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Title: A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus *Megophrys sensu lato* (Anura: Megophryidae)

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ABSTRACT

The horned toad assemblage, genus *Megophrys sensu lato*, currently includes three groups previously recognized as the genera *Atympanophrys*, *Xenophrys* and *Megophrys sensu stricto*. The taxonomic status and species composition of the three groups remain controversial due to conflicting phenotypic analyses and insufficient phylogenetic reconstruction; likewise, the position of the monotypic *Borneophrys* remains uncertain with respect to the horned toads. Further, the diversity of the horned toads remains poorly understood, especially for widespread species. Herein, we evaluate species-level diversity based on 45 of the 57 described species from throughout southern China, Southeast Asia and the Himalayas using Bayesian inference trees and the Generalized Mixed Yule Coalescent (GMYC) approach. We estimate the phylogeny using both mitochondrial and nuclear DNA data. Analyses reveal statistically significant mito-nuclear discordance. All analyses resolve paraphyly for horned toads involving multiple strongly supported clades. These clades correspond with geography. We resurrect the genera *Atympanophrys* and *Xenophrys* from the synonymy of *Megophrys* to eliminate paraphyly of *Megophrys s.l.* and to account for the morphological, molecular and biogeographic differences among these groups, but we also provide an alternative option. Our study suggests that *Borneophrys* is junior synonym of *Megophrys sensu stricto*. We provide an estimation of timeframe for the horned toads. The mitochondrial and nuclear trees indicate the presence of many putative undescribed species. Widespread species, such as *Xenophrys major* and *X. minor*, likely have dramatically underestimated diversity. The integration of morphological and molecular evidence can validate this discovery. Montane forest dynamics appear to play a significant role in driving diversification of horned toads.

Keywords: *Atympanophrys*; *Borneophrys*; Gene tree discordance; GMYC; Sympatric distribution; *Xenophrys*

1. Introduction

Megophrys sensu lato (s.l.; Asian horned toads) is an enigmatic assemblage of considerable interest and it has a lengthy history of taxonomic debate. The assemblage currently includes three groups, previously recognized as genera or subgenera: *Atympanophrys*, *Megophrys sensu stricto* (s.s.) and *Xenophrys* (Dubois, 1986; Dubois and Ohler, 1998; Günther, 1864; Lathrop, 1997; Tian and Hu, 1983). The group contains 57 species (AmphibiaWeb, 2016; Frost, 2016). Some arrangements split the horned toads into the genera *Atympanophrys*, *Megophrys* and *Xenophrys*, yet many researchers retain usage of *Megophrys s.l.* for all species (e.g., Boulenger, 1908; Fei et al., 1990; Fei et al., 2009; Mahony et al., 2013; Orlov et al., 2015; Stuart et al., 2006). This conservative taxonomy is controversial and rejected by some authorities (Delorme et al., 2006; Frost et al., 2006; Li and Wang, 2008; Ohler, 2003; Rao and Yang, 1997). The absence of a comprehensive phylogenetic analysis prevents the resolution of this controversy. In addition, the monotypic genus *Borneophrys* (*B. edwardinae*) was separated from the other horned toads based on solely plesiomorphic characteristics for Megophryini (Delorme et al., 2006). Due to the overall morphological similarity between *Borneophrys* and nearby species of *Megophrys*, this separation requires confirmation. Thus, because recent molecular analyses indicated that *Megophrys s.l.* was not a monophyletic assemblage of species with respect to the genera *Brachytarsophrys* and *Ophryophryne* (Jiang et al., 2003; Pyron and Wiens, 2011; Wang et al., 2012), the higher-level phylogenetic relationships and taxonomy of horned toads remain in flux.

Assessments of biodiversity and evolutionary history of the horned toads are often challenging due to relatively conserved morphological evolution and rampant homoplasy (Mahony et al., 2013; Wang et al., 2014). Thus, results from previous phenotypic studies widely conflict (reviewed in Wei et al., 2010) and suggest that analyses of molecular data offer the best hope of untying this “Gordian knot.”

Only a few molecular phylogenetic analyses have focused on this assemblage,

and no consensus exists except for a general agreement on the paraphyly of the horned toads (e.g., Jiang et al., 2003; Pyron and Wiens, 2011; Wang et al., 2012). Previous studies primarily have focused on regional faunas and in doing so had limited sampling. For example, the most recent analysis included 16 species mostly from southeastern China (Li et al., 2014). Few phylogenetic studies have included representatives of *Atympanophrys*, *Megophrys* and *Xenophrys* (Pyron and Wiens, 2011), and none have included the large proportion of species that occur in western China, the Himalayas and Southeast Asia. Previous analyses leave an incomplete picture due to the paucity of considered species from these areas.

The current hypothesis of relationships in the horned toads is based on mitochondrial DNA (e.g. Jiang et al., 2003; Li et al., 2014; Zheng et al., 2004). This leaves open the possibility of mito-nuclear discordance owing to introgression and incomplete lineage sorting, which is not uncommon (Qi et al., 2014; Toews and Brelsford, 2012). Thus, the validity of *Atympanophrys* and *Xenophrys* requires a thorough reassessment, including genes from the nuclear genome.

Lower taxonomic levels of the horned toads are also wanting of analyses. Most descriptions of new species have been based solely on morphology, and the identification of many species remains challenging (Wang et al., 2014). DNA-based approaches play an increasingly important role in recognizing species via informing searches for subtle and previously overlooked morphological contrasts (Barley et al., 2013; Welton et al., 2013). This is especially true in cases of diverse, understudied taxa like horned toads. The inclusion of molecular assessments and material from remote, previously unexplored areas in the last ten years has led to the description of many new species of horned toads (e.g., Li et al., 2014; Mahony et al., 2013; Stuart et al., 2006), suggesting that overall species richness may be underestimated. The names of widespread species, such as *M. major*, *M. minor* and *M. parva*, may conceal a great proportion of the species diversity (Mahony 2011; Mahony et al., 2013; Ye and Fei, 1995). Such widespread species all require taxonomic reevaluations.

Given the considerable uncertainty within horned toads in both high-level

evolutionary relationships and species diversity, we use multilocus DNA sequence data to examine phylogenetic relationships and to identify putative unrecognized species throughout southern China, Southeast Asia, and the Himalayas. Specifically, we address several questions. (1) Are *Atympanophrys*, *Borneophrys* and *Xenophrys* valid genera? (2) Do molecular data correspond with recognized morphological species? And finally, (3) do most widely distributed species potentially consist of multiple unrecognized taxa?

2. Materials and methods

2.1 Taxon sampling

Samples of *Megophrys s.l.* were broadly collected from throughout southern China, Southeast Asia and the Himalayas. We generated 177 new partial DNA sequences of 16S rRNA that represented the range of morphological and geographic variation of our collection, including 37 described species and multiple potentially undescribed species. Additional homologous sequences of 16S rRNA were retrieved from GenBank to expand the number of species and localities. Our final dataset included 249 sequences of 16S rRNA, including 45 described species of *Megophrys s.l.* (Table 1, Fig. 1). When possible, species were collected at or near their type localities. In cases of geographically widespread species, we included multiple samples from different localities. This strategy maximized the chances of capturing unknown genetic diversity and bolstered assessments of phylogenetic relationships. Based on previous phylogenetic analyses (Jiang et al., 2003; Pyron and Wiens, 2011; Zheng et al., 2004), *Brachytarsophrys* and *Ophryophryne* were selected as primary outgroup taxa due to *Megophrys s.l.* having a close but unresolved relationship with them. We used *Leptobrachium* and *Leptolalax* as our secondary outgroup taxa. In addition, *Borneophrys edwardinae* was included in the analysis to clarify its taxonomic status.

2.2 DNA extraction, amplification and sequencing

Total genomic DNA was extracted using standard phenol/chloroform method

(Sambrook et al., 1989), and all samples were sequenced initially for 16S rRNA to evaluate species diversity and to perform preliminary phylogenetic analyses. Subsequent multilocus phylogenetic analyses were based on a subset of 118 samples of *Megophrys s.l.* and outgroups, which represented the range of genetic variation of the entire dataset (Table 1). For the multilocus phylogenetic reconstruction, we also sequenced a fragment of the mitochondrial gene encoding cytochrome oxidase subunit I (*COI*) as well as nuclear gene fragments encoding brain-derived neurotrophic factor (*BDNF*), recombination activating gene 1 (*RAG1*) and rhodopsin (*RHOD*). Primer sequences are in Table S1 (Supplementary Material).

Amplification consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 16S rRNA, 46 °C for *COI*, 53 °C for *BDNF*, 60 °C for *RAG1* and 59 °C for *RHOD* for 1 min, followed by extension at 72 °C for 1 min. The final extension was set at 72 °C for 10 min. PCR products were purified with a Gel Extraction Mini Kit (Watson BioTechnologies, Shanghai, China), and then sequenced in both directions with BigDye Terminator Cycle Sequencing Kit (v.2.0, Applied Biosystems, Foster City, CA, USA) using an ABI PRISM 3730 DNA Analyzer (Applied Biosystems).

2.3 Sequence alignment

New sequences were first assembled and edited using DNASTAR LASERGENE 7.1. Sequences were aligned using MUSCLE 3.6 (Edgar, 2004), and then checked by eye for accuracy and trimmed to minimize missing characters in MEGA6 (Tamura et al., 2013).

2.4 Species delimitation

We used Bayesian inference (BI) to reconstruct the 16S rRNA gene tree from all samples. Bayesian analyses were performed using MRBAYES 3.2 (Ronquist and Huelsenbeck, 2003). The best-fit evolution model for 16S rRNA was GTR+I+G determined by JMODELTEST 1.0.1 (Posada, 2008). Our 16S rRNA BI analyses were run for 50 million generations with a sampling frequency of 1000. The first 25% of the sampled trees were discarded as burn-in, and we used the remaining samples

(37500 trees) to generate the consensus tree. Convergence was judged based on the average standard deviation of split frequencies (less than 0.01) and the ESS values (more than 200) in TRACER 1.5.

To identify putative species boundaries beyond those recognized currently by taxonomists, the General Mixed Yule-coalescent (GMYC) approach (Pons et al., 2006) was applied to the 16S rRNA data from all samples. Identical haplotypes were first collapsed using ALTER (Glez-Peña et al., 2010). The best-fit model of evolution for the construction of the ultrametric trees was GTR+I+G as determined by JMODELTEST 1.0.1. The trees with divergences estimated under an uncorrelated lognormal relaxed molecular clock model in BEAST 1.8.0 (Drummond and Rambaut, 2007) were calculated. The Yule process was assumed for the tree prior. Markov chains were run for 50 million generations, and sampling occurred every 1000th generation. TRACER 1.5 was used to confirm when the output reached stationarity. Subsequently, the first 25% of the samples were discarded as burn-in using TREEANNOTATOR 1.5.3, and we used the remaining samples (37500 trees) to generate the majority-rule consensus tree. The relaxed-clock BEAST maximum clade-credibility (mcc) tree was used as input in single-threshold GMYC analysis, as implemented on the GMYC server (<http://species.h-its.org/gmyc/>). Genetic divergences among *Megophrys s.l.* taxa were calculated based on uncorrected *p*-distances as implemented in MEGA6 (Tamura et al., 2013).

2.5 Multilocus gene tree reconstruction

We used BI and maximum-likelihood (ML) approaches to reconstruct the mitochondrial gene tree (matrilineal genealogy) of *Megophrys s.l.* The analyses used a concatenation of *COI* and 16S rRNA sequences. We also estimated the phylogeny based on the concatenation of the nuclear genes *BDNF*, *RAG1* and *RHOD*. We performed the approximately unbiased (AU) test (Shimodaira, 2002) using the CONSEL package (Shimodaira and Hasegawa, 2001) to test whether the topologies of the mitochondrial and nuclear phylogenies differed statistically significantly.

The optimal partitioning strategy for molecular evolution of the concatenated

datasets for BI was determined by PARTITIONFINDER 1.1.1 (Lanfear et al., 2012) using Bayesian information criterion (BIC). Partitioned BI analyses were run for 20 million generations with a sampling frequency of 1000. The first 25% of the sampled trees were discarded as burn-in, and we used the remaining samples (15000 trees) to generate the consensus tree. Convergence was judged based on the average standard deviation of split frequencies (all less than 0.01) and the ESS values (more than 200) in TRACER 1.5. Maximum-likelihood (ML) analyses were conducted using RAxML 7.0.4 (Stamatakis et al., 2006). The analyses used the GTR substitution model with gamma-distributed rate heterogeneity among sites, the proportion of invariable sites estimated from the data, and used for all data with 1000 bootstrap pseudoreplicates. Nodes in the trees were considered well supported when Bayesian posterior probabilities (PP) were ≥ 0.95 and ML bootstrap support (BS) was $\geq 70\%$.

2.6 Species-tree reconstruction and divergence times

We conducted two separate species-tree analyses using two datasets based on *BEAST (Heled and Drummond, 2010): the combined nuclear plus mitochondrial sequences (total evidence) and nuclear sequences only. In the combined species-tree analysis, the two mitochondrial genes were considered as one locus. Nuclear genes were partitioned by codons. We estimated the species tree using the best-fitting combination of partitions and models according to the results from PARTITIONFINDER. Substitution models were linked within the identified partitions. Tree models were linked across partitions within each gene, and clock models were unlinked across all codon positions of each gene. We employed the Yule process as the divergence-time prior, and the Piecewise constant as the population-size prior. A relaxed molecular clock with an uncorrelated lognormal distribution was specified. We ran 200 million generations with sampling every 5000th generation. A total of 40,000 samples were collected, and the first 25% were discarded as burn-in. TRACER 1.5 was employed to inspect the convergence of posterior distributions. We used the nuclear species-tree topology for estimating divergence times because it better corresponded with the clustering of morphology than the topology of the

total-evidence species tree.

We pursued a broad understanding of the tempo of lineage divergence in the horned toads. No calibration points existed for megophryid frogs due to the poor fossil record. Thus, we used two calibration priors to accommodate ages that were estimated previously (Pyron, 2014): using a normal prior, the time to the most recent common ancestor (TMRCA) for Megophryidae was 96.82 Ma and for Megophryinae 49.26 Ma.

Results

3.1 Sequences data

The aligned length of the complete 16S rRNA data matrix was 547 bp, among which, 207 sites were variable and 175 were parsimony-informative (excluding outgroups). Edited and aligned sequence lengths for *COI*, *BDNF*, *RAG1*, and *RHOD* equaled 561 bp, 711 bp, 1002 bp and 339 bp, respectively. After concatenation of the two mitochondrial genes, our final aligned length equaled 1108 bp and consisted of 466 parsimony-informative characters. After concatenation of the three nuclear amplified genes, final aligned length equaled 2052 bp and consisted of 411 parsimony-informative characters. All *de novo* sequences were deposited in GenBank (Table 1). Results from our test of optimal partitioning strategy for multilocus phylogenetic analysis are summarized in Table S2 (Supplementary Material).

3.2 Putative species identification based on 16S rRNA data

The BI gene-tree resulting from our 16S rRNA provided an initial assessment of species-level relationships (Fig. 2 and Fig. S1). In most cases, the recognized species were reciprocally monophyletic and strongly supported units. However, *M. shapingensis* and *M. wawuensis* were not monophyletic and showed close relationships with lineages *M. gigantea* and *M. nankiangensis*. *Megophrys spinata* was also not monophyletic because *M. sangzhiensis* nested within it. The analyses suggested the occurrence of 20 putative unrecognized species (Fig. 2). For example, *M. major* was paraphyletic with respect to *M. mangshanensis*, *M. glandulosa*, *M.*

medoensis and *M. zhangii*. We denoted the taxon from northern Thailand as *M. cf. major* because it is morphologically similar to *M. major*. Three putative new taxa (sp. 12–14) and one previously recognized species, *M. maosonensis*, were detected in the *M. major* species complex. For the *M. parva* species complex, the taxon from western Yunnan, China was denoted as *M. cf. parva* because morphologically it is similar to the type series of *M. parva* from Myanmar. Five other new species-level lineages (sp. 15–19) previously considered *M. parva* were well differentiated. *Megophrys auralensis* and *M. lekaguli* nested in the *M. parva* species complex. The remaining 12 potentially new species were scattered over the phylogeny and involved widespread species such as *M. boettgeri*, *M. brachykolos*, *M. minor* and *M. spinata* (Fig. 2).

The likelihood of the GMYC model (=multiple species) was significantly higher ($p < 0.001$) than that of the null model of uniform coalescent (=a single species), indicating the presence of more than one species in the dataset. Results of the GMYC analyses were largely congruent with the BI gene tree regarding the numbers of species-level lineages (Fig. 2). Most described species of *Megophrys s.l.* were validated by GMYC. However, *M. sangzhiensis* and *M. spinata* merged into one species, as did *M. boettgeri* and *M. huangshanensis* as well as *M. gigantea*, *M. wawuensis* and *M. nankiangensis* (Fig. 2; Table S3). The BI tree and GMYC analyses both strongly indicated the presence of many unrecognized species. However, the GMYC approach further partitioned six species-level lineages identified from the BI tree: *M. aceras*, *M. jinggangensis*, *M. nasuta*, *M. sp. 14*, *M. sp. 15* and *M. spinata* were all partitioned into two OTUs rather than a single genetic entity (Fig. 2 and Table S3).

3.3 Multilocus phylogeny from the concatenated data

Bayesian inference and ML analyses of the concatenated nuclear data yielded similar topologies, except at weakly supported or collapsed nodes (Fig. 3). In both analyses, *Leptobranchium* and *Leptolalax* rooted at the base of the tree, and *Megophrys s.l.*, *Ophryophryne*, *Borneophrys* and *Brachytarsophrys* formed a monophyletic group with strong support (PP=1.00; BS=100%). Our analyses revealed four well-supported

clades, denoted A–D within *Megophrys s.l.* (PP>0.95; BS>70; Fig. 3). However, *Megophrys s.l.* was not monophyletic: *Ophryophryne* grouped together with clades A–C of *Megophrys s.l.* with strong support in BI and moderate support in ML analyses (PP=0.96, BS=65), whereas Clade D seemed to be more closely related to *Brachytarsophrys* than to *Megophrys s.l.* (Fig. 3). The results strongly nested *Borneophrys* within Clade C and as the sister species of *M. ligayae* (Fig. 3). Clades A, B and C grouped together with strong support in the BI analysis (PP=0.95). Clade A and B had a sister-group relationship, but this was weakly supported (Fig. 3). Clades A and B had three well-supported sublineages A1–A3 and B1–B3, respectively, and Clade C contained C1–C2 (Fig. 3).

The BI and ML topologies based on concatenated mitochondrial data were similar (Fig. 4), and in some respects these were compatible with the nuclear phylogeny (Fig. 3). Analyses of the mtDNA data strongly recovered (PP>0.95; BS>70; Fig. 4) the clades of *Megophrys s.l.* lettered A, B1–B3, C1–C2, and D revealed by the nuclear data. The mitochondrial gene tree also showed paraphyly of *Megophrys s.l.* with respect to *Ophryophryne* and *Brachytarsophrys*. Except for *Leptobrachium* and *Leptolalax*, Clade C2 of *Megophrys s.l.* and *Brachytarsophrys* rooted at the base of the tree, whereas other *Megophrys s.l.*, *Ophryophryne* and *Borneophrys* assembled together with high support in the BI analysis (PP=0.99). Within this group, the basal split separated Clade D from a common ancestor of the remaining taxa. Clades A, B3 and *Ophryophryne* grouped together with high support in BI and moderate support in ML analysis (PP=0.98, BS=62). Clades B2 and C1 were sister groups with moderate to high support (PP=1.00, BS=61). Within Clade C1, *Borneophrys* and *M. ligayae* had a strongly supported sister-group relationship (Fig. 4).

The multiple lettered clades of *Megophrys s.l.* corresponded with the geographic distributions of their members (Table 1; Fig. 1; Fig. S2). Clades A, B, C and D of *Megophrys s.l.* were largely separated geographically from one another. Clade A contained many species of *Megophrys s.l.* and was distributed throughout southern China, but with a minor extension into Indochina. Relationships among subclades A1,

A2 and A3 remained unresolved (Fig. 3 and 4). Clades A1 and A2 had allopatric distributions. A1 was composed mainly of species from western China and A2 was composed mostly of species from eastern China. Limited overlapping occurred between A1 and A2 in several localities in central China: Badagongshan Nature Reserve, Hunan, Leigong Shan (Mountain), Guizhou, and Mao'er Shan, Guangxi. Clade A3 included *M. minor* from the type locality and two putative new taxa (previously referred to *M. minor*) from several localities around Sichuan Basin, China (Fig. S2).

Clade B was distributed throughout Indochina, the Malay Peninsula and the Himalayas, but with a minor extension into southern China (Table 1; Fig. 1; Fig. S2). Specifically, Clade B1 comprised all species morphologically assigned to the *M. major* (B1a) and *M. parva* (B1b) species complexes. These species occurred primarily in Indochina, but with a little extension into southern China and the Himalayas. Clade B2 included two species identified morphologically as *M. aceras* and *M. longipes* from the Malay Peninsula. Clade B3 included two species-level, narrowly distributed lineages from the Hengduan Mountains and Himalayas.

Clade C included species found only in areas of the Sunda shelf. In addition, although species in Clade D were distributed within the range of Clade A, all were high-elevation endemics whose distributions occupied a narrow band along the eastern escarpment of Hengduan Mountains (Table 1; Fig. 1; Fig. S2).

3.4 Mito-nuclear discordance

The conservative AU test detected significant incongruence ($P < 0.05$) between the phylogenies based on nuclear versus mitochondrial genes. The incongruence owed to basal relationships. For example, whereas clades B1–B3 strongly grouped together in the nuclear data, the mitochondrial data grouped Clade B2 with Clade C1 while excluding Clade C2, and Clade B3 grouped with Clade A and *Ophryophryne*. Sister subclades C1 and C2 from the nuclear data did not form a clade in the mitochondrial data (Fig. 3 and 4).

3.5 Species tree and divergence times

Topology of the nuclear species tree (Fig. 5) differed from the total-evidence tree (Fig. S3). Both trees strongly recovered clades A, B1–B3, C1–C2, and D, yet the total evidence tree was most similar to the topology of mitochondrial gene tree (Fig. 4). It disagreed in several respects with morphological evolution. For example, species in sister clades C1 and C2 of the nuclear data (Fig. 3 and 5) are broadly similar morphologically, but the total-evidence species tree separates them (Fig. S3).

The species-tree based on nuclear data (Fig. 5) was largely comparable with previous analyses (Fig. 3 and 4). *Megophrys s.l.* was not monophyletic and contained major strongly supported clades A–D. The sister-group relationship between Clade D and *Brachytarsophrys* was strongly supported (PP=0.96). Again, clades A and B contained multiple distinct sublineages and a sister-group relationship for them was somewhat supported (PP=0.90). According to *BEAST results, major diversification events among clades A–D of *Megophrys s.l.* and its allies occurred from ~19 to 49 Ma (Fig. 5). Specifically, median and 95% highest posterior density intervals (HPDs) for inferred splits were 33.8 (22.2–46.6) Ma for Clade D and *Brachytarsophrys*, 32.0 (21.2–45.5) Ma for clades C1 and C2, and 32.8 (22.1–41.9) Ma for clades A and B. The deepest splits within clades A and B dated to ~19 Ma and ~29 Ma, respectively. Further, the divergence times of the potential new species to their sister species all exceeded 1.5 Ma and most exceeded 2 Ma (Fig. 5). The smallest divergence time of 1.7 Ma occurred between *M. sp. 7* and *M. sp. 9*.

4. Discussion

4.1 Statistically significant topological difference

The significant incongruence ($P < 0.05$) between the phylogenies based on nuclear versus mitochondrial genes more likely owes to mitochondrial introgression, although we cannot rule out the possibility of incomplete lineage sorting. The topologies of the total-evidence (Fig. S3) and nuclear species trees (Fig. 5) differ, and the latter reflects the morphology, supporting our inference of mitochondrial introgression. Significant incongruence owes to conflict at some basal nodes (Fig. 3 and 4). Clade B3 has an

overlapping geographic range with Clade A, and this likely corresponds with an ancient hybridization event.

The species tree from *BEAST allows for incomplete lineage sorting but excludes introgression (Meyer et al., 2016). Thus, we mainly use the species tree from nuclear data for discussion because it potentially overcomes the effects of both introgression and incomplete lineage sorting. These two factors have been shown to be the main causes for conflicting trees (Edwards, 2009).

4.2 Phylogeny

Our analyses represent the most comprehensive phylogeny to date for *Megophrys s.l.* We directly assess numerous taxa using genetic data for the first time. Our general findings reinforce earlier suspicions (Jiang et al., 2003; Pyron and Wiens, 2011; Wang et al., 2012) that *Megophrys s.l.* is a paraphyletic taxon (Fig. 3–5). Although the basal relationships remain obscure, all analyses resolve multiple mutually exclusive, geographically cohesive major clades (Fig.1; Fig. 3–5; Fig. S2).

The species-tree analysis resolves three independent groups of *Megophrys s.l.* that have largely non-overlapping distributions (Fig. 1 and 5): clades A–B; Clade C; and Clade D. Although Clades A and B group together with moderate support only (PP=0.90), their similar morphologies compared with other megophrines gives credence to this result (Fei et al., 2009; Li and Wang, 2008; Rao and Yang, 1997). The assemblage of clades A–B and Clade C might overlap in the southern part of the Malay Peninsula (Taylor, 1962).

The geographic separation among clades of *Megophrys s.l.* reflects several important biogeographic barriers. For example, the Isthmus of Kra (Fig. S2) biogeographically divides clades B1 and B2. Similar species transitions in this area have been observed for a number of other taxa (e.g., de Bruyn et al., 2005; Hughes et al., 2003; Inger, 1999; Parnell, 2013). Divergence of clades B1 and B2 at the Isthmus of Kra dates to ~25 Ma (Fig. 5). This date generally corresponds to a marine transgression event in the Early Miocene, which dissected the Thai-Malay Peninsula and in doing so produced distinct assemblages of the taxa (de Bruyn et al., 2005;

Woodruff, 2003). Subclades A1 and A2 occur in western and eastern China, respectively. Analogous distributions in several other taxa, such as plants (Zhang et al., 2006) and mammals (Wu et al., 2013), suggest a common vicariance event. The initial separation of Chinese horned toads (Clade A) dates to ~19 Ma (Fig. 5). This associates with the second stage of Himalayan movements, which was suggested to drive similar distribution patterns and divergence times in other taxa, including plants (Zhang et al., 2006).

4.3 Implications for classification

Considerable uncertainty and disagreement as to generic recognition within *Megophrys s.l.* currently exists. Clearly, *Megophrys s.l.* is not monophyletic with respect to *Brachytarsophrys* and *Ophryophryne* (Fig. 3–5). Taxonomy should reflect the evolutionary history of the group and confer stability. To avoid a paraphyletic *Megophrys s.l.* and to consider morphological distinctiveness and biogeography, we recognize three genera within the *Megophrys* assemblage. This also serves to retain the recognition of *Ophryophryne* and *Brachytarsophrys*. Considering the nuclear-gene species tree (Fig. 5), we remove *Atympanophrys* from the synonymy of *Megophrys* for Clade D. Further, we resurrect genus *Xenophrys* for the assemblage consisting of clades A–B. Thus, *Megophrys s.s.* applies to Clade C only. These genera are morphologically distinguishable (Boulenger, 1908; Delorme et al., 2006; Fei et al., 2009; Günther, 1864; Lathrop, 1997; Li and Wang, 2008; Li, 2007; Rao and Yang, 1997; Tian and Hu, 1983; Wei et al., 2010; reviewed in Table S4).

Our phylogeny necessitates placement of monotypic *Borneophrys* as a junior synonym of *Megophrys s.s.* because it nests within Clade C. Morphologically, *B. edwardinae* is very similar to *Megophrys s.s.*, and only subtle differences separate it from *Megophrys s.s.* (personal observation). For example, both genera bear similar palpebral projections ("horns") above the eyes, have broad and depressed heads, possess transverse skin folds at the base of the head, have narrow and distinct dorsolateral folds, and lack enlarged neural spines on vertebrae (Delorme et al., 2006). All things considered, we return *B. edwardinae* to *Megophrys* as *M. edwardinae* Inger,

1989.

Our taxonomy leaves subfamily Megophryinae with the following five monophyletic genera: *Ophryophryne*, *Brachytarsophrys*, *Xenophrys*, *Atympanophrys* and *Megophrys*. Currently, *Ophryophryne* and *Brachytarsophrys* have largely overlapping distributions and are distributed in Indochina and southern China. *Xenophrys* has widespread distribution encompassing southern China, Southeast Asia and the Himalayas, whereas *Atympanophrys* and *Megophrys* are mostly restricted to the eastern escarpment of Hengduan Mountains and Sundaland, respectively. The 57 recognized species of *Megophrys s.l.*, the resurrected taxa *maosonensis* and *Borneophrys edwardinae* are reassigned based on the revised taxonomy (Table S5). Our taxonomy involves the following new arrangements: *Xenophrys vegrandis* **new combination**, *Xenophrys oropedion* **new combination**, *Xenophrys obesa* **new combination**, *Xenophrys acuta* **new combination**, *Xenophrys ancrae* **new combination**, *Xenophrys latidactyla* **new combination**, *Xenophrys maosonensis* **new combination**, *Atympanophrys nankiangensis* **new combination** and *Atympanophrys wawuensis* **new combination**.

Within *Xenophrys*, it is possible to recognize the subgenera *Panophrys* for Clade A and nominate subgenus *Xenophrys* for Clade B. *Panophrys* was established previously with the type species *X. omeimontis* (Rao and Yang, 1997). Later, it was synonymized with *Xenophrys* (Dubois and Ohler, 1998). Diagnostic morphological characters await future research.

Our analyses include the representative samples of all genera previously recognized in the Megophryinae and the type species of *Atympanophrys*, *Brachytarsophrys*, *Borneophrys*, *Megophrys* and *Ophryophryne*. Unfortunately, our analyses do not include *X. parva* (District of Karin Bia-po, Myanmar), the type species of *Xenophrys*, owing to the absence of tissue samples. We include *X. cf. parva* from western Yunnan, but future analyses of material from the type locality of *X. parva* is desirable.

Recognizing five genera would properly reflect phylogenetic history and

biogeographic differentiation of *Megophrys s.l.* frogs, but would complicate diagnostics solely from morphological characters. Some of the proposed genera are morphologically clearly separated from each other (like *Brachytarsophrys* and *Ophryophryne* vs. *Xenophrys*), whereas less morphological divergence is known between *Megophrys s.s.* and *Xenophrys*. Studies into morphological variation across a broader sampling of species will likely enrich diagnoses of the taxa and reveal morphological synapomorphies for them.

An alternative option for taxonomy exists. It would include genus *Megophrys* with the subgenera *Atympanophrys*, *Brachytarsophrys*, *Megophrys*, *Ophryophryne* and *Xenophrys*. This arrangement would create a genus containing 68 currently recognized species (Frost, 2016) of strikingly distinct morphology and ecology, and a distribution ranging throughout southern China, the Himalayas, and Southeast Asia. However, such a large and diverse clade is diagnosed with unambiguous synapomorphies, such as presence in tadpole of a distinctively funnel-shaped oral disc.

4.4 Unrecognized species diversity within the horned toads

Our analyses consider approximately 80% (45 species) of the currently known species of horned toads from throughout their range. It excludes species from Northeast India owing to restrictions in the exportation of samples.

Our results conservatively indicate the occurrence of 20 putative new species of horned toads (Fig. 2). Our determination considers the limited numbers of voucher specimens available for some taxa and that the GMYC approach has been found to overestimate putative species diversity (Kekkonen and Hebert, 2014; Hamilton et al., 2014; Miralles and Vences, 2013). Further study can determine which of the OTUs involve new species or denote intraspecific diversity. The potential new species separate from their genetically most similar species by a nucleotide distance comparable to the divergence between recognized species (Table S6). For the 16S rRNA dataset, the commonly used threshold of 2% *p*-distance discriminates all

potential new species from genetically most similar species, except for sp. 8, which has an uncorrected p -distance of 1.74%. For the *COI* dataset, all potential new species exhibit p -distances of more than 6% to their genetically most similar species (Table S6). Our analyses include 19 of the 20 potentially new taxa (except sp. 5) detected by mitochondrial data in the nuclear gene phylogeny. Analyses resolve most of the potentially new taxa, and age estimates of their speciation are old (Fig. 3 and 5), also suggesting that these are undescribed species.

Widespread species of horned toads conceal undescribed diversity. It is now necessary to evaluate each one using independent types of data in a second follow-up “delimitation-stage” to the initial “discovery/identification-stage” (Barley et al., 2013). Our sorting of voucher specimens based on DNA lineages reveals potentially diagnostic morphological characters for the putative new taxa, such as the presence or absence of vomerine teeth, the presence or absence of metacarpal and metatarsal tubercles and their coloration, the nature of interdigital webbing on the foot, body size, the size and numbers of dermal glands and conditions of tubercles. Such comparisons can serve to test our species hypotheses.

Widespread “ubiquitous” species (e.g. *X. minor*) appear to be composites of several morphologically distinct species, as also revealed previously (Mahony et al., 2013; Wang et al., 2014). The distribution of these species reflects the long-confused taxonomic history, indiscriminant application of names in systematic revisions and inadequate comparisons of phenotypic variation (Mahony et al., 2013; Wang et al., 2014). *Xenophrys major*, *X. minor* and *X. parva* are three notoriously “widespread” species in *Xenophrys*. Our study includes samples only for *X. minor* from the type locality. Further study including samples from the type locality of *X. major* and *X. parva* is necessary prior to revising these species groups.

The occurrence of co-distributed species also contributes to the underestimation of species diversity. Our analyses document apparent range overlaps of two or more species at 21 localities. These species often occur on the same mountain, and sometimes in sympatry (Table S7), as revealed previously (e.g. Li et al., 2014; Wang

et al., 2014). In many cases, the co-distributed species are phylogenetically distantly related. However, sister or closely related species also occur in sympatry. The prevalence of different co-distributed species on the same mountain rejects the hypothesis that sympatric samples are conspecifics, and calls attention to the closer scrutiny of montane species. Species diversification in mountains may act at a much more local scale than previously anticipated. The low dispersal ability of horned toads and rugged mountain terrain raise the possibility of localized vicariance and the existence of other unknown species.

Finally, molecular analyses discriminate most morphological species. However, analyses of 16S rRNA data do not clearly distinguish *X. spinata* from *X. sangzhiensis*, *X. boettgeri* from *X. huangshanensis*, or among the species in *Atympanophrys* (Fig. 2 and Table S3). When speciation events are recent and the evolved lineages have not accumulated enough mutational differences for high speciation probabilities, GMYC may fail to establish clear support for their divergence (Bagley et al., 2015; Reid and Carstens, 2012). This factor likely contributes to the lumping of species of horned toads by GMYC. We refrain from evaluating the validity of these species until larger sample sizes are available.

5. Conclusions

Herein, we make the first effort to present a comprehensive phylogeny of Asian horned toads, genus *Megophrys s.l.*, using broad sampling and a multilocus approach. Our results indicate that *Megophrys s.l.* is paraphyletic with respect to *Brachytarsophrys* and *Ophryophryne*. Multiple mutually exclusive, geographically cohesive major clades exist in *Megophrys s.l.* The phylogenetic relationships, biogeographic pattern and reappraised morphological characters form the basis of a revised classification. Our results also indicate that *Borneophrys* is not a valid genus but rather a junior synonym of *Megophrys*. Analyses strongly indicate the presence of many undescribed species. The artificial assemblage of multiple species into nominal “widespread” species and the neglect of co-distributed species contribute to

underestimating the diversity of horned toads. Further resolution of the phylogeny within subfamily Megophryinae is desirable to test our taxonomic hypothesis.

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770 **Table 1**

771 Samples of *Megophrys sensu lato* and outgroups used in this study. Sequences generated for the present study are KX811741–KX812403
772 (indicated by bold fonts), with the other sequences downloaded from GenBank; N/A indicates that data are lacking for that gene of the taxon.
773 Museum abbreviations for samples newly sequenced here are as follows: KIZ, Kunming Institute of Zoology, Chinese Academy of Sciences,
774 China; ROM, Royal Ontario Museum, Toronto, Canada; NCSM, North Carolina Museum of Natural Sciences, USA; ZMMU, Zoological
775 Museum of Moscow State University, Russia; CIB: Chengdu Institute of Biology, Chinese Academy of Sciences, China; ITBCZ, Institute of
776 Tropical Biology Collection of Zoology, Vietnam; KU, University of Kansas Biodiversity Institute, USA; FMNH, Field Museum of Natural
777 History, USA; LSUMZ, Museum of Natural Science, Louisiana State University, USA.

Sample ID	Species	Voucher ID	Clade ID	Locality ID	Locality	GenBank No.				
						16S rRNA	COI	BDNF	RAG1	RHOD
Ingroup										
1	<i>Megophrys sangzhiensis</i>	Tissue ID: YPX11006	A	23	Badagongshan Nature Reserve, Hunan, China	KX811856	KX812117	KX811961	KX812226	KX812343
2	<i>M. sangzhiensis</i>	Tissue ID: YPX8541	A	23	Badagongshan Nature Reserve, Hunan, China	KX811857	N/A	N/A	N/A	N/A
3	<i>M. sangzhiensis</i>	KIZ023177	A	24	Yuanbao, Hubei, China	KX811858	N/A	N/A	N/A	N/A
4	<i>M. spinata</i>	CIB ZYC644	A	21	Fanjing Shan, Guizhou, China	AY526205	N/A	N/A	N/A	N/A
5	<i>M. spinata</i>	KIZ03079	A	22	Qinghua Forest Farm, Chongqing, China	KX811859	N/A	N/A	N/A	N/A
6	<i>M. spinata</i>	KIZ021895	A	21	Fanjing Shan, Guizhou, China	KX811860	N/A	N/A	N/A	N/A
7	<i>M. spinata</i>	KIZ07734	A	19	Mao'er Shan, Guangxi, China	KX811861	KX812121	KX811965	KX812229	KX812345
8	<i>M. spinata</i>	KIZ019350	A	19	Mao'er Shan, Guangxi, China	KX811862	KX812122	KX811966	KX812230	KX812346
9	<i>M. spinata</i>	KIZ016097	A	20	Leigong Shan, Guizhou, China	KX811863	KX812118	KX811962	KX812227	KX812348
10	<i>M. spinata</i>	KIZ016100	A	20	Leigong Shan, Guizhou, China	KX811864	KX812119	KX811963	KX812228	KX812349
11	<i>M. spinata</i>	XM 1058	A	27	Kuankuoshui Nature Reserve, Guizhou, China	AY526204	N/A	N/A	N/A	N/A
12	<i>M. spinata</i>	Tissue ID: YPX28756	A	27	Kuankuoshui Nature Reserve, Guizhou, China	KX811855	KX812120	KX811964	KX812231	KX812344
13	<i>M. spinata</i>	Tissue ID: YPX24639	A	27	Kuankuoshui Nature Reserve, Guizhou, China	KX811854	N/A	N/A	N/A	N/A
14	<i>M. binlingensis</i>	KIZ025807	A	33	Wawu Shan, Sichuan, China	KX811852	KX812115	KX811970	KX812221	KX812341

15	<i>M. binlingensis</i>	KIZ025809	A	33	Wawu Shan, Sichuan, China	KX811853	KX812116	KX811971	KX812222	KX812342
16	<i>M. jingdongensis</i>	1999.5688	A	57	Sa Pa, Lao Cai, Vietnam	KR828082	N/A	N/A	N/A	N/A
17	<i>M. jingdongensis</i>	2001.0278	A	58	Lushun, Yunnan, China	KR828083	N/A	N/A	N/A	N/A
18	<i>M. jingdongensis</i>	KIZ-LC0805067	A	59	Huanglianshan National Nature Reserve, Yunnan, China	KX811872	KX812131	KX811973	KX812232	KX812350
19	<i>M. jingdongensis</i>	KIZ-YN0705230	A	43	Huangcaoling, Yunnan, China	KX811873	KX812134	KX811975	KX812225	KX812352
20	<i>M. jingdongensis</i>	KIZ011821	A	41	Wenlong, Yunnan, China	KX811874	KX812132	KX811974	KX812224	KX812351
21	<i>M. jingdongensis</i>	KIZ048763	A	52	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811875	KX812133	KX811955	KX812233	KX812353
22	<i>M. jingdongensis</i>	KIZ048850	A	55	Dawei Shan, Yunnan, China	KX811876	KX812135	KX811956	KX812234	KX812354
23	<i>M. jingdongensis</i>	KIZ022439	A	41	Wenlong, Yunnan, China	KX811877	N/A	N/A	N/A	N/A
24	<i>M. sp. 1</i>	1999.5698	A	57	Sa Pa, Lao Cai, Vietnam	KR828090	N/A	N/A	N/A	N/A
25	<i>M. sp. 1</i>	ROM 19077	A	57	Sa Pa, Lao Cai, Vietnam	KX811887	KX812149	KX811972	KX812246	KX812365
26	<i>M. sp. 1</i>	K685	A	57	Sa Pa, Lao Cai, Vietnam	JN848362	N/A	N/A	N/A	N/A
27	<i>M. daweimontis</i>	ROM 28272	A	57	Sa Pa, Lao Cai, Vietnam	KX811865	N/A	N/A	N/A	N/A
28	<i>M. daweimontis</i>	KIZ016066	A	52	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811866	KX812124	KX811968	KX812247	KX812359
29	<i>M. daweimontis</i>	KIZ048997	A	55	Dawei Shan, Yunnan, China	KX811867	KX812125	KX811969	KX812248	KX812360
30	<i>M. daweimontis</i>	KIZ048920	A	55	Dawei Shan, Yunnan, China	KX811869	N/A	N/A	N/A	N/A
31	<i>M. daweimontis</i>	KIZ048938	A	55	Dawei Shan, Yunnan, China	KX811870	KX812126	KX811977	KX812249	KX812361
32	<i>M. daweimontis</i>	KIZ020413	A	54	Yangjie, Yunnan, China	KX811868	KX812127	KX811976	KX812250	KX812362
33	<i>M. wuliangshanensis</i>	KIZ011509	A	63	Mengyang Farm, Yunnan, China	KX811879	N/A	N/A	N/A	N/A
34	<i>M. wuliangshanensis</i>	KIZ046769	A	43	Huangcaoling, Yunnan, China	KX811880	N/A	N/A	N/A	N/A
35	<i>M. wuliangshanensis</i>	KIZ046812	A	43	Huangcaoling, Yunnan, China	KX811881	KX812129	KX811958	KX812258	KX812357
36	<i>M. wuliangshanensis</i>	KIZ049149	A	64	Mengyang, Yunnan, China	KX811882	KX812130	KX811960	KX812259	KX812358
37	<i>M. wuliangshanensis</i>	KIZ-YN0705150	A	66	Mengla, Yunnan, China	KX811878	KX812128	KX811959	KX812257	KX812356
38	<i>M. omeimontis</i>	/	A	34	Emei Shan, Sichuan, China	AY561307	N/A	N/A	N/A	N/A
39	<i>M. omeimontis</i>	ROM 40462	A	34	Emei Shan, Sichuan, China	EF397242	N/A	N/A	N/A	N/A
40	<i>M. omeimontis</i>	/	A	32	Hongya, Sichuan, China	KP728257	N/A	N/A	N/A	N/A
41	<i>M. omeimontis</i>	KIZ09072	A	34	Emei Shan, Sichuan, China	KX811883	N/A	N/A	N/A	N/A
42	<i>M. omeimontis</i>	KIZ025765	A	34	Emei Shan, Sichuan, China	KX811884	KX812136	KX811967	KX812223	KX812347

43	<i>M. binchuanensis</i>	KIZ011261	A	39	Huafo Shan, Yunnan, China	KX811848	KX812111	KX811951	KX812218	KX812337
44	<i>M. binchuanensis</i>	KIZ019441	A	38	Jizu Shan, Yunnan, China	KX811849	KX812112	KX811952	KX812219	KX812338
45	<i>M. binchuanensis</i>	KIZ019442	A	38	Jizu Shan, Yunnan, China	KX811850	KX812113	KX811953	KX812220	KX812339
46	<i>M. binchuanensis</i>	Tissue ID: YPX32023	A	40	Ailao Shan, Yunnan, China	KX811851	KX812114	KX811954	KX812235	KX812340
47	<i>M. sp. 2</i>	KIZ-YN070507	A	47	Bangwa, Yunnan, China	KX811845	N/A	N/A	N/A	N/A
48	<i>M. sp. 2</i>	KIZ048520	A	46	Husa, Yunnan, China	KX811846	KX812141	KX812032	KX812239	KX812366
49	<i>M. sp. 2</i>	Tissue ID: YPX33610	A	45	Tongbiguan Nature Reserve, Yunnan, China	KX811847	KX812142	KX812035	KX812238	KX812367
50	<i>M. palpebralespinosa</i>	KIZ011603	A	74	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KX811888	KX812137	KX811994	KX812236	KX812363
51	<i>M. palpebralespinosa</i>	KIZ011650	A	74	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KX811889	KX812138	KX811995	KX812237	KX812364
52	<i>M. palpebralespinosa</i>	ROM 18385	A	71	Tam Dao, Vinh Phuc, Vietnam	KX811890	N/A	N/A	N/A	N/A
53	<i>M. sp. 3</i>	1999.5717	A	57	Sa Pa, Lao Cai, Vietnam	KR828091	N/A	N/A	N/A	N/A
54	<i>M. sp. 3</i>	KIZ020425	A	56	Maandi, Yunnan, China	KX811871	KX812123	KX811957	KX812245	KX812355
55	<i>M. sp. 4</i>	KIZ046938	A	41	Wenlong, Yunnan, China	KX811885	KX812139	KX812033	KX812240	KX812368
56	<i>M. sp. 4</i>	KIZ047082	A	41	Wenlong, Yunnan, China	KX811886	KX812140	KX812034	KX812241	KX812369
57	<i>M. sp. 5</i>	ROM 28044	A	57	Sa Pa, Lao Cai, Vietnam	KX811843	N/A	N/A	N/A	N/A
58	<i>M. sp. 5</i>	ROM 30899	A	57	Sa Pa, Lao Cai, Vietnam	KX811844	N/A	N/A	N/A	N/A
59	<i>M. sp. 5</i>	1999.5703	A	57	Sa Pa, Lao Cai, Vietnam	KR828088	N/A	N/A	N/A	N/A
60	<i>M. sp. 5</i>	1998.3406	A	57	Sa Pa, Lao Cai, Vietnam	KR828089	N/A	N/A	N/A	N/A
61	<i>M. huangshanensis</i>	KIZ022002	A	4	Huang Shan, Anhui, China	KX811816	N/A	N/A	N/A	N/A
62	<i>M. huangshanensis</i>	Tissue ID: YPX12664	A	1	Longwang Shan, Zhejiang, China	KX811817	KX812105	KX811981	KX812214	KX812306
63	<i>M. huangshanensis</i>	Tissue ID: YPX6168	A	2	Tianmu Shan, Zhejiang, China	KX811818	N/A	N/A	N/A	N/A
64	<i>M. huangshanensis</i>	KIZ025854	A	3	Qingliang Peak Nature Reserve, Anhui, China	KX811819	KX812106	KX811982	KX812215	KX812308
65	<i>M. huangshanensis</i>	KIZ022003	A	4	Huang Shan, Anhui, China	KX811820	N/A	N/A	N/A	N/A
66	<i>M. huangshanensis</i>	KIZ022004	A	4	Huang Shan, Anhui, China	KX811821	KX812107	KX811983	KX812216	KX812307
67	<i>M. huangshanensis</i>	KIZ022034	A	1	Longwang Shan, Zhejiang, China	KX811822	N/A	N/A	N/A	N/A
68	<i>M. boettgeri</i>	Tissue ID: YPXJK033	A	5	Wuyi Shan, Fujian, China	KX811814	KX812104	KX811980	KX812213	KX812305
69	<i>M. boettgeri</i>	SYS A0000378	A	5	Wuyi Shan, Fujian, China	JX867340	N/A	N/A	N/A	N/A
70	<i>M. boettgeri</i>	KIZ-FJ08080223	A	5	Wuyi Shan, Fujian, China	KX811815	N/A	N/A	N/A	N/A
71	<i>M. jinggangensis</i>	SYS a001860	A	9	Taoyuandong Nature Reserve, Hunan, China	KJ560400	N/A	N/A	N/A	N/A

72	<i>M. jinggangensis</i>	SYS a002131	A	9	Taoyuandong Nature Reserve, Hunan, China	KJ560401	N/A	N/A	N/A	N/A
73	<i>M. jinggangensis</i>	TDZ 2012	A	8	Jinggang Shan, Jiangxi, China	JX867336	N/A	N/A	N/A	N/A
74	<i>M. jinggangensis</i>	SYS a001859	A	9	Taoyuandong Nature Reserve, Hunan, China	KJ560399	N/A	N/A	N/A	N/A
75	<i>M. jinggangensis</i>	Tissue ID: YPX8506	A	10	Sifangshan Forest Farm, Hunan, China	KX811841	KX812109	KX811987	KX812243	KX812329
76	<i>M. jinggangensis</i>	KIZ07113	A	6	Chashan Forest Farm, Jiangxi, China	KX811839	N/A	N/A	N/A	N/A
77	<i>M. jinggangensis</i>	KIZ07132	A	6	Chashan Forest Farm, Jiangxi, China	KX811840	KX812108	KX811986	KX812242	KX812328
78	<i>M. cheni</i>	SYS a001427	A	8	Jinggang Shan, Jiangxi, China	KJ560391	N/A	N/A	N/A	N/A
79	<i>M. cheni</i>	SYS a002124	A	9	Taoyuandong Nature Reserve, Hunan, China	KJ560397	N/A	N/A	N/A	N/A
80	<i>M. cheni</i>	SYS a001871	A	8	Jinggang Shan, Jiangxi, China	KJ560394	N/A	N/A	N/A	N/A
81	<i>M. obesa</i>	SYS a001956	A	16	Heishiding Nature Reserve, Guangdong, China	KJ579117	N/A	N/A	N/A	N/A
82	<i>M. obesa</i>	SYS a002272	A	16	Heishiding Nature Reserve, Guangdong, China	KJ579122	N/A	N/A	N/A	N/A
83	<i>M. obesa</i>	SYS a002271	A	16	Heishiding Nature Reserve, Guangdong, China	KJ579121	N/A	N/A	N/A	N/A
84	<i>M. baolongensis</i>	Tissue ID: YPX47798	A	25	Baolong, Chongqing, China	KX811812	N/A	N/A	N/A	N/A
85	<i>M. baolongensis</i>	KIZ019216	A	25	Baolong, Chongqing, China	KX811813	KX812093	KX811945	KX812202	KX812318
86	<i>M. tuberogranulatus</i>	Tissue ID: YPX10987	A	23	Badagongshan Nature Reserve, Hunan, China	KX811823	KX812095	KX811979	KX812209	KX812331
87	<i>M. tuberogranulatus</i>	Tissue ID: YPX10988	A	23	Badagongshan Nature Reserve, Hunan, China	KX811824	N/A	N/A	N/A	N/A
88	<i>M. wushanensis</i>	Tissue ID: YPX31752	A	30	Guangwu Shan, Sichuan, China	KX811837	N/A	N/A	N/A	N/A
89	<i>M. wushanensis</i>	KIZ045469	A	30	Guangwu Shan, Sichuan, China	KX811838	KX812094	KX811984	KX812203	KX812319
90	<i>M. wushanensis</i>	Tissue ID: YPX47799	A	26	Dangyang, Chongqing, China	KX811835	N/A	N/A	N/A	N/A
91	<i>M. wushanensis</i>	Tissue ID: YPX47800	A	26	Dangyang, Chongqing, China	KX811836	N/A	N/A	N/A	N/A
92	<i>M. lini</i>	SYS a001420	A	8	Jinggang Shan, Jiangxi, China	KJ560405	N/A	N/A	N/A	N/A
93	<i>M. lini</i>	SYS a002370	A	7	Suichuan, Jiangxi, China	KJ560412	N/A	N/A	N/A	N/A
94	<i>M. lini</i>	SYS a002128	A	9	Taoyuandong Nature Reserve, Hunan, China	KJ560416	N/A	N/A	N/A	N/A
95	<i>M. lini</i>	KIZ07053	A	8	Jinggang Shan, Jiangxi, China	KX811842	KX812110	KX811988	KX812244	KX812394
96	<i>M. sp. 6</i>	KIZ07738	A	19	Mao'er Shan, Guangxi, China	KX811829	KX812099	KX811947	KX812206	KX812334
97	<i>M. sp. 6</i>	KIZ046647	A	19	Mao'er Shan, Guangxi, China	KX811830	KX812100	KX811948	KX812207	KX812336
98	<i>M. sp. 6</i>	KIZ03388	A	19	Mao'er Shan, Guangxi, China	KX811831	KX812101	KX811949	KX812208	KX812335
99	<i>M. sp. 7</i>	KIZ05197	A	17	Dayao Shan, Guangxi, China	KX811832	KX812096	KX811991	KX812210	KX812320
100	<i>M. sp. 7</i>	KIZ05198	A	17	Dayao Shan, Guangxi, China	KX811833	KX812097	KX811992	KX812211	KX812321

101	<i>M. sp. 7</i>	Tissue ID: YPX9911	A	17	Dayao Shan, Guangxi, China	KX811834	N/A	N/A	N/A	N/A
102	<i>M. brachykolos</i>	SYS A0001502	A	14	Hong Kong, China	JX867339	N/A	N/A	N/A	N/A
103	<i>M. brachykolos</i>	ROM 16634	A	14	Hong Kong, China	KX811897	KX812150	KX811985	KX812217	KX812395
104	<i>M. brachykolos</i>	SYS a002258	A	14	Hong Kong, China	KJ560403	N/A	N/A	N/A	N/A
105	<i>M. acuta</i>	SYS a001957	A	16	Heishiding Nature Reserve, Guangdong, China	KJ579118	N/A	N/A	N/A	N/A
106	<i>M. acuta</i>	SYS a002266	A	16	Heishiding Nature Reserve, Guangdong, China	KJ579119	N/A	N/A	N/A	N/A
107	<i>M. sp. 8</i>	KIZ049172	A	20	Leigong Shan, Guizhou, China	KX811825	KX812102	KX811946	KX812204	KX812332
108	<i>M. sp. 8</i>	KIZ049177	A	20	Leigong Shan, Guizhou, China	KX811826	KX812103	KX811950	KX812205	KX812333
109	<i>M. sp. 9</i>	KIZ018494	A	15	Nankunshan Nature Reserve, Guangdong, China	KX811827	N/A	N/A	N/A	N/A
110	<i>M. sp. 9</i>	KIZ018496	A	15	Nankunshan Nature Reserve, Guangdong, China	KX811828	KX812098	KX811993	KX812212	KX812322
111	<i>M. kuatunensis</i>	SYS a001579	A	5	Wuyi Shan, Fujian, China	KJ560376	N/A	N/A	N/A	N/A
112	<i>M. kuatunensis</i>	SYS a001590	A	5	Wuyi Shan, Fujian, China	KJ560377	N/A	N/A	N/A	N/A
113	<i>M. sp. 10</i>	SYS a001804	A	35	Laojun Shan, Sichuan, China	KJ560386	N/A	N/A	N/A	N/A
114	<i>M. sp. 10</i>	KIZ07022	A	34	Emei Shan, Sichuan, China	KX811891	N/A	N/A	N/A	N/A
115	<i>M. sp. 10</i>	KIZ025770	A	34	Emei Shan, Sichuan, China	KX811892	KX812146	KX811989	KX812254	KX812326
116	<i>M. sp. 10</i>	KIZ025788	A	34	Emei Shan, Sichuan, China	KX811893	KX812147	KX811990	KX812255	KX812327
117	<i>M. sp. 10</i>	SYS a002166	A	35	Laojun Shan, Sichuan, China	KJ560390	N/A	N/A	N/A	N/A
118	<i>M. minor</i>	Tissue ID: YPX37544	A	31	Qingcheng Shan, Sichuan, China	KX811894	KX812143	KX812043	KX812251	KX812323
119	<i>M. minor</i>	Tissue ID: YPX37545	A	31	Qingcheng Shan, Sichuan, China	KX811895	KX812144	KX812044	KX812252	KX812324
120	<i>M. minor</i>	KIZ019391	A	31	Qingcheng Shan, Sichuan, China	KX811896	KX812145	KX812045	KX812253	KX812325
121	<i>M. sp. 11</i>	KIZ08868	A	28	Jianba, Guizhou, China	KX811910	N/A	N/A	N/A	N/A
122	<i>M. sp. 11</i>	KIZ03076	A	22	Qinghua Forest Farm, Chongqing, China	KX811911	KX812148	KX811978	KX812256	KX812330
123	<i>M. sp. 11</i>	KIZ021889	A	21	Fanjing Shan, Guizhou, China	KX811912	N/A	N/A	N/A	N/A
129	<i>M. maosonensis</i>	MVZ 223691	B	71	Tam Dao, Vinh Phuc, Vietnam	AY236800	N/A	N/A	N/A	N/A
130	<i>M. maosonensis</i>	AMNH A161506	B	71	Tam Dao, Vinh Phuc, Vietnam	DQ283374	N/A	N/A	N/A	N/A
131	<i>M. maosonensis</i>	2000.2935	B	71	Tam Dao, Vinh Phuc, Vietnam	KR828086	N/A	N/A	N/A	N/A
132	<i>M. maosonensis</i>	KIZ016045	B	52	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811780	KX812080	KX812024	KX812195	KX812375
133	<i>M. maosonensis</i>	ROM 26311	B	73	Quang Thanh, Cao Bang, Vietnam	KX811781	N/A	N/A	N/A	N/A
134	<i>M. maosonensis</i>	ROM 35558	B	53	Malipo, Yunnan, China	KX811782	N/A	N/A	N/A	N/A

135	<i>M. maosonensis</i>	ROM 6930	B	70	Na Hang Nature Reserve, Tuyen Quang, Vietnam	KX811783	N/A	N/A	N/A	N/A
136	<i>M. maosonensis</i>	ROM 16679	B	71	Tam Dao, Vinh Phuc, Vietnam	KX811784	KX812081	KX812023	KX812196	KX812376
137	<i>M. maosonensis</i>	ROM 30830	B	57	Sa Pa, Lao Cai, Vietnam	KX811786	N/A	N/A	N/A	N/A
138	<i>M. maosonensis</i>	KIZ048765	B	52	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811785	N/A	N/A	N/A	N/A
139	<i>M. maosonensis</i>	KIZ048900	B	52	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811787	N/A	N/A	N/A	N/A
140	<i>M. sp. 12</i>	NCSM 78470	B	83	Dakchung, Xekong, Laos	KX811776	N/A	N/A	N/A	N/A
141	<i>M. sp. 12</i>	KIZ010213	B	82	Phong Dien Nature Reserve, Thua Thien Hue, Vietnam	KX811777	KX812078	KX812022	KX812193	KX812385
142	<i>M. sp. 12</i>	ROM 7471	B	85	Tram Lap, Gia Lai, Vietnam	KX811778	N/A	N/A	N/A	N/A
143	<i>M. sp. 12</i>	ROM 23827	B	86	Krong Pa, Gia Lai, Vietnam	KX811779	N/A	N/A	N/A	N/A
144	<i>M. sp. 12</i>	NCSM 77768	B	84	Paksong, Champasak, Laos	KX811775	N/A	N/A	N/A	N/A
145	<i>M. sp. 12</i>	NCSM 77355	B	87	Khanh Vinh, Khanh Hoa, Vietnam	KX811771	N/A	N/A	N/A	N/A
146	<i>M. sp. 12</i>	KIZ00263	B	89	Bi Doup Nui Ba National Park, Lam Dong, Vietnam	KX811772	KX812077	KX812021	KX812192	KX812374
147	<i>M. sp. 12</i>	ZMMU ABV-00529	B	88	Chu Yang Sinh National Park, Dak Lak, Vietnam	KX811773	N/A	N/A	N/A	N/A
148	<i>M. sp. 12</i>	ZMMU NAP-01431	B	89	Bi Doup Nui Ba National Park, Lam Dong, Vietnam	KX811774	N/A	N/A	N/A	N/A
149	<i>M. mangshanensis</i>	SYS a002119	B	11	Jiulian Shan, Jiangxi, China	KJ560375	N/A	N/A	N/A	N/A
150	<i>M. mangshanensis</i>	KIZ012053	B	12	Chebaling National Nature Reserve, Guangdong, China	KX811791	N/A	N/A	N/A	N/A
151	<i>M. mangshanensis</i>	SYS a001564	B	13	Nanling National Forest Park, Guangdong, China	KJ560371	N/A	N/A	N/A	N/A
152	<i>M. mangshanensis</i>	SYS A0000496	B	13	Nanling National Forest Park, Guangdong, China	JX867335	N/A	N/A	N/A	N/A
153	<i>M. mangshanensis</i>	KIZ018430	B	15	Nankunshan Nature Reserve, Guangdong, China	KX811788	N/A	N/A	N/A	N/A
154	<i>M. mangshanensis</i>	KIZ021681	B	18	Hongtangying, Hunan, China	KX811789	N/A	N/A	N/A	N/A
155	<i>M. mangshanensis</i>	KIZ021786	B	13	Nanling National Forest Park, Guangdong, China	KX811790	KX812079	KX812025	KX812194	KX812377
156	<i>M. sp. 13</i>	KIZ011945	B	49	Myitkyina, Myanmar	KX811808	KX812092	KX812028	KX812199	KX812382
157	<i>M. sp. 14</i>	NCSM 76243	B	80	Vilabouli, Savannakhet, Laos	KX811741	N/A	N/A	N/A	N/A
158	<i>M. sp. 14</i>	NCSM 76249	B	80	Vilabouli, Savannakhet, Laos	KX811742	N/A	N/A	N/A	N/A
159	<i>M. sp. 14</i>	Tissue ID: YPX18352	B	81	Bac Huong Hoa Nature Reserve, Quang Tri, Vietnam	KX811743	N/A	N/A	N/A	N/A
160	<i>M. sp. 14</i>	ROM 35980	B	62	Pu'er, Yunnan, China	KX811755	KX812088	KX812014	KX812189	KX812381
161	<i>M. sp. 14</i>	ROM 35938	B	62	Pu'er, Yunnan, China	KX811756	N/A	N/A	N/A	N/A

162	<i>M. sp. 14</i>	KIZ01535	B	61	Mengxian, Yunnan, China	KX811757	KX812089	KX812020	KX812190	KX812380
163	<i>M. sp. 14</i>	ZMMU ABV-00774	B	72	Xuan Son, Phu Tho, Vietnam	KX811758	N/A	N/A	N/A	N/A
164	<i>M. sp. 14</i>	NCSM 79803	B	77	Xaysomboun, Vientiane, Laos	KX811747	N/A	N/A	N/A	N/A
165	<i>M. sp. 14</i>	RH06001	B	79	Phong Nha-Ke Bang National Park, Quang Binh, Vietnam	HQ588953	N/A	N/A	N/A	N/A
166	<i>M. sp. 14</i>	Tissue ID: YPX21459	B	79	Phong Nha-Ke Bang National Park, Quang Binh, Vietnam	KX811748	N/A	N/A	N/A	N/A
167	<i>M. sp. 14</i>	ROM 14576	B	78	Nghe An, Vietnam	KX811749	N/A	N/A	N/A	N/A
168	<i>M. sp. 14</i>	KIZ01464	B	66	Mengla, Yunnan, China	KX811750	KX812087	KX812019	KX812188	KX812379
169	<i>M. sp. 14</i>	RH06163	B	79	Phong Nha-Ke Bang National Park, Quang Binh, Vietnam	HQ588952	N/A	N/A	N/A	N/A
170	<i>M. sp. 14</i>	TZ797	B		North Vietnam, no exact locality	AF285195	N/A	N/A	N/A	N/A
171	<i>M. sp. 14</i>	NCSM 77946	B	76	Phou Khoun, Louangphabang, Laos	KX811744	N/A	N/A	N/A	N/A
172	<i>M. sp. 14</i>	NCSM 79807	B	77	Xaysomboun, Vientiane, Laos	KX811745	N/A	N/A	N/A	N/A
173	<i>M. sp. 14</i>	ROM 13226	B	78	Nghe An, Vietnam	KX811746	N/A	N/A	N/A	N/A
174	<i>M. sp. 14</i>	KIZ01555	B	65	Menglun Twon, Yunnan, China	KX811751	N/A	N/A	N/A	N/A
175	<i>M. sp. 14</i>	2004.0285	B	68	Long Nai Khao, Phongsaly, Laos	KR828085	N/A	N/A	N/A	N/A
176	<i>M. sp. 14</i>	NCSM 80545	B	67	Boun Tai, Phongsaly, Laos	KX811752	N/A	N/A	N/A	N/A
177	<i>M. sp. 14</i>	KIZ011609	B	74	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KX811753	KX812086	KX812018	KX812187	KX812378
178	<i>M. sp. 14</i>	ZMMU NAP-04439	B	60	Muong Nhe, Dien Bien, Vietnam	KX811754	N/A	N/A	N/A	N/A
179	<i>M. sp. 14</i>	0025Y	B	94	Phu Soi Dao waterfall, Phitsanulok, Thailand	KR828084	N/A	N/A	N/A	N/A
180	<i>M. cf. major</i>	KIZ07223	B	92	Pang Num Poo, Chiang Mai, Thailand	KX811769	KX812090	KX812015	KX812186	KX812372
181	<i>M. cf. major</i>	KIZ024336	B	93	Ban Pang Kamphaeng Hin, Chiang Mai, Thailand	KX811770	KX812091	KX812016	KX812191	KX812373
182	<i>M. glandulosa</i>	KIZ046978	B	41	Wenlong, Yunnan, China	KX811759	N/A	N/A	N/A	N/A
183	<i>M. glandulosa</i>	Tissue ID: YPX33511	B	46	Husa, Yunnan, China	KX811760	N/A	N/A	N/A	N/A
184	<i>M. glandulosa</i>	KIZ013609	B	41	Wenlong, Yunnan, China	KX811761	KX812074	KX812010	KX812183	KX812298
185	<i>M. glandulosa</i>	KIZ048439	B	46	Husa, Yunnan, China	KX811762	KX812075	KX812012	KX812184	KX812299
186	<i>M. glandulosa</i>	KIZ048516	B	46	Husa, Yunnan, China	KX811763	N/A	N/A	N/A	N/A
187	<i>M. glandulosa</i>	KIZ048501	B	44	Dahaoping, Yunnan, China	KX811764	KX812076	KX812013	KX812185	KX812300
188	<i>M. medogensis</i>	KIZ06621	B	50	Beibeng, Xizang, China	KX811767	KX812082	KX812017	KX812197	KX812301
189	<i>M. medogensis</i>	KIZ06657	B	50	Beibeng, Xizang, China	KX811768	KX812083	KX812011	KX812198	KX812302

190	<i>M. zhangii</i>	KIZ014278	B	51	Zhangmu, Xizang, China	KX811765	KX812084	KX812026	KX812200	KX812303
191	<i>M. zhangii</i>	KIZ011131	B	51	Zhangmu, Xizang, China	KX811766	KX812085	KX812027	KX812201	KX812304
192	<i>M. sp. 15</i>	ROM 35937	B	62	Pu'er, Yunnan, China	KX811804	N/A	N/A	N/A	N/A
193	<i>M. sp. 15</i>	ROM 35689	B	62	Pu'er, Yunnan, China	KX811805	N/A	N/A	N/A	N/A
194	<i>M. sp. 15</i>	Tissue ID: YPX27640	B	63	Mengyang Farm, Yunnan, China	KX811801	KX812066	KX812004	KX812178	KX812291
195	<i>M. sp. 15</i>	Tissue ID: YPX27643	B	63	Mengyang Farm, Yunnan, China	KX811802	KX812067	KX812003	KX812179	KX812292
196	<i>M. sp. 15</i>	ZMMU NAP-05005	B	60	Muong Nhe, Dien Bien, Vietnam	KX811803	N/A	N/A	N/A	N/A
197	<i>M. sp. 15</i>	2004.0283	B	68	Long Nai Khao, Phongsaly, Laos	KR828097	N/A	N/A	N/A	N/A
198	<i>M. sp. 15</i>	2004.0285	B	69	Phoufa Mountain, Phongsaly, Laos	KR828096	N/A	N/A	N/A	N/A
199	<i>M. sp. 15</i>	K3359	B	75	Ban Keng Koung, Luang Prabang, Laos	JN848364	N/A	N/A	N/A	N/A
200	<i>M. sp. 15</i>	K3296	B	75	Ban Keng Koung, Luang Prabang, Laos	JN848365	N/A	N/A	N/A	N/A
201	<i>M. sp. 15</i>	K3359	B	75	Ban Keng Koung, Luang Prabang, Laos	KR828094	N/A	N/A	N/A	N/A
202	<i>M. sp. 15</i>	LEL82	B	91	Doi Chiang Dao, Chiang Mai, Thailand	JN848363	N/A	N/A	N/A	N/A
203	<i>M. sp. 15</i>	K3120	B	75	Ban Keng Koung, Luang Prabang, Laos	KR828093	N/A	N/A	N/A	N/A
204	<i>M. sp. 16</i>	K3053	B	91	Doi Chiang Dao, Chiang Mai, Thailand	KR828092	N/A	N/A	N/A	N/A
205	<i>M. sp. 16</i>	KIZ07227	B	92	Pang Num Poo, Chiang Mai, Thailand	KX811800	KX812065	KX812002	KX812182	KX812290
206	<i>M. auralensis</i>	NCSM 79599	B	90	Aural, Kampong Speu, Cambodia	KX811807	N/A	N/A	N/A	N/A
207	<i>M. sp. 17</i>	KIZ048503	B	45	Tongbiguan Nature Reserve, Yunnan, China	KX811793	KX812068	KX812007	KX812175	KX812293
208	<i>M. sp. 17</i>	KIZ048504	B	45	Tongbiguan Nature Reserve, Yunnan, China	KX811794	N/A	N/A	N/A	N/A
209	<i>M. sp. 17</i>	KIZ048505	B	45	Tongbiguan Nature Reserve, Yunnan, China	KX811795	KX812069	KX812008	KX812176	KX812294
210	<i>M. sp. 17</i>	KIZ011940	B	49	Myitkyina, Myanmar	KX811792	KX812070	KX812009	KX812177	KX812297
211	<i>M. cf. parva</i>	KIZ048508	B	48	Mengyue, Yunnan, China	KX811797	KX812072	KX812006	KX812181	KX812295
212	<i>M. cf. parva</i>	KIZ048572	B	48	Mengyue, Yunnan, China	KX811798	N/A	N/A	N/A	N/A
213	<i>M. cf. parva</i>	KIZ048507	B	45	Tongbiguan Nature Reserve, Yunnan, China	KX811796	KX812071	KX812005	KX812180	KX812296
214	<i>M. sp. 18</i>	2000.241	B	99	Namtok Raman Forest Park, Phang Nga, Thailand	KR828098	N/A	N/A	N/A	N/A
215	<i>M. sp. 18</i>	ZMMU NAP-04137	B	97	Khao Sok, Surat Thani, Thailand	KX811799	KX812064	KX812000	KX812173	KX812370
216	<i>M. sp. 19</i>	KIZ024524	B	95	Thong Pha Phum, Kanchanaburi, Thailand	KX811806	KX812073	KX812001	KX812174	KX812371
217	<i>M. lekaguli</i>	FMNH 266341	B	96	Pang Si Da National Park, Sa Kaeo Province, Thailand	DQ860092	N/A	N/A	N/A	N/A

218	<i>M. lekaguli</i>	FMNH 265959	B	96	Pang Si Da National Park, Sa Kao Province, Thailand	DQ860093	N/A	N/A	N/A	N/A
219	<i>M. montana</i>	LSUMZ 81916	C	110	Sukabumi, Java, Indonesia	KX811927	KX812163	KX811940	KX812281	KX812390
220	<i>M. nasuta</i>	/	C		pet trade, no exact locality	AB612070	N/A	N/A	N/A	N/A
221	<i>M. nasuta</i>	KIZ019419	C		Malaysia, no exact locality	KX811921	KX812054	KX811935	KX812171	KX812289
222	<i>M. nasuta</i>	ZMH A13127	C	103	Gunung Mulu National Park, Sarawak, Malaysia	KJ831311	N/A	N/A	N/A	N/A
223	<i>M. nasuta</i>	ZMH A13126	C	103	Gunung Mulu National Park, Sarawak, Malaysia	KJ831312	N/A	N/A	N/A	N/A
224	<i>M. nasuta</i>	KIZ019418	C		Malaysia, no exact locality	KX811920	KX812053	KX811934	KX812170	KX812288
225	<i>M. nasuta</i>	KUHE 53577	C	104	Tenom, Sabah, Malaysia	AB719248	N/A	N/A	N/A	N/A
226	<i>M. nasuta</i>	FMNH 236525	C	105	Crocker Range National Park, Sabah, Malaysia	DQ283342	N/A	N/A	N/A	N/A
227	<i>M. baluensis</i>	S52	C	106	Kinabalu, Borneo	DQ642121	N/A	N/A	N/A	N/A
228	<i>M. baluensis</i>	ZMH A13124	C	107	Gunung Kinabalu National Park, Sabah, Malaysia	KJ831309	N/A	N/A	N/A	N/A
229	<i>M. baluensis</i>	ZMH A13125	C	107	Gunung Kinabalu National Park, Sabah, Malaysia	KJ831310	N/A	N/A	N/A	N/A
230	<i>M. stejnegeri</i>	KU 314303	C	109	Pasonanca Natural Park, Zamboanga City, Philippines	KX811922	KX812052	KX811936	KX812172	KX812399
231	<i>M. stejnegeri</i>	ROM RMB631	C	109	Pasonanca Natural Park, Zamboanga City, Philippines	KX811923	N/A	N/A	N/A	N/A
232	<i>M. kobayashii</i>	UNIMAS 8148	C	107	Gunung Kinabalu National Park, Sabah, Malaysia	KJ831313	N/A	N/A	N/A	N/A
233	<i>M. ligayae</i>	ZMMU NAP-05015	C	108	Palawan, Philippines	KX811919	KX812051	KX811933	KX812169	KX812287
235	<i>M. dringi</i>	ZMH A09364	C	103	Gunung Mulu National Park, Sarawak, Malaysia	KJ831314	N/A	N/A	N/A	N/A
236	<i>M. dringi</i>	UNIMAS 8948	C	103	Gunung Mulu National Park, Sarawak, Malaysia	KJ831316	N/A	N/A	N/A	N/A
237	<i>M. nankiangensis</i>	CIB ZYC517	D	29	Nanjiang, Sichuan, China	KX811900	N/A	N/A	N/A	N/A
238	<i>M. nankiangensis</i>	CIB ZYC519	D	29	Nanjiang, Sichuan, China	KX811901	N/A	N/A	N/A	N/A
239	<i>M. nankiangensis</i>	CIB XM835	D	29	Nanjiang, Sichuan, China	EF397243	N/A	N/A	N/A	N/A
240	<i>M. wawuensis</i>	KIZ025799	D	33	Wawu Shan, Sichuan, China	KX811902	KX812062	KX812041	KX812271	KX812314
241	<i>M. wawuensis</i>	Tissue ID: YPX37516	D	33	Wawu Shan, Sichuan, China	KX811903	KX812063	KX812042	KX812272	KX812315
242	<i>M. gigantea</i>	Tissue ID: YPX32083	D	42	Wuliang Shan, Yunnan, China	KX811898	KX812058	KX811941	KX812270	KX812312
243	<i>M. gigantea</i>	KIZ04892	D	40	Ailao Shan, Yunnan, China	KX811899	KX812059	KX811942	KX812273	KX812313
244	<i>M. shapingensis</i>	XM 967	D	37	Mianning, Sichuan, China	AY526203	N/A	N/A	N/A	N/A
245	<i>M. shapingensis</i>	Tissue ID: YPX37515	D	33	Wawu Shan, Sichuan, China	KX811905	KX812061	KX811944	KX812275	KX812317
246	<i>M. shapingensis</i>	KIZ014512	D	36	Liziping Nature Reserve, Sichuan, China	KX811904	KX812060	KX811943	KX812274	KX812316

250	<i>M. aceras</i>	KIZ025467	B	98	Khao Nan National Park, Nakhon Si Thammarat, Thailand	KX811925	KX812159	KX812030	KX812262	KX812310
251	<i>M. aceras</i>	KIZ025468	B	98	Khao Nan National Park, Nakhon Si Thammarat, Thailand	KX811926	KX812160	KX812031	KX812261	KX812311
252	<i>M. aceras</i>	ZMMU NAP-03994	B	100	Thale Ban National Park, Satun, Thailand	KX811924	KX812158	KX812029	KX812260	KX812309
253	<i>M. aceras</i>	LSUHC 7038	B	101	Temenggor Forest, Perak, Malaysia	GQ995534	N/A	N/A	N/A	N/A
254	<i>M. longipes</i>	IABHU 21101	B	102	Genting highland, Malaysia	AB530656	N/A	N/A	N/A	N/A
255	<i>M. sp. 20</i>	KIZ048517	B	46	Husa, Yunnan, China	KX811906	KX812151	KX811996	KX812263	KX812386
256	<i>M. sp. 20</i>	KIZ048518	B	46	Husa, Yunnan, China	KX811907	KX812152	KX811997	KX812264	KX812387
257	<i>M. pachyproctus</i>	KIZ010978	B	50	Beibeng, Xizang, China	KX811908	KX812153	KX811998	KX812265	KX812383
258	<i>M. pachyproctus</i>	KIZ011175	B	50	Beibeng, Xizang, China	KX811909	KX812154	KX811999	KX812266	KX812384
Outgroup										
124	<i>Ophryophryne microstoma</i>	KIZ048799			Xiaoqiaogou Nature Reserve, Yunnan, China	KX811914	KX812156	KX812039	KX812277	KX812397
125	<i>Ophryophryne microstoma</i>	KIZ048800			Xiaoqiaogou Nature Reserve, Yunnan, China	KX811915	KX812157	KX812040	KX812278	KX812398
126	<i>Ophryophryne hansi</i>	KIZ010360			Phong Dien Nature Reserve, Thua Thien Hue, Vietnam	KX811913	KX812155	KX812036	KX812276	KX812396
127	<i>Ophryophryne cf. gerti</i>	ITBCZ 1103			Nui Chua National Park, Ninh Thuan, Vietnam	KX811916	KX812161	KX812037	KX812279	KX812388
128	<i>Ophryophryne cf. gerti</i>	ITBCZ 1108			Nui Chua National Park, Ninh Thuan, Vietnam	KX811917	KX812162	KX812038	KX812280	KX812389
234	<i>Borneophrys edwardinae</i>	FMNH 273694			Bintulu, Sarawak, Malaysia	KX811918	KX812050	KX811932	KX812168	KX812286
247	<i>Brachytarsophrys feae</i>	KIZ-YN070570			Longchuan, Yunnan, China	KX811809	KX812055	KX811937	KX812267	KX812391
248	<i>Brachytarsophrys feae</i>	KIZ046706			Huangcaoling, Yunnan, China	KX811810	KX812056	KX811938	KX812269	KX812393
249	<i>Brachytarsophrys carinense</i>	Tissue ID: YPX20455			Dayao Shan, Guangxi, China	KX811811	KX812057	KX811939	KX812268	KX812392
259	<i>Leptolalax oshanensis</i>	KIZ025778			Emei Shan, Sichuan, China	KX811928	KX812166	KX812048	KX812284	KX812402
260	<i>Leptolalax ventripunctatus</i>	KIZ046940			Wenlong, Yunnan, China	KX811929	KX812167	KX812049	KX812285	KX812403
261	<i>Leptobranchium huashen</i>	KIZ049025			Mengyang, Yunnan, China	KX811931	KX812165	KX812047	KX812283	KX812401
262	<i>Leptobranchium boringii</i>	Tissue ID: YPX37539			Emei Shan, Sichuan, China	KX811930	KX812164	KX812046	KX812282	KX812400

Figure captions

Fig. 1. Sampling localities of *Megophrys sensu lato* used in this study. Four major clades (A–D) correspond to those identified in the phylogeny. Locality numbers correspond to Locality IDs in Table 1.

Fig. 2. Putative species limits in *Megophrys sensu lato* obtained from Bayesian inference and GMYC based on 16S rRNA gene sequences. Each shaded bar represents a putative species identified by Bayesian inference. Outermost lines correspond to additional OTUs and species hypothesized to be merged by GMYC. Delimitation results of GMYC that are congruent with Bayesian inference are not shown. Numbers at terminals correspond to Samples IDs in Table 1. The capital letters (A–D) near branch nodes correspond to Clade IDs in Table 1, Figure 1 and Figure S2. Putative new species are represented by purple fonts. *Ophryophryne*, *Borneophrys* and *Brachytarsophrys* are represented by bold. Outgroups (*Leptobrachium* and *Leptolalax*) are represented by bar of light-cyan color.

Fig. 3. Phylogram of *Megophrys sensu lato* resulting from Bayesian inference of the combined nuclear data (*BDNF*, *RAG1*, *RHOD*). Nodal support values are Bayesian posterior probabilities (above) and maximum likelihood bootstraps (below). Asterisks denote Bayesian posterior probabilities (BPP) ≥ 0.95 and bootstrap support (BS) $\geq 70\%$. “-” denotes low support (BPP $< 85\%$ or BS $< 60\%$). Numbers at terminals correspond to Samples IDs in Table 1. The capital letters (A–D) above branches correspond to Clade IDs in Table 1, Figure 1 and Figure S2. Non-horned toads are denoted by a red rectangle.

Fig. 4. Phylogram of *Megophrys sensu lato* resulting from Bayesian inference of the concatenated mitochondrial data (*COI*, 16S rRNA). Nodal support values are Bayesian posterior probabilities (above) and maximum likelihood bootstraps (below). Asterisks denote Bayesian posterior probabilities (BPP) ≥ 0.95 and bootstrap support (BS) $\geq 70\%$. “-” denotes low support (BPP $< 85\%$ or BS $< 60\%$) in our analysis. Numbers at terminals correspond to Samples IDs in Table 1. The capital letters (A–D) above branch correspond to Clades IDs in Table 1, Figure 1 and Figure S2. Non-horned toads are denoted by a red rectangle.

Fig. 5. Bayesian species tree of *Megophrys sensu lato* inferred using *BEAST of the

810 three nuclear genes (*BDNF*, *RAG1*, *RHOD*). Numbers near branches are posterior
811 probabilities (PP). Asterisks denote $PP \geq 0.95$ and “-” denotes low support ($PP < 0.85$)
812 in our analysis. The capital letters (A–D) above branch correspond to Clade IDs in
813 Table 1, Figure 1 and Figure S2.

● ≤ 84
● 85~94
● 95~100

Xenophrys

Megophrys

Ophryophryne

Atympanophrys

Brachytarsophrys

Xenophrys

Megophrys

Ophryophryne

Atympanophrys

Brachytarsophrys