The reanalysis of biogeography of the Asian tree frog, *Rhacophorus* (Anura: Rhacophoridae): Geographic shifts and climatic change influenced the dispersal process and diversification

Tao Pan 1, Yanan Zhang 1, Hui Wang 1, Jun Wu 2, Xing Kang 1, Lifu Qian 1, Jinyun Chen 3, Dingqi Rao 4, Jianping Jiang 5, Baowei Zhang 6

1 Anhui Key Laboratory of Eco-engineering and Bio-technique, School of Life Sciences, Anhui University, Hefei, Anhui Province, China
2 Ministry of Environmental Protection, Nanjing Institute of Environmental Sciences, Nanjing, Jiangsu, China
3 Department of Life Science, Huainan Normal University, Huainan, Anhui, China
4 Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China
5 Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China
6 School of Biosciences, Cardiff University, Cardiff, United Kingdom

Corresponding Author: Baowei Zhang
Email address: zhangbw@ahu.edu.cn

Rapid uplifts of the Tibetan Plateau and climate change in Asia are thought to have profoundly modulated the diversification of most of the species distributed throughout Asia. The ranoid tree frog genus *Rhacophorus*, the largest genus in the Rhacophoridae, is widely distributed in Asia and especially speciose in the areas south and east of the Tibetan Plateau. Here, we infer phylogenetic relationships among species and estimate divergence times, asking whether the spatiotemporal characteristics of diversification within *Rhacophorus* were related to rapid uplifts of the Tibetan Plateau and concomitant climate change. Phylogenetic analysis recovered distinct lineage structures in *Rhacophorus*, which indicated a clear distribution pattern from Southeast Asia toward East Asia and India. Molecular dating suggests that the first split within the genus date back to the Middle Oligocene (approx. 30 Ma). The *Rhacophorus* lineage through time (LTT) showed that there were periods of increased speciation rate: 14 – 12 Ma and 10 – 4 Ma. In addition, ancestral area reconstructions supported Southeast Asia as the ancestral area of *Rhacophorus*. According to results of the molecular dating, ancestral area reconstructions and LTT, we think the geographic shifts, the staged rapid rises of the Tibetan Plateau with parallel climatic changes and reinforcement of the Asian monsoons (15 Ma, 8 Ma and 4 – 3 Ma), possibly prompted a burst of diversification in *Rhacophorus*.
The reanalysis of biogeography of the Asian tree frog, *Rhacophorus* (Anura: Rhacophoridae): geographic shifts and climatic change influenced the dispersal process and diversification

Tao Pan¹, Yanan Zhang¹*, Hui Wang¹, Jun Wu², Xing Kang¹, Lifu Qian¹, Jinyun Chen³, Dingqi Rao⁴, Jianping Jiang⁵, and Baowei Zhang¹,6*

¹ Anhui Key Laboratory of Eco-engineering and Bio-technique, School of Life Sciences, Anhui University, Hefei, Anhui, China

² Ministry of Environmental Protection, Nanjing Institute of Environmental Sciences, Nanjing, Jiangsu, China

³ Department of Life Science, Huainan Normal University, Huainan, Anhui, China

⁴ Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China

⁵ Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan, China

⁶ School of Biosciences, Cardiff University, Cardiff, CF103AX, United Kingdom.

¶ These authors contributed equally to this work.

*Corresponding author

E-mail address: zhangbw@ahu.edu.cn (Baowei Zhang)
ABSTRACT

Rapid uplifts of the Tibetan Plateau and climate change in Asia are thought to have profoundly modulated the diversification of most of the species distributed throughout Asia. The ranoid tree frog genus *Rhacophorus*, the largest genus in the Rhacophoridae, is widely distributed in Asia and especially speciose in the areas south and east of the Tibetan Plateau. Here, we infer phylogenetic relationships among species and estimate divergence times, asking whether the spatiotemporal characteristics of diversification within *Rhacophorus* were related to rapid uplifts of the Tibetan Plateau and concomitant climate change. Phylogenetic analysis recovered distinct lineage structures in *Rhacophorus*, which indicated a clear distribution pattern from Southeast Asia toward East Asia and India. Molecular dating suggests that the first split within the genus date back to the Middle Oligocene (approx. 30 Ma). The *Rhacophorus* lineage through time (LTT) showed that there were periods of increased speciation rate: 14 – 12 Ma and 10 – 4 Ma. In addition, ancestral area reconstructions supported Southeast Asia as the ancestral area of *Rhacophorus*. According to results of the molecular dating, ancestral area reconstructions and LTT, we think the geographic shifts, the staged rapid rises of the Tibetan Plateau with parallel climatic changes and reinforcement of the Asian monsoons (15 Ma, 8 Ma and 4 – 3 Ma), possibly prompted a burst of diversification in *Rhacophorus*.

INTRODUCTION

Abiotic factors like climatic and tectonic events, and biotic factors like inter- or intraspecific
interactions, competition and predation, may be the predominant driving factors during the evolution and diversification of organisms (Antonelli & Sanmartín 2011; Benton 2009). These factors can also affect the diversification at different temporal and geographical scales (Benton 2009). Understanding the processes of diversification and which factors have driven the evolution and diversification of organisms, may help guide prioritization in conservation and forecast the population demography under future climate conditions (Avise 2000; Frankham et al. 2002). It had been shown that orogenic activity during recent geological history was linked to the formation of hotspots of biodiversity (Myers et al. 2000). Consequently, the study of the origin and evolution of biodiversity in mountain systems has experienced a growing scientific interest (Klaus et al. 2016; van der Meijden et al. 2007; Zhang et al. 2006). In Asia, the uplift of the Tibetan Plateau was the most intense orogenic movement leading to the formation of several biomes (Favre et al. 2015; Klaus et al. 2016; Myers et al. 2000; Yang et al. 2009). Therefore, research has focused on the uplift of the Tibetan Plateau, based on a temporal (molecular dating) and spatial (biogeographic) framework, which may have triggered a series of evolutionary changes in different biological groups (Klaus et al. 2016), such as in plants (Gao et al. 2013; Jabbour & Renner 2012; Tu et al. 2010; Wang et al. 2009), birds (Lei et al. 2014; Tietze & Borthakur 2012; Tietze et al. 2013), mammals (Deng et al. 2011) and amphibians (Che et al. 2010; Guo et al. 2011; Li et al. 2013; Vijayakumar et al. 2016; Zhang et al. 2006).

The ranoid treefrog genus, *Rhacophorus*, is the largest genus in the Rhacophoridae, currently containing 88 species (Frost 2016), which are widely distributed across India, China, Japan, mainland South-east Asia, the Greater Sunda Islands and the Philippines (Frost 2016). A
previous study disclosed that Rhacophoridae underwent an early dispersal from India to Asia between 46 and 57 Ma, that a transient faunal exchange ceased during the Middle Eocene, and a subsequent increase of Rhacophorid dispersal events between Asia and the Indian subcontinent during the Oligocene that continued until the Middle Miocene (Li et al. 2013). Uplift of the Tibetan Plateau and a series of climatic and environmental changes led to many speciation events on a very large scale (Favre et al. 2015; Myers et al. 2000; Yang et al. 2009). Rhacophorus taxa are widely distributed across the areas around the Tibetan Plateau, according to previous study (Li et al. 2013), the speciation process in this genus may be linked to the uplift of Tibetan Plateau during the Miocene and Pliocene.

To gain a better understanding of the diversification processes in biomes around the Tibetan Plateau, we herein provide a historical biogeographic pattern of Rhacophorus. In the present study, we collected all the sequences datasets of Asian Rhacophorus that have been reported in addition to newly sequenced DNA from Rhacophorus specimens collected from the Dabie Mountains in Anhui, China. We infer the phylogenetic relationships within the genus and estimate the divergence times. Further, the correlation between diversification events within Rhacophorus and the geographic shifts in the Tibetan Plateau are explored.

MATERIALS AND METHODS

Ethical Statement

The collection of samples was performed within a long-term investigation project on
amphibians of Dabie mountains. This investigation project and the sample collection were approved by Anhui Tianma National Nature Reserve, Anhui Province, China. The relevant document of field permit was provided in the supplementary material.

Data Collection

For the phylogenetic analyses, sequences of about half the species of *Rhacophorus* were used in combination with sequences of two outgroup species, *Polypedates megacephalus* (Rhacophoridae, *Polypedates*) and *Spinomantis peraccae* (Mantellidae, *Spinomantis*) (Li et al. 2013; Li et al. 2012a; Li et al. 2012b). Sequence data were obtained from GenBank (the GenBank Accession numbers are given in Table S1). In total, there were 149 individuals of 57 species of *Rhacophorus* involved (Fig. 1 and Table S1). All the taxonomic revisions within *Rhacophorus* were follow previous studies (Biju et al. 2013; Li et al. 2013; Orlov et al. 2012).

Sampling, DNA Extraction, PCR Amplification, and Sequencing

Between 2012 to 2015, nine specimens of *R. zhoukaiyae* were collected from the Dabie Mountains, China (Pan et al. 2017). Muscle tissue from each individual was sampled and preserved in 100% ethanol for DNA extraction. Total DNA was extracted from the samples using a standard proteinase K/phenol-chloroform protocol (Sambrook et al. 1989). An EasyPure PCR Purification Kit (TransGene) was used to purify the DNA extractions. The sequences of 12S and 16S ribosome RNA (rRNA) of *R. zhoukaiyae* were collected from Pan et al. (2017). In
addition, we also amplified and sequenced five nuclear gene fragments with the indicated primer pairs (Table S2), including brain-derived neurotrophic factor (BDNF), proopiomelanocortin (POMC), recombination activating gene 1 (RAG-1), rhodopsin exon 1 (RHOD) and tyrosinase exon 1 (TYR) (Bossuyt & Milinkovitch 2000; Li et al. 2009; van der Meijden et al. 2007; Vieites et al. 2007; Wiens et al. 2005). Polymerase chain reactions (PCR) were performed using a reaction mixture (25 μL) containing 1 μL genomic DNA (concentration 10 – 50 ng/μL), 2.5 μL 10×buffer, 1 μL of 2.5 mM MgSO4, 2 μL of 2 mM dNTPs, 1 U Taq polymerase (Meridian Bioscience, Singapore) and 0.3 mM of each of the primers. Pure molecular biology grade water was added to reach the appropriate volume. The amplification protocol included an initial denaturation step of 95°C for 5 min; this was followed by 32 cycles of denaturation at 95°C for 30 s, primer annealing at 51°C – 57°C for 30 s, and an extension at 72°C for 40 s – 80 s, with a final extension at 72°C for 10 min. PCR products were purified using an EasyPure PCR Purification Kit (TransGene) and sequenced using previous primers and the BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) following the manufacturer’s instructions on an ABI Prism 3730 automated sequencer. All the sequences obtained in this study were deposited into GenBank (Table S1). For the analyses, the sequences were trimmed to match data downloaded from GenBank, then all the sequences were aligned automatically using Clustal X version 1.83 (Thompson et al. 1997), followed by visual confirmation and manual adjustments. Nucleotide sites with ambiguous alignments were removed from the analyses, and gaps were analyzed as missing data.
Phylogenetic Analyses

Two different datasets were generated for the different analyses. Dataset 1 was used for a phylogenetic analysis of *Rhacophorus* by Maximum Likelihood (ML) and Bayesian methods, was comprised of the 12S and 16S rRNA gene together with the complete t-RNA for the valine sequence of the *Rhacophorus* species and the outgroups (Table S1). Dataset 2 contained more genes (12S, 16S, Val, BDNF, POMC, RAG-1, RHOD, TYR) of more individual and species than Dataset 1 (Table S1). However, it was only used to calculate a Bayesian consensus tree. The best-fit model of evolution was calculated by MrModeltest 1.0 b under the AIC criterion (Nylander 2003). ML analyses were performed in RAxML version 8 (Stamatakis 2014) and a general time reversible model of nucleotide substitution under the Gamma model of rate heterogeneity (i.e., GTRCAT). Support for the internal branches for the best-scoring tree was evaluated via the bootstrap test with 1,000 iterations. A Bayesian inference of phylogeny was performed using the MrBayes 3.1.2 software program (Huelsenbeck & Ronquist 2005), using the best-fit substitution model. Two Markov Chain Monte Carlo (MCMC) models were run to provide additional confirmation of the convergence of posterior probability distributions. Analyses were run for 3,000,000 generations. Chains were sampled every 1000 generations. The first 25% of the total trees were discarded as “burn-in” and the remaining trees were used to generate a majority-rule consensus tree and to calculate Bayesian posterior probabilities.
Divergence Time Analyses

To estimate divergence times of *Rhacophorus*, we applied a Bayesian MCMC method with mitochondrial genes (Dataset 1), which employs a relaxed molecular clock approach, as implemented in BEAST 1.7.4 (Drummond et al. 2012). We assumed a relaxed uncorrelated log normal model of lineage variation and a Yule Process prior to the branching rates based on the GTR + I + G model as recommended by MrModeltest 1.0 b (Nylander 2003). Four replicates were run for 10,000,000 generations with tree and parameter sampling every 1,000 generations. The first 25% of samples were discarded as burn-in. All parameters were assessed by visual inspection using Tracer v. 1.5 (Rambaut & Drummond 2007). The tree was generated and visualized with TreeAnnotator v. 1.6.1 (Rambaut & Drummond 2010) and FigTree v. 1.3.1 (Rambaut 2009), respectively. Calibration points were taken from Li et al. (2013) (Table 1). In addition, to visualizing the temporal accumulation of species, a log-transformed lineage-through-time (LTT) (Nee et al. 1994) plot was constructed and compared with the null distribution for the LTT line simulated under the empirical pure-birth model. For visualizing diversification rate changes, we plotted the number of newly appearing species against the fixed time intervals of 2 million years (Ma) (Venditti et al. 2010).

Ancestral Area Reconstructions

Ancestral area reconstructions were inferred by the program RASP 3.2 (Yu et al. 2015) for speciational evolution in phylogenetic trees, using the Bayesian Binary MCMC (BBM) method
(Ronquist & Huelsenbeck 2003) and the statistical dispersal-vicariance method (S-DIVA) (Yu et al. 2010). To reconstruct ancestral areas on the basis of the topography, the distributional range of Asian *Rhacophorus* was divided into four regions, W, X, Y and Z (Fig. 2). W represents Southeast Asia, including the Indochinese Peninsula, Sundaland and the south margin of the Tibetan Plateau, X contains the Hengduan mountains and the mountains around the Sichuan Basin, Y refers to South China and Japan and Z represents India (Fig. 2). The tree data sets and the condensed tree were generated by BEAST 1.7.4 (Drummond et al. 2012). The distribution of each species was collected from http://maps.iucnredlist.org. For all analyses, the maximum number of ancestral areas at each node was constrained to three. The frequencies of an ancestral range at a node were averaged over all trees and each alternative ancestral range at a node was weighted by the frequency of occurrence for the node.

**RESULTS**

**Molecular Phylogenetic Analyses**

The aligned mtDNA gene fragments from *Rhacophorus* consisted of 1935 bp nucleotide positions before trimming (Dataset 1). After trimming, 1851 nucleotide positions were retained for genealogical reconstructions. The fragments contained 934 constant and 917 potentially phylogenetically informative characters. The ML or BI phylogenetic approaches based on Dataset 1 resulted in virtually identical topology, and all terminal clades obtained relatively high-
supporting values (Fig. S1). The genus *Rhacophorus* was supported as monophyletic containing four major clades (Fig. S1). For further probing of the dispersal process and diversification of the Asian tree frog, the molecular dating and ancestral area reconstructions were carried out. The phylogenetic tree, collected from the molecular dating, showed three distinct clades (A, B and C) in the genus of *Rhacophorus* (Fig. 2). There were some difference in the species distribution areas among the three clades. Species in clade A were mostly distributed in Southeast Asia and East Asia, species in Clade B were distributed in Southeast Asia and India, and species in lineage C only found in Southeast Asia. Clade A contained six groups, A1 to A6 (Fig. 2). The phylogenetic tree, based on Dataset 2, was largely consistent with the results from Dataset 1 (Fig. S2). However, there were some minor differences between them, such as the polyphyletic of clade B and C in Fig. S2. But, generally, it did not affect the results of ancestral area reconstructions of *Rhacophorus*.

**Molecular Dating, Ancestral Area Reconstructions and Lineage Through Time**

Dating analyses based on Dataset 1 suggested that the most recent common ancestor (MRCA) of *Rhacophorus* dates back to 29.51Ma (median value; 95% of the highest posterior density [HPD] = 25.00 – 34.07Ma) (Table 1 and Fig. 3). The MRCA of Clade A and Clade B was estimated at 27.38Ma (95% HPD = 22.44 – 32.17 Ma). The MRCA of Clade A was 21.56 Ma (95% HPD = 17.92 – 25.22 Ma) and the MRCA of Clade B was 26.73 Ma (95% HPD = 21.56 – 31.83 Ma).
Ancestral area reconstructions from S-DIVA and BBM analyses were largely similar with some minor differences (Fig. 2). All analyses supported Southeast Asia (Area W, Fig. 2) as the ancestral area of *Rhacophorus* and most speciation events were attributed to dispersal. The empirical LTT plot of *Rhacophorus* showed that, after a lengthy period of constant diversification, the diversification rate of the genus had increased during the middle Pliocene. The cumulative curve of species-birth per time interval showed that the diversification of *Rhacophorus* fluctuated through time, especially during 14 – 12 Ma and 10 – 4 Ma (Fig. 4).

**DISCUSSION**

The Dispersal Process of *Rhacophorus* and Its Spread toward East Asia and India

Previous studies have indicated that the diversification of Rhacophoridae was closely linked to the India-Asia collision (57 Ma – 35 Ma) (Li et al. 2013). Southeastern Asia houses three globally significant hot spots divided by sharp, yet porous biogeographic boundaries (Evans et al. 2003; Favre et al. 2015; Schmitt et al. 1995; Wallace 1860). Studies have shown that the dynamics of the formation of biodiversity in Southeastern Asia is assumed to be interrelated with many geological events and a unique climatic history.. Events such as the continuing processes of volcanic uplift and the emergence of many new islands in Indo-Australian Archipelago during the Miocene-Pliocene (Fig. 3iii) (Esselstyn et al. 2009; Hall 1996; Hall 1998; Hall 2002; Lohman et al. 2011), the rapid uplifts of the Tibetan Plateau (Shi et al. 1999), repeated sea level fluctuations during the Pleistocene (Bird et al. 2005; Esselstyn et al. 2009; Hall 1998; Heaney...
1985; Heaney 1986; Jansa et al. 2006; Voris 2000) and the onset of the Asian monsoon system (An et al. 2001; Qiang et al. 2001; Sun & Wang 2005; Zhisheng et al. 2001). Many phylogeographical studies of plants and animals support this assumption (Deng et al. 2011; Klaus et al. 2016; Shi et al. 1999), such as those on *Lilium* (Gao et al. 2013), Delphinieae (Jabbour & Renner 2012), Hyoscyameae (Tu et al. 2010), Mandragoreae (Tu et al. 2010), *Saussurea* (Wang et al. 2009), birds (Lei et al. 2014; Tietze & Borthakur 2012; Tietze et al. 2013; Yang et al. 2009), Hynobiidae (Zhang et al. 2006), lizards (Guo et al. 2011) and Spiny Frogs (Che et al. 2010), so the diversification and speciation in *Rhacophorus* may also be related to the special geological formations and the climatic history.

The phylogenetic analysis shows that *Rhacophorus* is composed of multiple lineages. In the phylogenetic tree with timescale, calculated by BEAST, *Rhacophorus* is composed of three major clades, A, B and C (Fig. 2). Among these clades, Clade C was the basal branch of *Rhacophorus*, which contained ten species from Southeast Asia, and the age of the MRCA of *Rhacophorus* was estimated at 29.51 Ma (i.e., 95% CI, 25 – 34.07 Ma, Fig. 2 and Table 1). The MRCA of Clades B and Clade A was 27.38 Ma (95% CI, 22.44 Ma – 32.17 Ma) during the Oligocene (Fig. 2, Table 1). The members of Clades A and B are mainly distributed in the south of the Tibetan Plateau margin, India and Eastern Asia (Fig. 1). Clade A contained six groups which were distributed in three areas: Southeast Asia (group A1), the south of the Tibetan Plateau margin (group A2) and an Eastern Asia (group A3 to A6) (Fig. 1, 2). The MRCA of Clade A occurred 21.56 Ma ago (95% CI, 17.92 – 25.22 Ma; Fig. 2) and the time of the split of different groups was estimated at 14.09 Ma (A2 vs A3 ~A6, 95% CI, 10.96 – 17.41 Ma), 11.39
Ma (A3 vs A4 ~ A6, 95% CI, 8.89 – 14.16 Ma), 8.56 Ma (A4 vs A5 ~ A6, 95% CI, 6.43–10.88 Ma) and 5.33 Ma (A5 vs A6, 95% CI, 3.92 – 6.99 Ma) respectively (Table 1). In addition, the LTT plot analysis indicated an increased diversification rate during two periods (14 – 12 Ma and 10 – 4 Ma) (Fig. 4). Basically, the above mentioned phylogeographical information reflected the trend of diversification and the speciation process. Obviously, the distribution of these species expanded continuously from Southern Asia to India and Eastern Asia, reaching as far as Japan (Fig. 2).

During the Oligocene and Miocene the uplift progressed, causing the extension of the Tibetan Plateau (Harrison et al. 1992; Mulch & Chamberlain 2006). The start of the uplift of the northern Tibetan Plateau occurred at about 30 Ma BP (Sun & Wang 2005) or slightly earlier (Wang et al. 2012b). Then, the eastern parts of the Tibetan Plateau likely reached an elevation comparable to the present-day elevation in the Mid to Late Miocene (from 15 to 5 Ma) (Axelrod 1997; Currie et al. 2005; Jacques et al. 2011; Spicer et al. 2003; Tapponnier et al. 2001; Valdiya 1999; Zhang et al. 2013). The southeastern edge of the Tibetan Plateau, the Hengduan mountain range, experienced rapid uplift only after the Miocene (5.33 Ma), reaching a peak elevation shortly before the Late Pliocene (5.33 – 2.66 Ma) (Li & Fang 1999; Mulch & Chamberlain 2006; Sun et al. 2011; Zheng et al. 2000), which separated several major rivers that ran in parallel (the Yangtze, Mekong, and Salween valleys) (Clark et al. 2004). This series of rapid Tibetan Plateau uplifts dramatically changed the terrain and landform in this area, which resulted in speciation, especially in animal groups (Che et al. 2010; Deng et al. 2011; Gao et al. 2013; Jabbour & Renner 2012; Lei et al. 2014; Li et al. 2013; Shi et al. 1999; Tietze & Borthakur 2012; Tietze et
al. 2013; Tu et al. 2010; Wang et al. 2009; Zhang et al. 2006). Zhang et al. (2006) found that the origin and phylogenetic divergence of the Hynobiidae had a correlation to the uplift of the Tibetan Plateau (Zhang et al. 2006). The phylogenetic history of Paini (Anura: Dicroglossidae) illuminates a critical aspect of the timing of geological events, especially for the uplift of the Tibetan Plateau (Che et al. 2010). On the other hand, the Tibetan Plateau and its adjacent mountain ranges acted as an orographic barrier to atmospheric circulation in Asia and consequently contributed to the formation of the Asian monsoon system, which was one of the major climatic changes in this region (Early Miocene, 24 Ma) due to the Tibetan Plateau’s considerable size and altitude (Guo et al. 2008; Kutzbach et al. 1993; Liu & Yin 2002; Ruddiman & Kutzbach 1991; Song et al. 2010; Sun & Wang 2005; Tang et al. 2013; Zhisheng et al. 2001). In the following millions of years, the East Asian monsoon intensified three times (~15 Ma, ~8 Ma and 4 – 3 Ma) (An et al. 2001; Jacques et al. 2011; Molnar et al. 2010; Song et al. 2010; Sun & Wang 2005; Valdiya 1999; Wan et al. 2007; Zhisheng et al. 2001). The development of the Asian monsoon system directly gave birth to the warm and humid climate in the south of China (Sun & Wang 2005), which was maybe favorable for the geographical spread and speciation of amphibians (Che et al. 2010; Thorn & Raffaëlli 2001; Wu et al. 2013; Zhang et al. 2006). In addition, the climate oscillations that began about 2.8 million years ago, in the Late Pliocene (Deng et al. 2011), also provided the chance for diversification and speciation of many species (Zhang et al. 2000), such as birds (Lei et al. 2014), the Tibetan woolly rhino (Coelodonta thibetana) (Deng et al. 2011) and stream-dwelling frog (Feirana quadranus) (Wang et al. 2012a). Molecular dating suggested that the TMRC of Clade A and Clade B was during the Oligocene
(22.44 Ma – 32.17 Ma) (Fig. 3, Table 1). At same time, ancestral area reconstructions supported Southeast Asia (W) as the ancestral area of *Rhacophorus* and the dispersal events happened from ancestral area of Clade A and Clade B (Fig. 2, node b). In addition, the land and sea in the Indo-Australian Archipelago changed greatly during this period (Lohman et al. 2011), which may promote the dispersal events from Southeast Asia. In Clade A, the time of the split of subgroups was estimated from 14.09 to 5.33 Ma (Table 1). In addition, the time of most nodes in Clade B also occurred during this period (Fig. 3). Based on the LTT plot analysis, there were two increased diversification rate periods (14 – 12 Ma, 10 – 4 Ma) in *Rhacophorus* (Fig. 4). The series of Tibetan Plateau rapid uplifts (from 15 to 2.66 Ma) dramatically changed the landscape, which resulted in the diversification of species or speciation in this area (Che et al. 2010; Deng et al. 2011; Gao et al. 2013; Jabbour & Renner 2012; Lei et al. 2014; Li et al. 2013; Shi et al. 1999; Tietze & Borthakur 2012; Tietze et al. 2013; Tu et al. 2010; Wang et al. 2009; Zhang et al. 2006) and the biotic interchange between the Indian subcontinent and mainland Asia (Klaus et al. 2016). In addition, the intensified East Asian monsoon (~15 Ma, ~8 Ma and 4 – 3 Ma) directly gave birth to the warm and humid climate in the south of China, which was favorable for the geographical spread and speciation of amphibians (Che et al. 2010; Thorn & Raffaëlli 2001; Wu et al. 2013; Zhang et al. 2006). Obviously, the diversification events in *Rhacophorus* were in line with the time frame of the orogenic movement and climatic histories, especially the staged rapid uplift of the Tibetan Plateau and the enhanced Asian monsoon system (Figs. 2 and 3). Therefore, we think that the diversification and speciation events in Clade A and Clade B, are related to the staged uplift of the Tibetan Plateau and the subsequent chain-reaction events, such as the
establishment of the Asian monsoon system, which facilitated the radiations and speciation of amphibians (Che et al. 2010; Thorn & Raffaëlli 2001; Wu et al. 2013; Zhang et al. 2006).

Overall, the evolutionary history of *Rhacophorus* originated approx 30 Ma Bp (Oligocene). Basically, it is the dispersal process from its ancestral area, Southeast Asia, toward India and East Asia. During the process, *Rhacophorus* diversified by multiple factors, such as geographic shifts, the staged rapid rises of the Tibetan Plateau with parallel climatic changes, the reinforcement of the Asian monsoons (15 Ma, 8 Ma and 4–3 Ma) and alternating glacial-interglacial oscillations.

**ACKNOWLEDGEMENTS**

We thank Wenliang Zhou, Zhonglou Sun, Zhaojie Peng and Xiaonan Sun for their help in sample collecting, John Bailey (Department of Genetics, University of Leicester) and Martin Burrows for input concerning the quality of the writing as regards the English language, Jiatang Li (Chengdu Institute of Biology, Chinese Academy of Sciences) for the study design, and thank the reviewers (Nguyen Tao, Gururaja Kotambylu Vasudeva and an anonymous reviewer) for their suggestions. We thank Tianma National Nature Reserve in Anhui Province for the investigation project and the sample collection.
REFERENCES

An ZS, Kutzbach JE, Prell WL, and Porter SC. 2001. Evolution of Asian monsoons and phased uplift of the Himalaya–Tibetan plateau since Late Miocene times. *Nature* 411:62-66.

Antonelli A, and Sanmartin I. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60:403-414.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Boston: Harvard University Press.

Axelrod DI. 1997. Paleoelevation estimated from Tertiary floras. *Int Geol Rev* 39:1124-1133.

Benton MJ. 2009. The Red Queen and the Court Jester: species diversity and the role of biotic and abiotic factors through time. *Science* 323:728-732.

Biju SD, Kamei RG, Mahony S, Thomas A, Garg S, Sircar G, and Suyesh R. 2013. Taxonomic review of the tree frog genus *Rhacophorus* from the Western Ghats, India (Anura: Rhacophoridae), with description of ontogenetic colour changes and reproductive behavior. *Zootaxa* 3636:257–289.

Bird MI, Taylor D, and Hunt C. 2005. Palaeoenvironments of insular Southeast Asia during the Last Glacial Period: a savanna corridor in Sundaland? *Quaternary Sci Rev* 24:2228-2242.

Bossuyt F, and Milinkovitch MC. 2000. Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *P Natl Acad Sci* 97:6585-6590.

Che J, Zhou WW, Hu JS, Yan F, Papenfuss TJ, Wake DB, and Zhang YP. 2010. Spiny frogs
(Paini) illuminate the history of the Himalayan region and Southeast Asia. *P Natl Acad Sci* 107:13765-13770.

Clark MK, Schoenbohm LM, Royden LH, Whipple KX, Burchfiel BC, Zhang X, Tang W, Wang E, and Chen L. 2004. Surface uplift, tectonics, and erosion of eastern Tibet from large-scale drainage patterns. *Tectonics* 23:1-20.

Currie BS, Rowley DB, and Tabor NJ. 2005. Middle Miocene paleoaltimetry of southern Tibet: implications for the role of mantle thickening and delamination in the Himalayan orogen. *Geology* 33:181-184.

Deng T, Wang XM, Fortelius M, Li Q, Wang Y, Tseng ZJ, Takeuchi GT, Saylor JE, Säilä LK, and Xie GP. 2011. Out of Tibet: Pliocene woolly rhino suggests high-plateau origin of Ice Age megaherbivores. *Science* 333:1285–1288.

Drummond AJ, Suchard MA, Xie D, and Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29:1969–1973.

Esselstyn JA, Timm RM, and Brown RM. 2009. Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. *Evolution* 63:2595-2610.

Evans BJ, Brown RM, McGuire JA, Supriatna J, Andayani N, Diesmos A, Iskandar D, Melnick DJ, and Cannatella DC. 2003. Phylogenetics of fanged frogs: testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. *Syst Biol* 52:794-819.

Favre A, Päckert M, Pauls SU, Jähnig SC, Uhl D, Michalak I, and Muellner - Riehl AN. 2015.
The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas.

*BioRev* 90:236-253.

Frankham R, Briscoe DA, and Ballou JD. 2002. *Introduction to conservation genetics*. Oxford: Cambridge University Press.

Frost DR. 2016. Amphibian Species of the World: an Online Reference. Version 6.0 (27 May, 2016). American Museum of Natural History, New York, USA.

Gao YD, Harris AJ, Zhou SD, and He XJ. 2013. Evolutionary events in Lilium (including Nomocharis, Liliaceae) are temporally correlated with orogenies of the Q–T plateau and the Hengduan Mountains. *Mol Phylogenet Evol* 68:443-460.

Guo XG, Dai X, Chen D, Papenfuss TJ, Ananjeva NB, Melnikov DA, and Wang YZ. 2011. Phylogeny and divergence times of some racerunner lizards (Lacertidae: Eremias) inferred from mitochondrial 16S rRNA gene segments. *Mol Phylogenet Evolution* 61:400-412.

Guo ZT, Sun B, Zhang ZS, Peng SZ, Xiao GQ, Ge JY, Hao QZ, Qiao YS, Liang MY, and Liu JF. 2008. A major reorganization of Asian climate by the early Miocene. *Clim Past* 4:153-174.

Hall R. 1996. *Reconstructing Cenozoic SE Asia*. Pages 153-184 in Tectonic evolution of southeast Asia (R. Hall and D. Blundell, eds.). London: Geological Society of London Special Publication.

Hall R. 1998. *The plate tectonics of Cenozoic SE Asia and the distribution of land and sea*. Pages 99-131 in Biogeography and geological evolution of SE Asia (R. Hall and J. D.
Holloway, eds.). Leiden: Backhuys Publishers.

Hall R. 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *J Asian Earth Sci* 20:353-431.

Harrison TM, Copeland P, Kidd WSF, and Yin AN. 1992. Raising tibet. *Science* 255:1663-1670.

Heaney LR. 1985. Zoogeographic evidence for middle and late Pleistocene land bridges to the Philippine Islands. *Mod Quat Res SE Asia* 9:127-144.

Heaney LR. 1986. Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. *Biol J Linn Soc* 28:127-165.

Huelsenbeck JP, and Ronquist F. 2005. MrBayes. Version 3.1. 2.<http://mrbayes.scs.fsu.edu/download.php>.

Jabbour F, and Renner SS. 2012. A phylogeny of Delphinieae (Ranunculaceae) shows that Aconitum is nested within Delphinium and that Late Miocene transitions to long life cycles in the Himalayas and Southwest China coincide with bursts in diversification. *Mol Phylogenet Evol* 62:928-942.

Jacques FMB, Guo SX, Su T, Xing YW, Huang YJ, Liu YS, Ferguson DK, and Zhou ZK. 2011. Quantitative reconstruction of the Late Miocene monsoon climates of southwest China: a case study of the Lincang flora from Yunnan Province. *Palaeogeogr Palaeocl* 304:318-327.

Jansa SA, Barker FK, and Heaney LR. 2006. The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Syst Biol* 55:73-88.
Klaus S, Morley RJ, Plath M, Zhang YP, and Li JT. 2016. Biotic interchange between the Indian subcontinent and mainland Asia through time. *Nat Commun* 7:12132.

Kutzbach JE, Prell WL, and Ruddiman WF. 1993. Sensitivity of Eurasian climate to surface uplift of the Tibetan Plateau. *J Geol* 101:177-190.

Lei FM, Qu YH, and Song G. 2014. Species diversification and phylogeographical patterns of birds in response to the uplift of the Qinghai-Tibet Plateau and Quaternary glaciations. *Current Zoology* 60:149-161.

Li JJ, and Fang XM. 1999. Uplift of the Tibetan Plateau and environmental changes. *Chinese Sci Bull* 44:2117-2124.

Li JT, Che J, Murphy RW, Zhao H, Zhao EM, Rao DQ, and Zhang YP. 2009. Evolution of reproduction in the Rhacophoridae (Amphibia: Anura) inferred from a phylogenetic analysis of five nuclear and three mitochondrial genes. *Mol Phylogenet Evol* 53:509-522.

Li JT, Li Y, Klaus S, Rao DQ, Hillis DM, and Zhang YP. 2013. Diversification of rhacophorid frogs provides evidence for accelerated faunal exchange between India and Eurasia during the Oligocene. *Proceed Nat Acad Sci* 110:3441-3446.

Li JT, Li Y, Murphy RW, Rao DQ, and Zhang YP. 2012a. Phylogenetic resolution and systematics of the Asian tree frogs, *Rhacophorus* (Rhacophoridae, Amphibia). *Zool Scr* 41:557-570.

Li JT, Liu J, Chen YY, Wu JW, Murphy RW, Zhao EM, Wang YZ, and Zhang YP. 2012b. Molecular phylogeny of treefrogs in the *Rhacophorus dugritei* species complex (Anura: Rhacophoridae), with descriptions of two new species. *Zool J Linn Soc* 165:143-162.
Liu XD, and Yin ZY. 2002. Sensitivity of East Asian monsoon climate to the uplift of the Tibetan Plateau. *Palaeogeogra Palaeocl* 183:223-245.

Lohman DJ, Bruyn MD, Page T, Rintelen KV, Hall R, Ng PKL, Shih HT, Carvalho GR, and Rintelen TV. 2011. Biogeography of the Indo-Australian Archipelago. *Annu Rev Ecol Evol S* 42:205-226.

Molnar P, Boos WR, and Battisti DS. 2010. Orographic controls on climate and paleoclimate of Asia: thermal and mechanical roles for the Tibetan Plateau. *Annu Rev Earth Pl Sc* 38:77.

Mulch A, and Chamberlain CP. 2006. Earth science: The rise and growth of Tibet. *Nature* 439:670-671.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, and Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853-858.

Nee S, May RM, and Harvey PH. 1994. The reconstructed evolutionary process. *Philosophical Transactions of the Royal Society B: Biological Sciences* 344:305-311.

Nylander JAA. 2003. MrModeltest 1.0 b. a simplified version of David Posada’s ÔÔModeltest 3.06 ‘‘. Uppsala, Sweden: Department of Systematic Zoology.

Orlov NL, Poyarkov NA, Vassilieva AB, Ananjeva NB, Nguyen T, Sang N, and Geissler P. 2012. Taxonomic notes on Rhacophorid frogs (Rhacophorinae: Rhacophoridae: Anura) of southern part of Annamite Mountains (Truong Son, Vietnam), with description of three new species. *Russ J Herpetol* 19:23–64.

Pan T, Zhang YN, Wang H, Wu J, Kang X, Qian LF, Li K, Zhang Y, Chen JY, Rao DQ, Jiang JP, and Zhang BW. 2017. A New Species of the Genus *Rhacophorus* (Anura:
Rhacophoridae) from Dabie Mountains in East China. *Asian Herpetol Res* 8:1–13.

Qiang XK, Li ZX, Powell CMA, and Zheng HB. 2001. Magnetostratigraphic record of the Late Miocene onset of the East Asian monsoon, and Pliocene uplift of northern Tibet. *Earth Planet Sc Lett* 187:83–93.

Rambaut A. 2009. FigTree version 1.3. 1. *Computer program distributed by the author*<http://tree.bio.ed.ac.uk/software/figtree/[accessed January 4, 2011]>.

Rambaut A, and Drummond AJ. 2007. Tracer. Version 1.5.

Rambaut A, and Drummond AJ. 2010. TreeAnnotator version 1.6. 1.

Ronquist F, and Huelsenbeck JP. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*:1572–1574.

Ruddiman WF, and Kutzbach JE. 1991. Plateau uplift and climatic change. *Sci Am* 264:66-75.

Sambrook J, Fritsch EF, and Maniatis T. 1989. *Molecular cloning*. New York: Cold spring harbor laboratory press.

Schmitt LH, Kitchener DJ, and How RA. 1995. A genetic perspective of mammalian variation and evolution in the Indonesian Archipelago: biogeographic correlates in the fruit bat genus *Cynopterus*. *Evolution* 49:399–412.

Shi YF, Li JJ, Li BY, Yao TD, Wang SM, Li SJ, Cui ZJ, Wang FB, Pan BT, Fang XM, and Zhang QS. 1999. Uplift of the Qinghai-Tibetan plateau and East Asia environmental change during late Cenozoic. *Acta Geographical Sinica* 54:10–20.

Song JH, Kang HS, Byun YH, and Hong SY. 2010. Effects of the Tibetan Plateau on the Asian summer monsoon: a numerical case study using a regional climate model. *Int J Climatol*
Spicer RA, Harris NBW, Widdowson M, Herman AB, Guo SX, Valdes PJ, Wolfe JA, and Kelley SP. 2003. Constant elevation of southern Tibet over the past 15 million years. *Nature* 421:622-624.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.

Sun BN, Wu JY, Liu YS, Ding ST, Li XC, Xie SP, Yan DF, and Lin ZC. 2011. Reconstructing Neogene vegetation and climates to infer tectonic uplift in western Yunnan, China. *Palaeogeogra Palaeocl* 304:328-336.

Sun XG, and Wang PX. 2005. How old is the Asian monsoon system?—Palaeobotanical records from China. *Palaeogeogra Palaeocl* 222:181-222.

Tang H, Micheels A, Eronen JT, Ahrens B, and Fortelius M. 2013. Asynchronous responses of East Asian and Indian summer monsoons to mountain uplift shown by regional climate modelling experiments. *Clim Dynam* 40:1531-1549.

Tapponnier P, Xu ZQ, Roger F, Meyer B, Arnaud N, Wittlinger G, and Yang JS. 2001. Oblique stepwise rise and growth of the Tibet Plateau. *Science* 294:1671-1677.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, and Higgins DG. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882.

Thorn R, and Raffaëlli J. 2001. *Les salamandres de l'ancien monde*. Paris: Editions Boubée.

Tietze DT, and Borthakur U. 2012. Historical biogeography of tits (Aves: Paridae, Remizidae).
Tietze DT, Päckert M, Martens J, Lehmann H, and Sun YH. 2013. Complete phylogeny and historical biogeography of true rosefinches (Aves: *Carpodacus*). *Zool J Linn Soc* 169:215-234.

Tu T, Volis S, Dillon MO, Sun H, and Wen J. 2010. Dispersals of Hyoscyameae and Mandragoreae (Solanaceae) from the New World to Eurasia in the early Miocene and their biogeographic diversification within Eurasia. *Mol Phylogenet Evol* 57:1226-1237.

Valdiya KS. 1999. Rising Himalaya: Advent and intensification of monsoon. *Curr Sci* 76:514-524.

van der Meijden A, Vences M, Hoegg S, Boistel R, Channing A, and Meyer A. 2007. Nuclear gene phylogeny of narrow-mouthed toads (Family: Microhylidae) and a discussion of competing hypotheses concerning their biogeographical origins. *Mol Phylogenet Evol* 44:1017-1030.

Venditti C, Meade A, and Pagel M. 2010. Phylogenies reveal new interpretation of speciation and the Red Queen. *Nature* 463:349-352.

Vieites DR, Min M-S, and Wake DB. 2007. Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proceed Nat Acad Sci* 104:19903-19907.

Vijayakumar SP, Menezes RC, Jayarajan A, and Shanker K. 2016. Glaciations, gradients, and geography: multiple drivers of diversification of bush frogs in the Western Ghats Escarpment. *P Roy Soc B* 283.

Voris HK. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and
time durations. *J Biogeogr* 27:1153-1167.

Wallace AR. 1860. On the zoological geography of the Malay Archipelago. *Proc Linn Soc* 4:172-184.

Wan SM, Li AC, Clift PD, and Stuut JBW. 2007. Development of the East Asian monsoon: mineralogical and sedimentologic records in the northern South China Sea since 20 Ma. *Palaeogeogra Palaeocl* 254:561-582.

Wang B, Jiang JP, Xie F, and Li C. 2012a. Postglacial colonization of the Qinling Mountains: phylogeography of the Swelled Vent frog (*Feirana quadranus*). *PloS one* 7:1-14.

Wang YD, Zheng JJ, Zhang WL, Li SY, Liu XW, Yang X, and Liu YH. 2012b. Cenozoic uplift of the Tibetan Plateau: Evidence from the tectonic–sedimentary evolution of the western Qaidam Basin. *Geoscience Frontiers* 3:175-187.

Wang YJ, Susanna A, Von Raab-Straube E, Milne R, and Liu JQ. 2009. Island-like radiation of *Saussurea* (Asteraceae: Cardueae) triggered by uplifts of the Qinghai–Tibetan Plateau. *Biol J Linn Soc* 97:893-903.

Wiens JJ, Fetzner JW, Parkinson CL, and Reeder TW. 2005. Hylid frog phylogeny and sampling strategies for speciose clades. *Syst Biol* 54:778-807.

Wu YK, Wang YZ, Jiang K, and Hanken J. 2013. Significance of pre-Quaternary climate change for montane species diversity: insights from Asian salamanders (Salamandridae: *Pachytriton*). *Mol Phylogenet Evol* 66:380-390.

Yang SJ, Dong HL, and Lei FM. 2009. Phylogeography of regional fauna on the Tibetan Plateau: a review. *Prog Nat Sci* 19:789-799.
Yu Y, Harris AJ, Blair C, and He XJ. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Mol Phylogenet Evol* 87:46-49.

Yu Y, Harris AJ, and He XJ. 2010. S-DIVA (statistical dispersal-vicariance analysis): a tool for inferring biogeographic histories. *Mol Phylogenet Evol* 56:848-850.

Zhang DF, Fengquan L, and Jianmin B. 2000. Eco-environmental effects of the Qinghai-Tibet Plateau uplift during the Quaternary in China. *Environ Geol* 39:1352–1358.

Zhang P, Chen YQ, Zhou H, Liu YF, Wang XL, Papenfuss TJ, Wake DB, and Qu LH. 2006. Phylogeny, evolution, and biogeography of Asiatic Salamanders (Hynobiidae). *P Nat Acad Sci* 103:7360-7365. DOI 10.1073/pnas.0602325103

Zhang WL, Fang XM, Song CH, Appel E, Yan MD, and Wang YD. 2013. Late Neogene magnetostratigraphy in the western Qaidam Basin (NE Tibetan Plateau) and its constraints on active tectonic uplift and progressive evolution of growth strata. *Tectonophysics* 599:107-116.

Zheng HB, Powell CMA, An ZS, Zhou J, and Dong GG. 2000. Pliocene uplift of the northern Tibetan Plateau. *Geology* 28:715-718.

Zhisheng A, Kutzbach JE, Prell WL, and Porter SC. 2001. Evolution of Asian monsoons and phased uplift of the Himalaya–Tibetan plateau since Late Miocene times. *Nature* 411:62-66.
**Figure 1** (on next page)

Sample sites of *Rhacophorus* species used in this study.
Figure 2 (on next page)

Chronogram and ancestral area reconstructions of *Rhacophorus* with outgroup species, *Polypedates megacephalus* and *Spinomantis peraccae* based on Dataset 1.

Branches in the tree are proportional to absolute ages (Ma). Node charts showed the relative probabilities of alternative ancestral distributions obtained by integrating the statistical dispersal-vicariance analysis (S-DIVA; above branches) and a Bayesian Binary MCMC method (BBM; below branches), and the first two areas with highest probability are shown corresponding to their relative probability on the area of one circle. Areas are divided for reconstructing ancestral areas. (W) Southeast Asia, including the Indochinese Peninsula, Sundaland, and the south margin of the Tibetan Plateau; (X) Hengduan mountains and the mountains around the Sichuan Basin; (Y) South China and Japan; (Z) India.
Biogeographical history of *Rhacophorus*.

(i) Time-calibrated phylogeny of the genus *Rhacophorus* inferred from the mitochondrial dataset with an outgroup species, *Polypedates megacephalus* and *Spinomantis peraccae*. The light-blue bars through the nodes indicate 95% credibility intervals. Detailed time estimates for nodes with letter labels are given in Table 1; (ii) Climatic sequence of events including a global average δ¹⁸O curve (right-hand axis) derived from benthic foraminifera which mirrors the major global temperature trends from the Paleocene to the Pleistocene (red line) [modified from Zachos et al. (2001), Zachos et al. (2008) and Favre et al. (2015)]. The establishment of ice sheets in the Northern Hemisphere is indicated by grey to black bars on top. The onset and development of the monsoon is symbolised by a blue polygon and its intensification by grey bars (I, II and III) (Wan et al. 2007; Jacques et al. 2011). The climate oscillations during the Quaternary are represented by a grey bar (IV) (Deng et al. 2011); (iii) Geological sequences of events are related to the diversification of *Rhacophorus* including the reconstructions historical land and sea in Southeast Asia and a graphical representation of the extent of the uplift of the TP through time. ① and ② show two Cenozoic reconstructions of land and sea in the Indo-Australian Archipelago [modified from Lohman et al. (2011)]. Red shading in ③ and ④ indicates the portion of the TP that had achieved altitudes comparable to the present day for each given time [modified from Mulch & Chamberlain, (2006) and Favre et al. (2015)].
(i) Diversification

(ii) Climate

(iii) Geology
**Figure 4** (on next page)

Visualization of diversification rate shifts of *Rhacophorus*.

(A) Lineage-through-time plot (logarithmic scale) and 95% confidence intervals of lineage diversification; (B) Cumulative curve of diversification rate per million years. The dashed line represents the period of rapid diversification in *Rhacophorus*. 
Table 1 (on next page)

Detailed results of molecular dating using BEAST 1.7.4, and the calibration points.

Labels for nodes correspond to Fig. 3. Unit: one million years. The abbreviation of time to most recent common ancestor is TMRC.
Table 1. Detailed results of molecular dating using BEAST 1.7.4, and the calibration points.

Labels for nodes correspond to Fig. 3. Unit: one million years. The abbreviation of time to most recent common ancestor is TMRC.

| Node     | TMRC  | Mean (95%)       | Mean(95%) (Li et al. 2013) |
|----------|-------|------------------|-----------------------------|
| Root     | ---   | 33.27 (25.11-40.20) | 36.5 (31.2-40.9) |
| a        | Clade A, B, C | 29.51 (25.34-34.07) | 30.6 (25.2-34.7) |
| b        | Clade A, B | 27.38 (22.44-32.17) | --- |
| c        | Clade A   | 21.56 (17.92-25.22) | 21.6 (17.5-25.1) |
| d        | Groups A2-A6 | 14.09 (10.96-17.41) | --- |
| e        | Groups A3-A6 | 11.39 (8.89-14.16) | --- |
| f        | Groups A4-A6 | 8.56 (6.43-10.88) | --- |
| g        | Groups A5, A6 | 5.33 (3.92-6.99) | --- |
| h        | Group A6   | 2.9 (1.78-4.29) | --- |
| i        | ---     | 8.4 (6.43-10.29) | 8.6 (5.5-9.8) |