TAXONOMIC CHAOS IN ASIAN RANID FROGS: AN INITIAL PHYLOGENETIC RESOLUTION

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The taxonomy of ranid frogs is in a state of chaos, and Asian ranids are no exception. We undertook an investigation of the phylogenetic relationships of most major groups of Asian ranids using mitochondrial DNA sequences from the 12S, tRNAVal and 16S genes. The resulting phylogenetic hypothesis had varying correspondence with the current taxonomy of the frogs at the subfamilial and generic levels. In order to maintain a taxonomy that reflects phylogenetic history, a number of taxonomic changes are proposed. Within subfamily Raninae, we recognize the genera Rana, Amolops, Hylarana, Odorrana and Nidirana. Recognition of Huia is not supported by our data and the recognition of Pseudorana is equivocal. Tribe Limnonectini is elevated to subfamily Limnonectinae and it contains Limnonectes, Hoplobatrachus and Nanorana. Membership in Genus Limnonectes is redefined. Recognition of genera Paa and Chaparana results in a paraphyletic taxonomy.

Key words: Anura, Asia, mtDNA, phylogeny, Ranidae

INTRODUCTION

For many years following the major revisions of Boulenger (1882, 1918, 1920), the taxonomy of ranid frogs was stable. Now it is in a state of chaos. Numerous generic and subgeneric shifts have been proposed, usually without an examination of phylogenetic relationships. Dubois (1986 [1987]) recognized six tribes within the Raninae (=Ranidae by most authorities). Among ranids, his Ranini included the genera Alirana, Amolops, Batrachylodes, Micrixalus, Nanorana, Staurops, and Rana, with the subgenera Amietia, Hylarana, Paa, and Strongylopus, and Rana. Dubois' Tomopternini only included the genus Tomopterna. The tribe Ptychadenini had Ptychadena and Hildebrandtia. His Dicroglossini contained Ceratobatrachus, Conraua, Discodèles, Limnonectes (with five subgenera: Limnonectes, Bouretia, Fejervarya, Hoplobatrachus, and Taylorana), Occidozyga (with two subgenera: Occidozyga and Euphyllyctis), Palmatorappia, Phrynoglossus, Platymantis, and Ingerana (with Ingerana and Liurana as subgenera). The fifth tribe, Ptyxicephalini, included Ptyxicephalus. Finally, tribe Ranixalini had Ranixalus, Nannophrys, and Nyctibatrachus.

Higher taxonomy has continued to change. Dubois (1992) raised the tribe Dicroglossini to the subfamily level, Dicroglossinae. He recognized four tribes in this subfamily: (1) Ceratobatrachini (Ceratobatrachus, Discodèles, Ingerana, Palmatorappia, Platymantis, and Taylorana), (2) Conrauini (Conraua), (3) Dicroglossini (Euphyllyctis, Occidozyga, and Phrynoglossus), and (4) Limnonectini (Hoplobatrachus and Limnonectes). These arrangements have been controversial. Inger (1996) noted that tribe Limnonectini was demonstrably paraphyletic with respect to the Ceratobatrachini, Conraui, and Dicroglossini. No evidence supported the monophyly of Limnonectini. It may be paraphyletic with respect to the Mantellinae and through the mantellines to the Rhacophoridae. Laurent (1951, 1979) and Ford (1993) questioned the monophyly of the Ranidae (sensu stricto) with respect to the family Rhacophoridae.

Recently, Chinese authorities have proposed numerous other changes, in particular generic reallocations. Fei et al. (1990 [1991]) described or erected a number of new generic combinations for many Chinese species. Further generic changes were made by Ye et al. (1993) and Fei (1999). These changes were made in the absence of a phylogenetic evaluation. Thus, we undertook an investigation of ranid relationships, particularly for representative south-east Asian genera and species. When we initiated this study, no phylogenetic evaluation of the group had been attempted at a higher taxonomic level, although one distance-based evaluation had been made (Wallace et al., 1973). Subsequently, four other phylogenetic studies have reported on the relationships of ranid frogs, as discussed below.
TABLE 1. Primers used for amplifying and sequencing fragments of RNA genes in the subfamily Raninae. Sequence position indicates the starting position of the primer in the *Xenopus laevis* genome and is preceded by the amplification direction as indicated by (H) heavy or (L) light strand.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence 5’ to 3’</th>
<th>Sequence position</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12S1L</td>
<td>CAAACTGGGATTAGATACCCCCACTAT</td>
<td>L2484</td>
<td>Kocher et al. (1989)</td>
</tr>
<tr>
<td>12S2H</td>
<td>AGGTTGACGCGCGGTGTTGT</td>
<td>H2897</td>
<td>Kocher et al. (1989)</td>
</tr>
<tr>
<td>12S2L</td>
<td>ACACACGCGGTACCCCT</td>
<td>L2917</td>
<td>Fu (1999)</td>
</tr>
<tr>
<td>16S3H</td>
<td>GTATCCTCATATTTGCAGGG</td>
<td>H3341</td>
<td>Fu (pers. comm.)</td>
</tr>
<tr>
<td>16S3L</td>
<td>CCGAAATCTACTGAGCTAAC</td>
<td>L3362</td>
<td>Fu (pers. comm.)</td>
</tr>
<tr>
<td>16S1H</td>
<td>GCTATGTTTTTGTAACAG</td>
<td>H3958</td>
<td>Modified from Palumbi (1996)</td>
</tr>
<tr>
<td>16S5H</td>
<td>CCGGATCCCCGGCCGTCTGAACAT</td>
<td>L4040</td>
<td>Fu (2000)</td>
</tr>
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<td>16S1M</td>
<td>CCGACTTGTATACAAAAACAT</td>
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<td>Fu (1998)</td>
</tr>
<tr>
<td>16S2H</td>
<td>AGGGTGACGGGCGGTG</td>
<td>L2897</td>
<td>Palumbi (1996)</td>
</tr>
<tr>
<td>12S2H</td>
<td>AGGGTGACGGGCGGTG</td>
<td>L2897</td>
<td>Palumbi (1996)</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

SPECIMENS EXAMINED

Forty-five individuals, most of them south-east Asian ranines, were sequenced for three mitochondrial DNA genes. Additional sequence data from GenBank were used for the following species: *Rana pipiens* (X86247, X86318), *R. catesbeiana* (M57572), *R. temporaria* (Y11977), and *Xenopus laevis* (M10217). We used *X. laevis* as our initial outgroup taxon, and included an Asian treefrog (family Rhacophoridae, subfamily Rhacophorinae), *Polypedates megacephalus* (AF026350, AF026367), and an *African mantelline* (family Rhacophoridae, subfamily Mantellinae), *Lalostoma labrum* (AF026354, AF026374), in our study to evaluate the monophyly of the family Ranidae as questioned by Laurent (1951, 1979) and Ford (1993). GenBank accession numbers, collection locality and voucher data for specimens sequenced in this study are given in an electronic Appendix downloadable from the Journal’s website (http://biology.bangor.ac.uk/~bss166/HJ). These tissue samples and most voucher data are preserved in the Royal Ontario Museum (ROM), or in the tissue collections of Jinzhong Fu (JF) and James P. Bogart (JPB) (Department of Zoology, University of Guelph). We also incorporated sequences from an analysis of fanged ranids (Emerson & Ward, 1998). These species include *Limnonectes acanthi* (U66120-21), several populations of *L. blythii* (U55262-3, U55269-70, U66114-15, U66126-27, U66130-31, U66134-37), *L. gruminenis* (U66124-25), *L. ibanorun* (U66122-23), *L. ingeri* (U55268, U55275), *L. limnocharis* (U55265, U55272), *L. macrocephala* (66116-17), *L. macdonon* (U66132-33), *L. magna* (U66118-19), *L. paramacdon* (U55267, U55274), *Limnonectes* sp. ("duboisi," a nomen nudum; Dubois, 1999) (U66112-13), and *Occidozyga laevis* (U66138-39). Taxonomic assignment of examined species generally follows Frost (2004).

DNA AMPLIFICATION AND SEQUENCING

Three ribosomal RNA genes, 12S, 16S, and tRNA*Val* from the mitochondrial genome were selected to reconstruct the phylogeny. Total genomic DNA was extracted from frozen or alcohol preserved tissue samples of muscle or liver by digestion with proteinase K for 5-12 hr, and purified three times with phenol-chloroform-isoamyl alcohol (PCI), and then once with chloroform-isoamyl alcohol (CI). The mtDNA region of 12S through 16S was sequenced using the following method. Double stranded fragments were amplified in 33 cycles of the polymerase chain reaction (PCR; 92°C for 30 sec, 45-55°C for 30 sec 72°C for 1.5 min) performed in 25 μl reactions. Annealing temperatures were changed from 45°C to 55°C as needed in order to improve the quality of PCR products. Usually, PCR reactions amplified the entire fragment from 12S1L to 16S2H. Subsequently, several internal primers were used for sequencing. Infrequently, amplification of the larger fragment was not possible and thus the following primers were used: 12S1L, 12S2H, 16S3H, 16S3L, 16S5H, 16SML and 16S2H (Table 1). After amplification, the 25 l product was separated by electrophoresis on an agarose gel and stained with ethidium bromide. The bands containing DNA were excised and the DNA was eluted using Gene Clean II kit (Bio101) and suspended in distilled, deionized water. The cleaned DNA was sequenced directly with Thermo Sequenase 33P-labeled terminator cycle sequencing kit (Amersham). Locations of the primers are shown in Fig 1.

The products of the sequence reactions were resolved in a polyacrylamide 7M urea gel that was then dried and visualized on autoradiograph films (Kodak) within 24-48 hr. A few sequences were resolved using an ABI 377 automated DNA sequencer using the manufacturer’s protocols.

DNA SEQUENCE ANALYSIS

Sequences were initially aligned using ClustalW (Thompson et al., 1994) with gap-open and gap-extension penalties of muscle or liver by digestion with proteinase K for 5-12 hr, and purified three times with phenol-chloroform-isoamyl alcohol (PCI), and then once with chloroform-isoamyl alcohol (CI). The mtDNA region of 12S through 16S was sequenced using the following method. Double stranded fragments were amplified in 33 cycles of the polymerase chain reaction (PCR; 92°C for 30 sec, 45-55°C for 30 sec 72°C for 1.5 min) performed in 25 μl reactions. Annealing temperatures were changed from 45°C to 55°C as needed in order to improve the quality of PCR products. Usually, PCR reactions amplified the entire fragment from 12S1L to 16S2H. Subsequently, several internal primers were used for sequencing. Infrequently, amplification of the larger fragment was not possible and thus the following primers were used: 12S1L, 12S2H, 16S3H, 16S3L, 16S5H, 16SML and 16S2H (Table 1). After amplification, the 25 l product was separated by electrophoresis on an agarose gel and stained with ethidium bromide. The bands containing DNA were excised and the DNA was eluted using Gene Clean II kit (Bio101) and suspended in distilled, deionized water. The cleaned DNA was sequenced directly with Thermo Sequenase 33P-labeled terminator cycle sequencing kit (Amersham). Locations of the primers are shown in Fig 1.

The products of the sequence reactions were resolved in a polyacrylamide 7M urea gel that was then dried and visualized on autoradiograph films (Kodak) within 24-48 hr. A few sequences were resolved using an ABI 377 automated DNA sequencer using the manufacturer’s protocols.

DNA SEQUENCE ANALYSIS

Sequences were initially aligned using ClustalW (Thompson et al., 1994) with gap-open and gap-exten-
TABLE 2. Summary of RNA genes sequenced from the ingroup and outgroup taxa. NT = Total number of taxa analyzed; TS = total number of homologous sites resolved; AS = number of ambiguous sites removed; NSR = number of homologous sites retained; NVS = number of variable sites; NPPIS = number of potentially phylogenetically informative sites; NMPTs = number of most parsimonious trees resolved; LMPTs = Length of most parsimonious solution; CI = consistency index; RI = retention index. Trees for the tRNAVal gene were not calculated (n/a) owing to the limited number of characters (37) available to resolve nodes among the 52 taxa in the analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>NT</th>
<th>TS</th>
<th>AS</th>
<th>NSR</th>
<th>NVS</th>
<th>NPPIS</th>
<th>NMPTs</th>
<th>LMPTs</th>
<th>CI</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
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<td>546</td>
<td>29</td>
<td>517</td>
<td>335</td>
<td>236</td>
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<td>1664</td>
<td>0.276</td>
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<td>tRNAVal</td>
<td>52</td>
<td>72</td>
<td>2</td>
<td>70</td>
<td>48</td>
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<td>n/a</td>
</tr>
<tr>
<td>16S rRNA</td>
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<td>1509</td>
<td>25</td>
<td>1484</td>
<td>910</td>
<td>728</td>
<td>16</td>
<td>6279</td>
<td>0.237</td>
<td>0.530</td>
</tr>
<tr>
<td>All RNAs</td>
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<td>2127</td>
<td>56</td>
<td>2071</td>
<td>1301</td>
<td>1012</td>
<td>3</td>
<td>8512</td>
<td>0.239</td>
<td>0.527</td>
</tr>
</tbody>
</table>

RESULTS

Forty-five specimens were sequenced for 12S through 16S RNA genes. In total, 546 sites were sequenced for 12S, 72 for tRNAVal, and 1509 for 16S for a total of 2127 aligned sites. Among these sites, 56 were ambiguously aligned and 1012 were potentially phylogenetically informative (Table 2). All sequences were deposited in GenBank (12S = AF206072-AF206116; tRNAVal = AF206117-206161; 16S rRNA = 206453-206497).

PARSIMONY EVALUATION

For 12S, analysis of the 236 potentially informative sites yielded six most parsimonious trees (MPTS, Table 2). We did not attempt a separate phylogenetic analysis of the tRNAVal gene because there were too few potentially phylogenetically informative sites for a meaningful analysis. For 16S, 728 potentially informative sites resulted in 16 cladograms, the differences constrained to one subclade. Because of similarities in nucleotide proportions and levels of site divergence, all RNA gene sequence data were combined for a total evidence analysis.

Combining all RNA gene sequence data into a single data set resulted in 1012 potentially cladistically informative characters. Analysis of these data yielded three most parsimonious trees (8512 steps in length, CI=0.24, RI=0.53). Ptychodera was resolved as the sister group of Ptychiceps plus two major speciose clades (A and B) of ranids (Fig. 2): Clade A, included Amolops, Hylarana, Dirida, Odorrella, and Rana; Clade B was composed of Chaparana, Hoplobatrachus, Limnonectes, Nanorana, Occidozyga, and Paa, plus a mantelline, Laliostoma, and rhacophorine, Polypedates.

Clade A was treated as having two major subclades, A1 and A2. Diridea chapaeensis was resolved as the sister group to all other members of clade A1, including specimens of Odorrella and Rana. Rana (Pantherana) pipiens was resolved as the sister species of R. (Aquadura) caesibiana. Their sister group contained R. (Pseudorana) johnsi plus R. (Rana) temporaria. The clade containing Rana formed the sister group to a clade composed of Amolops (Huia) nasica and a paraphyletic Odorrella.

In Clade A2, a monophyletic Amolops (Amolops) was the sister group to a clade of Rana including subgenera Hylarana and Pelophylax, and a paraphyletic subgenus Sylvirana.

Clade B was treated as having four major subclades, B1-B4. In Clade B1, the mantelline, Laliostoma labrosum, and the rhacophorine, Polypedates megacephalus, were resolved as sister taxa. Together they formed the sister group of the three species of Occidozyga. Clade B1, in turn, was the sister group of clades B2-B4. Clade B2 consisted of Hoplobatrachus crassus, H. rugulosus, Limnonectes cancivorus, and a paraphyletic L. limnocharis. It was the sister group of clades B3 and B4. Clade B3 contained Paa (as a paraphyletic taxon), Chaparana and Nanorana. The sister group of Clade B3 was B4, which consisted of Limnonectes blythii and its relatives, with L. blythii being resolved as paraphyletic (Fig. 2).
FIG 2. The strict consensus tree of the two most parsimonious explanations of mtDNA sequence data for south-east Asian ranids. *Xenopus laevis* was used to root the tree. Taxonomy reflects current usage. Taxonomic groupings proposed by Dubois, 1992 appear to the right of the tree. Numbers above the line are bootstrap proportions (50) and those below are Bremer decay indices examined up to six steps longer than the most parsimonious trees.
 Values of nodal support are indicated on the tree (Fig. 2). Bootstrapping (BS) trials supported 43 nodes with a consistency greater than 70%. Decay analyses revealed that many nodes required a considerable number of additional steps to collapse, except those not generally supported by high BS proportions.

**DISCUSSION**

Because different portions of the mtDNA genome evolve at different rates, cladograms from different genes for the same set of organisms may differ. The relatively slowly evolving 12S and 16S rRNA genes seem appropriate for resolving older divergences, perhaps as old as 150 Ma (Mindell & Honeycutt, 1990).

The two mitochondrial genes evolved in similar ways. We assume that this conciliation is due to their phylogenetic history. Our phylogenetic analysis of the combined data resulted in three MPTs. Independent analyses for each gene revealed compatible branching patterns.

**PREVIOUS STUDIES**

The phylogenetic relationships of ranid frogs have been investigated in three recent molecular studies. Marmayou et al. (2000) evaluated a short, 305 bp segment of mtDNA 12S for 28 species of ranid and rhacophorid frogs using maximum parsimony, transversion weighting, and phenetic neighbour joining. Their unweighted parsimony evaluation resolved *Occidozyga* and *Phrynoglossus* as sister taxa, which together formed the sister group of all other ranids plus rhacophorids. The remaining taxa clustered into four groups whose relationships to each other were not resolved. Representative rhacophorids, including species of *Buergeria*, *Philautus*, *Polypedates* and *Chirixalus*, formed one monophyletic group. *Amolops* and *Rana chalconota* formed another group. The genera *Limnonectes*, *Fejervarya*, *Hoplobatrachus*, *Sphaeroteca*, and *Taylorana* formed a third clade and several species of *Rana* formed the fourth cluster. In this taxonomy, *Rana* was paraphyletic. Transversion weighting and the phenetic evaluation resolved paraphyly in *Philautus*, *Limnonectes*, and an additional example of paraphyly with respect to *Rana*. Given the small numbers of characters analyzed, it is not surprising that most nodes received low levels of branch support.

Bossuyt & Milinkovich (2000) evaluated 2692 bp of mitochondrial and nuclear homologous DNA sequence sites, excluding third position codon sites for cytochrome b. They constructed trees using maximum likelihood and BS consensus methods based on maximum parsimony. Because the initial outgroup was very divergent it was dropped from the analysis and Madagascan ranids and rhacophorids were used to root the network of Asian ranids and rhacophorids combined, and vice versa. Unfortunately, bootstrapping is problematic (Kluge & Wolf, 1993) and consensus methods themselves have long been known to be suspicious (Miyamoto, 1985; Miyamoto & Fitch, 1995). Maximum likelihood analyses are philosophically problematic (Kluge, 1997; Siddall & Kluge, 1997). This puzzle is exemplified, in part, by “Brooks’ conundrum” (D. R. Brooks, Univ. of Toronto, pers. comm., 2002): “Do you believe that evolution occurs in a most parsimonious manner? If not (which is demonstrably true-homoplasy exists), then why try to force a model of maximum parsimony on the analysis of your data, which is exactly what maximum likelihood does?” Maximum parsimony should be used as a criterion for selecting among all possible trees, and not as a model of evolution. Regardless, the basal relationships in the bootstrap consensus tree of Bossuyt and Milinkovich were unresolved. Asian treefrogs were monophyletic, as was a clade containing representative species of *Fejervarya*, *Hoplobatrachus*, *Nanophrys*, *Euphlyctis*, *Asian Tomopterna* (*Sphaeroteca*) and some *Limnonectes*.

Kosuch et al. (2001) investigated the monophyly of tiger frogs, *Hoplobatrachus*, which occur in both Asia and Africa. They evaluated 34 ranids using a total of 903 homologous nucleotide sites from 16SrRNA and 12SrRNA with 281 sites being potentially phylogenetically informative. Though their focus was on the biogeographical relationships of Asian and African *Hoplobatrachus*, representatives of *Fejervarya*, *Limnonectes*, *Nanophrys*, *Occidozyga*, *Phrynoglossus*, *Pychadena* and several species of *Rana* were also included. Support was found for a monophyletic *Hoplobatrachus*, which was resolved as the sister group to *Fejervarya*. Subfamily Dicroglossinae was not resolved as a monophyletic grouping in either of the two trees presented.

More recently, Roelants et al. (2004) evaluated DNA sequences of several groups of ranid frogs, though their focus was on the biogeography of these frogs rather than taxonomy. The taxonomic implications of their study are summarized below.

**PATTERNS OF RELATIONSHIPS**

Although the relationships we resolved among the putative subfamilies of ranid frogs were not entirely consistent with previous taxonomies, lower taxonomic groupings were congruent in a number of ways with those proposed by Dubois (1986[1987], 1992). However, our analysis discovered several problematic associations. For example, the genus *Rana* was not resolved as a monophyletic taxon and *Limnonectes limnocharis* appears to be paraphyletic with respect to *L. cancivorus*.

**MONOPHYLY OF THE RANIDAE AND RELATIONSHIPS AMONG SUBFAMILIES**

Family Ranidae was resolved as a paraphyletic taxon with respect to rhacophorids. Therefore, recognizing Family Rhacophoridae as a subfamily within family Ranidae, as suggested by Dubois (1992) and Blommers-Schlösser (1993), provides an acceptable solution.
Alternatively, in order to avoid having an extremely speciose Ranidae, multiple families could be recognized. The problem requires further investigation using sequences from more conserved genes and a broader array of taxa, especially African ranids and rhacophorids.

**RANINAE, CLADE A**

Clade A consisted of five potential genera of ranid frogs: *Amolops, Hylarana, Nidirana, Odorrana, and Rana*, although group membership did not mirror current taxonomy. These genera were distributed amongst two subclades (A1 and A2).

**CLADE A1**

*Genus Rana (part), subgenus Nidirana:* One species, *R. (Nidirana) chapaensis*, was used to represent this subgenus of *Rana*. It was resolved as the sister taxon to the following two subclades of Clade A1:

*Genus Rana (part), subgenera Aquarana, Pantherana, Rana, and Pseudorana:* One species each was used to represent four relatively speciose subgenera of *Rana*. The two North American species, *R. (Pantherana) pipiens* and *R. (Aquarana) catesbeiana*, were resolved as sister taxa. Their sister group contained the Asian species, *R. (Pseudorana) johnsi*, and its sister group represented by the European *R. (Rana) temporaria*.

*Genus Rana, subgenus Odorrana:* The group containing *Odorrana* and *Amolops (Huia)* forms the sister group to the clade containing *Rana catesbeiana* and *R. pipiens* plus *R. johnsi* and *R. temporaria*.

The large, odoriferous ranids sometimes referred to the genus *Odorrana* formed a paraphyletic lineage with respect to *Amolops (Huia)* *nasica*. *Amolops (Huia)* was resolved within a group of *Odorrana*, and not with other *Amolops* with which it is usually associated (Yang, 1991). *Amolops (Huia)* differs from *Odorrana* by its non-glandular skin and the absence of enlarged toe discs.

Our data also support the finding that *Odorrana chloronota* is a species complex (Murphy et al., 1997; Bain et al., 2003). As cryptic species are identified, the number of species of *Odorrana* will likely increase significantly.

In some regions, like the Khe Moi River, Nghe An Province, Vietnam, three large species of this clade occur in sympatry (Bain et al., 2003). Some sympatric species are derived from distant lineages, such as the cooccurrence of *O. chloronota* and a similar species, *O. bacoensis*. However, other sympatric species appear to be much more closely related, such as *O. chloronota* and *O. morafkai*. This pattern of sympatry repeats in most other areas in Vietnam, although the species composition changes.

**CLADE A2**

*Genus* Amolops, *subgenus* Amolops: the sampled species are monophyletic, and a larger survey of species is currently underway. The two most anatomically similar species included in this analysis, *A. ricketti* and *A. torrentis*, formed a terminal sister relationship, followed basely by *A. loloensis*, and the geographically more distant, but anatomically similar *A. hongkongensis*. *Amolops spinapectoralis* was resolved as the sister group of these species. *Amolops* formed the sister group of the remaining subclade containing *Rana maosonensis* and *R. erythraea*.

*Genus Rana, subgenera Hylarana, Pelophylax, and Sylvirana:* this clade includes a paraphyletic assemblage of subgenera within the genus *Rana*. The association of subgenera is as follows: (*Sylvirana((Sylvirana, Pelophylax)(Hylarana))).

**RANINAE/RHACOPHORIDAE, CLADE B**

The second major group of ranines contains relatively stocky, largely edible Asian frogs. Frogs within this clade belong to several genera, possibly reflecting, in part, their economic significance (and, hence, greater attention) and a greater amount of anatomical divergence. The frogs within clade B clustered into four serially arranged clades as follows: (B1, B2, B3, and B4)).

**CLADE B1**

*Genera* Occidozyga, Polypedates, and Laliostoma: The two representative rhacophorids, *Polypedates megacephalus*, a *rhacophorine*, and *Laliostoma labrosum*, a *mantelline*, were resolved as sister taxa. These taxa formed the sister group to a monophyletic Occidozyga. The sister group to this clade contains the dicroglossine frogs of the genera *Fejervarya*, *Hoplobatrachus*, and *Limnonectes*, separated by the nine frogs *Chaparana, Nanorana, and Paa* (Fig. 2).

**CLADE B2**

*Genera* Hoplobatrachus, Limnonectes (part) and *Fejervarya*: This subclade, sometimes considered to be three genera, has been particularly problematic. Kosuch et al. (2001) examined the biogeographic relationships of *Hoplobatrachus*, and found a monophyletic *Hoplobatrachus* to be the sister group to *Fejervarya*. We also found a monophyletic *Hoplobatrachus*, with *H. crassus* plus *H. rugulosus* being the sister group to the remainder of the clade.

The rice frog, *L. limnocharis*, is resolved as paraphyletic with respect to *L. cancrivorus*. A considerable amount of allozyme work in other parts of its extensive range suggests that it is a composite of many cryptic species (e.g. Dubois, 1984; Toda et al., 1994, 1998a,b). Our data and cladogram support this conclusion.

**CLADE B3**

*Genera* Paa, Chaparana, and Nanorana: This clade is a paraphyletic assemblage of genera. *Paa spinosas* is resolved as the sister group of a clade containing two other
species of *Paa* plus *Chaparana fansipani*, *Nanorana parkeri* and *N. pleskei*. Thus, the genus *Paa* is paraphyletic with respect to *Nanorana* and *C. fansipani*. The association of these species is particularly interesting, given that, though *Chaparana* and *Nanorana* are heavy-set, they are not large frogs like *Paa*. This association does not appear to be spurious since all nodes within this clade received substantial support. This clade, in turn, is resolved as the sister group to the remaining ranine clade.

**Clade B4**

*Genus Limnonectes* (part): The third subclade of Asian edible frogs includes species placed in this genus. Within this clade, paraphyly is the rule rather than exception. Populations of *L. blythii* are variously associated with *L. macrodon*, *L. ingeri*, and *L. paramacrodon*. The clades have a greater correspondence to geographic location than taxonomy. Sister taxa co-occur on a single island. Some species appear to be large complexes of morphologically similar species. For example, Inger *et al.* (1999) noted several anatomical differences between *L. blythii* from the Malay Peninsula and southern Vietnam. Thus, as with *L. limnocharis*, the taxonomy of this group needs to be revised as it undoubtedly represents far more species than previously thought. Our arrangement differs from that of Roelants *et al.* (2004) who resolved this group as the sister of clade B2+B3. However, both studies found weak support at the conflicting nodes. Whereas we included 19 specimens, Roelants *et al.* (2004) sequenced two representatives.

**Taxonomic Implications**

*Type species of Rana Linnaeus 1758*: Before undertaking revisions, it is first necessary to establish the relationships of the type species of the genus *Rana*. Fleming (1822) designated *Rana temporaria Linnaeus, 1758* as the type species of *Rana*. This species is the name-bearer of the genus, subgenus, tribe, subfamily, and family. *Genus Rana sensu* Frost (2004) has more than 240 species divided into 22 subgenera. It is one of the most speciose groups of vertebrates and contains many independent lineages. Taxonomically, recognition of these major lineages as genera would better summarize their phylogenetic history.

*Taxonomic chaos*: At virtually every hierarchical level, taxonomic problems exist. For example, tribe *Ranini* is a paraphyletic assemblage of genera with respect to the genus *Rana* and at the subfamilial level with the genus *Nanorana* Günther 1869.

The taxonomy of these frogs has been unstable. Not exhaustive, Table 3 briefly summarizes some of the changes for Asian groups from 1985 onward for many of the species included in this study. For most species, placement in one group or another has remained relatively stable, but the taxonomic rank accorded to the groups has been quite unstable. For example, the crab-eating frog, *Limnonectes cancrivorus*, was placed in *Genus Rana*, subgenus *Euphlyctis* Fitzinger 1843 by Frost (1985), then into genus *Limnonectes* Fitzinger 1843, subgenus *Hoplobatrachus* Peters 1863 by Dubois (1986 [1987]). Subsequently, it was assigned to genus *Euphlyctis* by Fei *et al.* (1990), then to genus *Hoplobatrachus*, subgenus *Fejervarya* Bolkay 1915 by Dubois (1992). Most recently, the species was placed in *Genus Fejervarya* (Fei, 1999). Yet others (e.g., Inger, 1996; Nguyê & Ho, 1996; Zhao, 1994; Zhao & Adler, 1993) have left the species in the genus *Rana*. Much of this taxonomic instability is due to the absence of a reasonable phylogeny upon which to identify membership within particular clades.

A phylogenetically based taxonomy reflects the greatest amount of information within a hierarchical system (Farris, 1967; Wiley, 1980; Brooks & McLennan, 1991, 2002). Below, we review the taxonomy of these frogs and make taxonomic changes that directly reflect phylogenetic history, albeit conservatively.

*Subfamily Dicroglossinae*, tribe *Dicroglossini*: This group was represented by three of 12 species from the genus *Occidozyga* Kuhl et Hasselt 1882: *O. laevis* and *O. lima*, and *O. martensi*. *Occidozyga laevis* and *O. martensis* have been placed in the genus *Phrynoglossus* Peters 1867 by many authorities (e.g., Peters, 1867; Smith & Chasen, 1931; Taylor, 1962; Dubois, 1986 [1987]). Our data do not refute this placement but recognition of *Phrynoglossus* could result in a paraphyletic *Occidozyga*.


*Genus Nidirana* Dubois 1992: In order to maintain recognition of genus *Odorrana* and not render genus *Rana* paraphyletic, subgenus *Nidirana* must be elevated to generic status for *N. adenopleura*, *N. caldwelli*, *N. chapaensis*, *N. daunchina*, *N. lini*, *N. pleuraden*, and *N. psaltes*.

*Genus Hylarana Tschudi 1838*: The genus *Rana* is paraphyletic with respect to *Amolops* Cope 1865. In order to maintain the genus *Amolops*, another ranine genus must be recognized. The group of ranids that form the sister group of *Amolops* contains the subgenera *Hylarana* Tschudi 1838, *Pelophylax* Fitzinger 1843, *Sylvirana* Dubois 1992, and *Tenuirana* Fei Ye et Huang 1991. On the basis of priority, we recognize genus...
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**TAXONOMY OF ASIAN FROGS**

**Hylarana.** It contains those species associated with the subgenera *Hylarana*, *Sylvirana*, *Tenuirana*, and *Pelophylax*. The type species of *Hylarana*, *H. erythraea*, was included in our evaluation.

Recognition of the subgenera within *Hylarana* requires a phylogeny and the current taxonomy results in paraphyletic groupings (Fig. 2). For example, Dubois (1992) included *H. guentheri*, *H. maosonensis* and *H. milleti* in genus *Rana*, subgenus *Sylvirana*. However, whereas *H. guentheri* is the sister group of subgenus *Pelophylax*, *R. maosonensis* plus *H. milleti* is the sister group of the clade containing *H. guentheri* (subgenus *Sylvirana* in part), subgenus *Hylarana*, and subgenus *Pelophylax*.

The subgenus *Tenuirana* is also a puzzle. *Tenuirana* contains only *R. taiphenensis* and *R. macrodactyla*. Although these two species are sister taxa, recognition of this subgenus results in the paraphyly of other subgenera. Thus, *Tenuirana* should not be elevated to generic status as it leaves *Hylarana* a paraphyletic taxon.

Given the large number of species in *Hylarana*, the apparent polyphyly within the subgenus *Sylvirana*, and the problems surrounding the recognition of *Tenuirana*, recognition of these or any other subgenera or genera is premature in the absence of a more complete phylogeny.

**GENUS RANA LINNAEUS 1758**

*Rana temporaria* is a member of the clade consisting of *R. johnsi* and the American frogs, *R. catesbeiana* and *R. pipiens*. Dubois (1992) included *R. johnsi* (as *R. sauteri*) in the subgenus *Pseudorana*, and *R. pipiens* in subgenus *Pantherana*. He placed *R. temporaria* in subgenus *Rana*, and *R. catesbeiana* in subgenus *Aquarana*. This subgeneric arrangement is phylogenetically acceptable from the perspective of our data. Taxonomically, these species have been closely associated with one another.

*Genera* *Odorrana* Fei Ye et Huang 1991 and *Huia* Yang 1991: The usually large, odoriferous frogs referred to the genus *Odorrana* are the sister group to *Rana*. The type species for the genus *Odorrana* is *Rana margaritae* Liu, 1950 by original designation. Unfortunately, we did not have tissue samples from this species and no sequences exist in GenBank. Nevertheless, for the moment, we recognize genus *Odorrana* and include within it *O. bachoensis*, *O. bannaorum*, *O. chloronotata*, *O. daorum*, *O. limnonothum*, *O. megatympanum*, *O. moraskai*, and *O. nasica*. This list of species is not exclusive and at least 13 additional species could belong to the genus, including: *O. andersonii*, *O. anlungensis*, *O. exiliversabalbis*, *O. grahami*, *O. hainanensis*, *O. jingdengensis*, *O. huangwuensis*, *O. livida*, *O. lungshengensis*, *O. margaritae*, *O. nasuta*, *O. schmackeri*, and *O. swinhoana*.

*Genus Amolops* Cope 1865: Few have questioned the validity or membership of genus *Amolops*, though our data reveal that *Amolops* (Huia) *nasica* occurs within the clade containing *Odorrana chloronotata*. Consequently, membership in one genus or another may be uncertain for many of the larger species referred to as either *Amolops* (Huia) or *Odorrana* (see above).

*Subfamily Limnonectinae* (new content/combination): Dubois placed genus *Paa* Dubois 1975 in subfamily Ranieae, tribe Paini. However, subfamily Ranieae is a paraphyletic group. Consequently, tribe Paini must be moved from subfamily Ranieae and placed in subfamily Dicroglossinae, tribe Limnomectini along with the genera *Hoplobatrachus* and *Limnomectes*. However, doing so still leaves subfamily Dicroglossinae a paraphyletic group with respect to the Rhaeophoridae and Mantellidae. Thus, to avoid paraphyly, tribe Limnomectini must be elevated to subfamily Limnomectinidae. Recognition of the families Rhaeophoridae and Mantellidae will necessitate recognition of the family Limnomictidae.

Limnomectinidae has three distinctive lineages (Fig. 3). One lineage contains genus *Hoplobatrachus* and some species of genus *Limnomectes* referred to genus *Fejervarya* by Fei (1999). These frogs are placed in the tribe Hoplobatrachini (new combination). Another lineage, tribe Paini, contains the genera *Chaparana* Bouret 1939, *Nanorana* and *Paa* (but see below). Finally, tribe Limnomectini contains genus *Limnomectes* excluding those species previously referred to *Fejervarya*.

*Genus Hoplobatrachus* Peters 1863: This genus was represented by the species *H. crassus* and *H. rugulosus*. Kosuch et al. (2001) found this genus to be monophyletic. Our data support their conclusion.

*Genus Fejervarya* Bolkay 1915: *Fejervarya* is represented, in this clade, by the two species *F. limnochares* and *F. cancrivora*. However, *F. limnochares* is paraphyletic with respect to *F. cancrivora*. Though both species are generally assigned to genus *Limnomectes*, Fei (1999) included both species in the genus *Fejervarya*. Our data and cladogram support this conclusion.

*Genera* *Chaparana* Bouret 1939, *Nanorana* Günther 1896, and *Paa* Dubois 1975: The subclade containing *Paa* also contains members of the genera *Chaparana* and *Nanorana*. The genus *Paa* contains more than 29 species (Frost, 2004) of which two were included in our study plus one undescribed species. The genus is paraphyletic. The genera *Nanorana* and *Chaparana* fall out as sister taxa within the genus *Paa*. Among the available generic names, *Nanorana* (type species *N. pleskei* by original designation) is the oldest available name having priority over *Alirana* Stejneger, 1927 (type species *N. parkeri* by original designation), *Chaparana* (type species *Rana* (Chaparana) fansipani by original designation), and *Paa* (type species *Rana liebigii* Günther, 1860, [named originally as a subgenus of *Rana*] by original designation). Paraphyletic relationships preclude retention of the subgenera within *Nanorana*. In addition to species already included in the genus *Nanorana*, we add those species previously recognized as *Paa*, as well as *Nanorana fansipani*, *Nanorana aenea*, *N. delacouri*, *N. quadranus*, *N.
sikimensis, N. unculuanus, N. parkeri, N. pleskei and N. ventripunctata.

Genus Limnonectes Fitzinger 1843: The type species of Limnonectes is L. kuhlii by original designation. We recognize Limnonectes for the following species included in our study, L. acanthi, L. blythii, Limnonectes sp. ("duboisii"), L. grunniens, L. ibanorum, L. ingeri, L. kuhlii, L. macrocephala, L. macrodon, L. magna, L. paramacron and L. toumanoffi, and exclusive of Fejervarya limnocharis and F. cancivirora. The tree of Roelants et al. (2004) does not conflict with this new taxonomy.

Although our analysis contains a small number of ranid frogs, major Asian groups are represented herein. No doubt the genus Rana remains a "megataxon" in that it is a paraphyletic assemblage of species. Our evaluation revealed that most assemblages of species contained paraphyletic grades of species, and not monophyletic assemblages. Consequently, in the interest of nomenclatorial stability we believe that further divisions of ranid frogs in the absence of a phylogenetic hypothesis will only result in additional confusion in an already incredibly complex history of names and species. We have initiated further biochemical studies on some genera, particularly Amolops, Odorrana and Paa, but also including Vietnamese species in the genus Hylarana. Future investigations using gene sequences from 12S and 16S rRNA of smaller subsets of species should prove equally fruitful for resolving relationships among the genera of ranid frogs.

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