



Molecular Phylogenetic Status of *Rhacophorus laoshan* and *Zhangixalus yinggelingsensis* (Anura: Rhacophoridae) from China

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ABSTRACT

Recently, *Rhacophorus sensu lato* was divided into three genera. In addition, *Rhacophorus laoshan* and *Zhangixalus yinggelingsensis* were placed in the genus *Rhacophorus sensu stricto* and *Zhangixalus*, respectively, only based on morphological characters. The research on the genus *Rhacophorus sensu lato* faces major challenges due to their complex interspecies relationships, and the phylogenetic status of many species are still unclear, which hampered the taxonomy and protection of these species. In this study, we investigated the molecular phylogenetic status of *R. laoshan* and *Z. yinggelingsensis* using mitochondrial DNA (12S rRNA, tRNA^{Val}, 16S rRNA and Cyt *b*) fragments and nuclear DNA (RAG-1, RHOD, TYR) fragments. Our results revealed that *Rhacophorus laoshan* is closely related to *R. verrucopus*, *R. orlovi* and *R. calcaneus*, and *Zhangixalus yinggelingsensis* should indeed be placed under *Zhangixalus*. This research clarified the phylogenetic positions of two species within the genus *Rhacophorus sensu lato*, and it also has an impact on the protection and biogeographic analysis of these species.

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Authors' Contribution

DCJ and KY conceived the study. LMY performed the analysis and prepared the initial manuscript draft. DCJ approved the final version of the manuscript.

Key words

Rhacophorus, *Zhangixalus*, Tree frog

INTRODUCTION

Old world tree frogs of the family Rhacophoridae include 428 species that are widely distributed across Sub-Saharan Africa and southern Asia (from Sri Lanka, Nepal, and India, to Japan, the Philippines and Sulawesi) (Frost, 2020). The genus *Rhacophorus sensu lato* is one of the most diverse genera of the family Rhacophoridae, which contains 94 species and is widely distributed across China, Japan, and India, and from the Philippines to Sulawesi (Frost, 2020).

Many new species of the genus *Rhacophorus sensu lato* have been discovered recently (Dehling, 2015; Hamidy and Kurniati, 2015; Kropachev *et al.*, 2019; Matsui *et al.*, 2013; Nguyen *et al.*, 2017; Pan *et al.*, 2017b; Streicher *et al.*, 2014; Yu *et al.*, 2019), and these studies have made valuable taxonomic contributions of the genus *Rhacophorus sensu lato*. In addition, in the last ten years, there have been many reports that have inferred the phylogenetic relationships within the genus *Rhacophorus sensu lato* (Chen *et al.*, 2019; Li *et al.*, 2008, 2009, 2012a,b, 2013; O'Connell *et al.*, 2018a,b; Pan *et al.*, 2017a; Yu *et al.*, 2008).

However, the molecular data of many species of *Rhacophorus sensu lato* are still poorly known and not publicly available, such as these cryptic species *R. laoshan* and *Z. yinggelingsensis* from southern China. A brown tree frog species, *R. laoshan*, which was first described in Mo *et al.* (2008) based on seven adult male specimens collected from Cenwanglaoshan Nature Reserve, Guangxi, China. This frog can be distinguished from all other Asian *Rhacophorus* Kuhl and van Hasselt, 1822 by the combination of: skin brown and smooth; Y-shaped cartilage visible dorsally on tips of fingers and toes; outer fingers one-third webbed; distinct dermal ridges present on forearms, above vent, and calcar present on heels; anterior and posterior surface of thighs tangerine in color without distinct dark or light spots; tympanum distinct and large, about 6.6% of SVL; dorsum brown with wide dark cross-shaped mark (Mo *et al.*, 2008). To date, *R. laoshan* is still known from type series only, the validity of this species is based on morphological evidence only (Mo *et al.*, 2008). Similarly, another species, *Z. yinggelingsensis*, was first described in Chou *et al.* (2007), and it is known from the type locality in the Yinggelings Mountains of Hainan, China (Frost, 2020). It can be distinguished from other species by the following combination of characteristics: snout rounded, without protruding process; green dorsum with few fine white spots, dorsolateral folds absent; skin on dorsum smooth, flanks and venter granulate; metatarsal

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skinfolds faint; fingers and toes webbed; front of thigh yellow and red-tinged, rear of thigh and inner side of tibia red; white-tipped tuberculous dermal ridge on cloaca present, not well developed; iris silvery, partly light golden in upper half (Chou *et al.*, 2007). In addition, no further specimens or new distribution record has been reported for these two poorly known tree frogs, to the best of our knowledge (Fei *et al.*, 2009, 2012; Frost, 2020).

Recently, Jiang *et al.* (2019) divided *Rhacophorus sensu lato* into three genera: genus *Rhacophorus sensu stricto* Kuhl and Van Hassalt, 1822, genus *Leptomantis* Peters, 1867, and a new genus erected, namely *Zhangixalus* Jiang *et al.*, 2019. Furthermore, *R. laoshan* and *Z. yinggelingsensis* were placed in the genus *Rhacophorus sensu stricto* and *Zhangixalus*, respectively, only based on morphological characters (Jiang *et al.*, 2019). Due to the lack of molecular data for the two species, their phylogenetic status remains unclear. Previous studies suggested that it is essential to clarify species' phylogenetic status using molecular data. We thus conducted molecular phylogenetic analyses of the two *Rhacophorus sensu lato* species to clarify their taxonomic status.

MATERIALS AND METHODS

Ethical statement

This study was performed in accordance with the approval of Experimental Animal Ethics Committee of Chengdu Institute of Biology, Chinese Academy of Sciences.

Taxon sampling and data collection

Two specimens were collected in this study, including one specimen of *Z. yinggelingsensis* (HN2018002), which was collected in Hainan, China on 2018 (19°3'3.84N, 109°32'20.60"E) at 1,569 m altitude (Fig. 1), and one specimen of *R. laoshan* (1705014), which was collected from Cenwanglaoshan Nature Reserve (Guangxi, China) (Fig. 1). After euthanization, liver tissues were preserved in 95% ethanol for DNA extraction. Except for the two individuals, sequences of 39 individuals were collected from GenBank in this study, including sequences for

eight species of *Rhacophorus sensu stricto*, one species of *Leptomantis*, and 18 species of *Zhangixalus* as ingroups and two species of *Polypedates* as outgroups according to previous studies (Jiang *et al.*, 2019; Li *et al.*, 2008, 2009). All novel sequences obtained in this study were deposited in GenBank (Accession No. are shown in Supplementary Table S1).

DNA extraction, amplification, and sequencing

Genomic DNA of *R. laoshan* was extracted from ethanol-preserved liver tissue samples using an Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech, China), according to the protocols of the manufacturer. A total of 1890 bp of the 12S rRNA, tRNA^{Val}, and 16S rRNA was targeted and amplified using the primers that are listed in Table I. Polymerase chain reaction (PCR) were performed at 25 µL volume, including 12.5 µL Taq PCR Master Mix (2 X blue dye), 9.5 µL ddH₂O, 1 µL FS01, 1 µL Rend (C=10 µL/L), and 1 µL DNA template. PCR cycles consisted of initial denaturation at 95°C for 3 min followed by 35 cycles: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min. A final elongation step of 10 min at 72°C was performed.

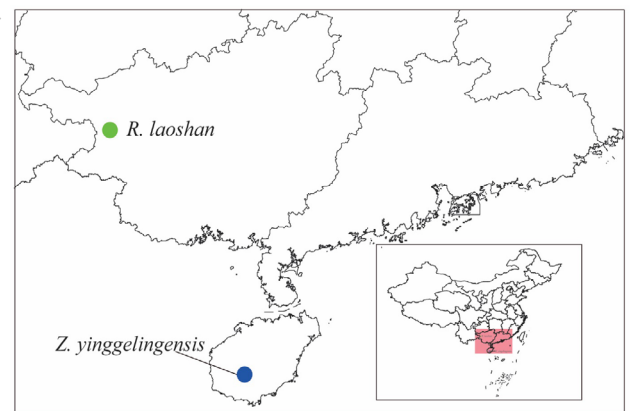


Fig. 1. Localities of *R. laoshan* and *Z. yinggelingsensis* in China.

Table I. Primers used in this study.

Primer	Primer sequence	Resource
FS01	5'-AAC GCT AAG ATG AAC CCT AAA AAG TTC T-3'	Wilkinson <i>et al.</i> , 2002
Rend	5'-GAC CTG GAT TAC TCC GGT CTG A-3'	Wilkinson <i>et al.</i> , 2002
F0483	5'-GAA GAG GCA AGT CGT AAC ATG G -3'	Wilkinson <i>et al.</i> , 2002
F1209	5'-CTC GTA CCT TTT GCA TCA TG-3'	Yang, 2018
R1209	5'-CAT GAT GCA AAA GGT ACG AG-3'	Yang, 2018

In order to conduct further research, we used high-throughput sequencing technology to extract genes of *Z. yinggelingsensis*. A total of 0.88 µg DNA extracted from *Z. yinggelingsensis* muscle tissue was sheared into short fragments (<500bp) using the Covaris system to construct pair-end DNA libraries. DNA libraries were constructed and sequenced using Illumina-Hi-Seq 2500. The sample was sequenced to a target 10×depth (30Gbp) of its genome. Reads containing more low-quality bases (phred score <30) than 20% were filtered out. Gene fragments of other species in this study were aligned using MEGA 7.0 (Kumar *et al.*, 2016) and adjusted manually. Then, we used the MEANGS (<https://github.com/YanCCscu/MEANGS>) to get the purpose genes. The process are as follows: The alignments of genes that aligned using MEGA 7.0 were used in construction profile HMM(s) with hmmer-build in HMMER V3.16 (Eddy, 2008). Hereafter, nhmmer were used to search reads that fitting the hmm profiler for each gene from high-throughout sequencing data. Finally, we assembled these matched reads with the de novo assembler SSAKE v2.2.1 (Warren *et al.*, 2007), and the assembled scaffolds were identified by blasting to the purpose DNA fragments, including four mitochondrial fragments, 12S rRNA, tRNA^{Val}, 16S rRNA, Cytochrome b (Cyt b), and three nuclear genes, Recombination activating gene 1 (RAG-1), Exon 1 of tyrosinase (RHOD) and exon 1 of tyrosinase (TYR). In order to evaluate the accuracy of the assembled genes of *Z. yinggelingsensis*, we mapped all reads back to the assembled genes, and statistic the mapping quality and average depth of these genes.

Phylogenetic analyses

Phylogenetic trees were reconstructed by only mitochondrial fragments (12S rRNA, tRNA^{Val}, 16S rRNA) and combined mitochondrial and nuclear fragments (12S rRNA, tRNA^{Val}, 16S rRNA, Cyt b, RAG-1, RHOD, TYR), respectively. Sequences were aligned using MEGA 7.0 (Kumar *et al.*, 2016) with default settings and rechecked in MAFFT version 7 (Katoh and Standley, 2013), then adjusted manually. Different gene fragments of each individual were concatenated by using Phylosuite (Zhang *et al.*, 2020), and positions of indels were treated as missing data for all datasets. The most optimum substitution model of evolution (GTR+I+G for mitochondrial fragments, HKY+I for RAG-1 gene and RHOD gene, and SYM+G for TYR gene) was calculated by Partition Finder v2.1.1 (Lanfear *et al.*, 2017) under Akaike Information Criterion (AIC) (lnL= -18812.57, AICc= 37858.23). We then reconstructed phylogenetic trees using the Bayesian inference (BI) and maximum likelihood (ML) methods, respectively.

The Bayesian phylogenetic tree was constructed using

MrBayes 3.2.0 (Ronquist *et al.*, 2012). Runs of Markov chains for 5,000,000 generations were summarized and sampled every 100 generations. The first 25% of samples as burn-in, and convergence was investigated using the parameter of average standard deviation of split frequencies ≤ 0.01 (Ronquist *et al.*, 2012). Nodes were considered strongly supported when BPP ≥ 0.95 . Software RAxML v8.2.10 (Stamatakis, 2014) was used to implement Maximum Likelihood (ML) analysis under the best-fit model of evolution (GTRGAMMA) based on the AIC criterion. And bootstrap proportions (BSP) were evaluated with 1000 nonparametric bootstrap pseudoreplications, and it were considered significantly supported when the node's BSP ≥ 70 . In addition, uncorrected pairwise distance (*p*-distances) of the four mitochondrial fragments (12S rRNA, tRNA^{Val}, 16S rRNA and Cyt b) and three nuclear genes (RAG-1, RHOD and TYR) dataset among species in *Rhacophorus sensu lato* were calculated using MEGA 7 (Kumar *et al.*, 2016), with the “*P*-distance” model and “Transitions + Transversions” substitutions to include.

RESULTS AND DISCUSSION

The mapping average depth of 12S rRNA, tRNA^{Val}, and 16S rRNA, Cyt-b, RAG-1, RHOD, and TYR for the *Z. yinggelingsensis* is 3331, 2315, 13.95, 5.847, 9.681, respectively. Simultaneously, the mitochondrial fragments consisted of 1890 bp, and the combined mitochondrial and nuclear fragments consisted of 3746 bp, including 1890 bp for 12S rRNA, tRNA^{Val}, and 16S rRNA, 546 bp for Cyt-b, 507 bp for RAG-1, 279 bp for RHOD, 524 bp for TYR. The present molecular phylogeny is concordant with previous molecular studies regarding the monophyly of *Rhacophorus sensu lato* (Chan *et al.*, 2018, 2019; Li *et al.*, 2008, 2009, 2012a, 2013; O'Connell *et al.*, 2018b; Pan *et al.*, 2017a). Both mitochondrial fragments (Supplementary Fig. S1) and combined mitochondrial and nuclear fragments (Fig. 2 and Supplementary Fig. S2) resulted in virtually identical topology. Although interspecific relationships were not fully resolved, the phylogenetic trees from both the BI and ML methods showed that all species contained in *Rhacophorus sensu lato* were clustered to three clades as monophyletic groups and each clade consisted of the same species. According to phylogenetic trees from combined mitochondrial and nuclear fragments, clade A was congruent with genus *Zhangixalus* and was strongly supported by a bootstrap proportion of 83 and a posterior probability of 0.96. Clade B was congruent with genus *Leptomantis* and it was sister group to *Zhangixalus* that supported by a bootstrap proportion of 80 and a posterior probability of 0.96, which was consistent with Jiang *et al.* (2019), but was different from Chen *et al.* (2019). Chen *et al.* (2019) indicated that *Leptomantis* was sister group

to *Rhacophorus sensu stricto*. They used anchored hybrid enrichment (AHE) targeted sequencing and their sequences were long larger than this study. Clade C was congruent with genus *Rhacophorus sensu stricto* and supported by a bootstrap proportion of 100 and a posterior probability of 1.00.

The phylogeny (Fig. 2 and Supplementary Fig. S2) depicted that *R. laoshan* belongs to *Rhacophorus sensu stricto* and *Z. yinggelingsensis* was nested within *Zhangixalus*. Jiang *et al.* (2019) propose the morphological diagnosis and difference of each genus of *Rhacophorus sensu lato*. They shed light on *Zhangixalus* differs from *Rhacophorus sensu stricto* by the absence of dermal folds along limbs and tarsal projections (vs. present), the absence of supracloacal fold (vs. present or not). And we found that *R. laoshan* and *Z. yinggelingsensis* corresponds with the morphological diagnosis of *Rhacophorus sensu stricto* and *Zhangixalus*, respectively. On the one hand, the morphological characteristics of *R. laoshan* similar to the characteristics diagnosis of *Rhacophorus sensu lato* in the presence of dermal folds along limbs (Mo *et al.*, 2008; Jiang *et al.*, 2019). On the other hand, the morphological characteristics of *Z. yinggelingsensis* similar to the following combination of characteristics diagnosis of *Zhangixalus*: Dermal folds along limbs absent; tarsal projections absent; body size relatively moderate or large, SVL 30–120 mm (mostly above 50 mm); dorsal coloration mostly green (Chou *et al.*, 2007; Jiang *et al.*, 2019). Consequently, based on our molecular results and published morphological data, we agree with Jiang *et al.* (2019), place *R. laoshan* and *Z. yinggelingsensis* into the genera *Rhacophorus* and *Zhangixalus*, respectively.

Furthermore, in the clade *Rhacophorus sensu stricto*, *R. laoshan* was the sister-taxon to the assembly of three species (BPP = 1.00; BSP = 99), including *R. verrucopus* (6254 RAO), *R. orlovi* (AMNH A161405) and *R. calcaneus* (FMNH 256465; Fig. 2). However, we cannot confirm the sister species of *R. laoshan*, and it may be related to *R. calcaneus*, which was recovered as polyphyletic and formed two distinct clades. Specifically, between the two *R. calcaneus* clades, one taxon (FMNH 256465, from Laos) was sister to *R. orlovi* (AMNH A161405; BPP = 1.00; BSP = 100) possessed a genetic divergence of 0.2% (Supplementary Table S2). Additionally, the second *R. calcaneus* (AMNH A163749, from Vietnam) and *R. robertingeri* (VNMN:4123, from Vietnam) formed a well-supported clade with short branch length (BPP = 1.00; BSP = 100), and this clade possessed a genetic divergence of 0.3% (Supplementary Table S2). However, on the one hand, the localities of the species *R. calcaneus* were certainty only from three places (Frost, 2020), and the localities of the two specimens of *R. calcaneus* (FMNH 256465, from Laos; AMNH A163749, from Quang Nam, Vietnam) didn't

fall within the known distribution sites of *R. calcaneus*. On the other hand, some species are poorly diagnosed yet and characters highly convergent in previous studies (David *et al.*, 2007, 2015a; Guo *et al.*, 2014). Consequently, we suspect that the two specimens (FMNH 256465, AMNH A163749) of *R. calcaneus* were misidentifications. Due to we do not have the two specimens, it is need to confirm whether the two specimens were misidentified in future study. Moreover, it may be that some sequences of the species *R. calcaneus* from the NCBI have problems, because taxonomic identification problems of existing Genbank sequences are very common (Guo *et al.*, 2014; Ren *et al.*, 2018).

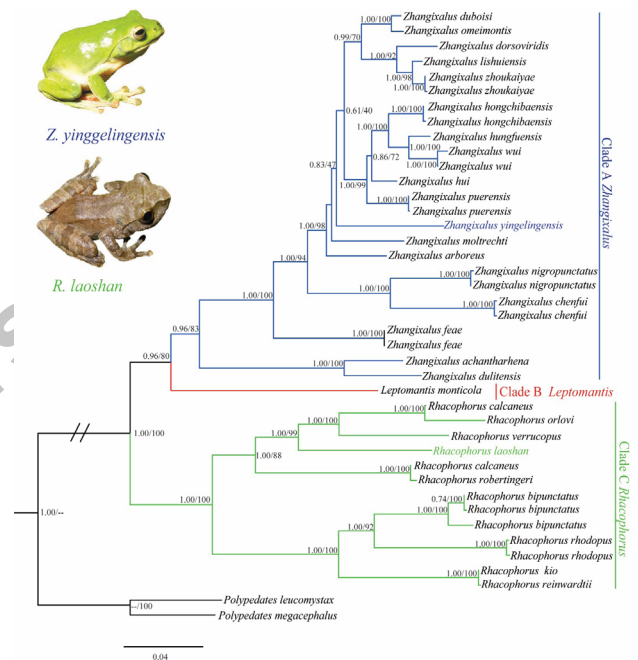


Fig. 2. Phylogenetic relationships of species of *Zhangixalus*, *Leptomantis* and *Rhacophorus* based on four mitochondrial genes (12S rRNA, tRNA^{Val}, 16S rRNA and Cyt b) and three nuclear DNA genes (RAG-1, RHOD, TYR). Different colors correspond to three clades: Clade A (blue), Clade B (red), Clade C (green). Numbers beside the nodes are given as Bayesian posterior probabilities (BPP) and bootstrap proportions (BSP) for maximum likelihood analyses. Photographs of *R. laoshan* and *Z. yinggelingsensis* are used from Mo *et al.* (2008) and Chou *et al.* (2007), respectively.

For *Z. yinggelingsensis*, the *p*-distances varied from 3.0% to 7.0% between it and other species of *Zhangixalus* that collected in this study [minimum distance to *Z. duboisi*; maximum distance to *Z. achantharhena* (Supplementary Table S2)]. Additionally, Chou *et al.* (2007) suggested that *Z. yinggelingsensis* seems to be closely related to *Z. arvalis*,

Z. aurantiventris, *Z. chenfui*, *Z. dorsovirens*, *Z. hungfuensis*, *Z. taipeianus*, and *Z. yaoshanensis*, *Z. moltrechti*, *Z. nigropunctatus* on the basis of morphological evidences. However, there are many morphologically similar and conservative species in *Zhangixalus* (Li *et al.*, 2012), and we can't distinguish them well. Thus, we suggest that it not only use morphological and molecular datasets, but also their distributions when we identified the species, which can reduce the probability of misidentifications.

Although phylogenetic status of *Z. yinggelingsensis* is not resolved well, we recognize the validity of it using the molecular data for the first time. However, why phylogenetic status of *Z. yinggelingsensis* is not resolved well? The possible reasons are as follows: (1) The sequence of *Z. yinggelingsensis* in this study is short; (2) There are only some species of *Zhangixalus* in this study; (3) the genus *Zhangixalus* evolution history is complex. As genomic data have become more common in phylogenetic studies, many studies have been shown that it can use genomic data resolving both shallow and deep-scale evolutionary relationships (Brandrud *et al.*, 2019; Burbrink and Gehara, 2018; Chen *et al.*, 2019; Godefroid *et al.*, 2019; Kuntner *et al.*, 2019; Spriggs *et al.*, 2019). Thus, it seems possible that we can use genomic data to resolve the phylogenetic status of *Z. yinggelingsensis* in future study. In addition, the research on amphibians in Southern China was scarce, and there have been some cryptic species were discovered in the past three years (Liu *et al.*, 2017; Pan *et al.*, 2017b; Yu *et al.*, 2019). Finally, we suggest that we can strengthen the investigation of amphibian diversity in southern China, which lay a foundation for the study of biogeography in southern China.

CONCLUSIONS

In this study, we have clarified the phylogenetic status of *R. laoshan* and *Z. yinggelingsensis* using mitochondrial fragments (12S rRNA, tRNA^{Val}, 16S rRNA and Cyt *b*) and nuclear DNA (RAG-1, RHOD, TYR) fragments. Our results suggest that *R. laoshan* and *Z. yinggelingsensis* belongs to *Rhacophorus sensu stricto* and *Zhangixalus*, respectively. Finally, this research play an important role in the further studies, such as protection, biogeographical patterns and the evolution of life history strategies of these species.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20200611050629>

Statement of conflict of interest

The authors have declared no conflict of interest.

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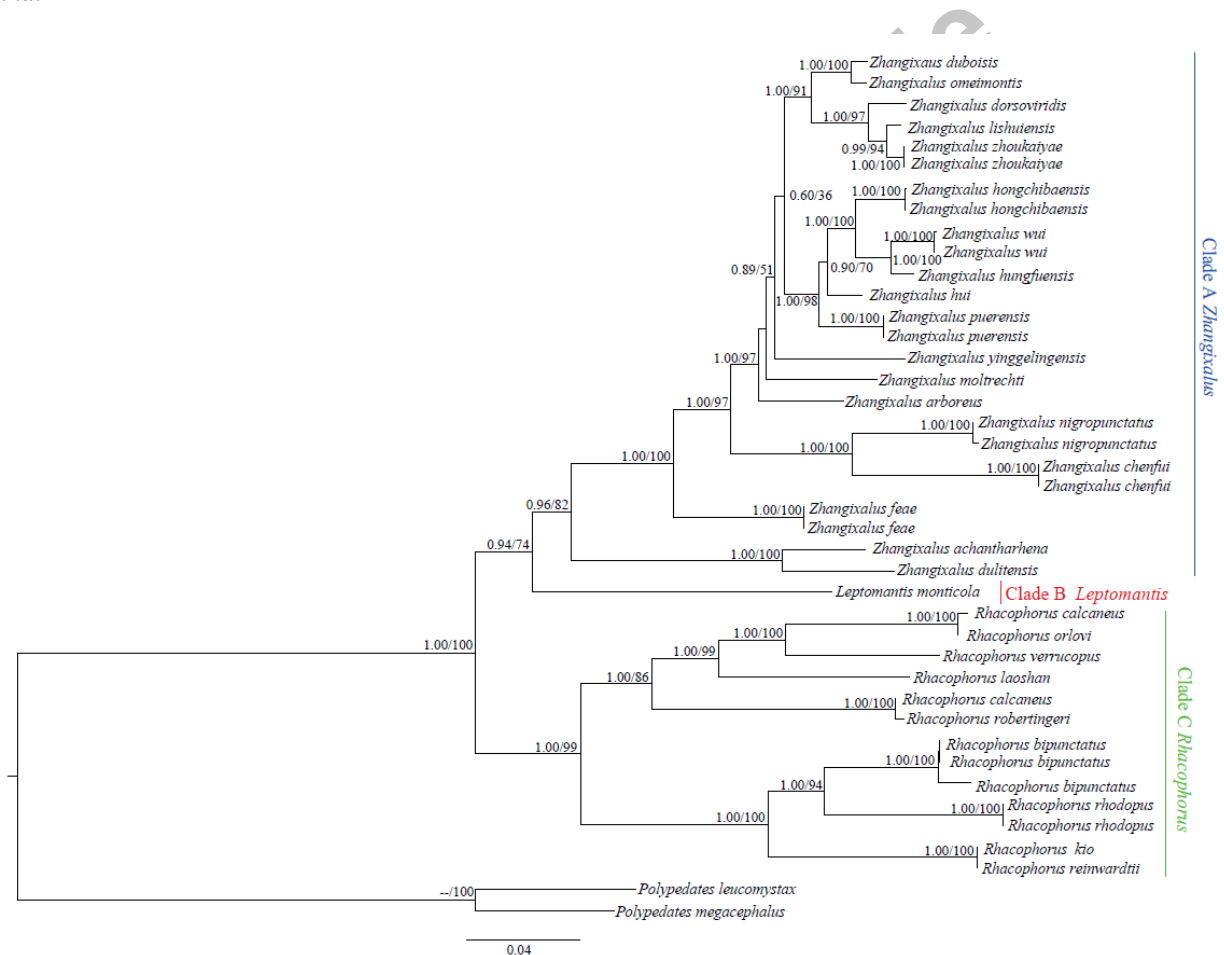
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Supplementary Material

Molecular Phylogenetic Status of *Rhacophorus laoshan* and *Zhangixalus yinggelingsensis* (Anura: Rhacophoridae) from China

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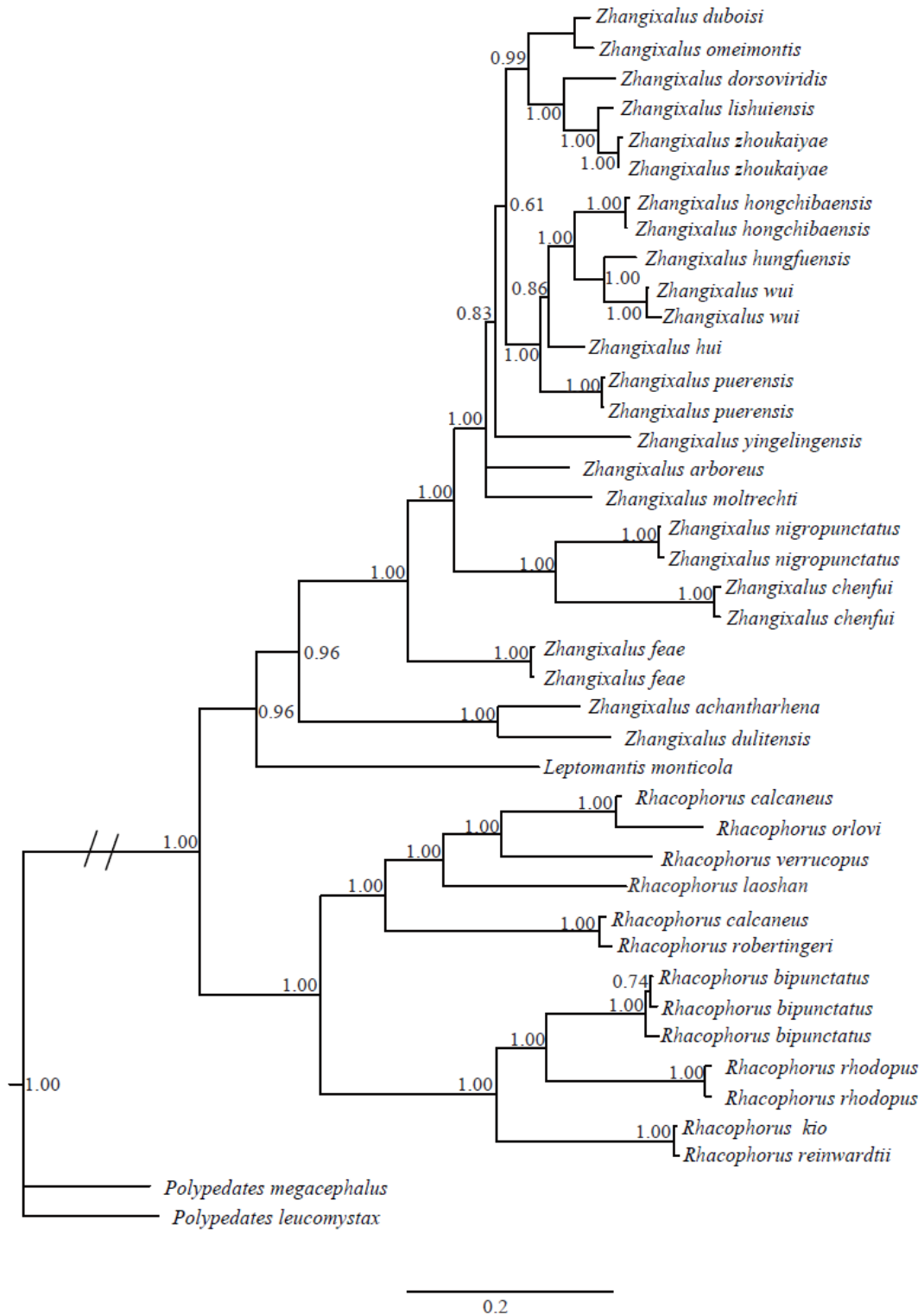
Supplementary Fig. S1. Phylogenetic relationships of species of *Zhangixalus*, *Leptomantis* and *Rhacophorus* based on three mitochondrial genes (12S rRNA, tRNA^{Val}, 16S rRNA).

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Supplementary Fig. S2. Bayesian phylogenetic relationships of species of *Zhangixalus*, *Leptomantis* and *Rhacophorus* based on four mitochondrial genes (12S rRNA, tRNA^{Val}, 16S rRNA and Cyt b) and three nuclear DNA genes (RAG-1, RHOD, TYR). Numbers beside the nodes are given as Bayesian posterior probabilities (BPP).

Supplementary Table S1. Samples, with their locality, museum voucher number, and GenBank accession No. of corresponding sequences. “---” represents missing data, and “*” represents the new sequences in this study.

Name	Locality	Voucher Number	Partial 12S rRNA and complete tRNA ^{val}	Cyt b	RAG-1	RHOD	TYR
<i>Zhangixalus achanharhena</i>	Indonesia, Sumatra, Jambi	ENS 14757	KX398867	---	---	---	---
<i>Zhangixalus arboreus</i>	Japan	TTU-R-11748	AF458142	---	---	AY880653	---
<i>Zhangixalus chentui</i>	China: Xichang, Sichuan	KIZ060821073	EF564465 EF564537	EU924603	EU924519	EU924547	EU924575
<i>Zhangixalus chentui</i>	China: Mt. Omei, Sichuan	SCUM 060404L	EU215534	---	---	EU215564	EU215594
<i>Zhangixalus dorsovittatus</i>	China: Pingbian, Yunnan	KIZ060821199	EF564490 EF564562	EU924610	EU924526	EU924554	EU924582
<i>Zhangixalus duboisi</i>	China: Mt. Dawei, Yunnan	SCUM 061104L	EU215536	---	---	EU215566	EU215596
<i>Zhangixalus duliensis</i>	Malaysia	RAO081201	JX219434	---	---	---	---
<i>Zhangixalus feae</i>	China: Pingbian, Yunnan	KIZ060821197	EF564474 EF564546	EU924606	EU924522	EU924550	EU924578
<i>Zhangixalus feae</i>	China: Pingbian, Yunnan	SCUM 050642W	EU215544	---	---	EU215574	EU215604
<i>Zhangixalus hongchibaensis</i>	China: Wuxi, Chongqing	CIB 097687	JN688883	---	---	JN688897	JN688906
<i>Zhangixalus hongchibaensis</i>	China: Wuxi, Chongqing	CIB 097696	JN688882	---	---	JN688898	JN688907
<i>Zhangixalus hui</i>	China: Zhaojue, Sichuan	Li01	JN688878	---	---	---	---
<i>Zhangixalus hungfuensis</i>	China: Wenchuan, Sichuan	SCUM 060425L	EU215538	---	---	EU215568	EU215598
<i>Zhangixalus lishuensis</i>	China, Lishui, Zhejiang	YPX47791	KY653718	---	---	---	---
<i>Zhangixalus molrechi</i>	China: Lianhuachi, Taiwan	SCUM 061106L	EU215543	---	---	EU215573	EU215603
<i>Zhangixalus nigropunctatus</i>	China: Weining, Guizhou	SCUM 070657L	EU215533	---	GQ285767	EU215563	EU215593
<i>Zhangixalus nigropunctatus</i>	China: Weining, Guizhou	KIZ07061001	EU924628 EU924623	EU924611	EU924527	EU924555	EU924583
<i>Zhangixalus omeimontis</i>	China: Pengxian, Sichuan	SCUM 0606137L	EU215535	---	---	EU215565	EU215595
<i>Zhangixalus puerensis</i>	China: Puer, Yunnan	SCUM 060648L	JN688884	---	---	JN688899	JN688908
<i>Zhangixalus puerensis</i>	China: Puer, Yunnan	SCUM 060649L	EU215542	---	---	EU215572	EU215602
<i>Zhangixalus wui</i>	China: Lichuan, Hubei	CIB 097685	JN688881	---	---	JN688896	JN688910
<i>Zhangixalus wui</i>	China: Lichuan, Hubei	CIB 097690	JN688880	---	---	JN688900	JN688909
<i>Zhangixalus yinggelingensis</i>	China: Hainan	HN2018002	MW192130*	MW192131*	MW192132*	MV192134*	MV192133*
<i>Zhangixalus zhoukaiyae</i>	China: Jinzhai, Anhui	AHU-RhaDb-150418-01	KU601494	---	KU601467	KU601476	KU601485
<i>Zhangixalus zhoukaiyae</i>	China: Jinzhai, Anhui	AHU-RhaDb-150418-02	KU601495	---	KU601468	KU601477	KU601486
<i>Leptomantis monticola</i>	Indonesia: Sulawesi	RMB 1236	AY326060	---	---	---	---
<i>Rhacophorus kio</i>	China: Xishuangbanna, Yunnan	SCUM 37941C	EU215532	---	---	---	---

Continue on next page.....

Name	Locality	Voucher Number	Partial 12S rRNA and complete 16S rRNA ^{vi}	Cyt b	RAG-I	RHOD	TYR
<i>Rhacophorus bipunctatus</i>	China: Hainan	SN 030035	EF564507 EF564579	EU924602	EU924518	EU924546	EU924574
<i>Rhacophorus bipunctatus</i>		FMNH 2531114	GQ204767 GQ204716	GQ204533	---	GQ204652	---
<i>Rhacophorus bipunctatus</i>	China: Hainan		EU215529	---	EU924574	EU924602	---
<i>Rhacophorus calcaneus</i>	Lao	FMNH 256465	GQ204770 GQ204719	GQ204536	- GQ204600- GQ204655	---	---
<i>Rhacophorus calcaneus</i>				-			
<i>Rhacophorus calcaneus</i>	Vietnam	AMNHA163749	DQ283380	---	---	Q283999	DQ282991
<i>Rhacophorus laoshan</i>	China: Guangxi	1705014	MW149528*	---	---	---	---
<i>Rhacophorus orlovi</i>	Vietnam	AMNHA161405	DQ283049	---	---	DQ283778	---
<i>Rhacophorus reinwardtii</i>	China: Luehuan, Yunnan	KIZ060821224	EF564498 EF564570	EU924615	EU924531	EU924559	EU924587
<i>Rhacophorus rhodopus</i>	China: Mengyang, Yunnan	SCUM 060692L	EU215531	---	---	EU215561	EU215591
<i>Rhacophorus rhodopus</i>	China: Yunnan,	KIZ060821037	EF564500 EF564572	EU924616	EU924532	EU924560	EU924588
<i>Rhacophorus robertingeri</i>	Vietnam: Gig Lai	VNMN:4123	LC010613	---	---	---	---
<i>Rhacophorus verrucopus</i>	China: Motuo, Xizang	6254 RAO	JX219436	---	---	---	---
<i>Polypedates leucomystax</i>	China: Luehuan, Yunnan	LC 0805113	KC465832	---	KC465846	KC465847	KC465848
<i>Polypedates megacephalus</i>	China: Mt. Daiyun, Fujian	SCUM 050508C	EU215552	---	GQ285771	EU215582	EU215612