahony et al. (2011): 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 border of the orbit (SL); distance from the nostril to the tip of the snout (SN); distance from the front of the orbit to the nostril (EN); eye length, the horizontal distance between the bony orbital borders of the eye (EL); inter-upper eyelid width, the shortest distance between the upper eyelids (IUE); maximum upper eyelid width (UEW); internarial distance (IN); internal front of eyes, the shortest distance between the anterior orbital borders of the eyes (IFE); internal back of eyes, 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 (FIIIL); 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); and length of the inner metatarsal tubercle (IMT). Sex and maturity were determined by the presence of nuptial pads on adult males and gonadal inspection of both sexes. Body mass was recorded in life (to the nearest 0.1 g) within 12 h of specimen collection using a Pesola® spring scale (Pesola AG, Schindellegi, Switzerland); we use mg/mm to assess the differences between specimens of the newly collected species and *M. fansipanensis*, *M. jingdongensis, M. hoanglienensis* and *M. rubri-mera* (the other *Panophrys* species that occur at elevations above 2000 m asl in the Hoang Lien Range). We acknowledge that these data may be skewed by diet and when the specimen was weighed.

To assess morphometric differences between male specimens from the new popula-tion ($N = 4$) and other *Panophrys* species that are present at elevations above 2000 m asl in the Hoang Lien Range, we assigned specimens to *a priori* groups informed by molecular data and compared morphometric data from the newly collected species and *M. fansipa-nensis* ($N = 13$; data from Tapley et al. 2018a), *M. jingdongensis* ($N = 4$; see Supplementary information III for measurements), *M. hoanglienensis* ($N = 11$; data from Tapley et al. 2018a) and *M. rubrimera* ($N = 8$; data from Tapley et al. 2017). All specimens were measured by a single author (BT).

We controlled for the allometric effects of body size after Lleonart et al. (2000): $yi* = yi (xo/xi)^b$, where $yi*$ is the scaled value after controlling for allometric effects, yi is the measured value for specimen i, xo is SVL to which measurements were scaled, xi is the observed SVL of specimen i, and b is the pooled within-group regression coefficient

calculated for logarithmically transformed measures of xi and yi for male specimens from each group of species. We performed linear discriminant analysis in PAST 4.02 (Hammer et al. 2001) between logged-transformed morphometric measurements of specimens from the new population and other *Panophrys* occurring above 2000 m asl in the Hoang Lien Range after these data had been controlled for allometric effects of body size. All the measured variables except SVL were included in the linear discriminant analysis.

We compared the newly collected specimens from the Hoang Lien Range with the following congeners reported from mainland Southeast Asia (Vietnam, Thailand, Myanmar, Laos and Cambodia, excluding the southern peninsula of Thailand south of the Isthmus of Kra) and nearby provinces of China (Yunnan, Guangxi and Guizhou): *Megophrys angka* (Wu, Suwannapoom, Poyarkov, Pawangkhanant, Xu, Jin, Murphy and Che, 2019); *Megophrys auralensis* Ohler, Swan and Daltry, 2002; *M. binchuanensis* Ye and Fei, 1995; *M. binlingensis* Jiang, Fei and Ye, 2009 (in Fei et al. 2009); *M. caobangensis*; *M. chishuiensis* Xu, Li, Liu, Wei and Wang, 2020; *M. damrei* Mahony, 2011; *M. daweimontis*; *M. elfina*; *M. fansipanensis*; *M. feae*; *M. gigantica*; *M. feii* Yang, Wang and Wang, 2018, *M. gerti*; *M. glandulosa* Fei, Ye and Huang, 1990; *M. hansi*; *M. hoanglienensis*; *M. intermedia*; *M. jiangi* Liu, Li, Wei, Xu, Cheng, Wang and Wu, 2020; *M. jingdongensis*; *M. koui; M. leishanensis* Li, Xu, Liu, Jiang, Wei and Wang, 2019"2018"; *M. lekaguli* Stuart, Chuaynkern, Chan-ard and Inger, 2006; *M. liboensis* (Zhang, Li, Xiao, Li, Pan, Wang, Zhang and Zhou, 2017); *M. major*; *M. maosonensis*; *M. microstoma*; *M. minor*; *M. mirabilis* Lyu, Wang and Zhao, 2020; *M. omeimontis* Liu, 1950; *M. pachyproctus*; *M. palpebralespinosa*; *M. parva*; *M. poilani*; *M. platyparietus* (Rao and Yang, 1997); *M. qianbeiensis* Su, Shi, Wu, Li, Yao, Wang and Li, 2020; *M. rubrimera; M. shapingensis* Liu, 1950; *M. shimentaina* Lyu, Liu and Wang, 2020; *M. shuichengensis* Tian and Sun, 1995; *M. spinata* Lui and Hu, 1973 (in Hu et al. 1973); *M. synoria; M. takensis* Mahony, 2011; and *M. wuliangshanensis* Ye and Fei, 1995. Specimens of congeners were examined from comparative material, focusing on type/topotype material (Appendix I) when available. When comparative material was not available, the following literature was used, which comprises descriptions of type specimens and, in some cases, reliably identified non-type specimens: Bourret (1937); Hu et al. (1973); Huang and Fei (1981); Tian and Sun (1995); Rao and Yang (1997); Tian et al. 2000; Fei and Ye (2001); Ohler et al. (2002); Fei et al. (2009, 2012); Neang et al. (2013); Li et al. (2014); Orlov et al. (2015); Poyarkov et al. (2017); Zhang et al. (2017); Li et al. (2019["2018"]); Yang et al. (2018); Wang et al. (2019b); Wu et al. (2019); Li et al. (2020); Liu et al. (2020); Lyu et al. (2020); Nguyen et al. (2020); Xu et al. (2020).

Acoustic data

Advertisement calls were recorded with a TASCAM (California, USA) DR–05 Linear PCM Recorder (44.1 kHz sampling rate and 16-bit encoding) with a Neewer® (Guangdong, China) 14.37-inch Shotgun Microphone. Calls were recorded at a distance of approximately 0.2 m and the ambient temperature at the calling site was taken immediately after recording using a Kestrel 3500 hand-held weather meter (Kestrel, Minneapolis, USA). Calls were analysed with Raven Pro© v. 1.5 software (http://www.birds.cornell.edu/raven). The audiospectrograms provided in figures were calculated by 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 and 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) per call group, number of pulses per call and dominant frequency (kHz). Comparative advertisement call characters for *Megophrys* species were obtained from published studies, with advertisement calls known for 11 of the 19 known species of *Megophrys* (subgenus *Panophrys*) found in mainland Southeast Asia, north of the Isthmus of Kra and nearby provinces of China (Yunnan, Guangxi and Guizhou): *M. boettgeri* (Wang et al. 2014), *M. chishuiensis* (Xu et al. 2020), *M. fansipanensis* (Tapley et al. 2018a), *M. hoanglienensis* (Tapley et al. 2018a), *M. jiangi* (Liu et al. 2020), *M. jingdongensis* (Cutajar et al. 2020), *M. leishanensis* (Li et al. 2019["2018"]), *M. minor* (Jiang et al. 2001), *M. qianbeiensis* (Su et al. 2020), *M. rubrimera* (Tapley et al. 2017) and *M. shimentaina* (Lyu et al. 2020). Not all published call descriptions reported the range of values for all call parameters consistently, and so bioacoustic comparisons were not possible for some parameters or calls for some species. The data included in our analyses were taken from topotypic material (Wang et al. 2014, 2019b; Tapley et al. 2017, 2018a; Li et al. 2019["2018"]), from specimens for which species identity had been confirmed with molecular analyses (Wang et al. 2014; Cutajar et al. 2020), or from specimens located at sites close to the type locality (Jiang et al. 2001).
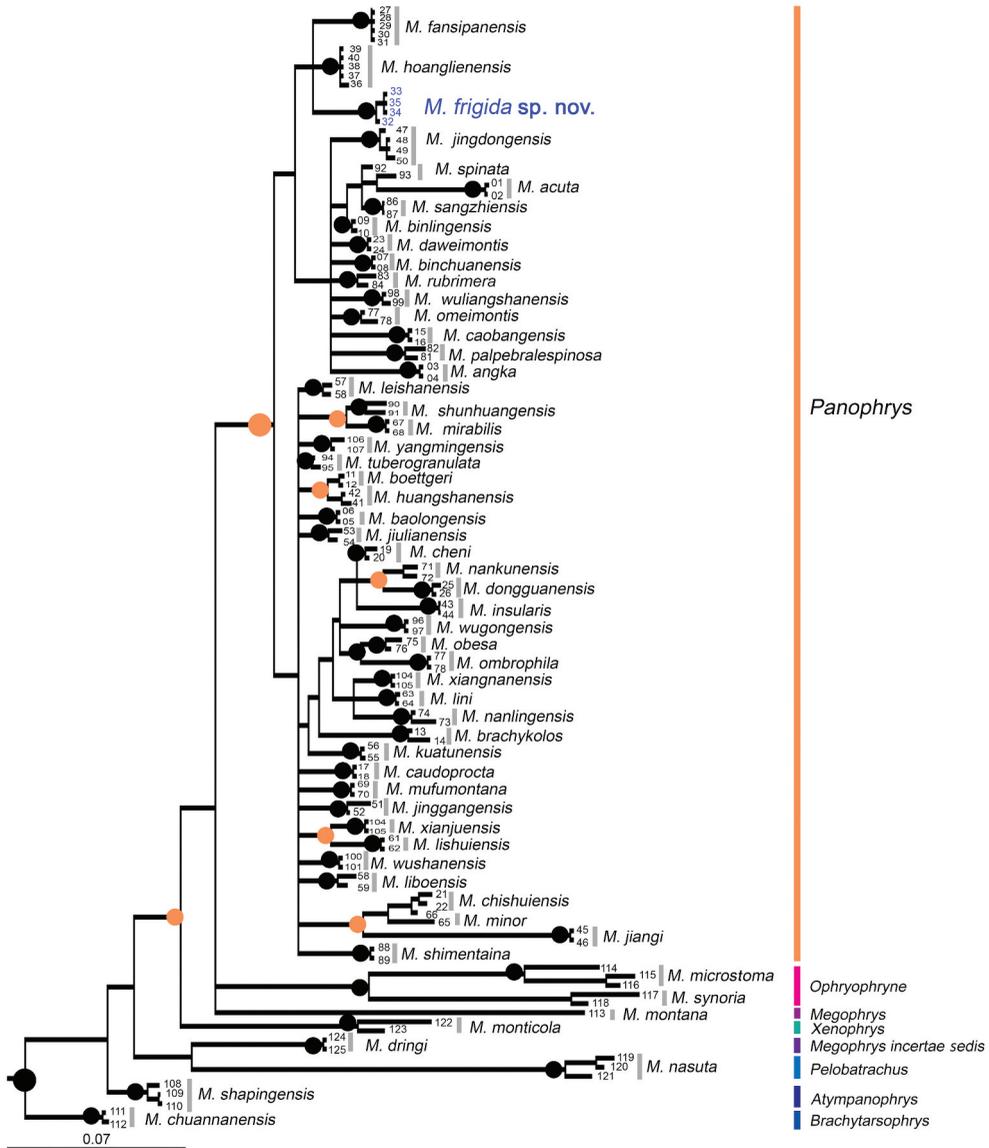
Species distribution mapping

The distribution map was created in ArcMap 10.2.2 (Esri, California, USA). The species' distribution was generated using the WorldClim altitude raster (Hijmans et al. 2005), the International Union for Conservation of Nature (IUCN) elevation raster (IUCN 2017) and ESRI World Topographic basemap layer to visualise topography, and the ESRI World Imagery basemap layer to determine local land cover.

The elevation range for the newly described species was estimated by adding a buffer to the lowest and highest known elevation records of the species following the standardised procedure developed by the mainland Southeast Asia Working Group of the Amphibian Red List Authority. The range of each buffer was determined by the distance between observations and the perceived likelihood of dispersal between known localities for the species. Areas of habitat were deemed suitable and included in maps if they are within species' estimated elevation range, are covered with forest, and are not separated from known localities by any continuous stretch of unsuitable habitat with a distance equal to or greater than 1 km. We followed the IUCN Red List (Red List Technical Working Group 2018) in dividing the species' estimated range into two categories of uncertainty: 'presumed range', defined as adjacent areas where the species is expected to occur, and 'potential range', defined as similar to presumed range but separated from known localities by a greater distance or possible minor barriers to dispersal, or occurring in a country in which the species has not been recorded. Extent of occurrence (EOO), defined as the area of a minimum convex polygon that encompasses all known and inferred sites occupied by the species, was measured using the IUCN EOO Calculator tool v. 1.2.
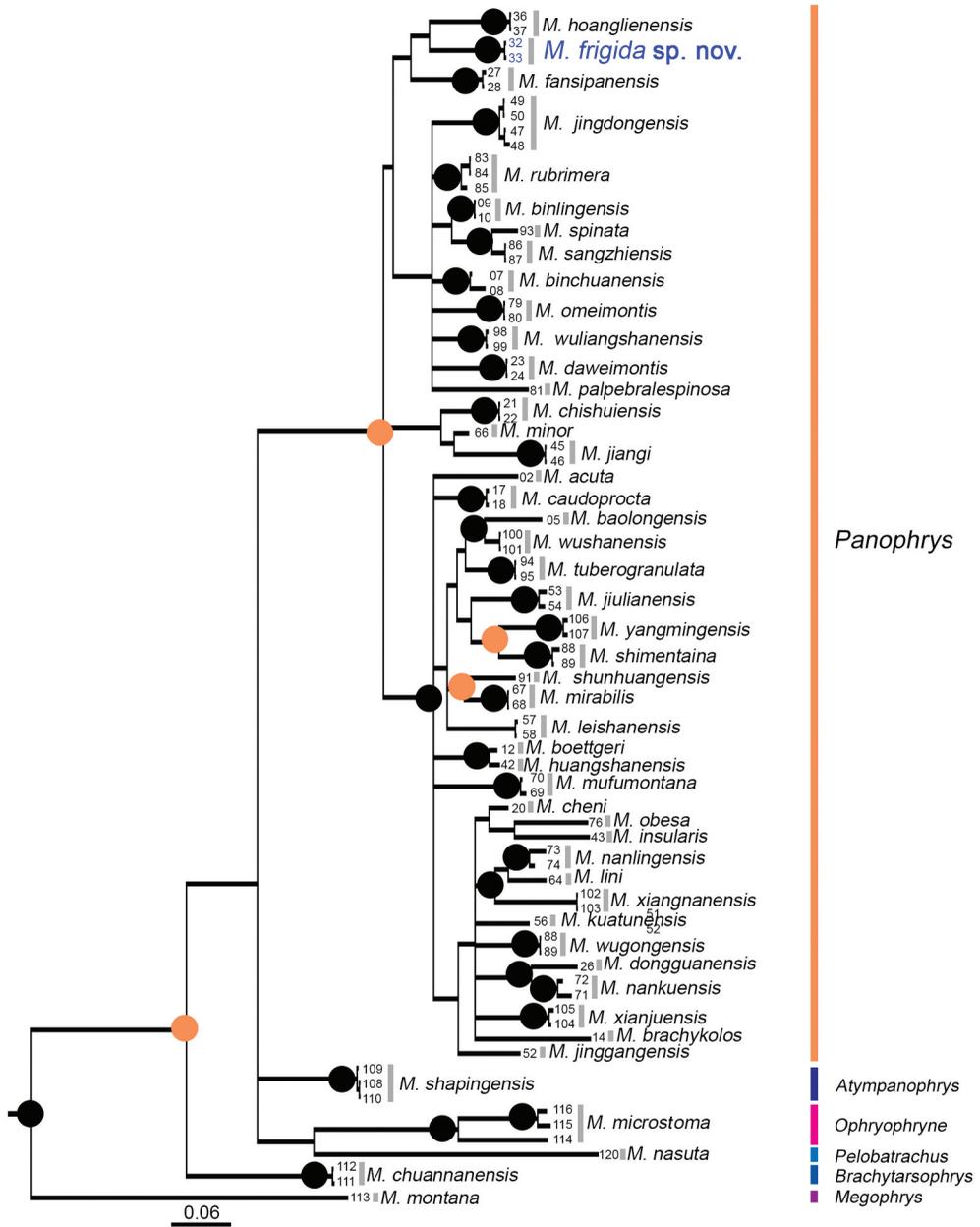
Results

Molecular analyses

Alignment length for the 12S–16S data set was 1353 bp, and for the CO1 data set it was 647 bp. The phylogenetic trees resulting from BI analyses are presented in Figures 2 and 3;

Figure 2. A BI phylogenetic tree for a 1353-bp section of 12S–16S (mtDNA) gene of *Megophrys frigida* sp. nov., along with representatives of all *Panophrys* species for which comparative sequences are available. Numbers at terminals correspond to Sample IDs in Supplementary information II. Bayesian posterior probability and ML bootstrap support for nodes are indicated on the tree: < 95, not indicated; ≥ 95, orange dot; 100%, black dot. Coloured bars denote subgenera. Outgroups (*Leptobrachella oshanensis* and *Leptobrachium boringii*) are not shown.

Figure 3. A BI phylogenetic tree for a 647-bp section of CO1 (mtDNA) gene for *Megophrys frigida* sp. nov., along with representatives of all *Panophrys* species for which comparative sequences are available. Numbers at terminals correspond to Sample IDs in Supplementary information II. Bayesian posterior probability and ML bootstrap support for nodes are indicated on the tree: < 95, not given; ≥ 95, orange dot; 100%, black dot. Coloured bars denote subgenera. The outgroup (*Leptobrachium boringii*) is not shown.
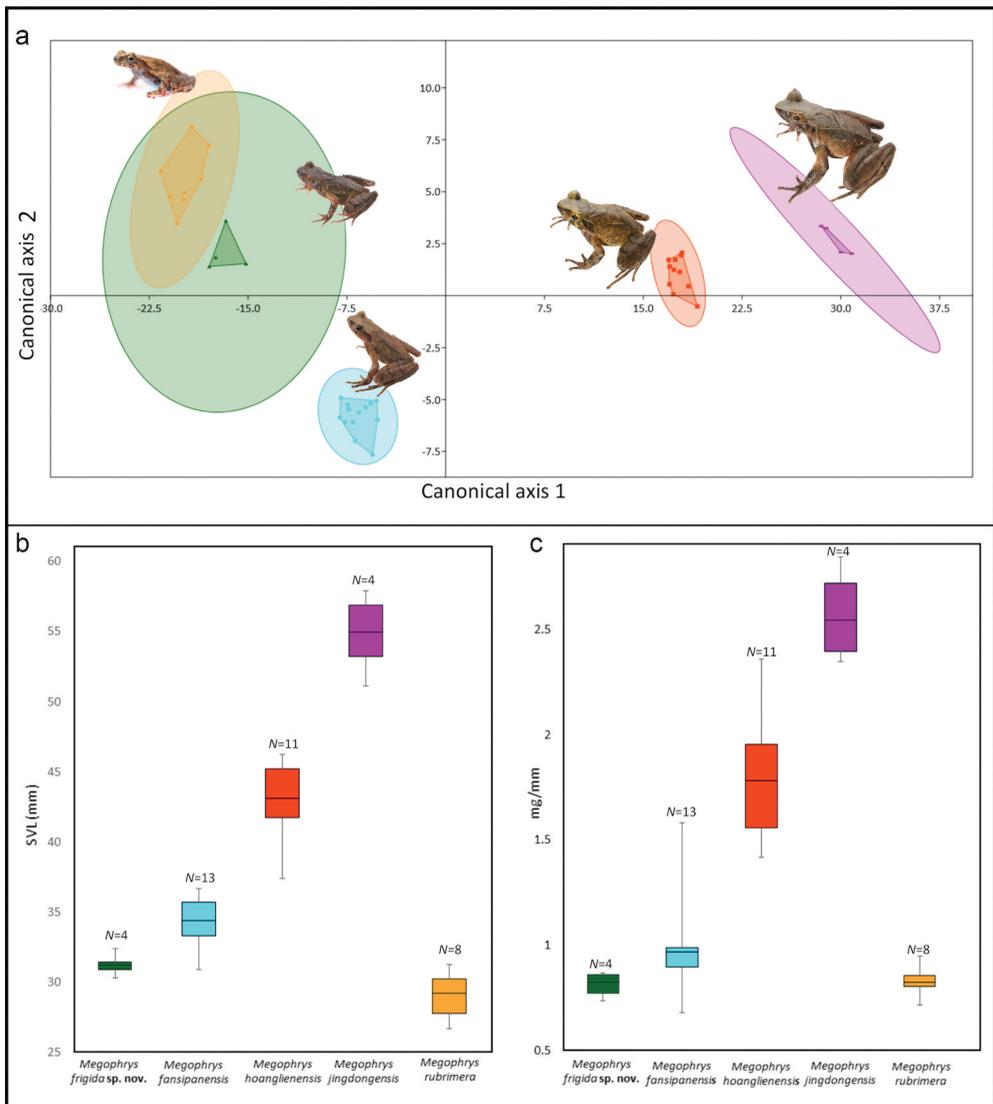
BS node support values for ML analyses are shown on the BI trees (the ML trees are presented in Supplementary information IV and V). Phylogenetic analyses placed all newly collected *Megophrys* specimens within the subgenus *Panophrys* with high support (BPP/

BS 0.99/97% in the 12S–16S trees and BPP/BS 0.99/96% in the CO1 trees). Phylogenetic analyses consistently placed the newly collected taxon within a poorly to moderately well-supported clade (BPP/BS 69/87% for the 12S–16S data set; BPP/BS 93/92% for the CO1 data set) along with *M. fansipanensis* and *M. hoanglienensis*. Relationships within this clade were not resolved on the 12S–16S trees; however, the newly collected taxon was consistently resolved as sister to *M. hoanglienensis* on the CO1 tree with moderate to high support (BPP/BS 75/94%).

Genetic diversity was low within the newly collected taxon (*p* distance 0.0–0.3% for 16S, 0.0% for CO1; Supplementary information VI and VII), demonstrating that the sequenced specimens represent a single operational taxonomic unit. Uncorrected *p* distance between the newly collected taxon and other species in the genus *Megophrys* ranged from 3.5% to 17.2% in the 16S data set and from 10.2% to 20.5% in the CO1 data set. The uncorrected *p* distance between the newly collected taxon and its closest relatives, *M. fansipanensis* and *M. hoanglienensis* (collected from their type localities 30 km south-east of our survey site), was 3.8–4.3% for *M. hoanglienensis* and 3.8–4.1% for *M. fansipanensis* in the 16S data set, and 10.2% for *M. hoanglienensis* and 11.4–11.6% for *M. fansipanensis* in the CO1 data set. The uncorrected *p* distance between the newly collected taxon and the sympatric *M. jingdongensis* was 3.5–4.1% in the 16S data set and 13.5–13.7% in the CO1 data set, despite the two taxa appearing distantly related on the phylogenetic tree. The uncorrected *p* distance between the newly sampled taxon and its closest relatives exceeded those observed between other closely related taxa within the *Megophrys* (*Panophrys*) subgenus, e.g. >2% for 16S and >6% for CO1 (Chen et al. 2017). We recognise the limitations of comparing *p* distances between studies and species, and so do not condone its use as a species delimitation tool in the absence of more reliable data (e.g. morphological, biological, etc.). However, the observed divergences were a result of mutations that were spread evenly across the length of the 16S and CO1 sequences (i.e. not the result of only a few large deletion/insertion events) between the new population and its closest relatives *M. fansipanensis* and *M. hoanglienensis*, which is indicative of long-term genetic isolation.

Morphological data

Linear discriminant analysis between morphometric measurements of male *M. fansipanensis* (N = 13), *M. hoanglienensis* (N = 11), *M. jingdongensis* (N = 4) and *M. rubrimera* (N = 8) and the new population (N = 4) that had been corrected for the effect of allometric size had an overall predictive accuracy of 100%. With jackknifed validation the predictive accuracy dropped to 95%; two *M. rubrimera* individuals were misclassified as specimens from the new population. Specimens representing the new population are readily distinguishable from *M. rubrimera* (see comparisons and bioacoustic comparison). When these data were plotted (Figure 4(a)) only data points for the new population and *M. rubrimera* exhibited any overlap. Canonical axis 1 explained 93% of the variance (eigenvalue = 336.2) and canonical axis 2 explained 5.9% of the variance (eigenvalue = 21.4). The factors with the highest positive loading variables for canonical axis 1 were HW, HAL and TL, and the traits with the highest positive loading variables for canonical axis 2 were FAL, HL and IBE. This analysis shows that the newly sampled population occupies a different morphospace from other taxa known to occur above

Figure 4. (a) Discriminant analysis of morphometric characters of male *Panophrys* in the Hoang Lien Range, with 95% ellipses. Blue = *Megophrys fansipanensis* (lateral view of AMS R186113 in life); red = *Megophrys hoanglienensis* (dorsolateral view of AMS R186122 in life); purple = *Megophrys jingdongensis* (dorsolateral view of AMS R185924 in life); orange = *Megophrys rubrimera* (lateral view of HNLP2016062200001 in life) and green = *Megophrys frigida* sp. nov. (dorsolateral view of AMS R186131 in life). Canonical axes 1 and 2 are linear combinations of the variables. Images of frogs are approximately to scale. (b) Boxplots of snout-to-vent length among adult male *Megophrys* species from the Hoang Lien Range. (c) Body weight differentiation (mg/mm). Horizontal lines within each box represent the median and boxes encompass the 75th and 25th quartiles.

2000 m asl in the Hoang Lien Range, particularly its two closest relatives, *M. hoanglienensis* and *M. fansipanensis*.

Molecular data combined with morphological and bioacoustic differences provide additional support that the newly collected specimens represent a new species.

Megophrys (Panophrys) frigida sp. nov.
(Figures 4–7; Tables 1, 2)

Holotype. Adult male (VNMN 010948; field tag BX009: Figures 5(a–e), 6(a,b,i,k), 7(a)) found beside a 1.5 m wide mountain stream in disturbed upper montane forest on Mount Ky Quan San, Bat Xat District, Lao Cai Province, Vietnam (22.499°N, 103.602°E, 2668 m asl; Figures 1, 8(a,b)), collected at 22:00 h on 9 September 2017 by Nguyen Thanh Chung, Luan Thanh Nguyen, Luke Harding, Timothy Cutajar, Jodi J. L. Rowley and Benjamin Tapley.

Paratypes. Adult male (AMS R186131; field tag BX011: Figures 5(f–h), 6(e–f,j)) same collection locality, date and collectors as holotype, collected at 21:00 h. Adult male (AMS R186132; field tag BX014: Figure 6(g,h,l)) found resting on soil substrate in dense vegetation beside a swampy stream headwater in disturbed broadleaf forest, Mount Ky Quan San, Bat Xat District, Lao Cai Province, Vietnam (22.508°N, 103.615°E, 2118 m asl; Figure 1), collected at 21:30 h on 10 September 2017, same collectors as holotype.
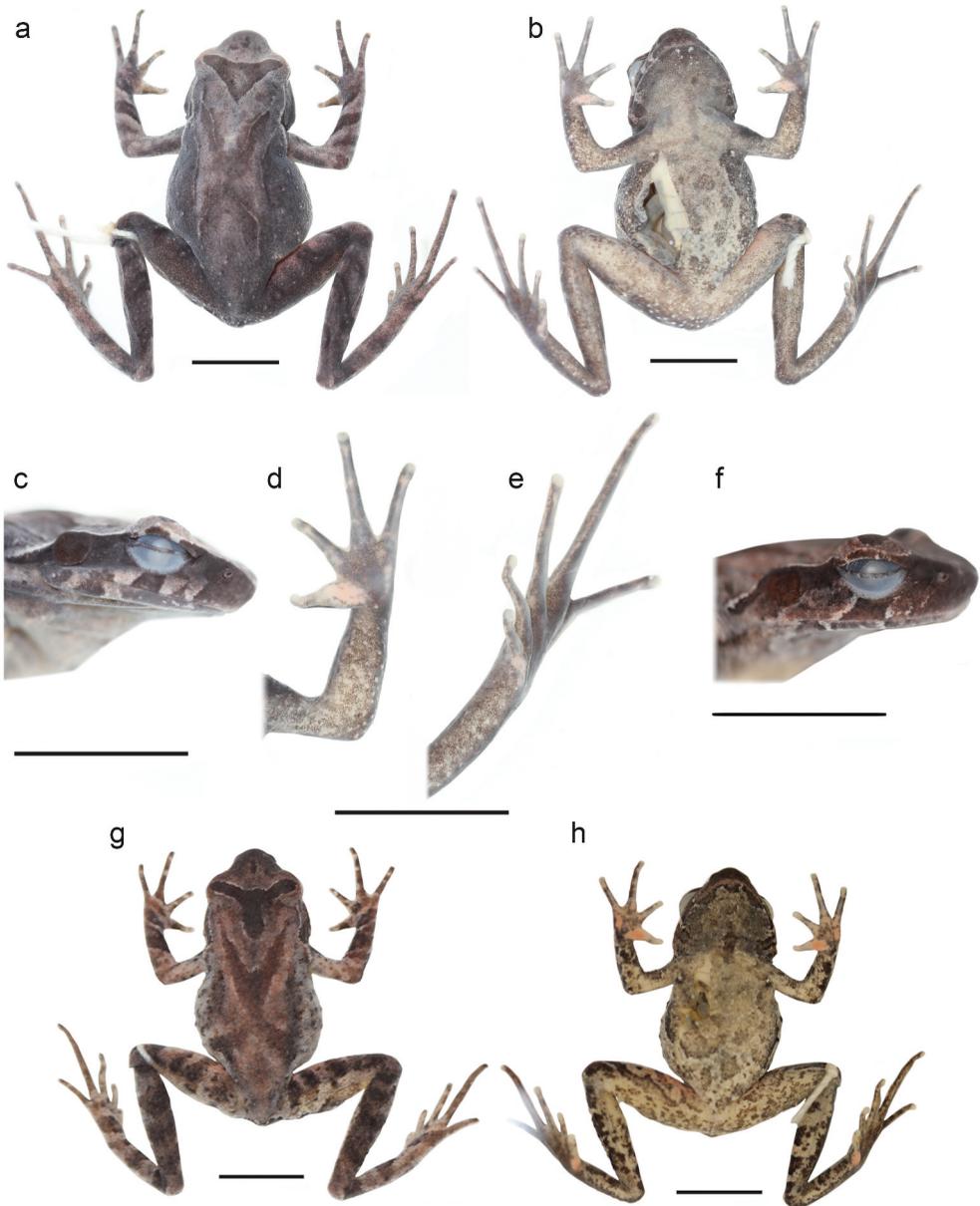
Referred specimen. Adult male (HLNP 20170910 00006; field tag BX010) found perched on a leaf, same collection locality, date and collectors as holotype, collected at 21:00 h. This specimen is not included in the type series due to it being deposited in a local collection. Its taxonomic identity is not in question.

Table 1. Measurements (mm) of *Megophrys frigida* sp. nov. All specimens are male. Abbreviations are defined in Materials and methods section. M: male; *: holotype; **: paratype; ^: referred specimen.

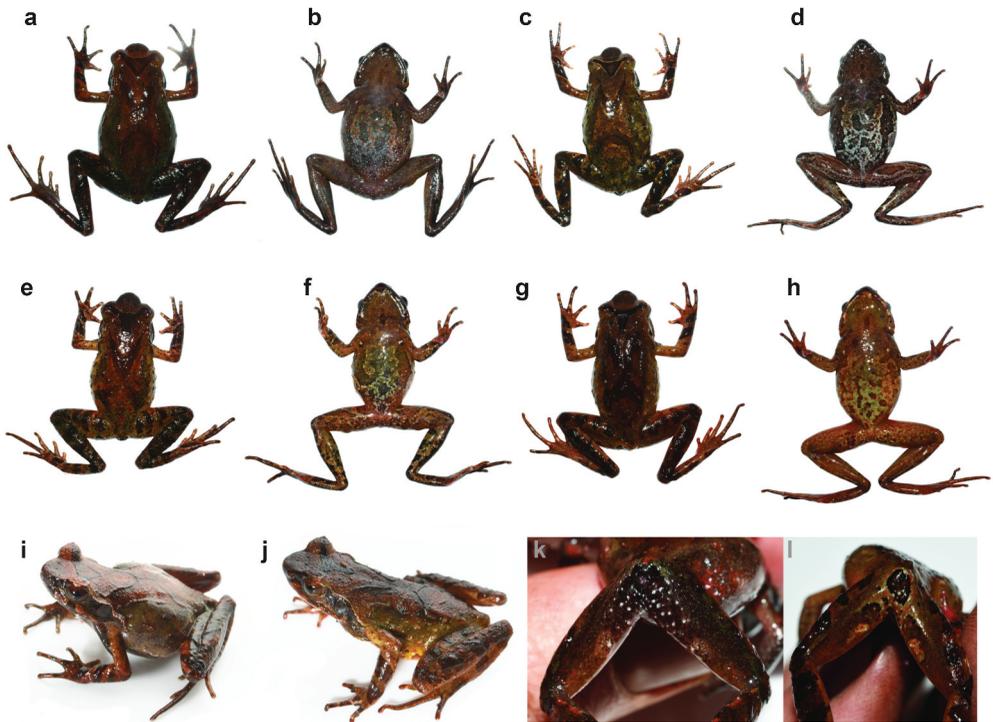| Voucher #	VNMN 010948*	HLNP 20170910 00006^	AMS R186131**	AMS R186132**
Sex	M	M	M	M
SVL	31.1	31.3	30.3	31.8
HW	11.4	11.2	11.6	11.8
HL	11.7	11.0	11.1	11.1
IFE	3.8	3.7	3.9	4.5
IBE	4.4	4.2	4.5	4.0
EL	3.8	3.4	3.7	3.6
TYD	2.9	2.5	3.0	2.9
TYE	1.9	1.7	2.1	1.4
SL	4.0	4.3	4.7	4.4
EN	2.4	2.0	2.2	2.2
SN	1.8	1.7	2.0	2.1
IUE	3.5	3.3	3.6	4.3
IN	3.2	2.9	3.7	3.9
UEW	3.4	3.2	3.8	3.5
FAL	7.9	7.3	6.9	7.4
HAL	9.3	8.8	9.2	8.7
FIL	3.4	3.3	3.1	3.5
FIIL	3.7	4.3	3.2	3.8
FIIIL	6.3	6.0	5.4	5.9
FIVL	4.2	4.1	3.8	3.4
SHL	17.4	16.6	16.9	16.9
TL	16.8	15.5	16.2	14.7
FOL	16.7	15.4	15.5	15.1
IMT	2.6	2.5	2.1	2.5
Mass in life (g)	2.7	2.3	2.6	2.5

Table 2. Measurements of advertisement call parameters for the holotype of *Megophrys frigida* sp. nov. compared to other *Panophrys* species occurring at elevations above 2000 m above sea level in the Hoang Lien Range: *M. fansipanensis*, *M. hoanglienensis*, *M. jingdongensis* and *M. rubrimera*. Parameter values are given as means (and ranges). Bold text indicates non-overlapping call parameters between compared species.

Species	*Megophrys frigida* sp. nov.	*M. fansipanensis*	*M. fansipanensis*	*M. fansipanensis*	*M. hoanglie- nensis*	*M. jingdongensis*	*M. rubrimera*	*M. rubrimera*	*M. rubrimera*
Specimen numbers	VNMN 010948	AMS R186115	VNMM 2018.01	AMS R186114	AMS R186122	AMS R185924	AMS R177677	Unvouchered	Unvouchered
Number of call groups measured	5	5	3	12	5	1	3	5	3
Total number of calls analysed	20	20	20	20	20	17	20	20	20
Call duration (ms)	47 (43–50)	42.0 (34–49)	43.8 (41–49)	40.8 (34–45)	**102.9 (96–108)**	**132.7 (117–147)**	**74.2 (56–83)**	**66.0 (62–70)**	79.6 (72–85)
Intercall interval (ms)	236.0 (214–279)	213.7 (197–245)	197.7 (180–227)	201.8 (168–290)	274.2 (178–565)	**113.0 (102–127)**	207.0 (190–235)	225.6 (208–324)	232.8 (217–261)
Call repetition rate (call/s) per call group	3.5 (3.5–3.6)	**3.8**	4	4	2.6	3.9	3.3	**3.4**	3.1
Calls/call group	15.4 (12–18)	22.2 (18–25)	34.7 (24–41)	11.4 (1–22)	17.0 (11–21)	17	39.3 (34–50)	31.2 (16–47)	45.6 (42–51)
Pulses/call	10.9 (10–11)	11.1 (10–12)	10.8 (10–11)	12.9 (12–14)	**18.7 (12–22)**	**Indistinct pulsation**	**Indistinct pulsation**	**23.1 (19–25)**	**Indistinct pulsation**
Dominant frequency (kHz)	3.6 (3.5–3.7)	3.6 (na)	3.9 (3.9–4.1)	4.0 (3.6–4.7)	**3.0 (2.8–3.0)**	**2.5 (2.4–2.6)**	**3.2 (3.2–3.4)**	**3.2 (3.2–3.4)**	3.4 (3.4–3.4)
Time of year reported calling	September	**June**	**June**	**June**	**June**	**June**	**June**	**June**	**June**
Temperature °C	16	15.3	18.3	15.3	18.5	18.5	22.9	22.1	21.0
Reference	This study	Tapley et al. 2018a	Tapley et al. 2018a	Tapley et al. 2018a	Tapley et al. 2018a	Cutajar et al. 2020	Tapley et al. 2017	Tapley et al. 2017	Tapley et al. 2017

Figure 5. *Megophrys frigida* sp. nov. in preservative: (a) dorsal view (holotype of VNMN 010948); (b) ventral view (holotype of VNMN 010948); (c) lateral view of head (holotype of VNMN 010948); (d) palmar surface of left hand (holotype of VNMN 010948); (e) plantar surface of left foot (holotype of VNMN 010948); (f, g, h) dorsal, ventral and profile view of *Megophrys frigida* sp. nov. paratype (AMS R186131). Scale bars: 10 mm.

Etymology. Specific epithet '*frigida*' is a femine adjective; the Latin word '*frigida*' meaning cold, in reference to the relatively cold temperature at the type locality of the species, Mount Ky Quan San. At some high-elevation sites in the Hoang Lien range, the climate is

Figure 6. *Megophrys frigida* sp. nov. in life: (a, b) dorsal and ventral view of holotype VNMN 010948 under sedation; (c, d) dorsal and ventral view of referred specimen HLNP 20170910 00006 under sedation; (e, f) dorsal and ventral view of paratype AMS R186131 under sedation; (g, h) dorsal and ventral view of paratype AMS R186132 under sedation; (i) dorsolateral view of holotype VNMN 010948; (j) dorsolateral view of paratype AMS R186131; (k) cloaca and femoral glands of holotype VNMN 010948; (l) cloaca and femoral glands of paratype AMS R186132. Not to scale.

almost temperate; temperatures range from −3 to +20°C. In the coldest months there is frequent ground frost (Nguyen and Harder 1996).

Suggested vernacular name. Mount Ky Quan San horned frog (English), Cóc sừng Ky Quan San (Vietnamese).

Diagnosis. Based on the type series and the referred specimen, which are all adult males (N = 4), *Megophrys frigida* sp. nov. differs from its congeners by a combination of the following characters: (1) small adult male size SVL 30.3–31.8 mm; (2) small blunt tubercle present on outer edge of upper eyelids; (3) dorsolateral ridges present; (4) toes lacking distinct interdigital webbing; (5) subarticular tubercles absent on fingers and toes; (6) palmar tubercles absent; (7) inner metatarsal tubercle present on feet; (8) tympanum clearly defined; (9) presence of vomerine ridges, vomerine and maxillary teeth; (10) nuptial pads covered with black microspinules; (11) advertisement call with a dominant frequency of 3.6 (3.5–3.7) kHz.

Figure 7. *Megophrys frigida* sp. nov. in life *in situ* at Mount Ky Quan San, Bat Xat District, Lao Cai Province, Vietnam, 2668 m above sea level: (a) holotype (VNMN 010948); (b) unvouchered individual.

Description of holotype. Figures 5(a–e), 6(a,b,i,k) and 7(a): Sexually mature male. Head small, greater in length than in width; snout rounded in dorsal view, obtusely protruding in lateral view, rostral appendage absent; loreal region vertical and concave; canthus rostralis angular; eye length 24% longer than maximum diameter of tympanum and subequal to snout length; eye to tympanum distance shorter than maximum tympanum diameter; tympanum oval, orientated vertically. Pupil in life oval, vertically orientated when dilated; nostril round and orientated laterally, closer to snout tip than to eye; eyelid width subequal to narrowest point between upper eyelids, and greater than internarial distance; vomerine ridges present, obliquely orientated and barely separated from choanae anteriorly with small vomerine teeth; maxillary teeth present. Tongue large and not clearly notched posteriorly. Fore limbs long and thin, forearms not significantly enlarged relative to upper arms, forearm length shorter than hand length; fingers long and narrow without lateral fringes, finger length formula FIL < FIIL < FIVL < FIIIL; interdigital webbing absent, subarticular, supernumerary and palmar tubercles absent; thenar tubercle weakly defined; finger tips slightly expanded relative to adjacent digit width and flattened to oval pads; terminal grooves absent. Hind limbs relatively long and thin; foot length subequal to thigh length, both shorter than shank length; toe tips slightly dilated relative to adjacent digit width and flattened to oval pads, terminal grooves absent; webbing and lateral fringes on toes absent; outer metatarsal, subarticular and supernumerary tubercles absent; inner metatarsal tubercle very weakly defined.

Skin of dorsal surfaces of body, limbs, and dorsal and lateral surfaces of head weakly granular; gular region, chest, abdomen and ventral surfaces of limbs smooth; tympanum surface with small raised granular bumps lacking black-tipped asperities; tympanum border slightly raised; small blunt tubercle present on outer edge of upper eyelids; very small, black-tipped asperities present on posterior half of upper eyelid; no asperities circummarginally on lower jaw; white tubercles, some with black asperities present on angle of jaw; supratympanic ridges with black asperities, supratympanic ridge narrows as it passes above tympanum, terminating above axilla; tubercles above fore limb insertion

with black-tipped asperities; flanks with small scattered tubercles lacking black-tipped asperities; thin dorsolateral ridge on each side, extending from behind supratympanic fold to approximately two-thirds of distance to groin, supratympanic fold with asperities along apex, these are black tipped on anterior half and without black tips on posterior half; 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 'V'-shaped sacral ridge present on mid-dorsum which does not join laterally with dorsolateral ridges; 'V'-shaped ridges joined at their apices by a weakly defined medial ridge; crest of parietoscapular and sacral ridge covered in asperities, which are black tipped on anterior half of parietoscapular ridge and without black tips on posterior half; small tubercles on dorsal surface of body lack black tips, except between parietoscapular ridge and supratympanic fold; small tubercles without black tips arranged into distinct transverse rows on dorsal surface of thighs, shanks and forearms. Large distinct tubercles present on dorsolateral surfaces of the body; slightly smaller tubercles present on dorsal and posterior surfaces of forearms, shanks and region surrounding cloaca, some tubercles surrounding cloaca with black-tipped asperities, small tubercles present on dorsal and ventral surfaces of thighs; ventral surfaces of fore limbs and shanks smooth; pectoral glands distinct, small, slightly raised, positioned level with axilla; femoral glands small, slightly raised, one positioned closer to knee than cloaca on posterior surface of each thigh.

Colour of holotype in life. Figures 6(a,b,i,k) and 7(a): Dorsally orange-brown; darker brown lines follow opposing 'V'-shaped parietoscapular-sacral ridges; darker brown triangular marking between eyes with a lighter central blotch; lateral surfaces of snout, anterior to orbits dark brown, tip of snout light brown; a vertical dark brown bar present below eyes; supratympanic ridges longitudinally bicoloured, cream above and dark brown below; tympanic region dark brown; inguinal region olive green; tubercles on flanks whitish-blue in colour, some bordered with irregular brown blotches; tubercles around cloaca, posterior surfaces of forearms, shanks and thighs white; gular and pectoral region dark brown with grey flecks; broad dark brown longitudinal stripes extend ventrolaterally on abdomen, darkest anteriorly and fading on posterior third of abdomen; centre of abdomen mottled with different hues of grey and orange-brown; three dark brown blotches on anterior lateral surface of forearms; dorsal surface of fingers with dark brown blotches; anterior lateral surfaces of thighs dark orange, ventral surfaces of thighs and forearms grey-brown, speckled with darker brown; white tubercles on ventral surface of thighs; white pectoral and femoral glands; ventral surface of hands dark brown; thenar and hyperthenar region of hands dark orange, giving illusion of tubercles; ventral surfaces of feet dark brown; inner metatarsal tubercle dark orange; iris metallic orange-brown with black reticulations throughout.

Colour of holotype in preservative. (Figure 5(a–e)): Majority of dorsal and lateral surfaces of head, body, fore limbs and hind limbs grey-brown; darker brown triangular marking with light central blotch between eyes; darker brown 'X'-shaped marking over opposing 'V'-shaped parietoscapular-sacral ridges; dorsolateral ridges, supratympanic ridges and flank tubercles brownish-cream; snout and lateral canthus rostralis dark brown; wide vertical dark brown bar below eyes and dark brown blotch covering tympanum and extending to posterior edge of eyes; three dark brown blotches on anterior

lateral surface of forearms; dorsal surface of fingers with dark brown blotches; inguinal region darker brown than dorsal surface; gular region, chest and anterior part of abdomen primarily creamy-grey, with brown speckling in gular region. Abdomen light grey, distinctly blotched with dark brown; ventral surfaces of thighs and shanks with pale brown mottling; ventral surfaces of feet grey-brown; inner metatarsal tubercle dark orange; fore limbs ventrally mottled and blotched with light and dark brown; ventral surface of hands grey; thenar and hyperthenar region of hands light pink, giving illusion of tubercles; pectoral and femoral glands white.

Variation. Morphometric measurements of type series are shown in Table 1. Paratypes and referred specimen generally agree with holotype morphologically, but with the following exceptions: head width greater than head length in HLNP 20170910 00006, AMS R186131 and AMS R186132 (vs head length greater than head width in holotype); EL/SL 79–82% in paratypes and referred specimen (vs EL/SL 95% in holotype); FL >TL in AMS R186132 but TL > FL in AMS R186131; finger length formula for AMS R186131 and AMS R186132 agrees with holotype (FIL < FIIL < FIVL < FIIIL), finger length formula of HLNP 20170910 00006 differs from holotype (FIL < FIVL < FIIL < FIIIL); tongue of AMS R186131 appears notched posteriorly, a notch absent in VNMN 010948, HLNP 20170910 00006 and AMS R186132, this apparent absence of notching may be an artefact of fixation. Colouration and markings in life are highly variable (see Figures 6 and 7), one unvouchered specimen had a brick red dorsal surface (Figure 7(b)); colouration of palmar surfaces of hands in life largely in agreement with holotype, though eminences over metacarpal regions observed in HLNP 20170910 00006, AMS R186131 and AMS R186132 were dark orange. Only holotype possessed 'V'-shaped parietoscapular and inverted 'V'-shaped sacral ridges; HLNP 20170910 00006 (Figure 6(c)) had one 'V'-shaped parietoscapular ridge and an opposing 'U'-shaped sacral ridge with apexes not connected by a middorsal ridge, and AMS R186131 and AMS R186132 had 'V'-shaped parietoscapular ridge and opposing 'U'-shaped sacral ridges with apexes not connected by a medial ridge. Distribution of asperities in AMS R186131 agreed with holotype, although asperities were far fewer and more dispersed on all surfaces than on holotype and none of these appeared to be black tipped; coverage of asperities in AMS R186132 and HLNP 20170910 00006 also agreed with holotype on all surfaces, but those on upper eyelids, supratympanic ridges, dorsolateral ridges, sacral ridge, parietoscapular ridge and dorsolateral to axilla lacked black tips; on AMS R186132 and HLNP 20170910 00006, black-tipped asperities were only present on dorsal surfaces of body and these were concentrated on posterior dorsum.

Secondary sexual characters. All specimens collected were male and had slightly raised nuptial pads covered with black microspinules; oval nuptial pads covering most dorsal surfaces of FI and FII at their base; fleshy projection posterior to cloaca absent, a secondary sexual character of some male *Megophrys*, e.g. *M. angka* (Wu et al. 2019), *M. caudoprocta* Shen, 1994, *M. koui* (Kou 1985) and *M. pachyproctus* Huang and Fei (1981).

Advertisement call. Call descriptions are based on the calls of the holotype (VNMN 010948); five call groups and 20 calls in total were analysed (Table 2; Figure 9(a–c)). Advertisement calls were recorded at 16.0°C ambient temperature. Refer to Table 2 for
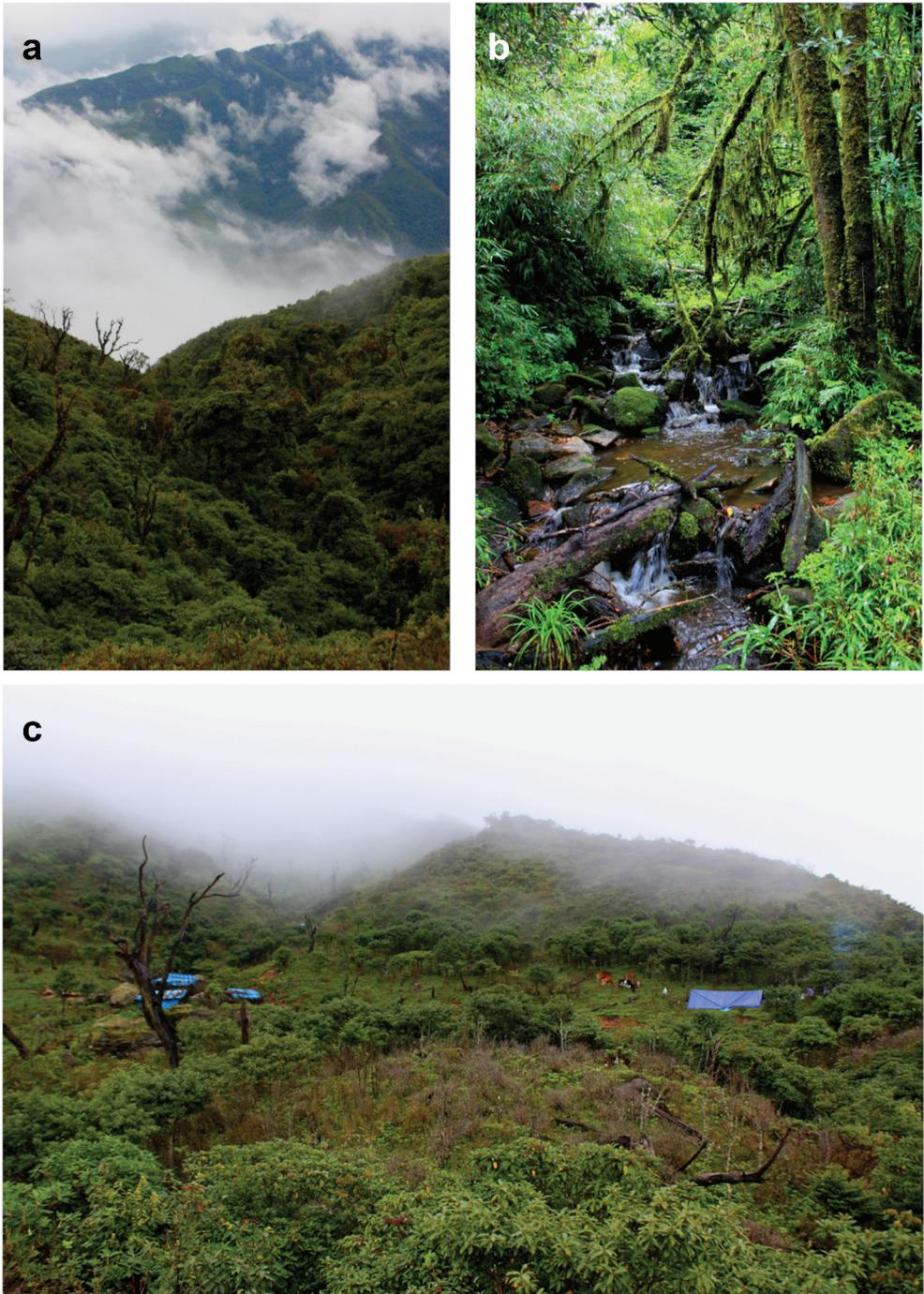
call characters. Call amplitude was relatively consistent within each call group, but the amplitude of pulses within each call dropped sharply after the first pulse (Figure 9(a–c)). Weak harmonics were visible above the dominant frequency (at approximately 7.4 kHz) and below the dominant frequency at approximately 2.3 kHz.

Natural history. All specimens of *Megophrys frigida* sp. nov. were associated with disturbed secondary broadleaf forest and upper montane forest with a relatively open canopy. All individuals were encountered at night and observed in riparian habitat along 1–2 m wide streams with clear water and rocky stream beds. Males were calling from stream-side vegetation in September 2017. Tadpoles and females were not observed. *Megophrys frigida* sp. nov. is syntopic with *M. jingdongensis, M. hoanglienensis* and *M. rubrimera* at 2118 m asl (Tapley et al. 2018b). At 2668 m, *Megophrys frigida* sp. nov. was the only *Megophrys* species encountered.
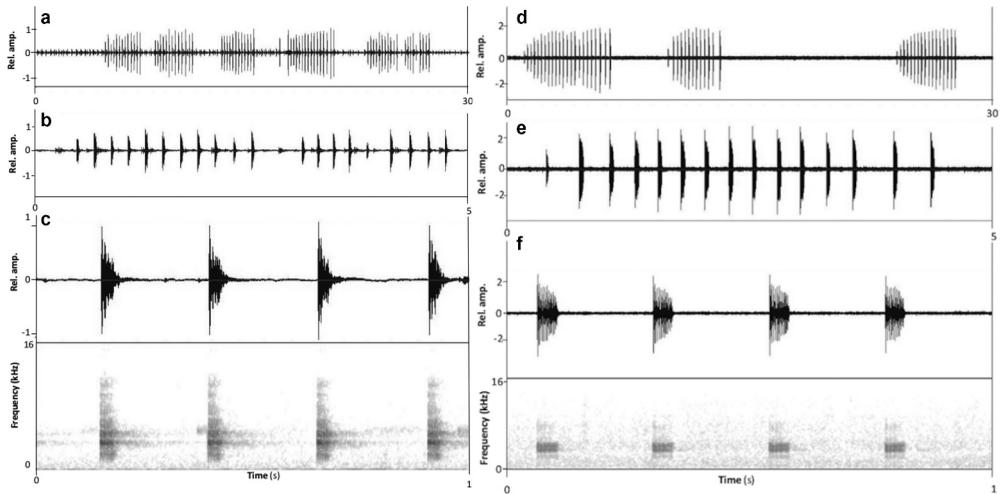
Distribution and conservation status. This species is currently only known from the recently designated Bat Xat Nature Reserve on Mount Ky Quan San at elevations between 2118 m asl and 2668 m (Figure 1). Despite an intensive survey effort at different times of the year (March, April, June, September and December), the species has not been encountered farther south on Mount Fansipan. The high-elevation sites where *Megophrys frigida* sp. nov. occurs face the immediate threat of habitat degradation; the forest in which this species occurs is being negatively impacted by fuelwood collection for the tourism industry and by the grazing of livestock (Figures 8(b,c)), particularly the site at 2668 m asl. There was no evidence of excessive water extraction or water pollution. The fungal pathogen, *Batrachochytium dendrobatidis*, was not detected from 15 samples collected from 15 different anuran amphibians on Mount Ky Quan in September 2017; including six samples from *Megophrys frigida* sp. nov. (Tapley et al. 2020a). If *Megophrys frigida* sp. nov. is restricted to a narrow, high-elevation band, it is likely that this species may be vulnerable to future climate change. The species' EOO is currently predicted to be 832 km^2. We recommend that *Megophrys frigida* sp. nov. is listed as Endangered in accordance with the IUCN Red List of Threatened Species categories and criteria B1ab (iii) (see IUCN 2012).

Comparisons. *Megophrys frigida* sp. nov. can be distinguished from all species in the subgenus *Panophrys* found in mainland Southeast Asia, north of the Isthmus of Kra and nearby provinces of China (Yunnan, Guangxi and Guizhou) on the basis of morphology, and from all species in the subgenus for which comparable data is available on the basis of molecular and acoustic data. Comparisons with each subgenus are discussed separately below. The following comparisons are based on four adult males of *Megophrys frigida* sp. nov.

Subgenus Panophrys. Phylogenetic analysis places *Megophrys frigida* sp. nov. in the subgenus *Panophrys*. *Megophrys frigida* sp. nov. differs from *M. angka* by vomerine teeth and vomerine ridges present (vs 'indistinct' vomerine ridges and vomerine teeth absent in *M. angka*; Wu et al. 2019) and subarticular tubercles absent (vs present on FI and FII in *M. angka*; Wu et al. 2019); from *M. binchuanensis* by subarticular tubercles absent (vs present in *M. binchuanensis*; Ye and Fei 1995) and dermal fringe on toes absent (vs present in *M.*

Figure 8. *Megophrys frigida* sp. nov. habitat: (a) view of the type locality on Mount Ky Quan San, Bat Xat District, Lao Cai Province, Vietnam, from 2700 m above sea level (asl); (b) type locality, 2668 m asl; (c) heavily grazed and deforested area very close to the type locality.

Figure 9. Comparison of advertisement calls of *Megophrys frigida* sp. nov. holotype (VNMN 010948: a–c) recorded at 16.0°C and *M. fansipanensis* (AMS R186115: d–f) recorded at 15.3°C: (a, d) 30 s waveform of relative amplitude (Rel. amp.) over time for several call groups; (b, e) 5 s waveform of Rel. amp. over time for one call group; (c, f) 1 s waveform and spectrogram of Rel. amp. and frequency for calls.

binchuanensis; Ye and Fei 1995); from *M. binlingensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 45.1–51.0 mm, *N* = 3, in *M. binlingensis*; Fei et al. 2009) and vomerine teeth present (vs absent in *M. binlingensis*; Fei et al. 2009); from *M. boettgeri* by vomerine ridges and vomerine teeth present (vs absent in *M. boettgeri*; Boulenger 1899; material examined), dorsolateral ridges present (vs absent in *M. boettgeri N* = 7; material examined) and male advertisement call (see Bioacoustic comparison); from *M. brachykolos* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 34.1–40.5 mm, *N* = 14, in *brachykolos*; Inger and Romer 1961; material examined), subarticular tubercles on fingers absent (vs present on base of all fingers in *M. brachykolos*; Inger and Romer 1961; material examined) and vomerine ridges and vomerine teeth present (vs absent in *M. brachykolos*; Inger and Romer 1961; material examined); from *M. caobangensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 34.9–38.9 mm, *N* = 11, in *M. caobangensis*; Nguyen et al. 2020), vomerine teeth present (vs absent in *M. caobangensis*) and male TYD/EL 74–81% (vs 40–47%, *N* = 11, in *M. caobangensis*; Nguyen et al. 2020); from *M. chishuiensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 43.4–44.1 mm, *N* = 3, in *M. chishuiensis*; Xu et al. 2020), vomerine teeth present (vs absent in *M. chishuiensis*; Xu et al. 2020) and male advertisement call (see Bioacoustic comparison); from *M. daweimontis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 34.0–37.0 mm, *N* = 18, in *M. daweimontis*; Rao and Yang 1997). The ranges in measurements of characters of *Megophrys frigida* sp. nov. largely overlap with those of *M. fansipanensis*, but *Megophrys frigida* sp. nov. differs from *M. fansipanensis* (Figure 4(a–c)) by an overlapping but typically smaller mean adult male size, SVL 31.1 (30.3–31.8) mm (vs 34.9 [30.9–44.3] mm, *N* = 13, in *M. fansipanensis*; material examined; Figure 4(b)), a typically overlapping but smaller mean weight/SVL, 0.81 (0.74–0.87) mg/mm (vs 1.01 [0.68–1.58] mg/mm, *N* = 13, in *M. fansipanensis*; Tapley et al. 2018a; Figure 4(c)), typically relatively shorter thigh length, mean TL/SVL 50.7 (46.2–54.0)% (vs mean 53.0 [47.2–59.8]%, *N* = 13, in *M. fansipanensis*; material examined), and advertisement

call (see Bioacoustic comparison); from *M. hoanglienensis* (Figure 4(a–c)) by having a smaller adult male body size, SVL 30.3–31.8 mm (vs 41.1–47.6 mm, *N* = 11, in *M. hoanglienensis*; material examined; Figure 4(b)), smaller weight/SVL, 0.81 (0.74–0.87) mg/mm (vs 1.79 [1.42–2.36] mg/mm, *N* = 11, in *M. hoanglienensis*; Tapley et al. 2018a; Figure 4(c)), and advertisement call (see Bioacoustic comparison); from *M. jiangi* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 34.4–39.2 mm, *N* = 9, in *M. jiangi*; Liu et al. 2020), vomerine teeth present (vs absent in *M. jiangi*; Liu et al. 2020) and male advertisement call (see Bioacoustic comparison); from *M. jingdongensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 51.1–59.7 mm, *N* = 4, in *M. jingdongensis*; material examined), wide dermal fringes on toes and interdigital webbing between toes absent (vs dermal fringes on toes present and toes being at least one-quarter webbed in *M. jingdongensis*; material examined), and male advertisement call (see Bioacoustic comparison); from *M. leishanensis* by vomerine teeth present (vs absent in *M. leishanensis*; Li et al. 2019["2018"]) and advertisement call (see Bioacoustic comparison); from *M. liboensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 60.5–67.7 mm, *N* = 5, in *M. liboensis*; Zhang et al. 2017), and by lateral fringes on toes absent (vs present in *M. liboensis*; Zhang et al. 2017); from *M. minor* by vomerine teeth present (vs absent in *M. minor*; Stejneger 1926), adult male TYD/EL 74–81% (vs 47–48%, *N* = 2, in *M. minor*; material examined) and male advertisement call (see Bioacoustic comparison); from *M. mirabilis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 55.8–61.4 mm, *N* = 2, in *M. mirabilis*; Lyu et al. 2020), vomerine teeth present (vs absent in *M. mirabilis*; Lyu et al. 2020), dermal fringes on fingers absent (vs present in *M. mirabilis*; Lyu et al. 2020); and dermal fringes on toes absent (vs present in *M. mirabilis*; Lyu et al. 2020); from *M. omeimontis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 51.6–60.9 mm, *N* = 5, in *M. omeimontis*; material examined) and dermal fringes on toes absent (vs present in *M. omeimontis*; material examined); from *M. palpebralespinosa* by dermal fringes on toes absent (vs present in *M. palpebralespinosa*; Bourret 1937; Orlov et al. 2015; material examined), interdigital webbing between toes absent (vs present in *M. palpebralespinosa*; Bourret 1937; Orlov et al. 2015; material examined) and a small blunt tubercle present on outer edge of upper eyelids (vs moderately large palpebral horn like structure on upper eyelids present in *M. palpebralespinosa*; Bourret 1937; Orlov et al. 2015; material examined); from *M. qianbeiensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 49.3–58.2 mm, *N* = 6, in *M. qianbeiensis*; Su et al. 2020), lateral fringes on toes absent (vs present in *M. qianbeiensis*; Su et al. 2020), interdigital webbing between toes absent (vs present in *M. qianbeiensis*; Su et al. 2020), large keratinised spines on nuptial pads of sexually mature males absent (vs present in in *M. qianbeiensis*; Su et al. 2020) and male advertisement call (see Bioacoustics comparison); from *M. rubrimera* by lateral fringes on toes absent (vs present in *M. rubrimera*; Tapley et al. 2017; material examined), absence of a red-orange groin, inner thighs and outer surface of shanks in life (vs presence in *M. rubrimera*; Tapley et al. 2017) and male advertisement call (see Bioacoustics comparison); from *M. shimentaina* by dermal fringes on fingers absent (vs present in *M. shimentaina*; Lyu et al. 2020), dermal fringes on toes absent (vs present in *M. shimentaina*; Lyu et al. 2020) and male advertisement call (see Bioacoustics comparison); from *M. shuichengensis* by interdigital webbing between toes absent (vs present, distinctly webbed in *M. shuichengensis*; Tian et al. 2000) and dermal fringes on toes absent (vs present in *M. shuichengensis*; Tian et al. 2000); from *M. shunhuangensis* by vomerine teeth present (vs absent in *M. shunhuangensis*; Wang et al. 2019b), male TYD/EL 74–81% (vs 47–70%, *N* = 10, in *M. shunhuangensis*; Wang et al. 2019b)

and advertisement call (see Bioacoustic comparison); from *M. spinata* by interdigital webbing between toes absent (vs present, distinctly webbed in *M. spinata*; Hu et al. 1973) and large keratinised spines on nuptial pads of sexually mature males absent (vs present in *M. spinata*; Hu et al. 1973); and from *M. wuliangshanensis* by having a larger tympanum on males, mean male TYD/EL 78% (vs mean male TYD/EL 49%, *N* = 10, in *M. wuliangshanensis*; Ye and Fei 1995), and vomerine ridges and vomerine teeth present (vs absent in *M. wuliangshanensis*; Fei et al. 2009, 2012).

Subgenus Xenophrys Günther, 1864. *Megophrys frigida* sp. nov. differs from *M. auralensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 60.1 – 76.9 mm, *N* = 20, in *M. auralensis*; Ohler et al. 2002; Neang et al. 2013); from *M. damrei* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 47.7–57.1 mm, *N* = 7, in *M. damrei*; Mahony 2011; Neang et al. 2013; material examined); from *M. glandulosa* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 76.7–81.6 mm, *N* = 10, in *M. glandulosa*; Fei et al. 2009; material examined), dermal fringes on toes absent (vs present in *M. glandulosa*; Huang et al. 1998; Mahony et al. 2018; material examined), distinct interdigital webbing between toes absent (vs present as basal webbing in *M. glandulosa*; Mahony et al. 2018; material examined) and a light-coloured upper lip stripe absent (vs present in *M. glandulosa*; Fei et al. 1990; Mahony et al. 2018; material examined); from *M. lekaguli* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 55.6–68.1 mm, *N* = 8, in *M. lekaguli*; Stuart et al. 2006; material examined); from *M. major* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 72.4–87.5 mm, *N* = 10, in *M. major*; Mahony et al. 2018; material examined), distinct interdigital toe webbing absent (vs present as distinct basal webbing in *M. major*; Mahony et al. 2018; material examined) and a light-coloured upper lip stripe absent (vs present in *M. major*; Boulenger 1908; Mahony et al. 2018; material examined); from *M. maosonensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 58.0–76.0 mm, *N* = 6, in *M. maosonensis*; Bourret 1937), distinct interdigital toe webbing absent (vs present, toes up to one-quarter webbed in *M. maosonensis*; Bourret 1937) and a light-coloured upper lip stripe absent (vs present in *M. maosonensis*; Bourret 1937); from *M. pachyproctus* by a larger TYD/EL 74–81% (vs 29%, *N* = 3, in *M. pachyproctus*; Huang and Fei 1981) and a protruding projection posterior to cloaca on male specimens absent (vs present in *M. pachyproctus* Huang et al. 1981); from *M. parva* by a larger TYD/EL 74–81% (vs 40–55%, *N* = 4, in *M. parva*; material examined); and from *M. takensis* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 47.3–53.0 mm, *N* = 3, in *M. takensis*; Mahony 2011; material examined).

Subgenus Atympanophrys Tian and Hu, 1983. *Megophrys frigida* sp. nov. can be distinguished from *M. gigantica* by having a distinct tympanum (vs obscured in *M. gigantica*; Liu et al. 1960), vomerine teeth present (vs absent in *M. gigantica*; Liu et al. 1960), lateral fringes on toes absent (vs present in *M. gigantica*; Liu et al. 1960) and smaller adult male size, SVL 30.3–31.8 mm (vs 80.5–107.0 mm in *M. gigantica*; Fei et al. 2012); and from *M. shapingensis* by having a distinct tympanum (vs obscured in *M. shapingensis*; Liu 1950) and vomerine teeth present (vs absent in *M. shapingensis*; Liu 1950).

Subgenus Brachytarsophrys Tian and Hu, 1983. *Megophrys frigida* sp. nov. can be distinguished from *M. carinense, M. feae, M. intermedia* and *M. platyparietus* by transverse

ridge at base of head absent (vs present in *M. carinense, M. feae, M. intermedia* and *M. platyparietus*), and by having a smaller adult male size, SVL 30.3–31.8 mm (vs >70.7 mm; material examined; Fei and Ye 2001; Li et al. 2020).

***Subgenus Ophryophryne* Boulenger, 1903.** *Megophrys frigida* sp. nov. can be distinguished from *M. elfina* by brown nuptial pads in life (vs bright orange; Poyarkov et al. 2017); from *M. gerti* by vomerine ridges present (vs absent in *M. gerti*; Poyarkov et al. 2017; material examined); from *M. hansi* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 33.4–44.4 mm, *N* = 12, in *M. hansi*; Poyarkov et al. 2017; material examined); from *M. koui* by protruding fleshy projection above cloaca in sexually mature males absent (vs present in *M. koui*; Poyarkov et al. 2017); from *M. poilani* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 32.6–38.1 mm, *N* = 14, in *M. poilani*; Poyarkov et al. 2017); from *M. synoria* by having a smaller adult male size, SVL 30.3–31.8 mm (vs 38.2–53.7 mm, *N* = 14, in *M. synoria*; Poyarkov et al. 2017; material examined), and microspinules on nuptial pads of sexually mature males (vs microgranules on nuptial pads of sexually mature male *M. synoria*; material examined); and from *M. microstoma* and all other species in the subgenus *Ophryophryne* due to maxillary teeth present (vs absent; Poyarkov et al. 2017; material examined).

Species not yet assigned to a subgenus. *Megophrys frigida* sp. nov. differs from *M. feii* by having a larger adult male body size, SVL 30.3–31.8 mm (vs 24.3–25.1 mm, *N* = 4, in *M. feii*; Yang et al. 2018), vomerine ridges and vomerine teeth present (vs absent in *M. feii*; Yang et al. 2018), lateral fringes on toes absent (vs present in *M. feii*; Yang et al. 2018), nuptial pads on breeding males present (vs absent in *M. feii*; Yang et al. 2018), and a protruding projection posterior to cloaca absent (vs present in both sexes of *M. feii*; Yang et al. 2018).

Bioacoustic comparison. The male advertisement call (*N* = 20 calls) of *Megophrys frigida* sp. nov. recorded at 16.0°C differs from those of its 10 congeners (subgenus *Panophrys*) found in mainland Southeast Asia, north of the Isthmus of Kra and nearby provinces of China (Yunnan, Guangxi and Guizhou) for which calls have been described. The male advertisement call of *Megophrys frigida* sp. nov. differs from that of *M. boettgeri* (data from Wang et al. 2014, *N* = 76 calls recorded at 15.0–18.0°C) by having an average call duration of 46.5 (43.0–50.0) ms (vs 54 ms [range not provided] in *M. boettgeri*), and weak harmonics at both 2.3 and 7.4 kHz (vs relatively clear harmonics at approximately 5.0 kHz in *M. boettgeri*); from *M. chishuiensis* (data from Xu et al. 2020, *N* = 7 calls recorded at 24.5°C) by having an average call duration of 47 (43–50) ms (vs 70–120 ms in *M. chishuiensis*) and an average dominant frequency of 3.6 (3.5–3.7) kHz (vs 5.9 [5.7–6.1] kHz in *M. chishuiensis*); from *M. fansipanensis* (data from Tapley et al. 2018a, *N* = 60 calls recorded at 15.3–18.3°C) by having an average call repetition rate of 3.5 (3.5–3.6) calls/s (vs 3.9 [3.8–4.0] calls/s in *M. fansipanensis*), and a consistent call amplitude within each call group and amplitude of pulses within each call dropping sharply after the first pulse (vs more variable amplitude within each call group and amplitude of pulses within each call declining gradually after the first pulse in *M. fansipanensis*; Figure 9(d)); from *M. hoanglienensis* (data from Tapley et al. 2018a, *N* = 20 calls recorded at 18.5°C) by having an average call duration of 47 (43–50) ms (vs 103 [96–108] ms in *M. hoanglienensis*), an average of 10.8 (10.0–11.0) pulses per call

(vs 18.7 [12.0–22.0] in *M. hoanglienensis*), and an average call repetition rate of 3.5 (3.5–3.6) calls/s (vs 2.6 calls/s in *M. hoanglienensis*); from *M. jiangi* (data from text in Liu et al. 2020, *N* = 90 calls recorded at 19.5°C) by having an average call duration of 47 (43–50) ms (vs 170–370 ms in *M. jiangi*) and an average dominant frequency of 3.6 (3.5–3.7) kHz (vs 5.8 [5.7–6.0] kHz in *M. jiangi*); from *M. jingdongensis* (data from Cutajar et al. 2020, *N* = 17 calls recorded at 18.5°C) by having an average call duration of 46.5 (43.0–50.0) ms (vs 132.7 [117.0–147.0] ms in *M. jingdongensis*), an average dominant frequency of 3.6 (3.5–3.7) kHz (vs 2.5 [2.4–2.6] kHz in *M. jingdongensis*), and an average call repetition rate of 3.5 (3.5–3.6) calls/s (vs 3.9 calls/s in *M. jingdongensis*); from *M. leishanensis* (data from Li et al. 2019["2018"], *N* = 36 calls recorded at 18.9°C) by having an average of 10.8 (10.0–11.0) pulses/call (vs 13.0 [12.0–14.0] pulses/call, *N* = 5, in *M. leishanensis*), and an average call repetition rate of 3.5 (3.5–3.6) calls/s (vs 2.6 [1.2–3.2] calls/s in *M. leishanensis*); from *M. minor* (data from Jiang et al. 2001, *N* = 14 calls recorded at 14.0°C) by having an average call repetition rate of 3.5 (3.5–3.6) calls/s (vs 4.0 [range not provided] calls/s in *M. minor*), and having weak harmonics at 2.3 and 7.4 kHz (vs relatively clear harmonics at approximately 7.2 kHz in *M. minor*); from *M. qianbeiensis* (data from Su et al. 2020, number of calls analysed not reported, calls recorded at 20.5°C) by having an average call duration of 46.5 (43.0–50.0) ms (vs 129.0–211.0] ms in *M. qianbeiensis*) and an average dominant frequency of 3.6 (3.5–3.7) kHz (vs 2.25–3.00 kHz in *M. qianbeiensis*); from *M. shimentaina* (data from Lyu et al. 2020, *N* = 96 calls recorded at 18.0–20.0°C) by having an average call duration of 46.5 (43.0–50.0) ms (vs 85.0 [64.0–101.0] ms in *M. shimentaina*) and an average dominant frequency of 3.6 (3.5–3.7) kHz (vs 4.9 [4.7–5.2] kHz in *M. shimentaina*); and from *M. rubrimera* (data from Tapley et al. 2017, *N* = 60 calls recorded at 21.0–22.9°C) by having an average dominant frequency of 3.6 (3.5–3.7) kHz (vs 3.3 [3.2–3.4] kHz in *M. rubrimera*), an average call duration of 46.5 (43–50) ms (vs 73.3 [62.0–85.0] ms in *M. rubrimera*), and a call repetition rate of 3.5 (3.5–3.6) calls/s (vs 3.3 [3.1–3.4] calls/s in *M. rubrimera*).

Discussion

Megophrys frigida sp. nov. is the fourth new species of *Megophrys* (subgenus *Panophrys*) described from the Hoang Lien Range in 4 years (Tapley et al. 2017, 2018a), and all 4 of the new species have relatively small ranges (< 4500 km^2; Tapley et al. 2017, 2018a, 2018b). All of the recently described *Megophrys* from the Hoang Lien Range (*Megophrys frigida* sp. nov., *M fansipanensis*, *M. hoanglienensis* and *M. rubrimera*) are likely to be highly threatened due to increasing anthropogenic habitat loss and degradation (Tapley et al. 2017, 2018a, 2018b), and formal IUCN Red List assessments are pending. Subsequent IUCN Red List assessments for newly described *Megophrys* species, as well as reassessment for species with out-of-date assessments, should also be a focus of future work so that species can be prioritised for conservation and incorporated into site management plans and wider conservation policy. Once assessed by the IUCN, we recommend that these species be added to Vietnam's Red Data Book as this would inform national legislation in Vietnam.

Megophryid species often occur in sympatry with congeners (Wang et al. 2014, 2019a; Chen et al. 2017, 2018; Liu et al. 2018; Mahony et al. 2018, 2020). Often, sympatric *Megophrys* are quite distantly related phylogenetically (Wang et al.

2019a). At lower elevations on Mount Ky Quan San, we found *Megophrys frigida* sp. nov. to be syntopic with *M. hoanglienensis*, a species that our phylogenetic analyses of the CO1 gene resolved as the sister taxon of *Megophrys frigida* sp. nov. with moderate to high support. In earlier work we reported the presence of two syntopic sister species elsewhere in the Hoang Lien Range on Mount Fansipan (*M. fansipanensis* and *M. hoanglienensis*; Tapley et al. 2018a).

Megophrys frigida sp. nov. is most similar to *M. fansipanensis* in terms of morphology, and many call parameters also overlap with this species; further surveys and the collection of additional genetic and bioacoustic data will further resolve species boundaries within this closely related, morphologically conserved, geographically proximate and highly imperilled group. *Megophrys frigida* sp. nov. likely occupies the equivalent niche of *M. fanispanensis* on Mount Ky Quan San, as both species occur at high elevation and they occupy similar habitats. During field work on Mount Ky Quan San we did not find evidence for the presence of *M. fansipanensis* at sites of a suitable elevation and with seemingly suitable habitat (e.g. clear rocky streams in evergreen forest). *Megophrys fansipanensis* may have a more restricted distribution than previously thought (Tapley et al. 2018a), occurring only in the southern part of the Hoang Lien Range. The presence of a valley 5.5 km north of Mount Fansipan may be a barrier to *Megophrys frigida* sp. nov. dispersing south and could mark the northernmost limit to the distribution of *M. fansipanensis*. The lowest reported elevations at which *Megophrys frigida* sp. nov. and *M. fansipanensis* have been observed are 2218 m and 2200 m, respectively; at 1950 m asl the valley floor is approximately 250 m below the lowest reported elevation at which these species have been observed. There is a growing body of literature showing that calls of congeneric and even sympatric congeneric amphibians are not always dissimilar (Pereyra et al. 2012; Park et al. 2013; Toledo et al. 2015). In cases of vicariant evolution, as suspected here, there would be little biological need for changes in bioacoustic signal in either *Megophrys frigida* sp. nov. or *M. fansipanensis*.

At elevations where *Megophrys frigida* sp. nov. is sympatric with *M. hoanglienensis*, we heard only the former calling during our survey in September. In our previous work (Tapley et al. 2018a), we reported that *M. hoanglienensis* is known to call in June and vocalisations were not heard during March 2017, September 2017 or May 2018 (surveys took place both in heavy rainfall and during dry nights). Given that these species are syntopic at some elevations, it could be that they avoid competition by breeding at different times of the year as well as having distinctive call parameters as observed between other syntopic *Megophrys* species (e.g. Wang et al. 2014; Poyarkov et al. 2017); however, further survey work on Mount Ky Quan San is needed to confirm this. Further surveys are also required to determine the distribution of *Megophrys frigida* sp. nov. Some species of *Megophrys* are seasonally active in the Hoang Lien Range, and post-metamorphic specimens may be difficult to detect if they are not calling (Tapley et al. 2020b). Surveys for tadpoles have been demonstrably successful in detecting the presence of *Megophrys* species in the Hoang Lien Range (Tapley et al. 2020b), and tadpole surveys undertaken at different times of year could assist in defining the range of *Megophrys frigida* sp. nov. and other *Megophrys* species. The species may be present in similar habitat on the western slopes of the Hoang Lien Range in Lai Chau Province and farther north into Yunnan, China.

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Disclosure statement

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ORCID

Benjamin Tapley (iD) http://orcid.org/0000-0002-9787-3793
Timothy Cutajar (iD) http://orcid.org/0000-0001-7841-9205
Luan Thanh Nguyen (iD) http://orcid.org/0000-0002-4663-125X
Christopher Portway (iD) http://orcid.org/0000-0003-1767-4282
Stephen Mahony (iD) http://orcid.org/0000-0002-8263-6832
Chung Thanh Nguyen (iD) http://orcid.org/0000-0001-8533-9721
Luke Harding (iD) http://orcid.org/0000-0003-2752-4966
Hao Van Luong (iD) http://orcid.org/0000-0002-7463-1118
Jodi J. L. Rowley (iD) http://orcid.org/0000-0002-2011-9143

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