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PHYLOGENETIC RELATIONSHIPS AMONG CHINESE RANIDS INFERRED FROM SEQUENCE DATA SET OF 12S AND 16S rDNA

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Phylogenetic relationships among twenty-nine species of Ranidae representing thirteen genera were investigated on the basis of 1005 base pairs of mitochondrial DNA sequences of 12S and 16S rRNA gene fragments. Sequence data were analyzed using maximum parsimony, likelihood maximum, and neighbour joining with all indel and missing/ambiguous sites deleted. Among the twenty-nine ranids studied, two clades are well supported by the results of the three analyses, the first consists of twenty-one species in the genera *Rana*, *Glandirana*, *Rugosa*, *Pelophylax*, *Amolops*, *Odorrana*, and *Hylarana*; and the second includes eight species in the genera *Fejervarya*, *Hoplobatrachus*, *Paa*, *Nanorana*, *Altirana*, and *Limnonectes*; the six genera with multi-species samples – including *Amolops*, *Pelophylax*, *Rugosa*, *Rana*, *Odorrana*, and *Paa* – are recognized as distinct lineages with higher bootstrap and quartet puzzling supports: the phylogenetic relationships between species within each lineage are resolved well. The results testify that the traditional genus *Rana* is heterogeneous. On the basis of the phylogenetic relationships of these taxa, it is suggested that the genera *Paa*, *Nanorana*, and *Altirana* should be removed from the subfamily Raninae and to be included in the subfamily Dicroglossinae. The torrent frog of the genus *Amolops* should be retained in the subfamily Raninae rather than in a distinct subfamily Amolopinae of its own. The inclusion of *Fejervarya limnocharis* in the genus *Limnonectes* is not supported.

Key words: China, molecular systematics, mtDNA, Ranidae

INTRODUCTION

With over 700 species, the family Ranidae is one of the most species-rich amphibian families. It is distributed throughout the world, except southern South America and most of Australia (Frost, 1985, Duellman, 1993). There are a few reports on the relationships of groups from lineages or regions, based on morphological and (or) molecular data sets, such as Boulenger (1920), Liu & Hu (1961), Wallace *et al*. (1973), Emerson *et al*. (1993), Marmayou *et al*. (2000), Emerson *et al*. (2000*a*), Sumida *et al*. (2003). Nevertheless, the taxonomy of the Ranidae is still very problematic (Dubois, 1999) because the phylogenetic relationships within the family are still poorly known (Duellman & Trueb, 1985).

On the basis of a phenetic analysis, Dubois (1992) placed the species in the family into seven subfamilies, 88 genera and subgenera including some new genera, (e.g. *Paa*) and reintroduced some genera previously named (e.g. *Limnonectes*, *Hoplobatrachus*). According to the classification of Dubois (1992), Chinese ranids should be grouped into two subfamilies – Dicroglossinae and Raninae – while Fei *et al*. (1990) classed them into three subfamilies – Raninae, Amolopinae Yang, 1989, and Occidozyginae Fei, Ye *et* Huang, 1990. In the subfamily Raninae, all the new genera proposed by Fei *et al*. (1990), including *Pseudorana*, *Rugosa*, *Glandirana*, and *Odorrana*, were treated as subgenera of the genus *Rana* by Dubois (1992), so were the genera reintroduced by Fei *et al*. (1990), including *Pelophylax*, *Hylarana*, and *Rana* consisting of only brown frogs. Zhao (1994, 1995) and Inger (1996) criticised the new taxonomy proposed by Fei *et al*. (1990) and Dubois (1992). Despite this, Dubois's classification is already being adopted in influential works, for example, *Additions and Corrections to Amphibian Species of the World* (Duellman, 1993) and *Amphibian Species of the World* (ver. 2.21 online, Frost, 2002).

The purpose of the present work was therefore threefold: (1) to clarify the phylogenetic relationships between some groups of the family Ranidae; (2) to test heterogeneity of the traditional genus *Rana*; (3) to further discuss the systematic issues of some ranid groups proposed by Dubois (1992) and Fei *et al*. (1990). All the topics will be addressed here using the sequence data set of 16S and 12S mitochondrial DNA.

MATERIALS AND METHODS

SPECIES STUDIED

Twenty-nine species (Table 1) representing 13 genera were examined. Tissue samples were all derived from thigh muscle preserved in either ethanol (95%) or in refrigerator (-20°C). Specimens were kept in the herpetological collection of the Institute of Genetic Resources, Nanjing Normal University (NJNU) and

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TABLE 1. Species studied in the present study.

Family	Subfamily (Fei *et al*., 1990)	Subfamily (Dubois, 1992)	Genus (Fei *et al*., 1990) (Fei, 1999)	Genus (Dubois, 1992)	Traditional Genus (Frost, 1985)	Species	Locality of collection
OUTGROUP							
Bufonidae			*Bufo*		*Bufo*	*gargarizans*	Nanjing, Jiangsu
Microhylidae			*Kaloula*		*Kaloula*	*pulchra*	Zhaoan,Fujian
Hylidae			*Hyla*		*Hyla*	*chinensis*	Zhaoan, Fujian
INGROUP							
Ranidae	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*chensinensis*	Yuzhong, Gansu
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*amurensis*	Mudanjiang, Heilongjiang
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*zhenhaiensis*	Zhenhai, Zhejiang
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*omeimontis*	Emei Mt., Sichuan
	Raninae	Raninae	*Rana*	*Rana*	*Rana*	*chaochiaoensis*	Muli, Sichuan
	Raninae	Raninae	*Pelophylax*	*`Rana*	*Rana*	*nigromaculata*	Huoqiu, Anhui
	Raninae	Raninae	*Pelophylax*	*Rana*	*Rana*	*hubeiensis*	Huoqiu, Anhui
	Raninae	Raninae	*Hylarana*	*Rana*	*Rana*	*adenopleura*	Nanjing, Fujian
	Raninae	Raninae	*Hylarana*	*Rana*	*Rana*	*guentheri*	Guangzhou, Guangdong
	Raninae	Raninae	*Rugosa*	*Rana*	*Rana*	*emeljanovi*	Dalian, Liaoning
	Raninae	Raninae	*Rugosa*	*Rana*	*Rana*	*tientaiensis*	Tianmushan,Zhejiang
	Raninae	Raninae	*Glandirana*	*Rana*	*Rana*	*minima*	Fuzhou, Fujian
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*margaretae*	Wawushan, Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*livida*	Hejiang, Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*schmackeri*	Emei Mt., Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*hejiangensis*	Hejiang, Sichuan
	Raninae	Raninae	*Odorrana*	*Rana*	*Rana*	*grahami*	Muli, Sichuan
	Raninae	Raninae	*Paa*	*Paa*	*Rana*	*boulengeri*	Wawushan, Sichuan
	Raninae	Raninae	*Paa*	*Paa*	*Rana*	*spinosa*	Tianmushan, Zhejiang
	Raninae	Raninae	*Paa*	*Paa*	*Rana*	*robertingeri*	Hejiang, Sichuan
	Raninae	Dicroglossinae	*Fejervarya*	*Limnonectes*	*Rana*	*limnocharis*	Nanjing, Jiangsu
	Raninae	Dicroglossinae	*Limnonectes*	*Limnonectes*	*Rana*	*fujianensis*	Nanjing, Fujian
	Raninae	Dicroglossinae	*Hoplobatrachus*	*Hoplobatrachus*	*Rana*	*rugulosus*	Haikou, Hainan
	Raninae	Raninae	*Nanorana*	*Nanorana*	*Nanorana*	*pleskei*	Songpan, Sichuan
	Raninae	Raninae	*Altirana*	*Nanorana*	*Altirana*	*parkeri*	Lasha, Xizang
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*daiyunensis*	Nanjing, Fujian
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*ricketti*	Hejiang, Sichuan
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*wuyiensis*	Huangshan, Anhui
	Amolopinae	Raninae	*Amolops*	*Amolops*	*Amolops*	*mantzorum*	Wawushan, Sichuan

Chengdu Institute of Biology, Chinese Academy of Sciences (CIB). Taxonomic assignment of examined species follows Fei *et al*. (1990) and Fei (1999), and the other two different classifications are also presented in Table 1.

CHOICE OF THE OUTGROUP

The family Ranidae is a member of the superfamily Ranoidea (Hay *et al*., 1995; Emerson *et al*., 2000*b*). Hay *et al*. (1995) reported that the Mantellidae was the sister group to the Ranidae, and they formed a sister group to the clade consisting of the Microhylidae and Hyperoliidae. Emerson *et al*. (2000*b*) indicated that the mantelline frogs may be nested within the Rhacophoridae; Dubois (1992) and Marmayou *et al*. (2000) suggested that the rhacophorids should be a lineage of the Ranidae. Since the relationships among the families mentioned above have not been resolved so far, three species of the three families Microhylidae, Bufonidae, and Hylidae respectively (see Table 1) were used as outgroup in this study.

DNA AMPLIFICATION AND SEQUENCING PROTOCOLS

Whole genomic DNA was extracted from alcohol-preserved or frozen tissue samples of thigh muscle using standard proteinase K/SDS digest extraction method followed by phenol-chloroform isolation and ethanol precipitation. Two regions of the mtDNA 12S and 16S rRNA genes were amplified and sequenced using the following protocols. Double stranded fragments were amplified in 35 cycles of PCR: 95°C for 30 s , 50-58°C for 30 s, 72°C for 60 s. It was pre-denatured at 95°C for

4 min before starting the cycles and elongated at 72°C for 7 min after ending the cycles. The PCRs were accomplished with the primer pairs of 12S (L2509: 5'-GCTTCAAACTGGGATTAGATACCCCACTAT-3' H2897:5'-TGACTGCAGAGGGTGACGGGCGGTGTGT-3') (Kocher *et al.,* 1989) that can amplify 388 base pairs, and 16S (L3975: 5'-CGCCTGTTTACCAAAAACAT-3', H4551: 5'-CCGGTCTGAACTCAGATCACGT-3') (Simon *et al*., 1994) that can amplify 576 base pairs. The capital L and H indicate the amplified directions of light and heavy strand, respectively. The numbers after L and H indicate the starting position of the 3'-end of the primers in the *Xenopus laevis* mitochondrial genome (Roe *et al*., 1985). After amplification, the PCR product was cleaned using Wizard® PCR Preps DNA Purification System (Promega) and suspended in distilled and deionized water. The cleaned DNA template was sequenced directly in both directions. The light strand was sequenced using SILVER SEQUENCE™ DNA sequencing Systems (Promega), and the heavy strand was sequenced using an ABI 310 with the BigDye kit (PE Applied Biosystems).

DNA Sequence Analysis

Sequence alignment was conducted using Clustal W (ver. 1.6; Thompson *et al*., 1994), and minor modifications were made by eye to correct the computer-aligned sequences. The sequences from the two genes were combined as one data set for further analyses. Indel sites resulting from the alignment and missing/ambiguous data were deleted all in phylogenetic analyses. For assessing character covariance in the data set, permutation tail probability (PTP; Faith and Cranston, 1991) and the skewness test (g1 statistic; Hills & Huelsenbeck, 1992) were used.

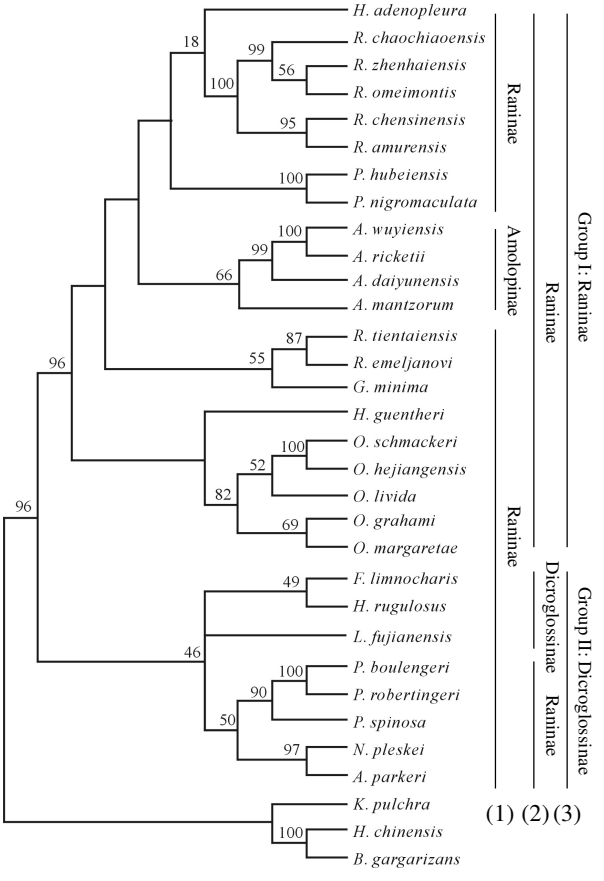

FIG. 1. Strict consensus of two trees recovered in maximum parsimony analysis (tree length=1485, CI=0.449, RI=0.561). Numbers above branches represent bootstrap support (100 replicates). (1) the subfamily classification of Fei *et al*. (1990); (2) Dubois (1992); (3) present study.

Maximum parsimony (MP) and Maximum likelihood (ML) as implemented in PAUP4.0b8a (Swofford, 1998) and Neighbour joining (NJ; Saitou & Nei, 1987) as implemented in MEGA (version 2.1, Kumar *et al*., 2001) were employed to infer relationships among taxa. MP analyses were conducted using 100 random replicates of the heuristic search option with ACCTRAN, MULPARS, and TBR options; only minimum-length trees were retained and zero-length branches were collapsed. A sequences evolution model was chosen using Modeltest 3.06 (Posada & Crandall, 1998) and used in the ML analysis. The robustness of the phylogenetic results was tested by bootstrap proportion (BSP; Felsenstein, 1985) with 1000 replicates in NJ analysis and with 100 replicates in MP analyses, and by the quartet puzzling replicates method (Strimmer & von Haeseler, 1996) with 1000 puzzling steps for ML analysis.

RESULTS

Sequence Characteristics and Genetic Distance Between Taxa

The sequences were deposited in GenBank, Accession numbers were AF315123 to AF315130 and AF315131 to AF315162. We added 24 sequences of 12S rDNA fragment retrieved from GenBank (AF205541 to AF 205565, Jiang & Zhou, 2001). Alignment resulted in a data matrix of 1005 unambiguously aligned characters, 582 of which were variable sites and 402 parsimony informative sites. The conserved and variable sites distributed alternately but not evenly. Nucleotide compositions were A 0.302, G 0.204, C 0.258, and T 0.236, and the ratio of transitions to transversions was average 1.32 with the range of 0.72 to 3.33.

The Kimura-2-parameter distances showed that the levels of divergence ranged from 0.199 (*N. pleskei* versus *K. pulchra*) to 0.357 (*F. limnocharis* versus *H. chinensis*) between outgroup and ingroup, the average is 0.261±0.031, and those within ingroup were from 0.029 (*O. hejiangensis* versus *O. schmacheri*) to 0.311 (*F. limnocharis* versus *R. amurensis*), of which the intrageneric divergence ranged from 0.029 to 0.178 (*H. adenopleura* versus *H. guentheri*) and the average was 0.095±0.038, and the inter-generic divergence ranged from 0.113 to 0.311, the averaged was 0.199±0.046.

The sequence evolution model chosen by Modeltest and used for ML analysis is the general time-reversal model plus I and G (GTR+I+G). Base frequencies were unequal (A=0.3168; C= 0.2517; G=0.1895; T=0.2419),

Nst=6, Rmat=(2.0855 4.7154 2.0659 0.6555 9.0422), Rates=gamma, Shape=0.4939, and Pinvar=0.1530. These parameters were set in ML analysis.

PHYLOGENETIC EVALUATION

The results of both permutation tail probability test (P=0.001, with 1000 replicates) and skewness test (g1= -0.586777) indicated that there was substantial structure in the data set.

The maximum parsimony analyses resulted in two trees of 1485 length (CI=0.449, RI=0.561; Fig. 1). Variation between the two MP trees occurred at the nodes where *L. fujianensis* presented, one was that it clustered with a clade including *F. limnocharis* and *H. rugulosus*; another was that it clustered with a clade containing *N. pleskei*, *A. parkeri* and three species of *Paa*. The 29 species of ingroup unambiguously formed a clade with a very high bootstrap support proportion (BSP) 96%, and they constituted two sister groups, the first (BSP=96%) was composed of 21 species belonging to seven genera

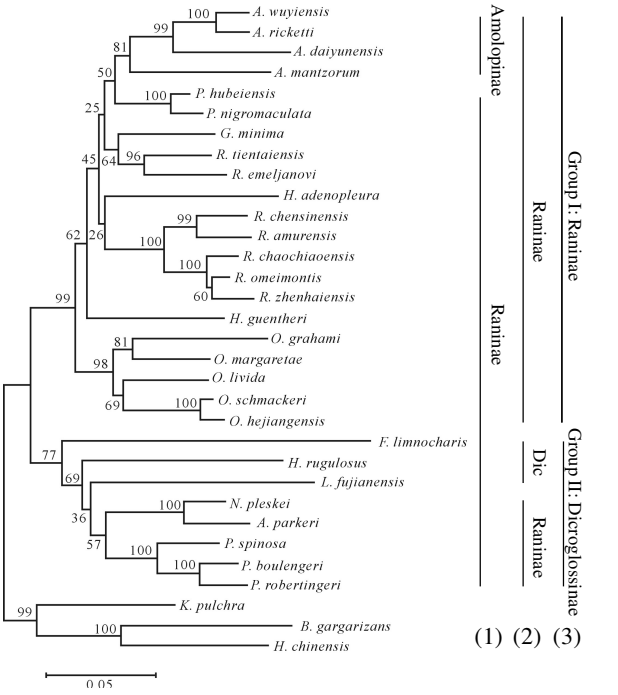

FIG. 3. The phylogenetic relationships among 29 species of ranids examined in the present study by NJ analysis. Numbers on branches are bootstrap proportions (1000 replications). (1) the subfamily classification of Fei *et al*. (1990); (2) Dubois (1992), Dic: Dicroglossinae; (3) present study.

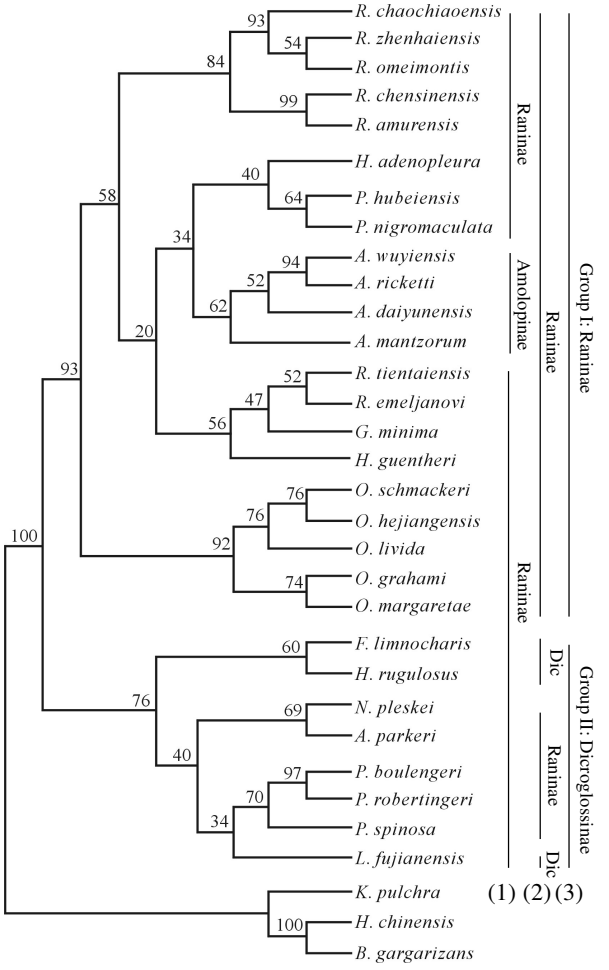

FIG. 2. The best tree derived by ML analysis. Addseq=asis, –lnL (unconstrained) = 611.50197. The number above branch is the quartet puzzling proportion with number of puzzling steps equal to 1000. (1) the subfamily classification of Fei *et al*. (1990); (2) Dubois (1992), Dic: Dicroglossinae; (3) present study.

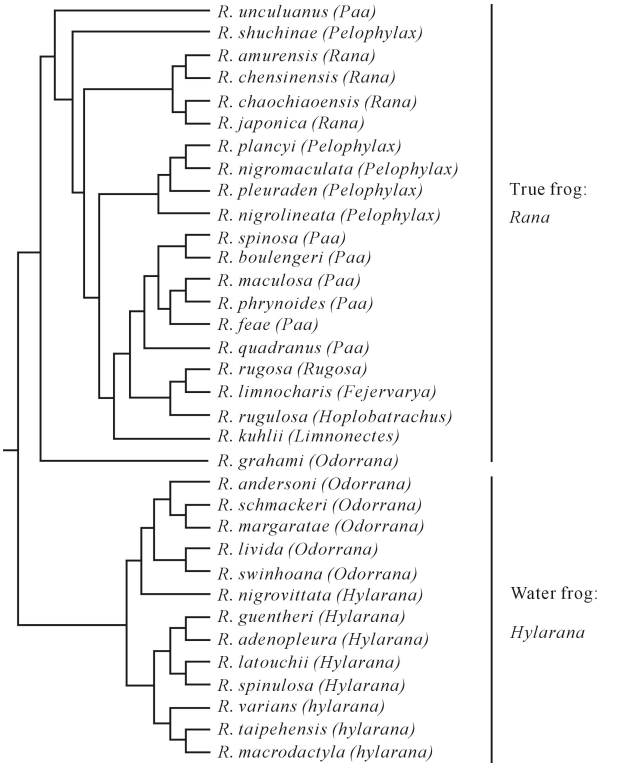

FIG. 4. The relationships between 34 species of the traditional genus *Rana* from China presented by Liu & Hu (1961, page 217). The genus name in parenthesis is proposed by Fei *et al*. (1990) & Fei (1999).

Odorrana, *Rana*, *Hylarana*, *Glandirana*, *Rugosa*, *Pelophylax*, and *Amolops*; the second (BSP=46%) consisted of eight species belonging to six genera *Paa*, *Nanorana*, *Altirana*, *Limnonectes*, *Fejervarya*, and *Hoplobatrachus*. Within the first group, monophyly of the five genera, including *Odorrana*, *Rugosa*, *Amolops*, *Pelophylax*, and *Rana*, respectively was well supported. The genus *Glandirana* (containing only one species, i.e. *G. minima*) has a sister relationship with the genus *Rugosa*. Within the second group, *Fejervarya* and *Hoplobatrachus* clustered together (BSP=49%), and the three species of the genus *Paa* clustered together as a sister group of a group containing *Altirana* and *Nanorana*.

Relationships differ somewhat under the ML analysis (Fig. 2) and NJ analysis (Fig. 3). Again, there was strong support for the two sister groups and monophyly of the six genera recognized in the MP analysis, the phylogenetic relationships among or between the species within each genus are resolved well except in the genus *Hylarana*. Within the first group, the two species of the genus *Hylarana* have no sister relationship with each other, and the relationships among genera are altered except that between the two genera *Glandirana* and *Rugosa*. Within the second group, the relationships among genera also are altered. However, the relationship between the two genera *Altirana* and *Nanorana* is similar to that of the MP tree.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

The MP, ML, and NJ analyses identified two major groups for the ranid species examined. This is consistent with the morphological data (Jiang, 1999). Group I has a smaller nasal bone and distinct space between the inner edge of the right and left nasal bones, and their sphenethmoid are visible from dorsal view; group II has a bigger nasal bone and almost no space between the inner edge of the right and left nasal bones, and the sphenethmoid is invisible from the dorsal side. The average of the Kimura-2-parameter distance between the two major groups is 0.238±0.031 (0.182~0.311), which is apparently bigger than that of inter-generic distance: 0.199±0.046 (0.113 to 0.311).

Generally, *Nanorana* and *Altirana* have a relatively close relationship with the genus *Paa* (Figs. 1 and 3). This is consistent with the results based on morphological data (Jiang, 1999). The precoracoid of the three genera is not forked at the basal end; spine patch or scattered spines can be found on the chest, fingers, belly, or lateral body, while the precoracoid of the genera *Hoplobatrachus*, *Limnonectes,* and *Fejervarya* is forked at the basal end.

The relationships between some genera studied here are not well supported by the quartet puzzling proportion or bootstrap value. Nevertheless, the results provide relatively strong evidence for resolving some taxonomic questions.

HETEROGENEITY OF THE TRADITIONAL GENUS *RANA*

As shown in Table 1, 23 of the 29 species were traditionally treated as members of the genus *Rana* since Boulenger (1920), and especially by Frost (1985). Liu & Hu (1961) presented a figure (redrawn as Fig. 4) showing a preliminary assessment of the relationships among 34 species of the genus *Rana* known in China on the basis of their own morphological study and Boulenger's monograph of 1920. They placed the 34 ranid species in two groups or subgenera, true frogs (*Rana*) and water frogs (*Hylarana*), depending on whether there are transverse grooves on the end of toes. On the basis of serum albumin data, Wallace *et al*. (1973) presented some clues for the heterogeneity of *Rana* thirty years ago. Marmayou *et al*. (2000) also provided some evidence for heterogeneity of the genus *Rana* in molecular phylogenetic relationships among ranid groups inferred from 12S rDNA fragment sequences. Our data provide further evidence that the traditional genus *Rana* is heterogeneous, and support the view of Dubois (1992) and Fei *et al*. (1990) that the traditional genus *Rana* should be split into several genera or taxa at other levels. Of course, all the split work should be based on relevant information about the phylogenetic relationships among these taxa.

CLASSIFICATION OF THE TAXA

Subfamily classification. The cladograms in figures 1, 2, and 3 support the two subfamilies, Raninae Rafinesque-Schmaltz, 1814 and Dicroglossinae Anderson, 1871, proposed by Dubois (1992). However, some modifications should be made, i.e. to move the genera *Paa*, *Nanorana* and *Altirana* from the subfamily Raninae to the subfamily Dicroglossinae and combined as tribe Paini proposed by Dubois (1992). Marmayou *et al*. (2000) introduced another subfamily name Ceratobatrachinae Boulenger, 1884 for the group at least including genera *Limnonectes*, *Taylorana*, *Sphaerotheca*, *Hoplobatrachus* and *Fejervarya* while restricting the use of the subfamily name Dicroglossinae to genera *Occidozyga* and *Phrynoglossus* (and maybe *Euphlyctis*). But the results of Bossuyt & Milinkovitch (2000) and this study do not support their suggestion.

The subfamily Amolopinae was built by Yang (1989) based mainly on the abdominal sucker of the tadpoles. This specialized character is an adaptation to a torrent stream habitat. Our data indicate that the abdominal sucker of *Amolops* tadpoles is not important enough to support building a super-generic level unit. The tadpoles of *Rana sauteri* are similar to those of *Amolops* in having abdominal suckers (Kuramoto *et al*., 1984; Yang, 1995). Based on this morphological evidence, Fei *et al*. (2000) established a new genus *Pseudoamolops* for *Rana sauteri* and placed it in the subfamily Amolopinae. However, based on molecular data, Tanaka-Ueno *et al*. (1998) found that *R. sauteri* was closely related to *R. longicrus*, suggesting that the sucker shared by *Amolops* and *R. sauteri* tadpoles is a case of convergence and may

have evolved from different evolutionary lines. Although the four species representing the genus *Amolops* constitute a monophyletic clade (Figs. 1, 2, and 3), the results of this study do not support the establishment of the subfamily Amolopinae Yang, 1989, and support their retention in the subfamily Raninae, as in Dubois (1992).

Generic Classification

Fei *et al*. (1990) and Fei (1999) held opinions different from that of Dubois (1992) on the rank of genus or subgenus for some ranid groups. Dubois (1992) placed about 213 species in the genus *Rana* consisting of 33 subgenera, of which, *Rana* (brown frog), *Pelophylax*, *Hylarana*, *Rugosa*, *Glandirana*, *Odorrana*, and *Pseudorana* were treated as different generic units by Fei *et al*. (1990) and Fei (1999). According to the point of view of the synthetic school (Mayr, 1969, 1974, 1981; Gisin, 1964), as a unit in the evolutionary history the genus can be recognized from three aspects at least: genetic unit, phylogenetic unit, and ecological unit. Usually, the first two aspects are consistent with each other because the phylogenetic analysis is mostly based on genetics, especially the molecular phylogenetic analysis. In this study, 13 genera were included, 10 of which were split from the traditional genus *Rana*. The phylogenetic cladograms identified six genera, as well as resolved monophyletic units, or in other words different phylogenetic units, with the exception of the genus *Hylarana*. The two members of the latter were placed in different subgenera of *Rana* by Dubois (1992), *H. adenopleura* in the subgenus *Nidirana* and *H. guentheri* in *Sylvirana*. In addition, these groups, like other groups including *Amolops*, *Limnonectes*, *Nanorana*, *Altirana*, *Paa*, have adapted to particular ecological environments of their own (Fei *et al*., 1990; Fei, 1999). As a whole, this study provides some evidence for the generic classification of ranids proposed by Fei *et al*. (1990), but more groups and genes need to be included for further analyses.

Taxonomic Status of *Odorrana grahami*

Liu & Hu (1961) discussed *Odorrana grahami* at length. Based on the absence of a transverse groove on the tip of the fingers, they removed the species from *Odorrana* group, and did not place it in the water frog group (Fig. 4). In the present study, *O. grahami* and *O. margaretae* represent a sister group to the clade consisting of the other three species of *Odorrana* (Figs. 1, 2, and 3). The skin of *O. grahami* can excrete poisonous liquid and produce a distinct odor, which is one of the most important characteristics of *Odorrana*. The poisonous secretion of the skin can kill other species of frogs kept temporarily in the same container during fieldwork. The evidence of both molecular phylogenetics and physiology indicate that *O. grahami* should be included in the genus *Odorrana*.

Taxonomic Status of *Fejervarya limnocharis*

Fejervarya limnocharis was placed in the genus *Limnonectes* in *Additions and Corrections to Amphibian Species of the World* (Duellman, 1993), and in the subgenus *Fejervarya* of the genus *Limnonectes* by Dubois (1987, 1992). Fei *et al*. (1990) elevated this species to its own genus *Euphlyctis*, which should be replaced by the generic name *Fejervarya* since *Euphlyctis* has applied to another group of species from the Indian region that is much more aquatic than *Fejervarya* and retains a lateral-line system in adults (see Dubois, 1992; Dubois & Ohler, 2000). Iskandar (1999), Fei (1999), Marmayou *et al*. (2000), and Dubois & Ohler (2000) considered this species should be included in the genus *Fejervarya* rather than in the genus *Limnonectes*. Their opinion was adopted in *Amphibian Species of the World* (version 2.21 online, Frost, 2002) . The results of this work indicate that *Fejervarya* is a distinct genus different from the genus *Limnonectes* and do not support the suggestion of Duellman (1993) to placed *F. limnocharis* in the genus *Limnonectes*.

Cladistic Relationships of *Nanorana* and *Altirana*

The genera *Nanorana* and *Altirana* have been treated as two distinct genera at least since Liu & Hu (1961) and Frost (1985). In this study, *N. pleskei* representing the genus *Nanorana*, and *A. parkeri* representing *Altirana* are resolved as well supported sister groups. In addition, the Kimura-2-parameter distance between them is as low as 0.062: this is within the range of intrageneric divergence. The results of this study support the view that these two genera should be combined as one, i.e. *Nanorana* (Dubois, 1992; Lu & Yang, 1995; Zhao, 1995; Fei, 1999).

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