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Phylogeny of Raninae (Anura: Ranidae) inferred from mitochondrial and nuclear sequences

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Abstract

Phylogenetic relationships among representative species of the subfamily Raninae were investigated using approximately 2000 base pairs of DNA sequences from two mitochondrial (12S rRNA, 16S rRNA) and two nuclear (tyrosinase, rhodopsin) genes. Phylogenetic trees were reconstructed using maximum parsimony, Bayesian, and maximum likelihood analyses. Comparison between the nuclear and mitochondrial findings suggested that our final combined data has higher resolving power than the separate data sets. The tribes Stauroini and Ranini formed a sistergroup relationship, and within Ranini, ten major clades were consistently resolved among all analyses based on the final combined data, although the phylogenetic relationships among the ten clades were not well resolved. Our result refuted several previous taxonomic divisions: the genus *Pseudoamolops* was invalid, and the monophyly of the genera *Amolops* and *Rana* were not supported. We suggest elevating Raninae to familial status, and recognizing within the family, at least twelve genera including *Staurois*, *Meristogenys*, *Clinotarsus*, *Amolops*, *Hylarana*, *Babina*, *Odorrana*, *Pseudorana*, *Rana*, *Lithobates*, *Glandirana*, and *Pelophylax*. A broader sampling of species and data from more molecular markers are needed to confidently resolve the phylogenetic relationships among Ranidae.

Keywords: Ranidae; Raninae; Mitochondrial DNA; Nuclear DNA; Phylogeny

1. Introduction

The family Ranidae is one of the most diverse and speciose amphibian groups. Since Frost (1985), the taxonomy of ranid species has been revised numerous times, and the monophyly of its subfamilies and many genera remains controversial. This is particularly true for members of the subfamily Raninae, which are found throughout the entire range of the family.

Dubois (1992) proposed a major taxonomic revision of Ranidae that contained over 700 species. Raninae, which included more than 300 species, was one of the seven subfamilies proposed by Dubois (1992). It was further divided into two tribes, Paini and Ranini. However, several molecular phylogenetic studies subsequently suggested a series of taxonomic rearrangements. For example, the tribe Paini including *Paa* and *Chaparana*, was transferred to the subfamily Dicroglossinae along with *Nanorana* of Ranini (Jiang and Zhou, 2005; Roelants et al., 2004). Other studies suggested that Raninae (sensu Dubois, 1992) was not a monophyletic group with respect to *Afrana* and *Strongylopus* (Bossuyt et al., 2006; Scott, 2005; Van der Meijden et al., 2005).

Recently, Dubois (2005) proposed a new revision of the classification of Ranidae. Within the subfamily Raninae, a new tribe, Stauroini, was erected, which included the genus *Staurois*. The genera *Rana* and *Amolops* were retained in the tribe Ranini, together with a new genus *Pseudoamolops*

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(Table 1). Three other genera, including *Nanorana*, *Micrixalus*, and *Batrachylodes* proposed by Dubois (1992) were removed from Ranini and relocated to other subfamilies. However, the lack of robust phylogenies made the proposed taxonomic revisions more speculations than well collaborated hypotheses (Inger, 1996). Monophyly of most of the high level taxa (Table 1; Dubois, 2005) remain to be tested phylogenetically. More recently, based on combined data of comparative anatomical and molecular data, Frost et al. (2006) proposed a new taxonomy across all living amphibians. The "Ranidae" were partitioned into eleven "family-group" taxa to avoid paraphyly with regard to the families Rhacophoridae and Mantellidae. Among them, Raninae (sensu Dubois, 2005) was elevated to family status, and 18 generic taxa were placed within it (Frost, 2006). Monophylies of the genera *Rana*, *Amolops*, and *Pseudoamolops* (sensu Dubois, 1992, 2005) were not supported, as suggested by several early studies (Chen et al., 2005; Marmayou et al., 2000; Matsui et al., 2006). However, it is clear that intensive taxa samplings are needed to provide a robust phylogeny for the subfamily Raninae, in particular for those related Chinese species. In addition to Dubois, a group of Chinese taxonomists also proposed several revisions of the family Ranidae (Fei, 1999; Fei et al., 1990, 2000, 2005; Ye et al., 1993). These revisions were mostly generic changes among ranid species, and were primarily for the Chinese species (Table 1). A wider implication of the Chinese ranid species with other regional species remains unclear.

Notably, the high homoplastic nature of the morphology among ranids determined that morphology alone will not be able to clarify these taxonomic controversies, and in this regard, molecular data could provide additional much needed information. Significant advances have been made in the past few years and a string of molecular phylogenetic studies on this group have been published (e.g. Bossuyt et al., 2006; Chen et al., 2005; Emerson et al., 2000; Frost et al., 2006; Hillis and Wilcox, 2005; Matsui et al., 2006).

In this study, we focused on broader taxonomic samplings within Raninae (sensu Dubois, 1992, 2005). Sequences from Genbank were also incorporated in our analysis. For Raninae, little prior research focused on nuclear genes at the DNA level (Bossuyt et al., 2006; Frost et al., 2006; Roelants et al., 2004). Thus, DNA sequences from two nuclear genes, in addition to two mitochondrial (mt) gene partitions with a total of approximately 2000 base pairs, were examined in the present study. Our objectives include: (1) examining the phylogeny and diversification of Raninae, (2) testing the differences between previous studies and current estimates of phylogeny, and providing a phylogenetic background for a revised classification.

2. Materials and methods

2.1. Specimens

The classifications of Dubois (1992, 2005) were followed mainly for convenience of discussion. New sequences from 30 species (Table 2) were analyzed, along with others from Genbank (Frost et al., 2006; Roelants et al., 2004). Furthermore, we used nine sequences of 16S rRNA from Che et al. (2006). Sequences were obtained from taxa representing the genera *Amolops*, *Pseudoamolops*, *Rana*, and *Staurois* of subfamily Raninae (Dubois, 2005), including the subgenera *Amerana*, *Amolops*, *Aquarana*, *Aurorana*, *Chalcorana*, *Clinotarsus*, *Eburana*, *Glandirana*, *Huia*, *Hydrophylax*, *Hylarana*, *Lithobates*, *Meristogenys*, *Nidirana*, *Odorrana*, *Pantherana*, *Papurana*, *Pelophylax*, *Pseudorana*, *Pulchrana*, *Rana*, *Rugosa*, *Sierrana*, *Sylvirana*, and *Trypheropsis* (Dubois, 1992). Nine Species were chosen as outgroup taxa based on the study of Frost et al. (2006). Table 2 lists our specimens examined, including species names, locality, specimen voucher no., and accession nos. in Genbank.

2.2. Extraction, amplification, and sequencing

Muscle or liver tissue samples were stored in 95 or 100% ethanol. DNA was extracted using the standard 3-step phenol/chloroform extractions. Two mitochondrial and two nuclear DNA fragments (Bossuyt and Milinkovitch, 2000) were PCR-amplified. The mtDNA fragments are as following: (1) 750 base pair (bp) region covering part of the 12S rRNA gene, the complete $tRNA^{Val}$ gene and part of the 16S rRNA gene; (2) 550 bp of the 16S rRNA gene. The nuDNA fragments are: (3) 532 bp of exon 1 of the tyrosinase gene; (4) 316 bp of exon 1of the rhodopsin gene. Amplification was performed in a 50 μl volume reaction with the following procedures for both 12S and 16S fragments: initial denaturation step with 4 min at 94 °C, 35 cycles of denaturation 1 min at 94 °C, annealing for 1 min at 55 °C, extension for 1 min at 72 °C. Final extension at 72 °C was conducted for 10 min. For rhodopsin and tyrosinase, the same procedure was used, but with annealing at 51 and 49 °C, respectively. Purified PCR products were directly sequenced with an ABI automated DNA sequencer and sequences were then determined in both directions for each species and submitted for BLAST searching in GenBank to ensure that required sequences had been amplified.

2.3. Data analysis

Alignments of the four data sets were first conducted separately using the program Clustalx 1.81 (Thompson et al., 1997) with default parameters, and were then verified by eye. Considering that all mtDNA gene sequences are virtually inherited as one linkage group, the two mtDNA gene segments were concatenated into a single partition at the beginning and analyzed simultaneously. For the two mtDNA genes, hyper-variable regions were excluded from further analysis due to the ambiguity of the alignment. Such exclusion increases the reliability of the phylogenetic analysis (Swofford et al., 1996). The data matrix has been deposited in TreeBase under accession number (SN3105). Before reconstructing phylogenetic relationships, we also took a plot of the number of transitions and transversions versus TN93

Table 1
A summary of different taxonomic assignments for ranid species investigated in present study

Specific epithet	Fei et al. (1990)	Dubois (1992)	Fei et al. (2005)	Dubois (2005)	Frost (2006)	Present study
	Ranidae	**Raninae**	**Ranidae**	**Raninae**	**Ranidae**	**Ranidae**
		Tribe Ranini		**Tribe stauroini**		
latopalmatus		*Staurois*		*Staurois*	*Staurois*	*Staurois*
	Amolopinae		**Amolopinae**			
				Tribe Ranini		
kinabaluensis		*Amolops* (*Meristogenys*)		*Amolops* (*Meristogenys*)	*Meristogenys*	*Meristogenys*
cf. orphnocnemis		*Amolops* (*Meristogenys*)		*Amolops* (*Meristogenys*)	*Meristogenys*	*Meristogenys*
lifanensis	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops*
loloensis	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops*
mantzorum	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops*
ricketti	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops* (*Amolops*)	*Amolops*	*Amolops*
chapaensis		*Amolops* (*Amolops*)		*Amolops* (*Amolops*)	*Huia*	*Odorrana*
nasica		*Amolops* (*Huia*)		*Amolops* (*Huia*)	*Huia*	*Odorrana*
	Raninae					
		Rana				
		Section *Pseudorana*				
sauteri	*Pseudorana*	*Rana* (*Pseudorana*)	*Pseudoamolops*	*Pseudoamolops*	*Rana*	*Rana*
			Raninae			
				Rana		
				Section *Pseudorana*		
weiningensis	*Pseudorana*	*Rana* (*Pseudorana*)	*Pseudorana*	*Rana* (*Pseudorana*)	*Rana*	*Pseudorana*
zhengi			*Pseudorana*		*Rana*	*Rana*
		Section *Rana*			*Rana*	*Rana*
amurensis	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana*
chaochiaoensis	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana*
chensinensis	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana*
huanrenensis	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana*
kunyuensis	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana*
temporaria		*Rana* (*Rana*)		*Rana* (*Rana*)	*Rana*	*Rana*
zhenhaiensis	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana* (*Rana*)	*Rana*	*Rana*
sylvatica (*us*)		*Rana* (*Rana*)		*Rana* (*Rana*)	*Lithobates*	*Lithobates*
		Section *Hylarana*		**Section *Hylarana***		
curtipes		*Rana* (*Clinotarsus*)		*Rana* (*Clinotarsus*)	*Clinotarsus*	*Clinotarsus*
galamensis		*Rana* (*Hydrophylax*)		*Rana* (*Hydrophylax*)	*Hydrophylax*	*Hylarana*
chalconota (*us*)		*Rana* (*Chalcorana*)		*Rana* (*Chalcorana*)	*Hydrophylax*	*Hylarana*
signata		*Rana* (*Pulchrana*)		*Rana* (*Pulchrana*)	*Pulchrana*	*Hylarana*
erythraea		*Rana* (*Hylarana*)		*Rana* (*Hylarana*)	*Hylarana*	*Hylarana*
macrodactyla	*Hylarana* (*Tenuirana*)	*Rana* (*Hylarana*)	*Hylarana* (*Tenuirana*)	*Rana* (*Hylarana*)	*Hylarana*	*Hylarana*
taipehensis	*Hylarana* (*Tenuirana*)	*Rana* (*Hylarana*)	*Hylarana* (*Tenuirana*)	*Rana* (*Hylarana*)	*Hylarana*	*Hylarana*
guentheri	*Hylarana* (*Hylarana*)	*Rana* (*Sylvirana*)	*Hylarana* (*Hylarana*)	*Rana* (*Sylvirana*)	*Sylvirana*[a]	*Hylarana*
spinulosa	*Hylarana* (*Hylarana*)	*Rana* (*Sylvirana*)	*Hylarana* (*Hylarana*)	*Rana* (*Sylvirana*)	*Sylvirana*	*Hylarana*
temporalis		*Rana* (*Sylvirana*)		*Rana* (*Sylvirana*)	*Sylvirana*	*Hylarana*
nigrovittata	*Hylarana* (*Hylarana*)	*Rana* (*Sylvirana*)	*Hylarana* (*Hylarana*)	*Rana* (*Sylvirana*)	*Sylvirana*	*Hylarana*
maosonensis		*Rana* (*Sylvirana*)		*Rana* (*Sylvirana*)	*Sylvirana*	*Hylarana*
daemeli		*Rana* (*Papurana*)		*Rana* (*Papurana*)	*Sylvirana*	*Hylarana*
livida	*Odorrana*	*Rana* (*Eburana*)	*Odorrana*	*Rana* (*Eburana*)	*Huia*	*Odorrana*
andersonii	*Odorrana*	*Rana* (*Odorrana*)	*Odorrana*	*Rana* (*Odorrana*)	*Huia*	*Odorrana*
grahami	*Odorrana*	*Rana* (*Odorrana*)	*Odorrana*	*Rana* (*Odorrana*)	*Huia*	*Odorrana*
hejiangensis	*Odorrana*	*Rana* (*Odorrana*)	*Odorrana*	*Rana* (*Odorrana*)	*Huia*	*Odorrana*
margaretae	*Odorrana*	*Rana* (*Odorrana*)	*Odorrana*	*Rana* (*Odorrana*)	*Huia*	*Odorrana*
schmackeri	*Odorrana*	*Rana* (*Odorrana*)	*Odorrana*	*Rana* (*Odorrana*)	*Huia*	*Odorrana*
versabilis	*Odorrana*	*Rana* (*Odorrana*)	*Odorrana*	*Rana* (*Odorrana*)	*Huia*	*Odorrana*
minima	*Glandirana*	*Rana* (*Glandirana*)	*Glandirana*	*Rana* (*Glandirana*)	*Glandirana*	*Glandirana*
		Section *Pelophylax*		**Section *Pelophylax***		
emeljanovi	*Rugosa*	*Rana* (*Rugosa*)	*Rugosa*	*Rana* (*Rugosa*)	*Glandirana*	*Glandirana*
tientaiensis	*Rugosa*	*Rana* (*Rugosa*)	*Rugosa*	*Rana* (*Rugosa*)	*Glandirana*	*Glandirana*
adenopleura	*Hylarana* (*Hylarana*)	*Rana* (*Nidirana*)	*Hylarana* (*Nidirana*)	*Rana* (*Nidirana*)	*Babina*	*Babina*
pleuraden	*Pelophylax*	*Rana* (*Nidirana*)	*Pelophylax*	*Rana* (*Nidirana*)	*Babina*	*Babina*
nigromaculata	*Pelophylax*	*Rana* (*Pelophylax*)	*Pelophylax*	*Rana* (*Pelophylax*)	*Pelophylax*	*Pelophylax*
lessonae		*Rana* (*Pelophylax*)		*Rana* (*Pelophylax*)	*Pelophylax*	*Pelophylax*
shuchinae	*Pelophylax*	*Rana* (*Pelophylax*)	*Pelophylax*	*Rana* (*Pelophylax*)	*Pelophylax*	*Rana*
heckscheri		*Rana* (*Aquarana*)		*Rana* (*Aquarana*)	*Lithobates*	*Lithobates*
catesbeiana (*us*)		*Rana* (*Aquarana*)		*Rana* (*Aquarana*)	*Lithobates*	*Lithobates*

(*continued on next page*)

Table 1 (*continued*)

Specific epithet	Fei et al. (1990)	Dubois (1992)	Fei et al. (2005)	Dubois (2005)	Frost (2006)	Present study
chiricahuensis		*Rana* (*Pantherana*)		*Rana* (*Pantherana*)	*Lithobates*	*Lithobates*
sphenocephala (*us*)		*Rana* (*Pantherana*)		*Rana* (*Pantherana*)	*Lithobates*	*Lithobates*
		Section*Lithobates*		**Section*Lithobates***		
palmipes		*Rana* (*Lithobates*)		*Rana* (*Lithobates*)	*Lithobates*	*Lithobates*
warszewitschii		*Rana* (*Trypheropsis*)		*Rana* (*Trypheropsis*)	*Lithobates*	*Lithobates*
maculata (*us*)		*Rana* (*Sierrana*)		*Rana* (*Sierrana*)	*Lithobates*	*Lithobates*
		Section*Amerana*		**Section*Amerana***		
muscosa		*Rana* (*Amerana*)		*Rana* (*Amerana*)	*Rana*	*Rana*
aurora		*Rana* (*Aurorana*)		*Rana* (*Aurorana*)	*Rana*	*Rana*

[a] *Sylvirana* should be changed into *Hylarana* (Frost, personal communication).

distance as a measure of detecting substitution saturation using DAMBE program (Xia, 2000). To examine possible incongruence between genes and gene combinations (tyrosinase + rhodopsin; mitochondrial DNA + tyrosinase + rhodopsin), we used an incongruence length difference (ILD) test (Farris et al., 1994, 1995) referred to as a partition homogeneity test in PAUP 4.0b10a (Swofford, 2003). We implemented 1000 replicates of the ILD test with 10 random addition sequences. ILD test presented no evidence for phylogenetic conflict between rhodopsin and tyrosinase gene partitions ($P = 0.789$) or among mitochondrial DNA, tyrosinase, and rhodopsin gene ($P = 0.773$).

The separate and combined dataset were both used to calculate maximum parsimony (MP) phylogenies using PAUP 4.0b10a (Swofford, 2003) and Bayesian inference (BI) with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Heuristic MP Searches were executed in 1000 replicates with all characters unordered and equally weighted, and using tree bisection reconnection (TBR) branch swapping. Bootstrap branch support (BBP) values were calculated with 100 MP replicates. The Bayesian posterior probabilities (BPP) used models estimated with Modeltest 3.06 (Posada and Crandall, 1998) under the Akaike Information Criterion (AIC) criterion, performing two separate runs with four Markov chains. Each run was conducted with 3,000,000 generations and sampled every 100 generations. When the log-likelihood scores were found to stabilize, a consensus tree was calculated after omitting the first 25% trees as burn-in. For the combined Bayesian analyses (tyrosinase + rhodopsin; mitochondrial DNA + tyrosinase + rhodopsin), the data were separated into different partitions, each following its own model.

Due to the limitation of time, ML analysis was only confined to the final combined data (mtDNA + tyrosinase + rhodopsin). The best fitting models of sequence ML analyses were determined by AIC in Modeltest. Heuristic Searches were executed in 10 replicates with all characters unordered and equally weighted, and using tree bisection reconnection (TBR) branch swapping. Bootstrap branch support (BBP) values were calculated with 10 ML replicates. In addition to BBP and BPP, Partitioned Bremer support analysis (PBS) was also conducted with the program TreeRot.v2 (Sorenson, 1999) to measure the respective contribution of each gene partition (mtDNA + tyrosinase + rhodopsin) toward the total Bremer support for nodes of multigene-based tree topology.

3. Results

3.1. Sequence characteristics

All sequences are deposited in GenBank (Accession Nos. are shown in Table 2). For the two nuclear genes, the result is about 316 bp region of coding sequence from the rhodopsin gene exon 1 and 521 bp from the tyrosinase gene exon 1. No indels was found in them. After the alignment, a total of 1242 base pairs of 12S gene were resolved. Four regions with 625 base pairs in total (1–475, 638–676, 866–914, and 1058–1119) were removed from further analysis due to ambiguous alignment and missing data at each end of section. Among the remaining 617 base pairs, 335 were variable and 261 were parsimony informative. The alignment of 16S gene sequences produced 578 base pairs in length, of which three regions with 100 base pairs in total (1–21, 248–304, and 557–578) were removed from further analyses due to ambiguous alignment and missing data at each end of section. Among the remaining 478 base pairs, 228 were variable and 184 were parsimony informative. Table 2 shows all the data used in this study, including those from Genbank. Transitions and transversions in the case of the four genes were accumulating linearly and gave no indication of saturation effect (data not shown), thus all substitutions in these genes were used for phylogenetic reconstructions.

3.2. Phylogenetic analyses of different genes and gene combinations

3.2.1. Mitochondrial genes

Maximum parsimony analysis resulted in eight equally most parsimonious trees, with 3428 steps, a consistency index (CI) of 0.262 and a retention index (RI) of 0.514. Fig. 1 shows the strict consensus tree. Bootstrap analyses revealed low nodal supports; only 32 nodes receive BBP greater than 70. The 50% majority consensus tree from the Bayesian analysis was not completely consistent with the MP tree (Fig. 1). Notably, all these topological differences were weakly supported (BBP < 50, BPP < 90). Bayesian

Table 2
Samples and sequences used in this study

Subfamily	Current genus and species name	Locality	Specimen voucher no.	Rhodopsingene	Tyrosinasegene	12S rRNA gene	16S rRNA gene
Ingroup							
Raninae	*Amolops* (*Amolops*) *cf. ricketti*	Vietnam	VUB 0701	AY322231	AY322352	AY322326	AY322286
	Amolops (*Amolops*) *chapaensis*	Vietnam	AMNH A161439	DQ283992	DQ282984	DQ283372	
	Amolops (*Amolops*) *lifanensis*	China: Lixian, Sichuan	SCUM0405177CJ	DQ360034[a]	DQ360065[a]	DQ359981[a]	DQ360003[a]
	Amolops (*Amolops*) *loloensis*	China: Xichang, Sichuan	SCUM0405178CJ	DQ360012[a]	DQ360043[a]	DQ359959[a]	DQ359990[a]
	Amolops (*Amolops*) *mantzorum*	China: Maoxian, Sichuan	SCUM030014GP	DQ360023[a]	DQ360054[a]	DQ359970[a]	DQ360000[a]
	Amolops (*Amolops*) *ricketti*	China: Hejiang, Sichuan	SCUM0405181CJ	DQ360009[a]	DQ360040[a]	DQ359956[a]	DQ359987[a]
	Amolops (*Huia*) *nasica*	Vietnam	AMNH A161169	DQ283971	DQ282970	DQ283345	
	Amolops (*Meristogenys*) *cf. orphocnemis*	Borneo	VUB0630	AY322222	AY322358	AY322319	AY322291
	Amolops (*Meristogenys*) *kinabaluensis*	Borneo	VUB 0627	AY322233	AY322357	AY322317	AY322292
	Rana (*Amerana*) *muscosa*	USA	BY	DQ283877	DQ282945	DQ283190	
	Rana (*Aquarana*) *catesbeiana*	China: Chengdu, Sichuan	SCUM0405176CJ	DQ360013[a]	DQ360044[a]	DQ359960[a]	DQ289127
	Rana (*Aquarana*) *heckscheri*	USA	AMCC 125635	DQ283878	DQ282946	DQ283191	
	Rana (*Aurorana*) *aurora*	USA	ARBT018	DQ283876	DQ282944	DQ283189	
	Rana (*Chalcorana*) *chalconota*	Borneo	VUB 0610	AY322232	AY322341	AY322313	AY322293
	Rana (*Clinotarsus*) *curtipes*	India	GenBank	AF249117	AF249180	AF249021	AF249058
	Rana (*Eburana*) *livida*	China: Hejiang, Sichuan	SCUM0405182CJ	DQ360022[a]	DQ360053[a]	DQ359969[a]	DQ359999[a]
		Vietnam	VUB 0711	AY322220	AY322353	AY322322	AY322285
	Rana (*Glandirana*) *minima*	China: Fuzhou, Fujian	CIB-HUI040003	DQ360021[a]	DQ360052[a]	DQ359968[a]	DQ359998[a]
	Rana (*Hydrophylax*) *galamensis*	Kenya	CAS 214840	AY322238	AY322337	AY322331	AY322303
	Rana (*Hylarana*) *macrodactyla*	China: Wenchang, Hainan	SCUMH004	DQ360025[a]	DQ360056[a]	DQ359972[a]	DQ360002[a]
	Rana (*Hylarana*) *erythraea*	Borneo	VUB 0610	AY322228	AY322356	AY322323	AY322294
	Rana (*Hylarana*) *taipehensis*	China: Sanya, Hainan	SCUMH019	DQ360036[a]	DQ360067[a]	DQ359983[a]	DQ360005[a]
	Rana (*Lithobates*) *palmipes*	Guyana	AMNH A166454	DQ284001	DQ282994	DQ283384	
	Rana (*Nidirana*) *adenopleura*	China: Mt. Omei, Sichuan	CIB-WU37990	DQ360010[a]	DQ360041[a]	DQ359957[a]	DQ359988[a]
	Rana (*Nidirana*) *pleuraden*	China: Kunming, Yunnan	SCUM0405185CJ	DQ360011[a]	DQ360042[a]	DQ359958[a]	DQ359989[a]
	Rana (*Odorrana*) *andersonii*	China: Jingdong, Yunnan	KIZ-RD02YNJD01	DQ360018[a]	DQ360049[a]	DQ359965[a]	DQ359995[a]
	Rana (*Odorrana*) *grahami*	China: Xichang, Sichuan	SCUM0405186CJ	DQ360016[a]	DQ360047[a]	DQ359963[a]	DQ359993[a]
	Rana (*Odorrana*) *hejiangensis*	China: Hejiang, Sichuan	SCUM0405180CJ	DQ360037[a]	DQ360068[a]	DQ359984[a]	DQ360006[a]
	Rana (*Odorrana*) *margaretae*	China: Mt. Omei, Sichuan	SCUM0403026CJ	DQ360017[a]	DQ360048[a]	DQ359964[a]	DQ359994[a]
	Rana (*Odorrana*) *schmackeri*	China: Mt. Omei, Sichuan	CIB-WU37943	DQ360020[a]	DQ360051[a]	DQ359967[a]	DQ359997[a]
	Rana (*Odorrana*) *versabilis*	China: Mt. Limu, Hainan	HNNU-A0019L	DQ360015[a]	DQ360046[a]	DQ359962[a]	DQ359992[a]
	Rana (*Pantherana*) *chiricahuensis*	USA	ASC 33310	DQ283934	DQ282962	DQ283270	
	Rana (*Pantherana*) *sphenocephala*	USA	VUB 0558	AY322223	AY322345	AY322312	AY322297
	Rana (*Papurana*) *daemeli*	Australia	SAMA R40355	DQ283884	DQ282948	DQ283201	
	Rana (*Pelophylax*) *lessonae*	Belgium	VUB 0940	AY322243	AY322347	AY322321	AY322276
	Rana (*Pelophylax*) *nigromaculata*	China	NJNU F97072	AY322241	AY322363	AY322305	AY322278
		China: Hongya, Sichuan	SCUM045199CJ	DQ360014[a]	DQ360045[a]	DQ359961[a]	DQ359991[a]
	Rana (*Pelophylax*) *shuchinae*	China: Xichang, Sichuan	CIB-HUI040009	DQ360026[a]	DQ360057[a]	DQ359973[a]	DQ289126
	Rana (*Pseudorana*) *sauteri*	China: Kaohsiung, Taiwan	SCUM0405175CJ	DQ360029[a]	DQ360060[a]	DQ359976[a]	DQ289109
	Rana (*Pseudorana*) *weiningensis*	China: Huili, Sichuan	SCUM0405174CJ	DQ360008[a]	DQ360039[a]	DQ359955[a]	DQ359986[a]
		China: Kunming, Yunnan	KIZ-RD05KMWN01	DQ360019[a]	DQ360050[a]	DQ359966[a]	DQ359996[a]
	Rana (*Pseudorana*) *zhengi*	China: Hongya, Sichuan	SCUM0405190CJ	DQ360027[a]	DQ360058[a]	DQ359974[a]	DQ289103
	Rana (*Pulchrana*) *signata*	Borneo	VUB 0606	AY322237	AY322354	AY322316	AY322296
	Rana (*Rana*) *amurensis*	China: Heilongjiang	SYNU040003	DQ360032[a]	DQ360063[a]	DQ359979[a]	DQ289110
	Rana (*Rana*) *chaochiaoensis*	China: Zhaojue, Sichuan	SCUM0405170CJ	DQ360028[a]	DQ360059[a]	DQ359975[a]	DQ289107

(*continued on next page*)

Table 2 (*continued*)

Subfamily	Current genus and species name	Locality	Specimen voucher no.	Rhodopsingene	Tyrosinasegene	12S rRNAgene	16S rRNAgene
	Rana (*Rana*) *chensinensis*	China: Qinling, Shaanxi	KIZ-RD05SHX001	DQ360030[a]	DQ360061[a]	DQ359977[a]	DQ289119
	Rana (*Rana*) *huanrenensis*	China: Liaoning	SYNU040006	DQ360031[a]	DQ360062[a]	DQ359978[a]	DQ289122
	Rana (*Rana*) *sylvatica*	USA	AMCC 108286	DQ284004	DQ282996	DQ283387	
	Rana (*Rana*) *kunyuensis*	China: Mt. Kunyu, Shandong	CIB-HUI040001	DQ360033[a]	DQ360064[a]	DQ359980[a]	DQ289111
	Rana (*Rana*) *temporaria*	Belgium	GenBank	AF249119	AF249182	AF249023	AF249048
	Rana (*Rana*) *zhenhaiensis*	China	NJNU F97004	AY322217	AY322346	AY322318	AY322279
	Rana (*Rugosa*) *emeljanovi*	China	NJNU 980073	AY322218	AY322362	AY322320	AY322281
	Rana (*Rugosa*) *tientaiensis*	China: Mt. Huang, Anhui	SCUM0405192CJ	DQ360007[a]	DQ360038[a]	DQ359954[a]	DQ359985[a]
	Rana (*Sierrana*) *maculata*	Honduras	USNM 559483	DQ283951	DQ282962	DQ283303	
	Rana (*Sylvirana*) *guentheri*	China: Sanya, Hainan	SCUMH002	DQ360024[a]	DQ360055[a]	DQ359971[a]	DQ360001[a]
		Vietnam	VUB 0693	AY322216	AY322351	AY322325	AY322287
	Rana (*Sylvirana*) *maosonensis*	Vietnam	AMNH A161487	DQ283993	DQ282985	DQ283373	
	Rana (*Sylvirana*) *nigrovittata*	China	VUB 0749	AY322242	AY322348	AY322324	AY322277
	Rana (*Sylvirana*) *spinulosa*	China: Mt. Limu, Hainan	SCUMH010	DQ360035[a]	DQ360066[a]	DQ359982[a]	DQ360004[a]
	Rana (*Sylvirana*) *temporalis*	India	GenBank	AF249118	AF249181	AF249022	AF249054
	Rana (*Trypheropsis*) *warszewitschii*	Panama	KRL823	DQ283925	DQ282958	DQ283256	
	Staurois latopalmatus	Borneo	VUB 0652	AY322239	AY322359	AY322327	AY322290
Outgroup							
Dicroglossinae	*Occidozyga laevis*	Philippines	TNHC (DLSUD002)	AY322227	AY322342	AY322329	AY322300
	Quasipaa boulengeri	China	NJNU F96030	AY322240	AY322349	AY322311	AY322280
Lankanectinae	*Lankanectes corrugatus*	Sri Lanka	GenBank	AF249115	AF249178	AF249019	AF249043
Mantellinae	*Boophis tephraeomystax*	Madagascar	GenBank	AF249105	AF249168	AF249009	AF249039
	Laliostoma labrosa	Madagascar	GenBank	AF249106	AF249169	AF249010	AF249037
	Mantella madagascariensis	Madagascar	GenBank	AF249101	AF249164	AF249005	AF249049
Nyctibatrachinae	*Nyctibatrachus major*	India	GenBank	AF249113	AF249176	AF249017	AF249052
Rhacophorinae	*Philautus charius*	India	GenBank	AF249128	AF249191	AF249032	AF249062
	Rhacophorus malabaricus	India	GenBank	AF249125	AF249188	AF249029	AF249050

[a] Sequences new to this study. CIB, Chengdu Institute of Biology, the Chinese Academy of Sciences; HNNU, Hainan Normal University; KIZ, Kunming Institute of Zoology, the Chinese Academy of Sciences; SCUM, Sichuan University Museum; SYNU, Shenyang Normal University.

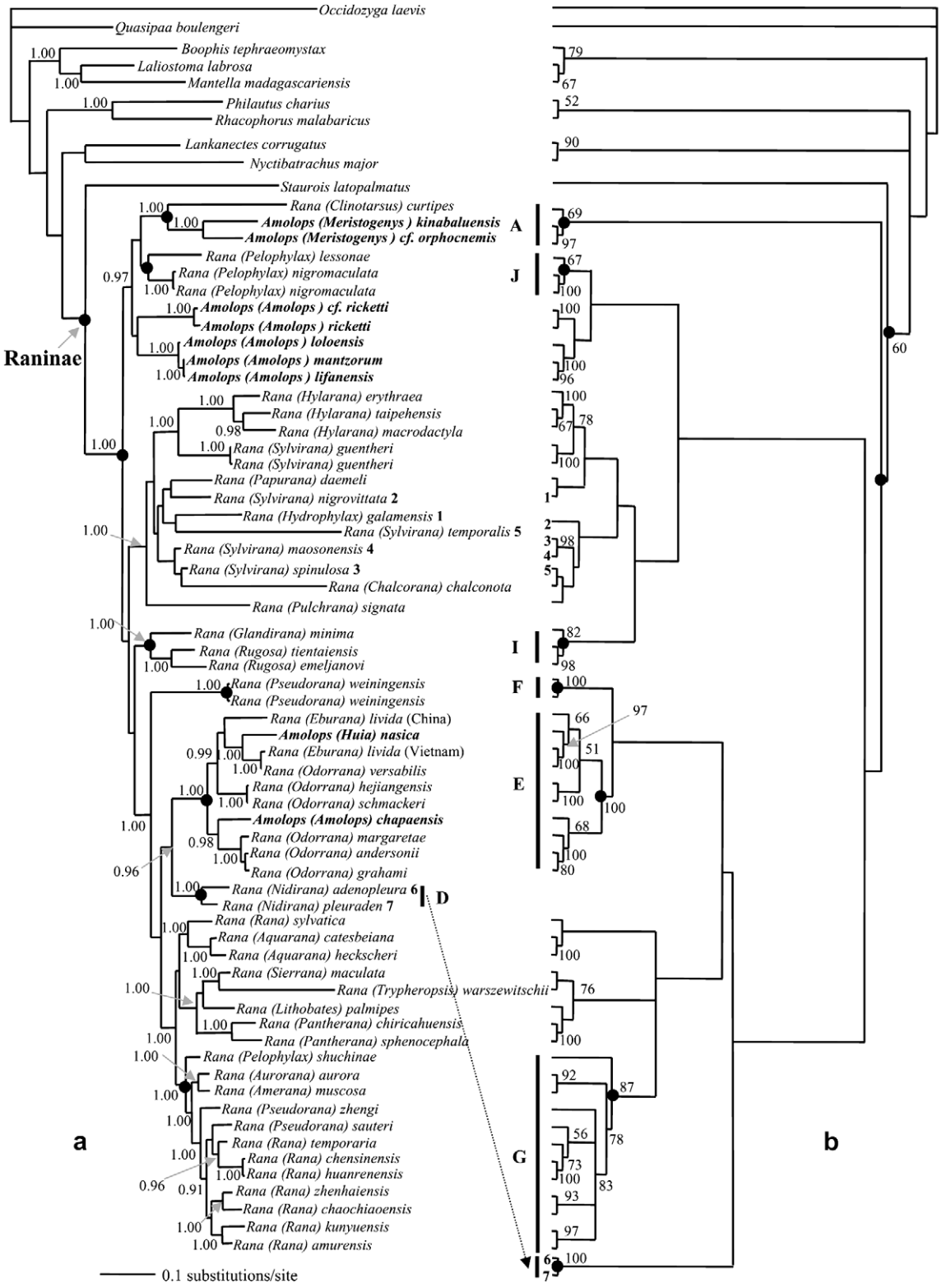

Fig. 1. The phylogenetic hypothesis derived from partial DNA sequences of the mitochondrial gene 12S and 16S. (a) The 50% majority rule consensus from the Bayesian analysis. The numbers above the lines or beside the nodes are Bayesian posterior probabilities ($\geqslant$90% retained). The branch lengths are shown to scale. (b) The strict consensus tree from the parsimony analysis. Numbers above the lines or beside the nodes are bootstrap proportions calculated with 100 replicates ($\geqslant$50% retained). The black circles correspond to the clade assignments as Figs. 2, 3. Species assigned to *Amolops* proposed by Dubois (1992) are shown by bold face as Fig. 2 and 3. 1–7 represent seven species taxa (1, *Rana galamensis*; 2, *Rana nigrovittata*; 3, *Rana spinulosa*; 4, *Rana maosonensis*; 5, *Rana temporalis*; 6, *Rana adenopleura*; 7, *Rana pleuraden*).

analysis revealed a topology with a higher nodal support. There are 43 nodes receiving BPP greater than 90.

3.2.2. Nuclear genes (tyrosinase + rhodopsin)

For rhodopsin, due to the limited sequence numbers, the phylogenetic relationships in general were poorly resolved. ILD test presented no evidence for phylogenetic conflict between two nuclear gene partitions ($P = 0.789$), then an alternative combined dataset was constructed for phylogenetic inferences (Fig. 2). Maximum parsimony analysis of the combined data of tyrosinase and rhodopsin resulted in 10045 most parsimonious trees, each with 1030 steps, a CI of 0.440, and RI of 0.612. The strict consensus tree is shown in Fig. 2. Bootstrap analysis revealed relatively low nodal support; only 37 nodes receive BBP greater than 70 (Fig. 2). The 50% majority rule consensus tree inferred from the Bayesian analysis essentially was compatible with the result of the parsimony analysis (Fig. 2). Topological differences between the Bayesian tree and the parsimony tree were merely in those nodes with weak resolutions (BBP < 50, BPP < 90). The Bayesian posterior probabilities (BPP) offered a slightly higher nodal support in comparison with the bootstrapping. There are 44 nodes received BPP greater than 90.

3.2.3. Concatenated data (mitochondrial DNA + tyrosinase + rhodopsin)

The partition homogeneity test revealed no conflict among mtDNA, tyrosinase, and rhodopsin gene ($P = 0.773$). Therefore, we performed a separate, alternative analysis of the final concatenated data. Parsimony analysis using equal weights only resulted in a single MPT (length, 4495; CI = 0.300, RI = 0.531), which is shown in Fig. 3. There are 42 MP nodes receiving BBP greater than 70. Bayesian inference and ML essentially were consistent with the MP tree. Incongruent nodes still were the basal ones, which received weak supports (BBP < 50, BPP < 90). In Bayesian analysis, 59 nodes receive BPP greater than 90.

Clearly, comparisons between the mtDNA (Fig. 1) and nuDNA (Fig. 2) findings suggested that our final combined data (Fig. 3) provides higher resolution than separate analyses. In the end we chose the phylogeny from the combined data as our preferred phylogenetic hypothesis. Here we reported the result of Fig. 3.

Monophyly of Raninae received strong supports (BPP = 100, BBP = 100, 100, PBS = 19). All phylogenetic analyses are consistent with recognizing *Staurois latopalmatus* (tribe Stauroini) as sister to the well-supported Ranini clade of the remaining taxa (BPP = 100, BBP = 90, 88, PBS = 21). In Ranini, *Amolops* (sensu lato: Dubois, 1992) was recovered as polyphyletic. The monophyly of the genus *Rana* (sensu stricto: Dubois, 1992) was also not well resolved (Fig. 3). However, ten major supported clades among Ranini (A, B, C, D, E, F, G, H, I, and J) were all recovered by Bayesian, ML and MP analyses (Fig. 3).

Unsurprisingly, species of subgenera *Meristogenys* and *Rana curtipes* constituted clade A (BPP = 100, BBP = 100, 98, PBS = 15), because we used the homologous sequences as Roelants et al. (2004). Monophyly of the subgenus *Amolops* (clade B), consisting of two well-supported subclades, was also supported (BPP = 100, BBP = 80, 76, PBS = 6). Clade C (BPP = 100, BBP = 70, 69, PBS = 11) contained the representatives of the subgenera *Hylarana*, *Sylvirana* (as one polyphyletic taxon), *Papurana*, *Chalcorana*, *Hydrophylax*, and *Pulchrana* with unresolved relationships. Among clade C, subclade C-1 received good nodal supports (BPP = 100, BBP = 100, 82, PBS = 11). Moreover, *Rana adenopleura* and *Rana pleuraden* constituted one strongly supported clade D (BPP = 100, BBP = 100, 100, PBS = 21). In our results, subgenera *Odorrana* and *Eburana* were treated as clade E, along with *Amolops* (*A.*) *chapaensis* and *Amolops* (*Huia*) *nasica*, with strong supports (BPP = 100, BBP = 100, 100, PBS = 22). The relationships among the three subclades (E-1, E-2, and E-3) were completely identical among parsimony and likelihood analyses. Clade F was only composed of *Rana weiningensis*. *Rana shuchinae* appearing in clade G (Fig. 3: BPP = 100, BBP = 100, 99, PBS = 8) was the earliest diverged taxa, then followed by the subclade NWII, including *Rana aurora* and *Rana muscosa* (BPP = 100, BBP = 100, 100, PBS = 11), and the monophyly of *Rana zhengi* and the brown frogs (BPP = 100, BBP = 100, 98, PBS = 9). *Rana sauteri* was well nested within the brown frogs. Clade H (NWI), another clade from the New World, included representatives of subgenera *Aquarana*, *Rana*, *Lithobates*, *Sierrana*, *Trypheropsis*, and *Pantherana* with strong nodal supports (BPP = 94, BBP = 100, 90, PBS = 6). A close relationship between the subgenus *Rugosa* and the monotypic subgenus, e.g., *Rana minima* was well supported (clade I: BPP = 100, BBP = 80, 90, PBS = 8). Clade J (BPP = 97, BBP = 84, PBS = 10) only included *Rana lessonae* and *Rana nigromaculata*.

4. Discussion

4.1. Phylogeny of Amolops sensu lato (Dubois, 1992)

Clearly, the nominal *Amolops* sensu lato (Dubois, 1992) is polyphyletic (Fig. 3). Yang (1991a) divided *Amolops* into three distinct genera (*Amolops* Cope, 1865; *Huia* Yang, 1991; *Meristogenys* Yang, 1991), and later Yang (1991b) proposed the subfamily Amolopinae within Ranidae, which has been accepted by Chinese herpetologists (Fei, 1999; Fei et al., 1990, 2005). However, Dubois (1992) merely retained the three genera proposed by Yang (1991a) as subgenera within one genus *Amolops*, along with subgenus *Amo*. Largely following the idea of Fei et al. (2000), Dubois (2005) accepted the genus *Pseudoamolops* with the type species as *R. sauteri* and placed it within the Raninae. Our present phylogeny, combined with Che et al. (2006), Matsui et al. (2006), and Tanaka-Ueno et al. (1998), distinctly suggests that *R. sauteri* is part of the brown frogs, and therefore *Pseudoamolops* is invalid. Matsui et al. (2006) questioned the monophyly of either Amolopinae or *Amolops* sensu lato based on the mtDNA data. Our data support this view. Except for *A.* (*A.*)

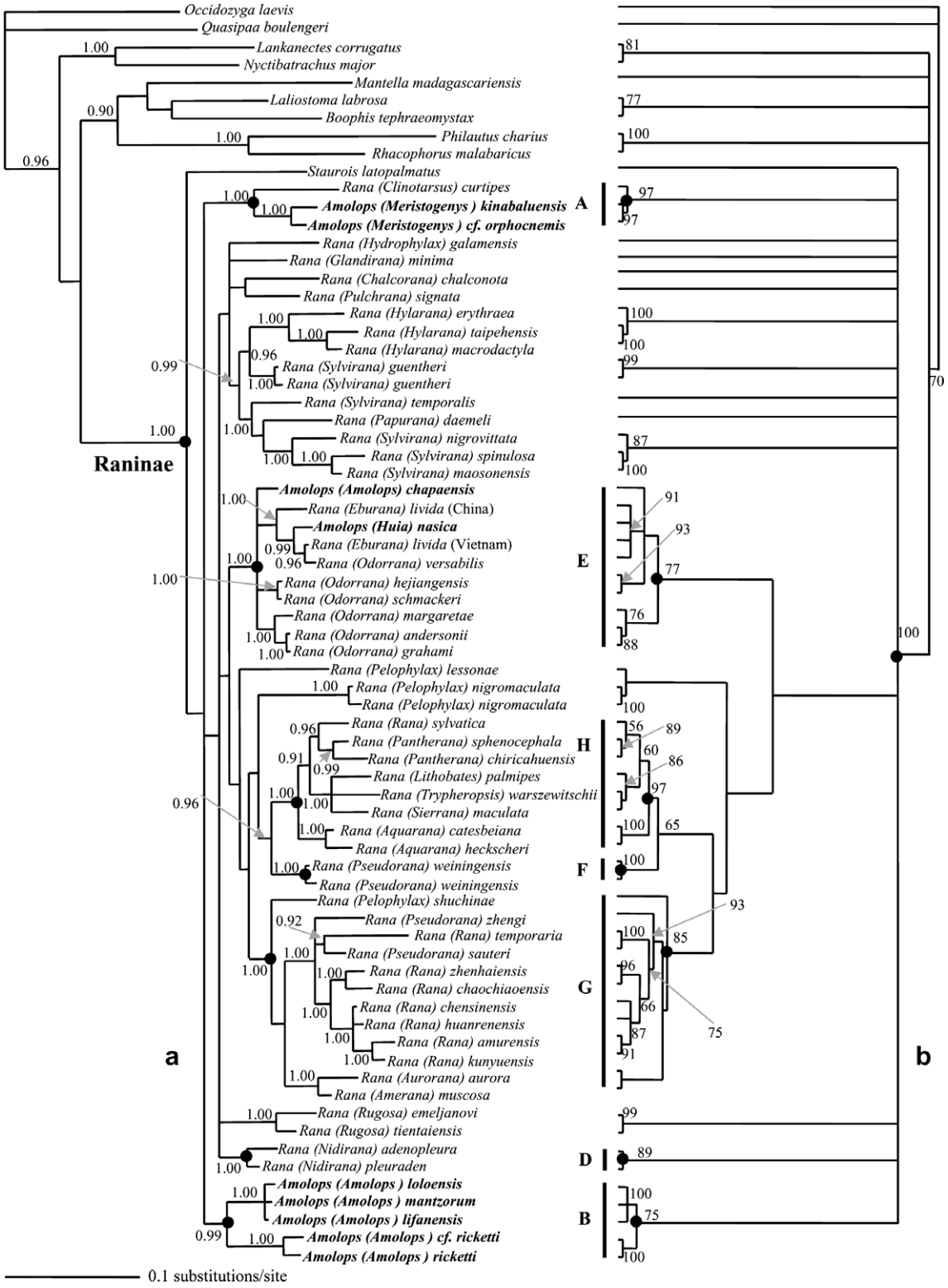

Fig. 2. The phylogenetic hypothesis derived from partial DNA sequences of the nuclear gene tyrosinase and rhodopsin. (a) The 50% majority rule consensus from the Bayesian analysis. The numbers above the lines or beside the nodes are Bayesian posterior probabilities ($\geqslant$90% retained). The branch lengths are shown to scale. (b) The strict consensus tree from the parsimony analysis. Numbers above the lines or beside the nodes are bootstrap proportions calculated with 100 replicates ($\geqslant$50% retained).

chapaensis and *A.* (*H.*) *nasica* which are well nested in clade E (Fig. 3), as indicated by Frost et al. (2006), *R. curtipes* and two *Meristogenys* species, and five *Amolops* species distinctly constituted clade A and B in the present study, respectively. Monophyly of *Meristogenys* is supported, as already suggested by Frost et al. (2006), Matsui et al. (2006), and

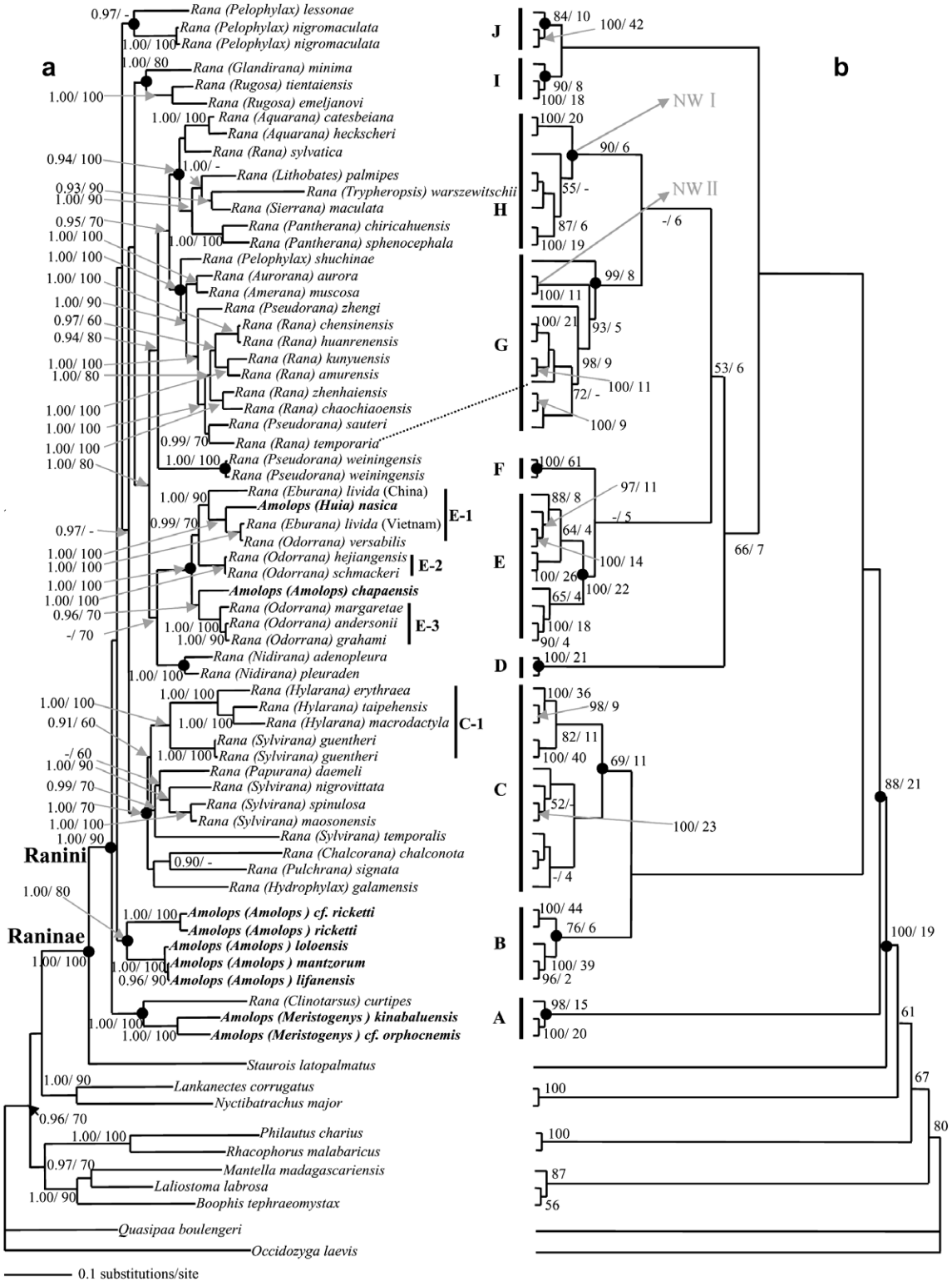

Fig. 3. The phylogenetic hypothesis derived from the combined gene fragments (mtDNA + tyrosinase + rhodopsin). (a) The 50% majority rule consensus from the Bayesian analysis. Posterior probabilities (⩾90% retained) and ML bootstrap proportions calculated with 10 replicates (⩾50% retained) are shown beside the branches (BPP/BBP). The branch lengths are shown to scale. (b) The single strict tree from the parsimony analysis. Numbers above the lines or beside the nodes are bootstrap proportions calculated with 100 replicates (⩾50% retained) and the partitioned Bremer support (BBP/PBS) analyses to each node.

Roelants et al. (2004). However, the phylogenetic relationships among *Amolops*, *Huia*, and *Meristogenys* are still poorly known based on the mtDNA evidence (Matsui et al., 2006). For example, Matsui et al. (2006) recognized two remote lineages of *Amolops* within China (e.g., lineage of South-western China and Southern China). To the contrary,

the representatives of the above two lineages in our study constituted one monophyletic group (Fig. 3: clade B). In this regard, our nuclear data (Fig. 2) has one higher nodal support to the clade B than the mtDNA data (Fig. 1). Furthermore, the combined analyses of nuclear and mtDNA (Bossuyt et al., 2006) indicated the close relationship between *Huia* and *Meristogenys*. Obviously, additional resolution should be afforded by sequencing more taxa (see Matsui et al., 2006) to better understand the nominal *Amolops* sensu lato (Dubois, 1992). Besides mtDNA, we suggest that more nuclear genes should be used in further study.

4.2. Phylogeny of *Rana* (Dubois, 1992, 2005)

Monophyly of *Rana* was not resolved (Fig. 3) based on either likelihood or parsimony analysis. Meanwhile, sections *Hylarana*, *Pelophylax*, *Rana*, *Pseudorana*, and *Lithobates* within *Rana* proposed by Dubois (1992) (Table 1) were all unwarranted. We used some sequences from Frost et al. (2006) and Roelants et al. (2004), here we only discussed those taxa related to our own data.

Dubois (1992) named the section *Hylarana*, which was further divided into two subsections, *Hydrophylax* and *Hylarana*. The monophyly of subsection *Hydrophylax*, including subgenera *Hydrophylax*, *Papurana*, *Pulchrana*, and *Sylvirana* was not supported (see clade C). Subgenera *Chalcorana*, *Hylarana*, *Eburana*, *Odorrana*, and *Glandirana* ascribing to subsection *Hylarana* were clearly polyphyletic and well nested in present three clades (Fig. 3: C, E, I). Matsui et al. (2005) indicated that the subsection *Hylarana* is problematic. After adding additional taxa, the present study further supports this view. Reporting only on Chinese frogs, Fei et al. (1990) established two subgenera (*Hylarana*, *Tenuirana*) within their genus *Hylarana* (Table 1). Subsequently, Fei et al. (2005) further recognized three subgenera and named *Nidirana* as the third subgenus (Table 1). Our present data contradict the division of Fei et al. (1990, 2005). The nominal subgenus *Hylarana* including *Rana guentheri*, *Rana nigrovittata*, and *Rana spinulosa* is paraphyletic with respect to the subgenus *Tenuirana* leading to *Rana macrodactyla* and *Rana taipehensis*. Furthermore, species of the nominal subgenus *Nidirana* proposed by Fei et al. (2005), e.g., *R. adenopleura* (within clade D) is remotely related to the taxa of subgenera *Hylarana* and *Tenuirana*.

Our present clade C included representatives of the genera *Hylarana*, *Hydrophylax*, *Sylvirana* and *Pulchrana* suggested by Frost (2006). Except for *Hylarana* (subclade C-1), monophylies of the genera *Hydrophylax* and *Sylvirana* proposed by Frost et al. (2006) were not recovered by our data. The type species *Rana signata* of *Pulchrana* (Frost, 2006) was also well nested in clade C. Regarding the present monophyly of clade C, our data was consistent with the result of Bossuyt et al. (2006). It is difficult to explain the incongruence between our data and Frost et al. (2006)'s, and the unresolved relationships among clade C in our analyses may be due to insufficient sampling. Therefore, more data are needed, including more DNA markers and species included in the genera *Hylarana*, *Hydrophylax*, *Sylvirana* and *Pulchrana* as suggested by Frost (2006).

Fei et al. (1990) erected *Odorrana*, and later Ye and Fei (2001) suggested four groups (*andersonii* group, *kuangwuensis* group, *schmacheri* group, and *livida* group) within *Odorrana* based on a morphological phylogenetic study. However, recently, Fei et al. (2005) (Table 1) further established two subgenera (*Odorrana*, *Bamburana*) within *Odorrana* and recognized *Odorrana versabilis* as type species of *Bamburana*. In our result, *O. versabilis* is well nested in subclade E-1 (Fig. 3: clade E), which indicates the invalidity of *Bamburana*. Thus, our present data are more consistent with the result of Ye and Fei (2001), not the new taxonomy as proposed by Fei et al. (2005). Except for *kuangwuensis* group (not available for our study), present Chinese species (subclades: E-1, E-2, and E-3) correspond well with the other three groups (*livida* group, *schmacheri* group, and *andersonii* group) as proposed by Ye and Fei (2001). Unsurprisingly, *Rana livida* from Vietnam was closer to *R. versabilis* from Hainan (China), than to the *R. livida* from Sichuan (China), because Bain et al. (2003) suggested Chinese *R. livida* should be *Rana chloronota*. Clearly, *R. livida* complex should be further revaluated. Furthermore, data here also rejected the validation of *Eburana* proposed by Dubois (1992), as already suggested by Matsui et al. (2005).

The section *Pelophylax* proposed by Dubois (1992), including the subgenera *Pelophylax* and *Rugosa*, was distinctly polyphyletic (Fig. 3). Two species of *Rugosa* and one species of *Glandirana* (Dubois, 1992: section *Hylarana*) constituted the monophyly as clade I, which was remotely related to other *Pelophylax* taxa consisting of *R. lessonae*, *R. nigromaculata* (clade J), as well as *R. shuchinae* (in clade G). Moreover, Fei et al. (1990) placed *R. pleuraden* in *Pelophylax*. However, data here clearly support the close relationships between *R. pleuraden* and *R. adenopleura* (clade D), which well correspond to *Nidirana* recognized by Dubois (1992).

Pseudorana was recognized by Fei et al. (1990) as one distinct genus including *R. sauteri*, *Rana sangzhiensis*, and *R. weiningensis*, which were placed in the subgenus *Pseudorana* (section *Pseudorana*) within *Rana* by Dubois (1992) (Table 1). However, *R. weiningensis*, the type species of *Pseudorana*, constituted one distinct clade F, which was remotely related to *R. sauteri* (Fig. 3). Other species assigned to *Pseudorana*, e.g., *Rana johnsi*, *R. sangzhiensis* need to be further evaluated.

Rana shuchinae is outside of the clade NWII from the New World and the group leading to *R. zhengi* and brown frogs (Fig. 3: clade G), then followed by another clade H from the New World (NWI), which has not been previously hypothesized. The two distinct clades of American frogs (Fig. 3: NWI, NWII) nesting within Eurasian species reveal their Eurasian origins, and two independent Eurasia to the New world dispersal events (Case, 1978) were supported based on present phylogenetic branching patterns. Alternatively, dispersal into the New Word and a back into Eurasia

as suggested by Bossuyt et al. (2006) and Macey et al. (2006) could be excluded.

4.3. Taxonomy implication

Taxonomy should reflect historical relationships, so these non-monophyletic genera, e.g., *Rana* and *Amolops*, as proposed by Dubois (1992, 2005) should be divided. Recently, Frost et al. (2006) proposed a new taxonomy of living amphibians based on the combined analyses of morphological and molecular data. "Ranidae" were placed in eleven "family-group" taxa to avoid paraphyly with regard to Rhacophoridae and Mantellidae, and accordingly, Raninae sensu Dubois (2005) was elevated to family status, viz., Ranidae sensu Frost (2006). As an alternative solution, in this regard, we prefer to accept the new taxonomy of Frost (2006).

Within Ranidae sensu Frost (2006), except for the genus *Staurois* Cope, 1865, our present results support some generic divisions: *Meristogenys* Yang, 1991 and *Clinotarsus* Mivart, 1869 (corresponding to clade A); *Amolops* Cope, 1865 (corresponding to clade B); *Babina* Thompson, 1912 (corresponding to clade D); *Rana* Linnaeus, 1758 (corresponding to clade G); *Lithobates* Fitzinger, 1843 (corresponding to clade H); *Glandirana* Fei et al., [1991 (1990)] (corresponding to clade I); *Pelophylax* Fitzinger, 1843 (corresponding to clade J). However, care must be taken to understand clades D and J. We do not have representatives from the subgenus *Babina* (Dubois, 1992), so our data neither supported nor rejected such idea of Frost et al. (2006) who transferred all members of Dubois' subgenus *Nidirana* to genus *Babina*. We also accepted *Pelophylax* Fitzinger, 1843 as the name of clade J as proposed by Frost et al. (2006), only including *R. lessonae* and *R. nigromaculata*, however, we must note that the present data do not include the type species of *Rana esculenta*.

Furthermore, data here preferred to propose three other genera, which contradicted the result of Frost (2006): *Hylarana* Tschudi, 1838 (corresponding to clade C); *Odorrana* Fei et al., [1991 (1990)] (corresponding to clade E); *Pseudorana* Fei et al., [1991 (1990)] (corresponding to clade F). Clearly, our *Hylarana* included representative species of the genera *Hylarana*, *Hydrophylax*, *Sylvirana* and *Pulchrana* suggested by Frost (2006), however, more data should be added to better elucidate the phylogenetic relationships. Frost et al. (2006) applied *Huia* Yang, 1991 as the generic name of present clade E, because *H. nasica* was well nested in the group including *Odorrana* and *Eburana*. However, we found the assigned name might be not appropriate. We used the sequences of species *Huia cavitympanum* Boulengeri, 1893 (type species of *Huia*) (see Matsui et al., 2006) from Genbank and found *Huia cavitympanum* was not closely related to the clade E including *Huia nasia* (not shown). Furthermore, *H. masoni* was shown to be more related to species of *Meristogenys* by Bossuyt et al. (2006). Thus, we suggest retaining *Odorrana* as the generic name for clade E. Certainly, the result still needs to be tested, and the position of *H. cavitympanum*, along with other species assigned to *Huia* was unresolved in Ranidae (Matsui et al., 2006). Furthermore, present data support *Pseudorana* Fei et al., [1991 (1990)] as the generic name of clade F. In conclusion, twelve genera within Ranidae sensu Frost (2006) are supported based on the present study (see Table 1).

Without question, the limits of Ranidae sensu Frost (2006) are far beyond the scope of this paper. More taxa from other regions and evidence from more additional molecular markers are still required to decisively evaluate the evolutionary history of Ranidae. Further generic definition among Ranidae must await a detailed phylogenetic study of these frogs.

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