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# Phylogenetic relationships of Oriental torrent frogs in the genus *Amolops* and its allies (Amphibia, Anura, Ranidae)

Masafumi Matsui [a,*], Tomohiko Shimada [a], Wan-Zhao Liu [a,b], Mohamed Maryati [c], Wichase Khonsue [d], Nikolai Orlov [e]

[a] *Graduate School of Human and Environmental Studies, Kyoto University, Sakyo, Kyoto 606-8501, Japan*
[b] *Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan*
[c] *Institute for Tropical Biology and Conservation, University Malaysia Sabah, Teluk Sepanggar, Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia*
[d] *Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[e] *Department of Herpetology and Ornithology, Zoological Institute, Russian Academy of Sciences, 119034, St. Petersburg, Russia*

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**Abstract**

We investigated the phylogenetic relationships among 20 species of Oriental torrent frogs in the genus *Amolops* and its allies from China and Southeast Asia based on 1346-bp sequences of the mitochondrial 12S and 16S rRNA genes. Oriental species of the tribe Ranini form a monophyletic group containing 11 clades (*Rana temporaria* + *Pseudoamolops*, *R. chalconota*, four clades of *Amolops*, *Meristogenys*, three clades of *Huia* species, and *Staurois*) for which the phylogenetic relationships are unresolved. The genus *Amolops* consists of southern Chinese, southwestern Chinese, Thai, and Vietnamese–Malaysian lineages, but their relationships are also unresolved. The separation of southern and southwestern lineages within China conforms to previous morphological and karyological results. Species of *Huia* do not form a monophyletic group, whereas those of *Meristogenys* are monophyletic. Because *P. sauteri* is a sister species of *R. temporaria*, distinct generic status of *Pseudoamolops* is unwarranted.


*Keywords:* *Amolops*; Biogeography; Classification; *Huia*; *Meristogenys*; Oriental torrent frogs; Phylogeny; *Pseudoamolops*

## 1. Introduction

The Asian ranid genus *Amolops* Cope, 1865 (sensu lato: Dubois, 1992) is characterized by the peculiar ecology and morphology of the larvae, which bear a large abdominal sucker and inhabit torrents (Inger, 1966). Members of this genus were long confused with the ranid genus *Staurois* Cope, 1865 (e.g., Liu, 1950) until Inger (1966) split them based on marked differences in their larval morphology and ecology.

Matsui (1986) suggested that Bornean and Chinese members [Liu and Hu, 1961 (as *Staurois*)] of this group represent two phylogenetically divergent lineages on the basis of clear differences in the size and pigmentation of the ova and in the number of rows of larval denticles. Yang (1991a) later revised *Amolops* (sensu lato) on the bases of adult and larval morphology. He divided the genus into three distinct genera: *Amolops* Cope, 1865, which inhabits mainland Southeastern Asia; *Huia* Yang, 1991; found in Java, Sumatra, Borneo, Thailand, and southern China; and *Meristogenys* Yang, 1991; which is endemic to Borneo. Dubois (1992), however, retained only one genus, *Amolops*, and divided it into four subgenera; he relegated the three genera proposed by Yang (1991a; *Amolops*, *Huia*, and *Meristogenys*) to subgenera, and added the new subgenus *Amo* (Dubois, 1992). In addition, Yang (1991b) further established the subfamily Amolopsinae within the Ranidae,

* Corresponding author. Fax: +81 75 753 2891.
*E-mail address:* fumi@zoo.zool.kyoto-u.ac.jp (M. Matsui).

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a system that was not followed by Dubois (1992), who placed *Amolops* (sensu lato) in the Raninae.

In his most recent review of world amphibians, Frost (2004) listed 34 species in the genus *Amolops* (sensu stricto) of which one species, *A. taiwanianus* (Otsu, 1973) should be removed because it is actually a member of *Rana* (Matsui, 2005). *Amo*, as proposed by Dubois (1992), was retained as a subgenus of *Amolops*; five other species were placed in the genus *Huia*, and eight species were included in *Meristogenys*. However, the generic assignment adopted by Frost (2004) is simply based on recent publications (e.g., Inger, 1999) and has no valid phylogenetic basis. Frost (2004) also listed *Pseudoamolops* Jiang, Fei, Ye, Zeng, Zheng, Xie, and Chen, 1997 as a distinct genus of the Ranidae, but this classification is questionable based on the results of phylogenetic analyses of mitochondrial cytochrome *b* (mt cyt *b*; Tanaka-Ueno et al., 1998; by implication).

In this paper, we attempted to reassess the validity of the subfamily Amolopsinae as proposed by Yang (1991b) and the generic or subgeneric status of *Amolops* (sensu lato) based on an analysis of partial sequences of mt DNA. Furthermore, we examined the validity of *Pseudoamolops* as a distinct genus by examining its relationships with *Amolops* and *Rana*.

## 2. Materials and methods

### 2.1. Sampling design

We examined DNA sequences of mitochondrial 12S and 16S rRNA genes of 22 specimens of 20 species of *Amolops* and its allies, including 14 species of *Amolops* and three species each of *Huia* and *Meristogenys* (Fig. 1, Table 1). Of these, *Amolops* sp. from Thailand resembles *A. marmoratus* in appearance, but differs in details of adult and larval morphology (unpublished data). *Huia* sp. from Sumatra differs substantially from *H. sumatrana* in adult morphology and is thought to be an undescribed species (unpublished data). We also sequenced one species of each of the genera *Rana*, *Pseudoamolops*, and *Staurois* of the tribe Ranini in the subfamily Raninae (Dubois, 1992) for comparison. Because Marmayou et al. (2000) suggested close relationships of *Amolops* (sp. from Thailand) and *R. chalconota* based on 12S rRNA sequences, we chose the latter species as a representative of *Rana*. We also added sequence data for the brown frog *R. temporaria* (AY326063) because close relationships of brown frogs with *Pseudoamolops sauteri* have been suggested by mt cyt *b* sequence analysis (Matsui et al., 2001). As hierarchical outgroups, we included *Paa quadrana* of the tribe Paini

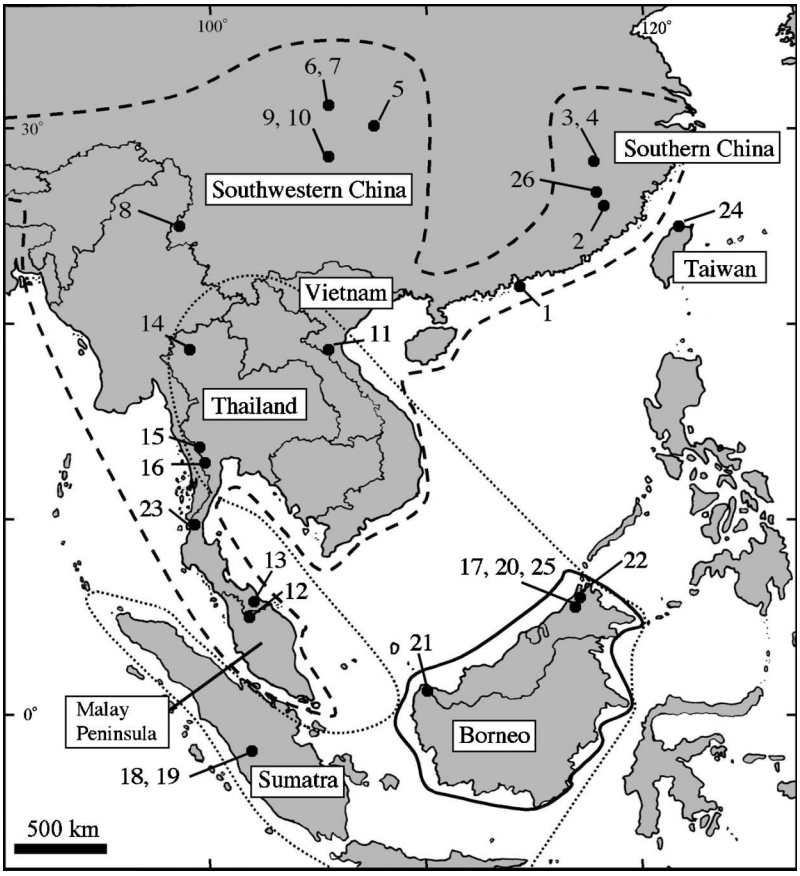


Fig. 1. Map of China and Southeast Asia showing sampling localities of *Amolops* and related species. For the locality number, refer to Table 1. Solid, dashed, and dotted lines indicate the distribution range of *Meristogenys*, *Amolops*, and *Huia*, respectively.

Table 1
Samples used in this study and GenBank accession numbers

| Species | Voucher, Locality | GenBank No. (12S; 16S) |
|---|---|---|
| *Amolops hongkongensis* | KUZ30210, China, Hong Kong (1) | AB211450; AB211473 |
| *Amolops daiyunensis* | C93075, China, Fujian (2) | AB211451; AB211474 |
| *Amolops ricketti* | C-F93006, China, Fujian, (3) | AB211452; AB211475 |
| *Amolops wuyiensis* | C-F93066, China, Fujian (4) | AB211453; AB211476 |
| *Amolops chunganensis* | KUHE27699, China, Sichuan (5) | AB211454; AB211477 |
| *Amolops loloensis* | C18, China, Sichuan (6) | AB211455; AB211478 |
| *Amolops mantzorum* | C62, China, Sichuan (7) | AB211456; AB211479 |
| *Amolops viridimaculatus* | C-green 05, China, Yunnan (8) | AB211457; AB211480 |
| *Amolops granulosus* | C93161, China, Sichuan (9) | AB211458; AB211481 |
| *Amolops lifanensis* | C93150, China, Sichuan (10) | AB211459; AB211482 |
| *Amolops cremnobatus* | N24538, Vietnam, Ha Tinh (11) | AB211460; AB211483 |
| *Amolops larutensis* | KUHE15488, Malaysia, Perak (12) | AB211461; AB211484 |
| | KUHE23166, Thailand, Narathiwat (13) | AB211462; AB211485 |
| *Amolops marmoratus* | KUHE19089, Thailand, Chieng Mai (14) | AB211463; AB211486 |
| *Amolops* sp. | KUHE19975, Thailand, Kanchanaburi (15) | AB211464; AB211487 |
| | KUHE20133, Thailand, Phetchaburi (16) | AB211465; AB211488 |
| *Huia cavitympanum* | BOR uncatalogued, Sabah, Crocker (17) | AB211466; AB211489 |
| *Huia sumatrana* | N6474, Indonesia, Sumatra, Jambi (19) | AB211467; AB211490 |
| *Huia* sp. | N6468, Indonesia, Sumatra, Jambi (18) | AB211468; AB211491 |
| *Meristogenys kinabaluensis* | BOR uncatalogued, Sabah, Crocker (20) | AB211469; AB211492 |
| *Meristogenys jerboa* | KUHE12028, Sarawak, Kuching (21) | AB211470; AB211493 |
| *Meristogenys orphnocnemis* | BOR22352, Sabah, Kinabalu (22) | AB211471; AB211494 |
| *Rana chalconota* | KUHE 23936, Thailand, Ranong (23) | AB200932; AB200956 |
| *Rana temporaria* | GenBank sequence, no locality data | AY32063; AY32063 |
| *Pseudoamolops sauteri* | KUHE18098, Taiwan (24) | AB211472; AB211495 |
| *Staurois latopalmatus* | BOR8098, Sabah, Crocker (25) | AB200942; AB200966 |
| *Paa quadrana* | C91, China, Fujian | AB200943; AB200967 |
| *Fejervarya limnocharis* | GenBank sequence, China | AY158705; AY158705 |
| *Microhyla fissipes* | KUHE35165, Thailand, Kanchanaburi | AB201175, AB201186 |

Locality numbers correspond to those of Fig. 1. Classification follows Frost (2004) and Matsui et al. (2005). BOR, BORNEENSIS Collection, University Malaysia Sabah; C, Kumming Institute of Zoology; KUHE, Graduate School of Human and Environmental Studies, Kyoto University; KUZ, Department of Zoology, Faculty of Science, Kyoto University; N, Nikolai Orlov tissue collection, Zoological Institute, Russian Academy of Sciences.

(Raninae: Dubois, 1992), *Fejervarya limnocharis* (AY158705) of the ranid subfamily Dicroglossinae, and *Microhyla fissipes* of the ranoid family Microhylidae (Table 1). The division of China into geographic regions follows Zhao (1999).

### 2.2. Preparation of DNA, PCR, and DNA sequencing

Tissues were obtained from either frozen or ethanol-preserved specimens. Total genomic DNA was extracted using standard phenol–chloroform extraction procedures (Hillis et al., 1996). PCR was used to amplify fragments of the mitochondrial 12S and 16S rRNA genes. The primers and cycling procedures were as follows: (i) 12S: 12Sh of Cannatella et al. (1998) and H1548 of Matsui et al. (2005) were used to amplify a ca. 860-bp section of the mitochondrial 12S ribosomal RNA gene. Amplification was carried out in a 25-μL reaction volume with Blend *Taq* (Toyobo). The PCR cycle included an initial denaturation step of 5 min at 94 °C and 25 cycles of denaturation for 1 min at 94 °C, primer annealing for 1 min at 45 °C, and extension for 3 min at 72 °C. (ii) 16S: L2188 (light chain; 5′-AAA GTG GGC CTA AAA GCA GCC A-3′), which was designed for this study, and 16H1 of Hedges (1994) were used to amplify a ca. 850-bp section of the mitochondrial 16S ribosomal RNA gene. The PCR cycle was the same as for 12S except that the primer annealing temperature was 50 °C.

PCR products purified using polyethylene glycol (PEG) purification procedures were used directly as templates for cycle sequencing reactions with fluorescent-dye-labeled terminators (ABI Prism Big Dye Terminators v.3.1 cycle sequencing kits). The sequencing reaction products were purified by ethanol precipitation following the manufacturer's protocol and then run on an ABI PRISM 3100 genetic analyzer. All samples were sequenced in both directions using the same primers as for PCR.

### 2.3. Sequence data process

Sequence data for each sample were first obtained and checked by eye using ABI PRISM Sequencing Analysis Software (V3.6.2). Alignment of data from all samples at this step was performed using the Clustal option in the BioEdit software (Hall, 1999). We obtained sequences of 1739 bp [855 bp (12S) and 884 bp (16S)]. However, to exclude gaps and ambiguous areas, we further revised the alignment with Gblocks 0.91b (Castresana, 2000), using the most stringent options and not allowing "smaller final blocks," "gap positions within a final block," "less strict

flanking positions," and "many contiguous non-conservative positions." The revised alignment was 1346 bp in length [665 bp (12S) and 681 bp (16S)].

To test for saturation in base substitutions, we plotted uncorrected *p* distances against the number of transitions and transversions, and confirmed that both 12S and 16S fragments reached only slight saturation. These plots are available from the authors upon request.

### 2.4. Phylogenetic analyses

Prior to phylogenetic analyses, we calculated a g1 test to assess the amount of phylogenetic signal. We generated 100,000 random trees and calculated the skewness (g1) of the resulting tree length distribution with PAUP 4.0b (Swofford, 2002).

The data were subjected to three different methods of phylogenetic reconstruction: maximum parsimony (MP) using a heuristic search with the tree bisection reconnection (TBR) branch-swapping algorithm, 100 random addition replicates, and transitions and transversions given equal weight; maximum likelihood (ML) analysis based on the substitution model and phylogenetic parameters, which was considered the best option in a hierarchical likelihood ratio test (hLRT) using the program Modeltest 3.06 (Posada and Crandall, 1998) for ingroup taxa of the combined data of 12S and 16S, a heuristic search with the TBR branch swapping algorithm, and 10 random addition replicates; and Bayesian inference (Huelsenbeck et al., 2001; Rannala and Yang, 1996) with the model derived from an hLRT in MrModeltest (Nylander, 2002) for ingroup taxa of each partition, running four simultaneous Metropolis-coupled Monte Carlo Markov chains for $1 \times 10^6$ generations, and sampling a tree every 100 generations. Stationary trees were determined visually, burn-in trees were discarded, and the remaining trees were used to estimate Bayesian posterior probabilities.

Except for the Bayesian approach (MrBayes: Huelsenbeck and Ronquist, 2001), all analyses were conducted using PAUP4.0b. Pairwise comparisons of corrected sequence divergences [Kimura-2 parameter (K2p) distances (Kimura, 1980)] were also calculated using PAUP. The robustness of MP and ML tree topologies was tested using bootstrap analyses (Felsenstein, 1985), with 2000 replicates for MP and 100 for ML (Hedges, 1992). We considered topologies with bootstrap values >70% to be sufficiently supported, and those with values between 50 and 70% to be weakly supported (Huelsenbeck and Hillis, 1993). For the Bayesian analyses, we used posterior probabilities as indicators of node confidence. Because these represent the true probabilities of the clades (Rannala and Yang, 1996), probabilities >95% were considered to be significant (Leaché and Reeder, 2002). Additionally, decay indices (Bremer, 1994) were estimated for all internal branches using TreeRot version 2 (Sorenson, 1999).

## 3. Results

### 3.1. Sequence and tree statistics

Sequence statistics for the two gene fragments and for the combined alignment are given in Table 2. From 100,000 random trees, we obtained a skewness (g1) of −0.777, which significantly supported that our data contained sufficient phylogenetic signals. Of 1346 bp, 593 were variable in the combined alignment, with 445 being parsimony informative. The average transition/transversion (Ts/Tv) ratio varied among genes and was 2.23 in the combined dataset when considering only ingroup taxa.

The best substitution model derived from Modeltest was a general time-reversible model with a proportion of invariant sites and a $\gamma$ shape parameter estimated as 0.360 and 0.507, respectively (GTR+I+G; Rodriguez et al., 1990). MrModeltest also concluded that GTR+I+G fit for both partitions of 12S and 16S, so we applied this model to each partition and calculated the phylogenetic parameters separately.

We obtained a single most parsimonious tree with 2079 evolutionary steps, with a consistency index (CI) of 0.421 and a retention index (RI) of 0.504. The likelihood values of the ML tree and the consensus tree in the Bayesian approach were $\ln L = -10742.81$ and $-10746.81$, respectively. The burn-in in the Bayesian analyses occurred before 10,000 generations (data not shown); we discarded the first 100,000.

### 3.2. Phylogenetic relationships

The three phylogenetic analyses yielded slightly different topologies (only the Bayesian tree is shown in Fig. 2). The following relationships were indicated by the three analyses as statistically reliable:

(i) Monophyly of the tribe Ranini with respect to the outgroup Dicroglossinae and Paini was supported (100, 99, and 85% support in Bayesian posterior probability and ML and MP bootstrap values, respectively).
(ii) *Pseudoamolops sauteri* and *R. temporaria* were grouped as sister species (100% support).
(iii) *Meristogenys* formed a monophyletic group (100, 99, and 99% support) within which *M. jerboa* and *M. orphnocnemis* were grouped as sister species (99, 87, and 86% support).

Table 2
Alignment statistics for fragments of 12S and 16S rRNA

| | bp | vs | pi | Ts/Tv |
|---|---|---|---|---|
| 12S rRNA | 665 | 305 | 227 | 2.645 |
| 16S rRNA | 681 | 288 | 218 | 1.908 |
| Combined | 1346 | 593 | 445 | 2.229 |

The number of base pairs (bp), variable sites (vs), and parsimony informative sites (pi), and the transition–transversion ratio (Ts/Tv) are given for ingroups only.

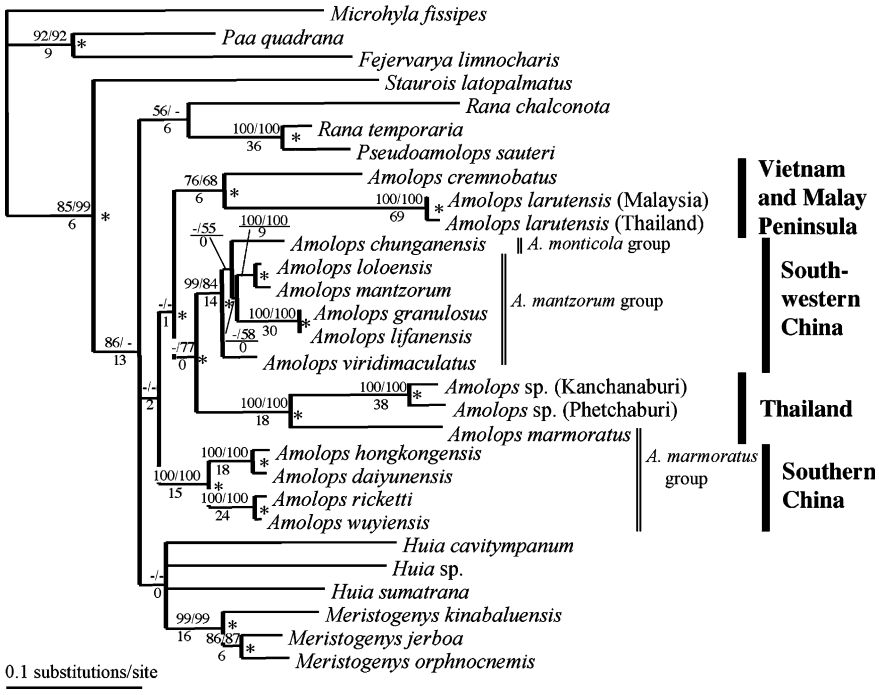


Fig. 2. Bayesian tree of a 1346-bp sequence of 12S and 16S rRNA for species of *Amolops* and related species. Numbers above branches represent bootstrap support for MP (2000 replicates)/ML (100) inference, and numbers below branches indicate the decay index for the respective clade. Nodes with asterisks indicate significant support (>95%) by Bayesian inference. Species group names designated by Fei et al. (2005) for Chinese *Amolops* species are shown.

(iv) Species of *Amolops* from southern China formed a monophyletic group (100% support).
(v) Within the southern Chinese group of *Amolops*, *A. hongkongensis* and *A. daiyunensis*, and *Amolops ricketti* and *A. wuyiensis*, respectively, were sister taxa (100% support).
(vi) Species of *Amolops* from Thailand (*A. marmoratus*) and *A.* sp. formed a monophyletic group (100% support).
(vii) Species of *Amolops* from southwestern China formed a monophyletic group (100, 84, and 99% support) within which interspecific relationships were not well resolved.
(viii) *Amolops loloensis* and *Amolops mantzorum*, and *Amolops granulosus* and *Amolops lifanensis*, respectively, were sister taxa (100% support).
(ix) The monophyly of a clade of *Amolops* comprising species from Vietnam and Malaysia (*Amolops cremnobatus* and *A. larutensis*) was supported by Bayesian analysis (100%), but only weakly by ML (68%) and MP (76%).

In contrast, the following relationships were supported only by Bayesian, ML, or MP analysis:

(x) monophyly of the genera *Rana*, *Pseudoamolops*, *Amolops*, *Huia*, and *Meristogenys* with respect to *Staurois* (51, <50, and 86% support);
(xi) monophyly of a clade of *Amolops* comprising species from Thailand and southwestern China (99, 77, and −% support);
(xii) sister relationships of a clade of *Amolops* from Vietnam and Malaysia with another clade of species from Thailand and southwestern China (96, <50, and −% support).

Monophyly of *Amolops* (64, 50, and <50% support) and sister relationships of *Rana chalconota* with *R. temporaria* + *P. sauteri* (94, <50, and 56% support) were not supported by any of the three analyses.

The relationships supported by all three methods (i–ix) constitute the basic framework for the discussion of the systematics and evolutionary history of this group of frogs.

## 4. Discussion

### 4.1. Phylogenetics and taxonomy at supraspecific levels

In recent studies of ranoid molecular phylogeny, species of *Amolops* (sensu lato) have been included only sporadically (Jiang and Zhou, 2001; Marmayou et al., 2000; Roelants et al., 2003; Wilkinson et al., 2002). Among the species of *Rana* studied by Marmayou et al. (2000), *R. chalconota* was closest to *Amolops* sp. from Thailand, but very low support of the branch did not support their monophyly. In our results, relationships of *R. chalconota* with other

clades were unresolved, and this species did not form a clade with any of the four *Amolops* clades. Jiang and Zhou (2001) showed monophyletic relationships of three Chinese *Amolops* species, but in this case also, the relationships with species of *Rana* were unresolved. Roelants et al. (2003) obtained monophyletic relationships of two *Meristogenys* species with *R. curtipes*, but relationships of that clade with one *Amolops* species and other *Rana* species were not resolved. The small number of *Amolops* (sensu lato) species sampled may have obscured the results of these previous studies.

Yang (1991b) established the distinct ranid subfamily Amolopsinae, with the type genus *Amolops*, and also placed the genera *Huia* and *Meristogenys* in this subfamily. Furthermore, Fei et al. (2005) included *Pseudoamolops* in the Amolopsinae. Our results, however, did not support the monophyly of either Amolopsinae or *Amolops* (sensu lato). Our results supported the ideas of Yang (1991a) and Dubois (1992), who recognized distinct lineages within *Amolops* (sensu lato), but at the same time, suggested further division of each genus or subgenus, except *Meristogenys*, whose monophyly is almost beyond doubt. However, we refrain here from establishing a new genus or subgenus for each clade discovered because this would merely complicate the classification of the tribe Ranini at this stage of study. Further, because we were able to examine only 14 of 34 known or well-established undescribed species of *Amolops* and three of six species of *Huia*, unresolved relationships among lineages of *Amolops* (sensu lato) in our results may have been affected by insufficient sampling of the taxa.

What is more reliable in our results is the taxonomic position of *Pseudoamolops*. Jiang et al. (1997) established this name for a subgenus of *Amolops* with *R. sauteri* from Taiwan as the type species, and Fei et al. (2000) later elevated the subgenus as a distinct genus. However, as clearly indicated in our results, the species is sister to the brown frogs of the genus *Rana* and has no close relationship with *Amolops*. Therefore, the taxonomic hypotheses of Jiang et al. (1997) and Fei et al. (2000) are not justified, and the two species now placed in *Pseudoamolops* should be treated as *Rana*. Unique *Amolops*-like characteristics of abdominal suckers found in *R. sauteri* can be viewed as the result of convergent evolution (Matsui et al., 2001; Tanaka-Ueno et al., 1998).

### 4.2. Phylogenetics and taxonomy at specific levels

On the basis of morphological characteristics, Fei (1999) recognized four groups of species within Chinese *Amolops* (sensu lato). More recently, Fei et al. (2005) slightly changed the grouping and divided Chinese *Amolops* (sensu stricto) into five groups (*A. tormotus*, *A. monticola*, *A. mantzorum*, *A. marmoratus*, and *Amolops hainanensis*). Of these, we were not able to study representatives of the *A. tormotus* and *A. hainanensis* groups.

We found that all species of the *A. mantzorum* group from Southwestern China formed a monophyletic group, which supports the morphological grouping proposed by Fei et al. (2005), although this clade also included *Amolops chunganensis* in our results. We could examine only *A. chunganensis* in the *A. monticola* group of Fei et al. (2005), but our results indicate close relationships of *A. chunganensis* with the *A. mantzorum* group. Of the five of six known species of the *A. marmoratus* group that we studied, four from southern China formed a monophyletic group, in agreement with the grouping by Fei (1999). However, the remaining species, *A. marmoratus* from Thailand, was not close to the Chinese *A. marmoratus* group. Thus, the older name used by Fei (1999); *A. ricketti* group would be more appropriate for the group of four species from southern China.

In the *A. mantzorum* group, *A. granulosus* and *A. lifanensis* showed very similar sequences (sequence divergence <1%), although they have been reported to differ in morphology (Fei, 1999), and the sequence divergence between *A. loloensis* and *A. mantzorum* was also low (1.9%). Likewise, in the *A. ricketti* group (see above), *A. ricketti* and *A. wuyiensis* did not show much divergence (sequence divergence = 2.1%). Jiang and Zhou (2001) also reported sister relationships of *A. ricketti* and *A. wuyiensis* from sequences of 12S rRNA. *Amolops daiyunensis* and *A. hongkongensis* of the same group also showed low sequence divergence (3.1%). The former species was once synonymized with the latter (Yang, 1991a), but they are now considered heterospecific (Fei et al., 2005; Zhao and Adler, 1993). Our results support the current taxonomy of these two species, but suggest their relatively recent differentiation.

An undescribed species of *Amolops* from Thailand has a distributional range between that of *A. marmoratus* (northern Thailand) and *A. larutensis* (southern Thailand to Malaysia: Fig. 1; Nabhitabhata et al., 2000), but our results indicated a sister relationship of *A.* sp. with *A. marmoratus*. Comparatively substantial sequence divergence was evident between the two populations of *A.* sp. studied (5.5%); thus, further detailed study is required.

*Amolops larutensis* from southern Thailand and Malaysia were very close to each other (sequence divergence = 1.3%) and together formed the sister clade of Vietnamese *A. cremnobatus*. Although Dubois (1992) established the subgenus *Amo* on the basis of unique morphological characteristics of *A. larutensis*, i.e., presence of ventral grooves on the disks, this condition was not reported for *A. cremnobatus* (Inger and Kottelat, 1998). Separation of *A. larutensis* as the distinct subgenus *Amo* is not justified.

*Huia* sp. from Sumatra is morphologically distinct from *H. sumatrana*. Our results strongly suggest their heterospecific relationship because they were not monophyletic and showed a large degree of genetic divergence (sequence divergence = 18.0%).

### 4.3. Biogeography

Our results indicate remote relationships of two lineages of *Amolops* from southern China and Vietnam (the *A. ricketti* group and *A. cremnobatus*, respectively), despite the geographic proximity of the two regions. Because we have no data on the species from intervening regions (i.e., the *A. hainanensis* group from Hainan), further study is required to investigate the validity of our results. Similarly, our study lacks species from far-western Nepal.

Nevertheless, our results indicate remote relationships of southern and southwestern lineages of *Amolops* within China. All but two of the Chinese *Amolops* species are mainly restricted to either the southern or southwestern region (Fei, 1999). Of the two exceptional species that occur in both regions, *A. chunganensis* shows a wide disjunction between the two regions, while *A. ricketti* shows a more continuous distribution south of the Chang Jiang River (Fei, 1999).

Our sample of *A. chunganensis* from Sichuan was in the southwestern clade, while that of *A. ricketti* from Fujian was in the southern clade. Although further studies are necessary to assess the geographic variation within each of these species, our results indicate different origins of southwestern and southern lineages of Chinese *Amolops*. This idea supports ideas proposed from lines of evidence from morphology (Pang and Liu, 1992) and karyology (Tan, 1992). Tan (1992) considered a group of *Amolops* species from the Hengduangshan mountain range (in the Southwestern region) to be monophyletic, although he could not resolve within-group relationships of those species. Zhao (1999) also indicated Hengduangshan to be the center of speciation for some groups of amphibians, including *Amolops*.

Southern and southwestern China are separated by central China, where the Nanling Mountains form the boundary of the southern and central regions (Zhao, 1999). Inger (1999) suggested that the lack of clear, rocky streams in flat terrain prevents the occurrence of torrent frogs, including *Amolops*, and this seems to have been the case in the wider region of central China during the dispersal of ancestral species of *Amolops*.

*Meristogenys* most probably arose in Borneo. In addition to eight known species, some more cryptic species of *Meristogenys* occur on this island (unpublished data). Finally, *R. sauteri*, which now occurs at the northern periphery of the Oriental region (i.e., Taiwan) appears to have originated as a Palearctic element because it has no close relationships to *Amolops* and its allies, which are purely Oriental in distribution.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2005.11.019.

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