Phylogenetic resolution and systematics of the Asian tree frogs, *Rhacophorus* (Rhacophoridae, Amphibia)

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The treefrog genus *Rhacophorus*, a large genus with 80 species, has a wide range, occurring eastward from India to China, Japan, South-east Asia, the Greater Sunda Islands and the Philippines. The phylogenetic relationships and taxonomic recognition of many species are very controversial. To stabilize the taxonomy, the phylogenetic relationships among about 52 species are investigated from 96 samples using mtDNA sequence data. Matrilineal relationships based on maximum likelihood and Bayesian inference methods resolve three well-supported lineages (A, B and C), although the phylogenetic relationships among three lineages remain ambiguous. Analyses support recognition of two previously assigned subgenera, *Leptomantis* and *Rhacophorus*, and these correspond to lineages A and B, respectively. Given that we have three strongly supported lineages, that these lineages are morphologically distinct, and the constrained geographic distributions of these groups, we recognize each lineage as a taxon. Subgenus *Leptomantis* includes species mainly from Malaysia, Indonesia and the Philippines. Subgenus *Rhacophorus* contains a mix of species occurring in India, Indochina and southern China. Lineage C accommodates species distributed mostly in East Asia, including Japan and China. Based on genetic and morphological data from type localities, the taxonomic recognition of some species needs to be reconsidered. *Rhacophorus pingbianensis* and *Polypedates spinus* are considered as junior synonyms of *Rhacophorus duboisi*. Specimens of *Rhacophorus rhodopus* from Vietnam and Hainan, China likely represent an undescribed, cryptic species.

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Introduction

The treefrog genus *Rhacophorus* Kuhl & Van Hasselt, 1822, which is widely distributed across India, China, Japan, mainland South-east Asia, the Greater Sunda Islands and the Philippines, contains 80 species (Frost 2011), which renders it the largest genus in the Rhacophoridae (Frost 2011; AmphibiaWeb 2012). These frogs are commonly referred to as parachuting or flying frogs because some species possess extensive digital webbing, which serves as parachutes when escaping predators (Davis 1965). Some species also have dermal extensions on their forearms and legs. However, not all treefrogs in this genus are arboreal and not all possess extensive digital webbing. Some species live in swamps or shrubby habitats. Species of *Rhacophorus* exhibit interesting reproductive modes. Eggs are deposited in self-produced foam nests, a strategy that may have evolved once only within the family Rhacophoridae (Wilkinson *et al.* 2002; Grosjean *et al.* 2008; Li *et al.* 2009).

As is typical, the extent of morphological variation differs between species. Some morphological studies analyse

a small number of species, and their results may not be representative of the diversity within *Rhacophorus*. The genus is defined by the following collection of morphological characters: vertebrae procoelus: *M. extensor radialis accessorius lateralis* originates near *crista ventralis*; *M. cutaneous pectoris* thin, with a few layers; anal folds are usually present; typically, extensive dermal folding occurs forearm and tarsus; and bright green or brown coloration usually present (Liem 1970; Jiang *et al.* 1987; Wilkinson & Drewes 2000). The content and monophyly of *Rhacophorus* remains controversial because members of this genus are easily confused with those in the rhacophorid genus *Polypedates* Tschudi, 1838. Rao *et al.* (2006) and Li *et al.* (2008, 2009) provided a diagnosis of the two genera using morphological and molecular characters.

Genus *Rhacophorus* has been variously subdivided, and often with controversy. Dubois (1986) resurrected *Leptomantis* Peters, 1867 as a subgenus and further subdivided subgenus *Rhacophorus* into ten groups. However, other herpetologists (e.g. Harvey *et al.* 2002) do not follow this division. Iskandar & Colijn (2000) raised subgenus *Leptomantis* to full genus rank, which they "provisionally consider as valid". Their *Leptomantis* contains *Rhacophorus appendiculatus* (Günther, 1858) (as *L. appendiculatus*) and species of *Philautus* Gistel, 1848 placed by Dubois (1986) and Bossuyt & Dubois (2001) into subgenus *Gorhixalus* (the *Philautus hosii* group of Dring, 1987). Iskandar & Colijn (2000) define *Leptomantis* by a collection of morphological characters, although many of these are not diagnostic because they are shared with species of the *Rhacophorus* sensu stricto (Liem 1970; Dring 1983; Inger *et al.* 1999), such as *Rhacophorus kajau* Dring, 1983; '*Rhacophorus*' *bisacculus* (Taylor, 1962) (transferred to genus *Kurixalus* by Li *et al.* 2008) and '*Rhacophorus*' *everetti* (Boulenger, 1894) (transferred to genus *Philautus* by Hertwig *et al.* 2012).

Drawing on the morphological characters of Liem (1970) and Channing (1989), Wilkinson & Drewes (2000) conducted a comprehensive phylogenetic analysis of the family Rhacophoridae. They do not resolve two monophyletic subgenera of *Rhacophorus*. Harvey *et al.* (2002) also questioned the validity of subgenus *Leptomantis* as constructed by Dubois (1986) and do not believe it to be a natural group. Further, they considered Dubois (1986) species groups of subgenus *Rhacophorus* to be undiagnosed.

Many species of *Rhacophorus* have been included in molecular phylogenetic assessments of the family Rhacophoridae (Wilkinson *et al.* 2002; Grosjean *et al.* 2008; Li *et al.* 2008, 2009; Yu *et al.* 2008, 2009). These studies consistently resolve two lineages within *Rhacophorus*, but, unfortunately, none includes Philippine species. Most recently, Hertwig *et al.* (2012) included Philippine species and resolved them as a clade. However, the type species of

Leptomantis, *Leptomantis bimaculata* (Peters, 1867), is from the Philippines, rendering the validity of the subgenera largely untested.

To the largest genus *Rhacophorus* within the family Rhacophoridae, the taxonomic status of some species remains unresolved, although some previous studies attempt to recommend numerous taxonomic rearrangements (Zhao *et al.* 2000; Frost *et al.* 2006; Rao *et al.* 2006; Li *et al.* 2008, 2009; Yu *et al.* 2009; Hertwig *et al.* 2012). Before any systematic work, it is necessary to identify the origins and identity of samples (Ohler 2007). Research conducted in isolation because of language barriers and access to literature may be responsible for two descriptions of one species in neighbouring countries. For example, Stejneger (1924) described the new species *Rhacophorus omeimontis* (as *Polypedates*) from the type locality Shin-kai-si, Mount Omei, Sichuan, China. The species occurs from Sichuan and Yunnan eastward to Hubei and Guangxi, China (Frost 2011). *Rhacophorus duboisi* is described by Ohler *et al.* (2000) from Fan Si Pan Mountain, near Sa Pa, Lao Cai, Vietnam. Kou *et al.* (2001) described the new species *Rhacophorus* (*Polypedates*) *pingbianensis* from Pingbian, Yunnan, China. Without discussion, Orlov *et al.* (2002) considered *R. pingbianensis* to be a junior synonym of *R. duboisi*, perhaps, because *R. duboisi* is morphologically similar to *R. omeimontis* (Ohler *et al.* 2000). Recently, Yang (2008) has described the new species *Rhacophorus* (*Polypedates*) *spinus* from Huang Lian Shan, Luchan, Yunnan, China, a species very similar to *R. pingbianensis*. The molecular analyses of Yu *et al.* (2009) support the synonymization of *R. pingbianensis* into *R. omeimontis* by Fei *et al.* (2005). However, the conclusions of Yu *et al.* (2009) are in conflict with those of Yu *et al.* (2008). The trees of Yu *et al.* (2008) depict *R. pingbianensis* from Jiuxiu and Jinping as the sister group of *R. omeimontis* from Hongya and Zhaotong. However, Yu *et al.* (2009) report that *R. pingbianensis* from Pingbian and Jinping is paraphyletic with respect to *R. omeimontis* from Hongya. Thus, the taxonomic status of *R. duboisi*, *R. omeimontis*, *R. pingbianensis* and *P. spinus* requires investigation.

The new subspecies *Rhacophorus schlegelii dorsoviridis* from Sa Pa, Lao Cai, Vietnam was described by Bourret (1937). Orlov *et al.* (2001) elevated it to full rank and assigned it to *Polypedates* without discussion. They also restricted its distribution to northern parts of the Hoang Lien Mountains in Lao Cai and Lai Chau provinces, Vietnam. It likely occurs in adjacent Yunnan, China (Frost 2011). Liu *et al.* (1962) describe the new species *Rhacophorus nigropunctatus* from Long-chu, Weining, western Guizhou, China. It occurs in central and south-western China including the provinces of Anhui, Guizhou, Yunnan and Hunan (Frost 2011). Orlov *et al.* (2001) stated that *Polype-*

dates dorsoviridis and *P. nigropunctatus* were closely related species, yet they refrained from making a taxonomic change. The validity of these species needs to be confirmed using molecular analyses of specimens from type localities.

Another interesting conundrum involves *Rhacophorus bipunctatus* Ahl, 1927 and *Rhacophorus rhodopus* Liu & Hu, 1960. The latter appears to be a junior synonym of the former (Inger *et al.* 1999). Recently, Bordoloi *et al.* (2007) compared similar species that have red webbing on the feet and offered a revision for them. Nowadays, *Rhacophorus rhodopus* is a valid species (Bordoloi *et al.* 2007; Li *et al.* 2008; Yu *et al.* 2008). Bordoloi *et al.* (2007) and Nguyen *et al.* (2008) considered all previous records of *R. bipunctatus* in Vietnam to be *R. rhodopus*. Thus, the distribution of *R. rhodopus* extends from north-eastern India to Myanmar, northern Thailand, Laos, Vietnam and southern China. This arrangement suggests the sympatric occurrence of both species in north-eastern India and Myanmar (Bordoloi *et al.* 2007). Molecular analyses of specimens from type localities are required to confirm the identity and validity of these species.

Herein, we expand on the phylogenetic relationships of *Rhacophorus* based on the taxonomically most comprehensive data set to date using partial sequences of mitochondrial DNA genes. We reassess the validity of the subgenera and re-evaluate the taxonomic status of some problematic species.

Materials and methods
Species sampling and data collection
Taxonomic sampling included 96 specimens representing 52 species. *Spinomantis peraccae* (Boulenger, 1896), *Polypedates megacephalus* Hallowell, 1861 and *Chiromantis xerampelina* Peters, 1854 were chosen as outgroup taxa (Frost *et al.* 2006; Li *et al.* 2008, 2009). Sequence data were obtained de novo and from GenBank, although the latter data were not as complete for some Indian and Malaysian species. GenBank accession numbers for all data, all sampled species, and locations and voucher specimen numbers are detailed in Table 1, and the localities are mapped in Fig. 1. Taxonomy followed those of Frost (2011), Li *et al.* (2008) and Ohler (2009) for the purposes of discussion.

DNA extraction, amplification and sequencing
Genomic DNA was extracted from toe clips, muscle, or liver tissues initially preserved in either 95% or 100% ethanol. Tissue samples were digested using proteinase K and then followed a standard 3-step phenol/chloroform extraction procedure (Sambrook *et al.* 1989; Hillis *et al.* 1996). The mitochondrial DNA (mtDNA) fragment included

2034 bp from the 12S and 16S together with the complete t-RNA for valine. The primers used in this study were taken from Wilkinson *et al.* (2002). Double stranded polymerase chain reaction (PCR) amplification for the mitochondrial genes was carried out using the following parameters: 95 °C initial hot start (5 min), 35 cycles of 94 °C denaturation (1 min), 55 °C annealing (1 min) and 72 °C extension (1 min). Final extension at 72 °C was conducted for 10 min. Purified PCR products were directly sequenced with an ABI 3730 automated DNA sequencer, and sequences were then determined in both directions for each species and submitted to a BLAST search (Altschul *et al.* 1997) in GenBank to ensure gene and taxon identity.

Sequence alignment
Alignments were initially achieved using Clustal X 1.81 (Thompson *et al.* 1997) with default parameters and subsequently adjusted by eye. Nucleotide sites having ambiguous alignments were removed from the analyses to increase the reliability of tree building (Swofford *et al.* 1996). Gaps resulting from the alignment were treated as missing data. Because all mtDNA gene sequences were inherited effectively as one locus, they were concatenated into a single fragment for analyses. Possible saturation of substitution types was checked by plotting the number of transitions (Ti) and transversions (Tv) vs. F84 distance using DAMBE (Xia 2000). Pairwise comparisons of uncorrected sequence divergences (P-distance) were made using PAUP* 4.0b 10a (Swofford 2003).

Phylogenetic analyses
Missing data and indels were coded as 'N' in the analyses. The inclusion of a limited amount of missing data was unlikely to distort the phylogenetic results in constructing phylogenetic analysis (Wiens 2003; Philippe *et al.* 2004; Wiens *et al.* 2005; Wiens & Moen 2008).

Because we were hypothesizing macroevolutionary relationships, we assumed that the matrilineal history based on mtDNA was indicative of the species' history. We did not assume that mtDNA indicated of gene flow. Genealogical history was hypothesized using Bayesian inference (BI) and maximum likelihood (ML). BI was implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The best-fitting nucleotide substitution models were selected by using the Akaike Information Criterion as implemented in Modeltest 3.7 (Posada & Crandall 1998). The model GTR+I+G was selected for the sequences. For BI and Bayesian posterior probabilities (BPP), the following settings were applied: number of Markov chain Monte Carlo (MCMC) generations = 3 000 000 and sampling frequency = 100. The first 7500 sampled trees were discarded

Table 1 Samples and sequences used in this study

Genus	Specific epithet	Specimen voucher no.	Locality	GenBank no.
Rhacophorus	*angulirostris*	GenBank	–	AF215360
	annamensis	KIZ1195	Ta Kou Mountain Natural Reserve, Binh Thuan, Vietnam	JX219447*
	annamensis	KIZ1196	Ta Kou Mountain Natural Reserve, Binh Thuan, Vietnam	JX219446*
	annamensis	KIZ64	Bu Gia Map National Park, Bina Phuoc, Vietnam	JX219448*
	annamensis	GenBank	Vietnam	DQ283047
	arboreus	GenBank	Japan	AF458142
	arboreus	GenBank	Japan	AY880610
	bipunctatus	CAS235303	Mindat District, Mindat township, Bee Hoe village, Chin State, Myanmar	JX219444*
	bipunctatus	CAS229913	Au Yin Ga camp, Nagmung Township, Putao District, Kachin State, Myanmar	JX219445*
	burmanus	GenBank	Mt. Gaoligong, Yunnan, China	EU215537
	burmanus	Rao6239	Motuo, Xizang, China	JX219422*
	calcaneus	GenBank	Tre Don Commune, Tre My, Quang Nam, Vietnam	DQ283380
	chenfui	RaoZT0806013	Zhaotong, Yunnan, China	JX219431*
	chenfui	Li05	Mt. Omei, Sichuan, China	JX219432*
	chenfui	GenBank	Mt. Omei, Sichuan, China	EU215534
	chuyangsinensis	KIZ528	Bi Doup National Park, Lam Dong, Vietnam	JX219450*
	chuyangsinensis	KIZ746	Bi Doup National Park, Lam Dong, Vietnam	JX219451*
	dennysi	GenBank	Vietnam	AY880611
	dennysi	GenBank	Shaoguan, Guangdong, China	EU215545
	dennysi	Li06	Hunan, China	JX219433*
	dorsoviridis	ROM38015	Sa Pa, Lao Cai, Vietnam	JX219423*
	dorsoviridis	ROM38011	Sa Pa, Lao Cai, Vietnam	JX219427*
	duboisi	ROM38771	Sa Pa, Lao Cai, Vietnam	JX219413*
	duboisi	ROM38758	Sa Pa, Lao Cai, Vietnam	JX219414*
	dugritei	GenBank	Hongya, Sichuan, China	EU215540
	dugritei	GenBank	Baoxing, Sichuan, China	EU215541
	dulitensis	Rao081201	Malaysia	JX219434*
	dulitensis	GenBank	–	AF215187
	feae	GenBank	China	EF564474
	feae	GenBank	Mt. Dawei, Pingbian, Yunnan, China	EU215544
	gauni	FMNH273928	Bintulu Division, Sarawak, Malaysia	JX219456*
	gauni	GenBank	–	AF215362
	hui	Li01	Zhaojue, Sichuan, China	JN688878
	hungfuensis	GenBank	Wenchuan, Sichuan, China	EU215538
	kio	GenBank	Vietnam	AF458147
	kio	GenBank	Xishuangbanna, Yunnan, China	EU215532
	kio	GenBank	Vietnam	AF215188
				AF215359
	lateralis	GenBank	India	AY880612
	malabaricus	GenBank	India	DQ346957
	maximus	Rao6241	Motuo, Xizang, China	JX219411*
	minimus	GenBank	Mt. Dayao, Guangxi, China	EU215539
	moltrechti	GenBank	Lianhuachi, Taiwan, China	EU215543
	monticola	GenBank	Mt. Lompo Batang, Sulawesi Island, Indonesia	AY326060
	nigropalmatus	Rao081203	Malaysia	JX219438*
	nigropalmatus	Rao081204	Malaysia	JX219437*
	nigropunctatus	Rao060821200	Jinping, Yunnan	JX219424*
	nigropunctatus	YN080446	Pingbian, Yunnan, China	JX219425*
	nigropunctatus	Rao060821199	Pingbian, Yunnan, China	JX219426*
	nigropunctatus	Rao3496	Longling, Yunnan, China	JX219428*
	nigropunctatus	Rao3494	Longling, Yunnan, China	JX219429*
	nigropunctatus	GZ070658	Weining, Guizhou, China	JX219430*
	nigropunctatus	GenBank	Weining, Guizhou, China	EU215533
	omeimontis	GenBank	Hongya, Sichuan, China	EF564492
	omeimontis	RaoZT0806010	Zhaotong, Yunnan, China	JX219419*

Table 1 Continued

Genus	Specific epithet	Specimen voucher no.	Locality	GenBank no.
	omeimontis	GenBank	Pengxian, Sichuan, China	EU215535
	omeimontis	Li02	Yaan, Sichuan, China	JX219420*
	omeimontis	SC080505	Mt. Omei, Sichuan, China	JX219421*
	orlovi	GenBank	Nga Doi region, Huon Son Reserve, Huong Son District, Ha Tinh, Vietnam	DQ283049
	pardalis	GenBank	–	AF215189
	pardalis	FMNH273245	Bintulu Division, Sarawak, Malaysia	JX219453*
	pardalis	FMNH273243	Bintulu Division, Sarawak, Malaysia	JX219454*
	pingbianensis	RaoYN080492	Jinxiu, Guangxi, China	JX219412*
	pingbianensis	RaoL060821289	Jinping, Yunnan, China	JX219415*
	pingbianensis	GenBank	Pingbian, Yunnan, China	EU215536
	pingbianensis	YN080484	Pingbian, Yunnan, China	JX219418*
	puerensis	GenBank	Yongde, Yunnan, China	EF564469
	puerensis	ROM37996	Lao Cai, Sa Pa, Vietnam	JN688891
	puerensis	GenBank	Puer, Yunnan, China	EU215542
	reinwardtii	Rao081205	Malaysia	JX219443*
	reinwardtii	GenBank	–	AY880614
	rhodopus	Loc08007018	Longchuan, Yunnan, China	JX219439*
	rhodopus	Lc0805109	Lvchun, Yunnan, China	JX219440*
	rhodopus	GenBank	Mengyang, Yunnan, China	EU215531
	rhodopus	L06245	Motuo, Xizang, China	JX219441*
	rhodopus	L062456	Motuo, Xizang, China	JX219442*
	rhodopus	GenBank	Vietnam	AY843750
	rhodopus	GenBank	Vietnam	AF458144
	rhodopus	GenBank	Hainan, China	EU215529
	rufipes	FMNH272858	Bintulu Division, Sarawak, Malaysia	JX219455*
	schlegelii	GenBank	Japan	AB202078
	schlegelii	GenBank	Japan	AY880615
	sp.	FMNH235741	Kota Marudu District, Sabah, Malaysia	JX219452*
	sp.	03309 Rao	Maguan, Wenshan, Yunnan, China	JX219435*
	sp.	Rao03308	Malipo, Wenshan, Yunnan, China	GQ285680
	sp.	Rao03324	Malipo, Wenshan, Yunnan, China	JX219408*
	sp.	Rao03321	Malipo, Wenshan, Yunnan, China	JX219410*
	sp.	Rao03326	Malipo, Wenshan, Yunnan, China	JX219409*
	translineatus	Rao6237	Motuo, Xizang, China	JX219449*
	verrucopus	6254 Rao	Motuo, Xizang, China	JX219436*
Polypedates	*megacephalus*	GenBank	China	AF458141
	spinus	LC0805089	Lvchun, Yunnan, China	JX219416*
	spinus	LC0805088	Lvchun, Yunnan, China	JX219417*
Chiromantis	*xerampelina*	GenBank	Africa	AF458132
Spinomantis	*peraccae*	GenBank	Africa	DQ283036

* Sequences new to this study. ROM, Royal Ontario Museum, Toronto, Canada; FMNH, Field Museum of Natural History, Chicago, USA; CAS, California Academy of Sciences, San Francisco, USA; KIZ, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, China; "–", unknown data.

as a conservative burn-in. The remaining samples were used to generate a majority rule consensus tree, and the frequency of nodal resolution was termed 'BPP'. All MCMC runs were repeated twice to confirm consistent approximation of the posterior parameter distributions.

Maximum likelihood (ML) analyses were performed using RAxML Web Servers (Stamatakis *et al.* 2008). Searches were initiated with 100 rapid bootstrap inferences and thereafter pursued in a thorough ML search on partitioned data sets. Non-parametric bootstrap proportions (BSP) were used to infer nodal reliability. RAxML estimated all model parameters.

Morphological data and analyses

The following measurements were taken to the nearest 0.01 mm with digital calipers: SVL, snout-vent length (from tip of snout to vent); HL, head length (from tip of snout to the hind border of the angle of the jaw and not measured parallel with the median line); HW, head width (at angle of jaw); IND, internarial distance (distance
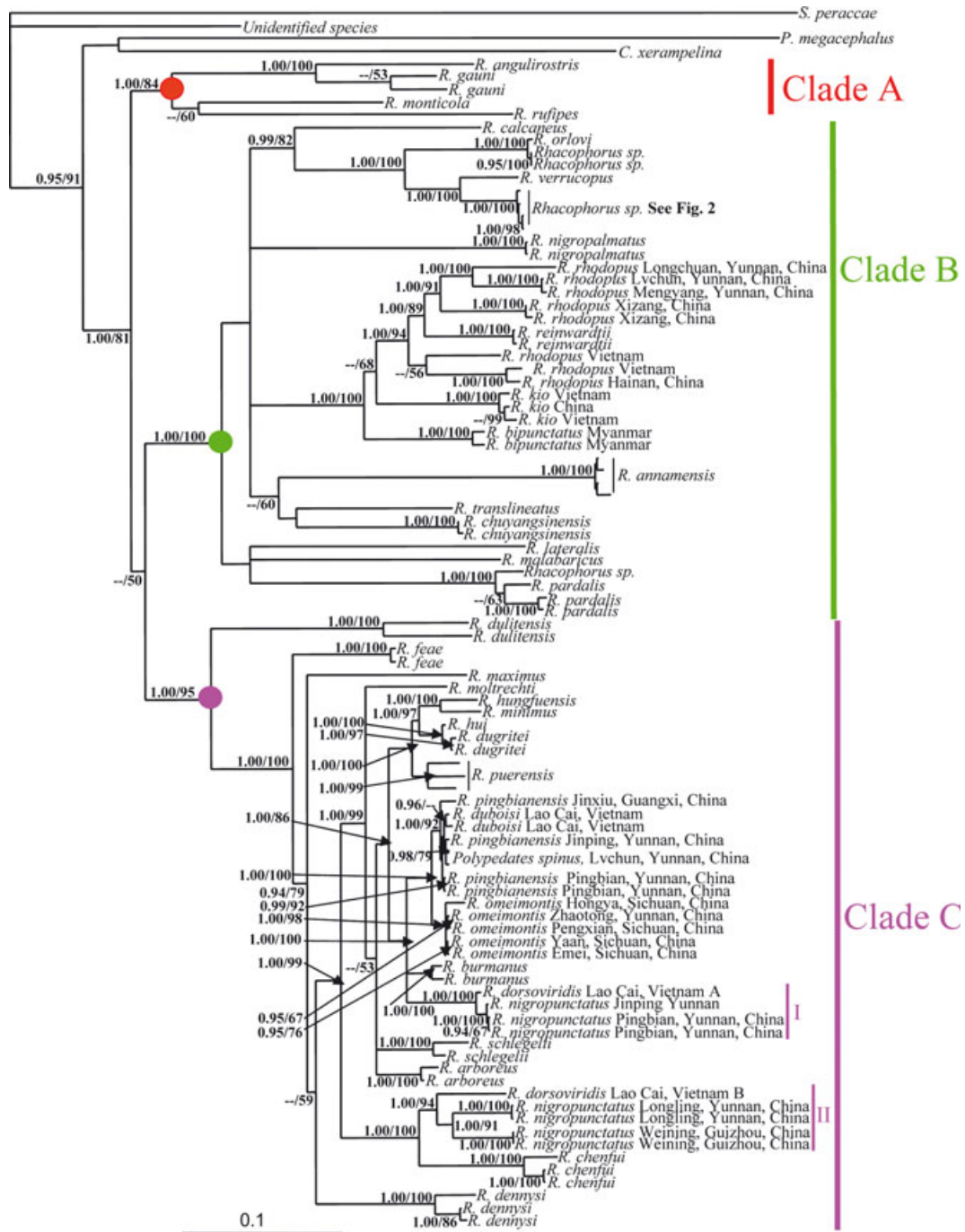
Fig. 1 The 50% majority consensus tree from Bayesian analysis derived from partial fragments of 12S and 16S ribosomal DNA genes together with the complete t-RNA^{valine}. Numbers above the lines or besides the nodes are given as Bayesian posterior probabilities (BPP) (≥90 retained)/bootstrap support for maximum likelihood analyses (≥50 retained); '–' represents BPP and bootstrap proportions lower than 90% and 50%, respectively.

between nares); IOD, interorbital distance (minimum distance between upper eyelids); ED, eye diameter (between anterior and posterior corners of eye); TD, tympanum diameter (the greatest diameter); DNE, distance from nostril to eye; HUM, humerus length(elbow to insertion point along posterior edge); FLL, forelimb length (from elbow

Table 2 Measurements (in mm) of specimens from males of *Rhacophorus pingbianensis*, *Polypedates spinus* and *Rhacophorus duboisi*

No.	SVL	HL	HW	IOD	IND	DNE	ED	TD	HUM	FLL	HLT	THBL	FEMUR	TIL	MET	FL
R. pingbianensis																
YN080485*	67.74	29.79	21.93	7.24	5.35	5.21	7.27	4.95	11.70	15.73	26.15	10.76	33.36	34.31	15.61	32.39
YN080423*	66.09	19.72	22.97	7.37	5.59	5.69	6.44	4.99	11.69	14.11	24.55	12.25	33.27	32.29	13.92	34.26
YN080430*	64.51	21.82	21.00	8.48	5.34	5.55	7.34	5.48	11.62	12.02	24.25	11.09	28.26	31.34	16.68	30.81
YN080484*	68.13	20.94	22.12	7.56	5.81	5.75	7.23	5.41	12.99	13.70	24.02	10.32	30.61	30.31	15.99	30.88
YN080486*	63.49	20.05	20.14	7.89	5.55	5.54	7.89	5.43	12.05	14.52	23.99	11.36	29.73	31.86	15.51	30.75
Unknown[a]	55.50	19.50	19.50	5.20	4.50	–	7.40	5.10	–	11.00	20.00	–	–	26.50	–	25.30
Unknown[a]	67.50	25.50	23.00	9.00	6.80	–	8.00	6.00	–	13.00	25.00	–	–	34.00	–	32.50
Polypedates spinus																
85I0180[b]	62.00	21.00	21.00	6.00	5.00	–	5.50	5.50	–	10.50	22.00	–	–	30.00	–	–
85I0193[b]	60.00	20.00	20.00	7.00	5.00	–	8.00	8.00	–	11.00	19.50	–	–	28.50	–	–
85I0195[b]	61.50	21.00	20.50	6.50	5.50	–	7.00	7.00	–	11.50	20.50	–	–	29.00	–	–
85I0181[b]	61.00	21.00	20.00	6.00	6.00	–	8.50	5.00	–	11.00	21.00	–	–	28.00	–	–
85I265[b]	62.00	21.00	20.00	7.00	6.00	–	8.00	5.00	–	10.00	22.00	–	–	29.50	–	–
85I0194[b]	60.00	21.80	21.50	6.50	6.00	–	9.00	4.50	–	11.00	22.00	–	–	29.00	–	–
85I0271[b]	61.00	21.00	21.00	6.00	6.00	–	8.50	4.00	–	11.50	21.50	–	–	27.50	–	–
85I0184[b]	63.50	23.00	21.50	6.50	5.00	–	8.00	4.50	–	12.00	22.00	–	–	30.00	–	–
85I0198[b]	64.00	21.00	20.50	7.00	5.00	–	8.00	5.00	–	11.00	21.00	–	–	29.50	–	–
85I0272[b]	59.00	21.00	20.00	6.50	5.50	–	7.00	4.50	–	8.00	20.00	–	–	27.00	–	–
85I0197[b]	65.50	24.50	22.00	6.00	6.50	–	7.50	5.00	–	12.00	22.00	–	–	31.50	–	–
85I0182[b]	60.00	21.50	20.50	5.00	6.00	–	7.00	4.50	–	11.00	20.00	–	–	29.00	–	–
85I0236[b]	60.00	20.00	20.00	5.00	6.00	–	7.50	5.00	–	11.00	21.00	–	–	28.00	–	–
R. duboisi																
MNHN 1999.5971[c]	61.50	19.37	19.31	–	–	–	–	–	–	–	–	28.11	–	–	–	–
Unknown[c]	65.70	21.88	22.21	–	–	–	–	–	–	–	–	32.59	–	–	–	–

*This study.
[a]From Kou *et al.* (2001); [b]From Yang (2008); [c]From Ohler *et al.* (2000).

to tip of third finger); HLT, hand length (from base of outer palmer tubercle to tip of third finger); THBL, thumb length (proximal edge inner palmar tubercle to tip of thumb); FEMUR, femur length (knee to insertion point, midline between two legs); TIL, tibia length (from knee to foot); MET, metatarsal length (proximal edge inner foot tubercle to joint with tibiofibula); FL, foot length (from proximal end of inner metatarsal tubercle to tip of fourth toe) Table 2.

We applied a principal component analysis (PCA) on log-transformed morphometric data to assess whether or not these continuous characters could form the basis of qualitatively detectable structure in the data.

Results
Sequence variation
The aligned mtDNA gene fragments consisted of 2034 sites, corresponding to sites 726 through 2666 of *P. megacephalus* mitochondrial genome (AY458598). The fragments contained 993 constant characters (CC) and 847 potentially phylogenetically informative (PPI) characters. Transitions and transversions in the sequence data set genes were accumulating linearly and gave no indication of saturation effect. Thus, all substitutions in these genes

were used for phylogenetic inference. These plots are available from the authors upon request.

Phylogenies analysis
For BI, the likelihood values of the majority rule consensus tree were lnL = −26303.17. The standard deviation of split frequencies among the four BI runs (Fig. 1) was 0.007671. Because the ML tree was consistent with the BI tree, it was not shown. The following relationships were indicated by both analyses as being reliable:

1. Monophyly of the genus *Rhacophorus* with respect to the outgroup was strongly supported (BPP = 1.00; BSP = 81).
2. *Rhacophorus* contained three well-supported lineages (lineages A, B and C).
3. In lineage A, *Rhacophorus angulirostris* and *R. gauni* formed a strongly supported group (BPP = 1.00; BSP = 100). Together with *R. monticola* and *R. rufipes*, they constituted well-supported lineage A (BPP = 1.00; BSP = 84).
4. Lineage B contained *R. pardalis*, *R. malabaricus*, *R. lateralis*, *R. chuyangsinensis*, *R. translineatus*, *R. annamensis*, *R. kio*, *R. bipunctatus*, *R. reinwardtii*, *R. rhodopus*, *R. nigropalmatus*, *R. orlovi*, *R. verrucopus* and *R. calcaneus*. Monophyly of this

lineage was strongly supported (BPP = 1.00; BSP = 100). *Rhacophorus rhodopus* from Vietnam and *R. rhodopus* from Hainan, China, formed a lineage, which was sister to the lineage including *R. rhodopus* from Yunnan and Xizang, China, and *R. reinwardtii*.

5. Lineage C included *R. dulitensis*, *R. feae*, *R. maximus*, *R. dennysi*, *R. nigropunctatus*, *R. dorsoviridis*, *R. chenfui*, *R. schlegelii*, *R. arboreus*, *R. dugritei*, *R. puerensis*, *R. minimus*, *R. hungfuensis*, *R. hui*, *R. burmanus*, *R. omeimontis*, *R. pingbianensis*, *R. duboisi*, *R. moltrechti* and *P. spinus* (BPP = 1.00; BSP = 95). *Rhacophorus nigropunctatus* from Longling, Yunnan and Weining, Guizhou, sample B of *R. dorsoviridis* from Hoang Lien Mountains and *R. chenfui* formed a lineage (BPP = 1.00; BSP = 100). *Rhacophorus nigropunctatus* from Jinping and Pingbian, Yunnan, and sample A of *R. dorsoviridis* from Hoang Lien Mountains constituted another lineage (BPP = 1.00; BSP = 100). They combined with *R. burmanus*, *R. omeimontis*, *R. pingbianensis*, *R. duboisi*, *R. moltrechti* and *P. spinus* to form a well-supported lineage (BPP = 1.00; BSP = 100). *Rhacophorus pingbianensis* from Jinping, Yunnan was the sister to *P. spinus* (BPP = 0.98; BSP = 79). These taxa combined with *R. duboisi*, and *R. pingbianensis* from Pingbian, Yunnan and Jinxiu, Guangxi, to form a strongly supported lineage (BPP = 1.00; BSP = 92). In turn, this group was the sister to a strongly supported lineage containing *R. omeimontis* (BPP = 1.00; BSP = 100). *Rhacophorus dugritei* and *R. hui*, *R. minimus* and *R. hungfuensis*, respectively, were sister taxa (both with BPP = 1.00; BSP = 100). The lineage containing *R. dugritei* and *R. hui* was sister to the lineage of *R. minimus* and *R. hungfuensis* (BPP = 1.00; BSP = 97).

Discussion

Systematics of the genus *Rhacophorus*

Often, one species has been described at least twice as new species in neighbouring countries (Ohler 2007). This coincidence likely relates to difficulties in scientific communication between countries, in particular language barriers and access to literature. This phenomenon commonly occurs in the genus *Rhacophorus*. Taxonomy is an international endeavour, and describing new taxa often requires knowing the fauna of neighbouring countries (Ohler 2007). Below, we review some of the species-level taxonomic issues, particularly as they relate to our analyses.

Systematics of *R. pingbianensis*, *R. omeimontis*, *P. spinus* and *R. duboisi*.

In historical order, Stejneger (1924) described the new species 'Polypedates' omeimontis with type specimen USNM 66548 and type locality Mt. Emei, Sichuan, China. Ohler et al. (2000) described the new species *Rhacophorus*

duboisi with type specimen MNHNP 1999.5971 and type locality Fan Si Pan Mountain, Sa Pa, Lao Cai, Vietnam. Kou et al. (2001) described the new species 'Polypedates' pingbianensis with holotype CIB 654003 and type locality nature conservation region of Pingbian, Yunnan, China. Yang (2008) described the new species 'Polypedates' spinus with holotype 03199 and type locality, Huang Lian Shan, Yunnan, China. Kou et al. (2001) described the new species 'Polypedates' pingbianensis because, unlike *R. omeimontis*, it did not have a *linea masculinae* and vocal sac. Rao et al. (2006) suggested that *P. pingbianensis* be assigned to *Rhacophorus* and this arrangement is supported by the molecular studies (Li et al. 2008, 2009; Yu et al. 2008, 2009). Without explanation, Orlov et al. (2002) and Fei et al. (2005, 2010) considered *R. pingbianensis* to be a junior synonym of *R. duboisi* and *R. omeimontis*, respectively. However, Frost (2011) did not follow these changes. Yu et al. (2008) and Li et al. (2008) tentatively suggested that *R. pingbianensis* is distinct and forms the sister taxon of *R. omeimontis*. Subsequently, Yu et al. (2009) discovered that *R. pingbianensis* from the type locality (Pingbian) and Jinping County are paraphyletic with respect to *R. omeimontis*. Consequently, they placed *R. pingbianensis* back into synonymy with *R. omeimontis*. Most recently, Yang (2008) described *Polypedates spinus*, which is very similar to *R. pingbianensis* according to the original description. However, he did not compare the two species.

Our analyses resolve *R. omeimontis* from the type locality (Emei Mountain, Sichuan) and other populations as the sister group of the lineage containing *R. pingbianensis* from the type locality Pingbian, Yunnan, and from Jinping, Yunnan and Jinxiu, Guangxi. The lineage also includes *Polypedates spinus* from the type locality (Lüchun, Yunnan), and *R. duboisi* from the type locality (Lao Cai, Vietnam). Orlov et al. (2001) summarized morphological measurements from the studies of Inger et al. (1990), Zhao & Yang (1997) and their own data and indicated that *R. duboisi*, *P. omeimontis*, *R. pingbianensis* and *R. spinus* are very similar in body proportions (male SVLs varies from 52.0 to 67.0 mm) and colour pattern (Ohler et al. 2000). However, *R. duboisi*, *R. pingbianensis* and *P. spinus* differ from *R. omeimontis* in having very granular dorsal skin, no linea masculinae and no vocal sac (Ohler et al. 2000; Kou et al. 2001). These data require recognition of *R. omeimontis* as a valid species, as is well supported by molecular analyses. Further, we resurrect *R. pingbianensis*, an action consistent with Yu et al. (2008) and Li et al. (2008). The study of Yu et al. (2009) conflicts with other prior work and this study, possibly because of misidentified species or an error in their data base.

Rhacophorus duboisi differs from *R. omeimontis* by having a very granular dorsal (Ohler et al. 2000). Further, *R. pingbianensis* is morphologically similar to *R. omeimontis*

(Kou *et al.* 2001) and *R. duboisi*. Owing to limited access to the literature and specimens, Kou *et al.* (2001) could not compare *R. pingbianensis* with *R. duboisi* from Vietnam. Similarly, no comparison exists for 'Polypedates' spinus with either *R. omeimontis* or *R. pingbianensis* (Yang 2008). Our PCA does not differentiate *R. duboisi*, *R. pingbianensis* and *P. spinus* (Fig. 2). The primary loadings in the PCA reflect size-based morphological variation, and this is not diagnostic for the tree species. Furthermore, *R. duboisi*, *R. pingbianensis* and *P. spinus* are very similar in that (i) webbing on hands is half-developed, (ii) the dorsum has small granules, and (iii) dorsum is green and brown in colour. Our molecular results resolve shared lineages with respect to *R. pingbianensis* and *P. spinus* and *R. duboisi*. The genetic distances (uncorrected *P*-distance) between any two taxa range from 0.02% to 0.89%. In comparison, interspecific *P*-distances within the genus *Rhacophorus* average 11.58%. Therefore, we agree with Orlov *et al.* (2002) that *R. pingbianensis* is a junior synonym of *R. duboisi*, because the latter has priority. Further, our results require that *P. spinus* be placed as a junior synonym of *R. duboisi*, considering rules of priority. This discovery is not surprising given that the type localities of these three taxa are geographically nearby and their habitat is continuous. According to this taxonomic arrangement, *R. duboisi* occurs in northern parts of the Hoang Lien Mountains in Lao Cai Province, Vietnam and adjacent Yunnan, China. This arrangement also results in a new Chinese record for *R. duboisi*.

Systematics of R. nigropunctatus and R. dorsoviridis Bourret (1937) described the new subspecies *Rhacophorus schlegelii dorsoviridis* with the type locality 'Chapa' (=Sa Pa), Lao
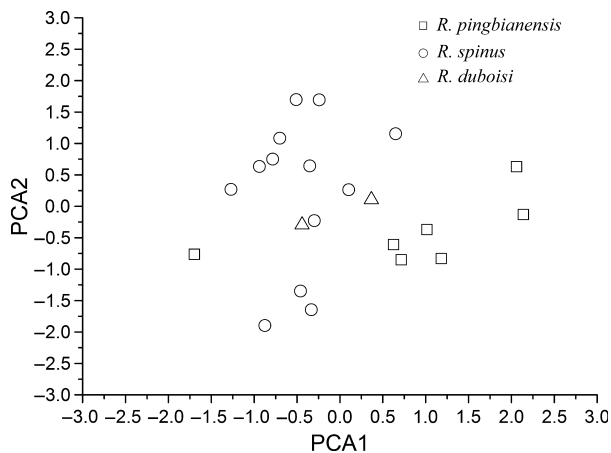

Fig. 2 Principal components analysis scores from males of *Rhacophorus duboisi*, *R. pingbianensis* and *Polypedates spinus*.

Cai, Vietnam and type specimens MNHNP 1948.149 (formerly LSNUH B.143). Later, because two diagnostic characters separated it from *Rhacophorus schlegelii dorsoviridis*, Liu *et al.* (1962) describe the new species *Rhacophorus nigropunctatus* from Weining, Guizhou, China, and holotype CIB 590405. This species has a conspicuous subgular external vocal sac and only 1/4 webbing between the 3rd and 4th fingers. Orlov *et al.* (2001) elevated *Rhacophorus schlegelii dorsoviridis* to full species rank and stated that *R. dorsoviridis* may be conspecific with *R. nigropunctatus*. They refrained from making the taxonomic change pending additional material from either Guizhou or Yunnan. The colour pattern and body size of the two species are very similar (Orlov *et al.* 2001). Both species have a bright green dorsum with small light-white spots; their flanks are white with variable black spotting; and the ventral surfaces do not have spots. The body size of two species varies from about 34.2 to 37.2 mm (Liu *et al.* 1962; Orlov *et al.* 2001). Morphologically, *R. dorsoviridis* and *R. nigropunctatus* from Yunnan, China, are distinctive as follows: *R. nigropunctatus* (from Weining, Guizhou, China) has a single external subgular vocal sac and only 1/4 webbing between the 3rd and 4th fingers; and *R. nigropunctatus* (from Pingbian, Yunnan, China) has an internal single subgular vocal sac and moderate webbing reaching between 1/3 and 1/2 the 3rd and 4th fingers.

Rhacophorus nigropunctatus occurs in western Guizhou, Yunnan, Anhui and Hunan provinces, China (Fei 1999; Fei *et al.* 2005, 2010; Frost 2011). Yu *et al.* (2009) have reported that *R. nigropunctatus* from the type locality (Weining, Guizhou) and Pingbian, Yunnan do not form a monophyletic lineage and suggested the latter locality may be a cryptic species. Our analyses of sequences for *R. nigropunctatus* from Weining, Guizhou, and Jinping, Longling, and Pingbian, Yunnan resolve a paraphyletic *R. nigropunctatus* with respect to *R. dorsoviridis* from the type locality (Sa Pa, Lao Cai, Vietnam). Further, samples A and B of *R. dorsoviridis* from Sa Pa do not cluster together. Well-supported sublineage II, including *R. nigropunctatus* from Weining and Longling, and sample B of *R. dorsoviridis* form the sister group of *R. chenfui* (Fig. 1). Sample A of *R. dorsoviridis* clusters in sublineage I with *R. nigropunctatus* from Pingbian and Jinping, which form a polytomy with *R. taronensis* and the lineage containing *R. duboisi* and *R. omeimontis*. In sublineage II, our samples are similar morphologically to the original descriptions of *R. nigropunctatus*. Further, based on type localities, sublineage II is *R. nigropunctatus*. Orlov *et al.* (2001) stated that one of their samples (ROM 38011) from Sa Pa, Lao Cai, Vietnam, differs from others in having a darkened vocal sac. This sample, which corresponds to our sample B of *R. dorsoviridis*, clusters within sublineage II with *R. nigropunctatus*. Sublin-

eage I is *R. dorsoviridi*s, based on the following characteristics: (i) geographically nearby the type locality of *R. dorsoviridi*s; (ii) moderate webbing between the 3rd and 4th fingers; and (iii) having single internal subgular vocal sac from Pingbian and Jinping Yunnan, China. Samples A and B of *R. dorsoviridi*s from Sa Pa do not share the same maternal lineage, which is consistent with the report by Orlov *et al.* (2001), especially for ROM 38011. Thus, both *R. dorsoviridi*s and *R. nigropunctatus* appear to have colonized Sa Pa, Vietnam, a place that contains other morphologically similar syntopic species (Ohler *et al.* 2000).

Rhacophorus *dorsoviridi*s and *R. nigropunctatus* have broad distributions, and yet their colour patterning varies little between populations of the species and across their wide ranges. Despite the trend, two distinct species are resolved in our molecular analysis. Our sampling focuses on the one group that exhibits differences in colour (Fig. 3), and we provisionally assume existence of the new species for the following discussion. Analyses of molecular data can test morphologically based hypotheses by pinpointing characters that reliably capture phylogenetic relationships vs. those that are consistently homoplastic (Jablonski & Finarelli 2009). Our genealogy does not resolve reciprocal monophyly in the two morphological patterns (Fig. 3). This leads to three possibilities: (i) colour pattern does not diagnose the species; (ii) the absence of monophyly reflects introgressive hybridization of mtDNA; and (iii) speciation is so recent that incomplete lineage sorting occurs. Nuclear gene data can separate these alternative explanations. Until then, colour-based taxonomy requires careful consideration.

Fig. 3 Colour-based patterns in the genus *Rhacophorus* (two green dorsal stripes and no green dorsal stripes) and their molecular phylogenetic relationships.

Systematics of R. rhodopus *and* R. bipunctatus. Anderson (1871) described *Rhacophorus maculatus* based on five primary synphoronts (ZSI 10291, 2753–2756) from Khasi Hills and specimens collected by Jerdon (1870). Boulenger (1882) provided the replacement name *Rhacophorus bimaculatus*, because the *Rhacophorus maculatus* Anderson, 1871 was a junior secondary homonym of *Hyla maculata* Gray, 1830. The replacement name was a junior secondary homonym of *Leptomantis bimaculata* Peters, 1867, and thus, Ahl (1927) provided the alloneonym *Rhacophorus bipunctatus*. The type locality of *Rhacophorus rhodopus* Liu & Hu, 1960 is Mengyang, Yunnan, China, and the holotype is CIB 571171. Inger *et al.* (1999) considered *R. rhodopus* to be a junior synonym of *R. bipunctatus*. Nowadays, *R. rhodopus* is a valid species (Bordoloi *et al.* 2007; Li *et al.* 2008; Yu *et al.* 2008). Following the study by Bordoloi *et al.* (2007), Nguyen *et al.* (2008) referred all previous records of *R. bipunctatus* in Vietnam to *R. rhodopus*. Accordingly, the distribution of *R. rhodopus* extends from north-eastern India to Myanmar, northern Thailand, Laos, Vietnam and southern China, and it occurs sympatrically with *R. bipunctatus* in north-eastern India and Myanmar (Bordoloi *et al.* 2007; Frost 2011). Our analyses cluster *R. rhodopus* from Vietnam and Hainan, China, in a lineage far removed from individuals from the type locality (Mengyang, Yunnan). Further, our samples from the type locality conform to the original description of *R. rhodopus* in having either reddish brown or brown dorsal coloration, distinct banding on the limbs, usually a single, rounded spot on flank, and all red webbing on the feet (Liu & Hu 1960; Bordoloi *et al.* 2007). Morphologically, *R. rhodopus* from Hainan, China, differ from specimens from the type locality as follows: (1) dorsal colour red in specimens from Hainan but reddish brown or brown from the type locality; and (ii) specimens from Hainan do not have large, moderate-sized ink-black spots on the flanks and specimens from the type locality have one or two. Both molecular and morphological evidence indicate that specimens from Hainan are not *R. rhodopus*. This leaves the question: Are the specimens assignable to *R. bipunctatus*?

True *R. bipunctatus* (type locality 'Khasi Hills', India) possess a green dorsal colour (violet when preserved) with a few, scattered, minute white and black spots (Bordoloi *et al.* 2007). It also has to a few large and moderate ink-black spots on the flanks (rarely without such spots) and orange webbing without black spots on the feet (whitish when fixed). Our *R. rhodopus* from Hainan differ from Indian specimens in the dorsal colour being all red rather than green with few scattered minute white and black spots in Indian specimens, and in having red webbing on the feet as opposed to orange. Our molecular analyses include samples of *Rhacophorus htunwini* Wilkinson, Thin,

Lwin and Shein, 2005 from the type locality, Putao District, Kachin State, Myanmar, which is a junior synonym of *R. bipunctatus* (Bordoloi *et al.* 2007). In our analyses, *R. rhodopus* from Vietnam and Hainan, China, form a lineage far removed from *R. bipunctatus*. *Rhacophorus bipunctatus* roots as the sister group of *R. bipunctatus*, *R. kio*, *R. reinwardtii* and *R. rhodopus*. Given these molecular and morphological differences and that the type locality of *R. bipunctatus* is relatively far from Hainan, China, and Vietnam, our specimens of *R. rhodopus* from Vietnam and Hainan, China, likely represent an undescribed, cryptic species requiring additional study.

Systematics of R. kio and R. reinwardtii. Ohler & Delorme (2006) divide *Rhacophorus reinwardtii* (Schlegel, 1840) into two species, *Rhacophorus kio* Ohler & Delorme 2006 and *Rhacophorus reinwardtii* (Schlegel, 1840), based on molecular, morphological and colour pattern evidence. Newly described *R. kio* extends from China to most of southern Vietnam, as well as eastern India, Laos and Cambodia. In contrast, *R. reinwardtii* occurs from Thailand to the Greater Sunda Islands and the Philippines (Ohler & Delorme 2006; Frost 2011). Bain *et al.* (2007) and Orlov *et al.* (2008) accepted this assignment for the Vietnamese species. In contrast, Yang & Rao (2008) recognized the Chinese species as *R. reinwardtii*. In our study, *R. reinwardtii* from Malaysia forms the sister group of *R. rhodopus*, and *R. kio* from China and Vietnam forms the sister group to these taxa plus *R. rhodopus* from Vietnam and Hainan, China (Fig. 1). Therefore, Chinese and Vietnamese samples of *R. reinwardtii* are *R. kio*.

Phylogeny and the subgenera of Rhacophorus. Our study has the most comprehensive taxonomic coverage to date for the genus *Rhacophorus*. It includes representatives from South Asia, East Asia and South-east Asia. There are three well-supported lineages (A, B and C), yet their phylogenetic relationships remain unresolved. Lineages B and C are largely consistent with previous molecular studies (Wilkinson *et al.* 2002; Li *et al.* 2008, 2009; Yu *et al.* 2008, 2009). A phylogenetic investigation of the *Rhacophorus everetti* species group (Hertwig *et al.* 2012) assigns the species to genus *Philautus* and reports a novel lineage, which is largely consistent with our lineage A. This lineage is restricted to Malaysia, Indonesia and the Philippines.

Dubois (1986) divided *Rhacophorus* sensu lato into two subgenera, *Leptomantis* and *Rhacophorus*, and this arrangement is consistent with our analyses. The type species of *Rhacophorus*, *R. reinwardtii*, nests within lineage B with strong support. Therefore, we recognize lineage B as being Genus *Rhacophorus* (Kuhl & Van Hasselt, 1822). Accordingly, *Rhacophorus* contains the following 15 species:

Rhacophorus annamensis Smith, 1924, *Rhacophorus bipunctatus* Ahl, 1927, *Rhacophorus calcaneus* Smith, 1924, *Rhacophorus chuyangsinensis* Orlov, Nguyen, and Ho, 2008, *Rhacophorus kio* Ohler & Delorme, 2006; *Rhacophorus lateralis* Boulenger, 1883, *Rhacophorus malabaricus* Jerdon, 1870; *Rhacophorus nigropalmatus* Boulenger, 1895, *Rhacophorus orlovi* Ziegler and Köhler, 2001, *Rhacophorus pardalis* Günther, 1858, *Rhacophorus reinwardtii* (Schlegel, 1840), *Rhacophorus rhodopus* Liu & Hu, 1960, *Rhacophorus translineatus* Wu, 1977, and *Rhacophorus verrucopus* Huang, 1983.

Subgenus *Leptomantis* is resurrected by Dubois (1986) to contain *R. bimaculatus*, *R. gauni* and *R. oxycephalus*. The type species of *Leptomantis*, *R. bimaculatus*, is not included in our analyses, but the remaining two species are. Liem (1970) suggested that *R. oxycephalus* belongs to *Buergeria* (Buergeriinae) and this arrangement is consistent with molecular studies (Wilkinson *et al.* 2002; Frost *et al.* 2006; Grosjean *et al.* 2008; Li *et al.* 2008; Yu *et al.* 2008, 2009). The distribution of *R. gauni* and *R. angulirostris* largely overlaps that of *R. bimaculatus*, which occurs in Malaysia, Indonesia and the Philippines. Geographic distributions help to identify the species. For example, Orlov (2001) lists *R. bimaculatus* as part of the Vietnamese herpetofauna, and Stuart (2005) suggests that published records of *R. bimaculatus* are *R. orlovi*. *Rhacophorus bimaculatus* is unlikely to occur in Vietnam because of its south-eastwardly distribution (Frost 2011). Dubois (1992) transferred *R. angulirostris* to *Leptomantis* based on tadpole morphology (Inger & Tan 1990). Iskandar & Colijn (2000) raised subgenus *Leptomantis* to full generic rank and provide three diagnostic characters: (i) species of small to medium size, (ii) occurrence in lowlands and (iii) eggs laid in a foam nest and known tadpoles with sucker-like mouths adapted to medium current. However, these characters are not diagnostic (Liem 1970; Dring 1983; Inger *et al.* 1999). Harvey *et al.* (2002) reported that *Leptomantis* is not a natural group and, thus, does not deserve recognition. Our BI and ML analyses resolve a strongly supported *Leptomantis*. Combined with the study by Hertwig *et al.* (2012), we recognize lineage A as containing the following nine species: *Rhacophorus angulirostris* Ahl, 1927, *Rhacophorus belalongensis* Dehling and Grafe, 2008, *Rhacophorus bimaculatus* (Peters, 1867), *Rhacophorus fasciatus* Boulenger, 1895, *Rhacophorus gauni* (Inger, 1966), *Rhacophorus harrissoni* Inger and Haile, 1959, *Rhacophorus monticola* Boulenger, 1896, *Rhacophorus penanorum* Dehling, 2008 and *Rhacophorus rufipes* Inger, 1966.

Lineage C is strongly supported as a monophyletic group by all analyses. The group occurs in East Asia, including China and Japan, and it includes 18 species as follows: *Rhacophorus arboreus* (Okada and Kawano, 1924), *Rhacophorus burmanus* (Andersson, 1939), *Rhacophorus*

chenfui Liu, 1945, *Rhacophorus dennysi* Blanford, 1881, *Rhacophorus dorsoviridis* Bourret, 1937; *R. duboisi* Ohler, Marquis, Swan, and Grosjean, 2000, *Rhacophorus dugritei* (David, 1872), *Rhacophorus dulitensis* Boulenger, 1892, *Rhacophorus feae* Boulenger, 1893, *Rhacophorus hui* Liu, 1945, *Rhacophorus hungfuensis* Liu and Hu, 1961, *Rhacophorus maximus* Günther, 1858, *Rhacophorus minimus* Rao, Wilkinson and Liu, 2006, *Rhacophorus moltrechti* Boulenger, 1908, *Rhacophorus nigropunctatus* Liu, Hu and Yang, 1962, *Rhacophorus omeimontis* (Stejneger 1924), *Rhacophorus puerensis* (He, 1999) and *Rhacophorus schlegelii* (Günther, 1858).

Morphological characters unambiguously serve to diagnose one of the three lineages, and to a lesser extent, the other two. Species in lineage B differ from those in lineages A and C in having (i) calcanar projections, (ii) a bluntly pointed vs. round snout, and (iii) an elongated vs. stout body. No morphological characters unambiguously diagnose lineages A and C. However, species in lineage A are usually small in body size (20–40 mm), and the dorsal colour of all species is predominantly brown. In contrast, species in lineage C are usually large (40–100 mm), and most species are green in dorsal coloration; brown is present, but uncommon. Further, whereas species in lineage A are usually associated with shrubs, species in lineage C live in a variety of habitats, including swamps, trees and shrubs.

Nowadays, higher level taxonomy almost always reflects a hypothesis of phylogenetic relationships. We have three strongly supported, morphologically distinct, largely geographically constrained distributions of these lineages and believe it desirable to recognize formally each as a taxon. This arrangement better serves to emphasize phylogenetic relationships and reflect hypothesized historical relationships. However, we refrain from making any taxonomic decisions, especially in erecting a genus or subgenus for lineage A, pending analyses that include sequences from the type species of *Leptomantis*, *R. bimaculatus*, from the type locality, Mindanao, Philippines.

Acknowledgements

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