

## Article

# From mainland to islands: colonization history in the tree frog *Kurixalus* (Anura: Rhacophoridae)

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## Abstract

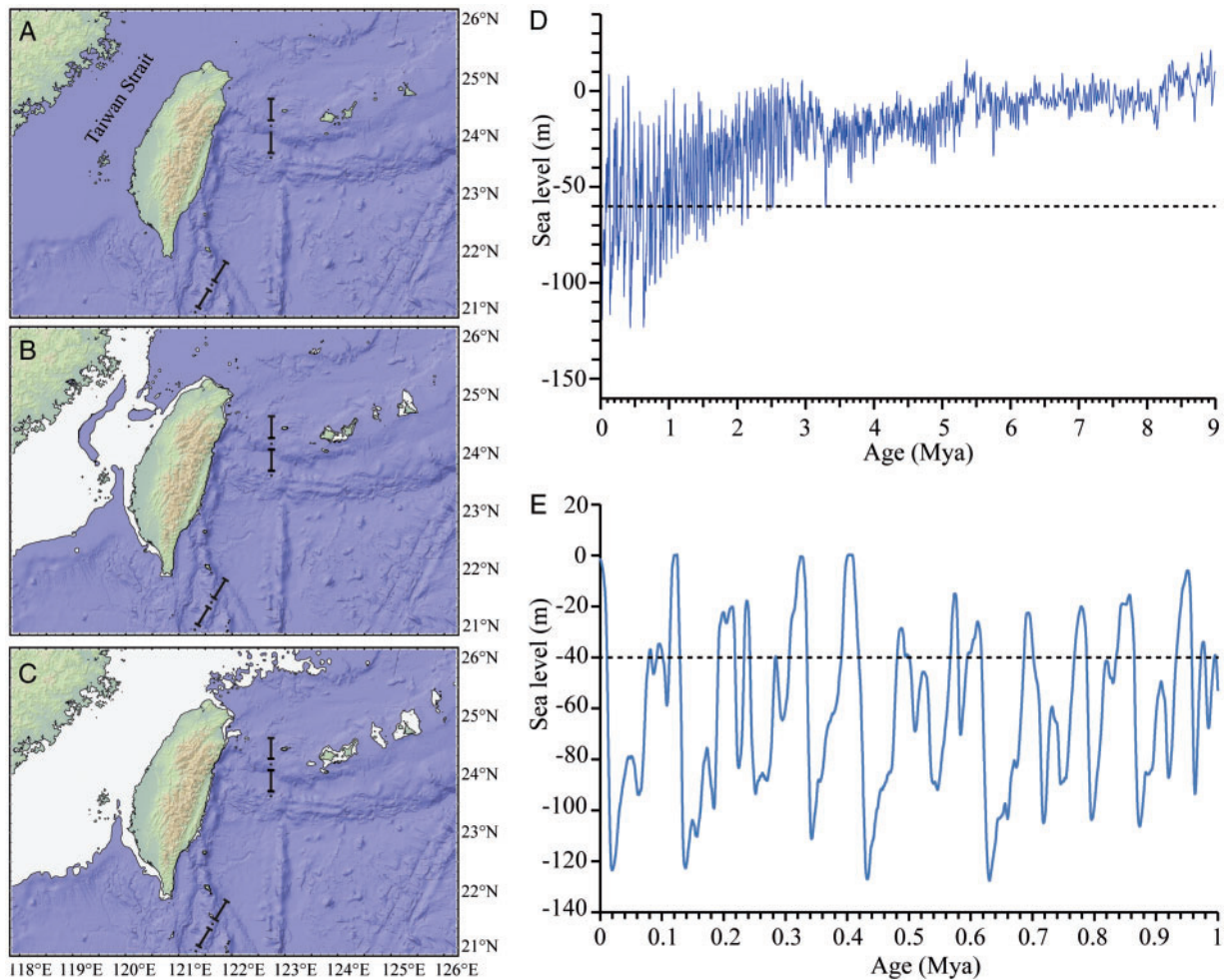
The origin and colonization history of *Kurixalus*, a genus of small arboreal tree frogs breeding exclusively in shallow swamps, is under disputed. On the basis of comprehensive sampling program, the evolutionary history of *Kurixalus* is investigated based on 3 mitochondrial genes. Our results indicate that the genus *Kurixalus* originated in the Asian mainland and subsequently arrived at its current distribution in Borneo, Taiwan, Ryukyu, and Hainan islands by a series of dispersal events. Moreover, the colonization of Taiwan from mainland Asia has occurred 2 times. The initial colonization of Taiwan occurred at 3.46–8.68 Mya (95% highest posterior density), which rejects the hypothesis that *Kurixalus* probably originated from Taiwan during the early Oligocene and favors the model of Neogene-origin rather than the model of Quaternary-origin for Taiwanese *Kurixalus*. *Kurixalus eiffingeri* has dispersed from Taiwan to the Ryukyus once or 2 times pending more data. Both transoceanic dispersal and land-bridge dispersal have played a role in the colonization process; the former resulted in the colonization of Taiwan and the Ryukyus and the latter led to the colonization of Borneo and Hainan.

**Key words:** colonization, *Kurixalus*, landbridge, Neogene-origin, Taiwan Island, transoceanic dispersal

Taiwan and Ryukyu islands are a part of the island-arc system along the western edge of the Pacific Ocean (Vita-Finzi 2000); the former is separated from Asian mainland by the Taiwan Strait, a shallow strait about 130-km-wide at narrowest point and 60 m in average depth (Figure 1A). These continental islands emerged from the waters almost simultaneously owing to the collision between the Luzon arc and Eurasian margins during the Late Miocene (Sibuet and Hsu 2004; Huang 2017). After the emergence of Taiwan Island in the Late Miocene (ca. 6.5 Mya; Huang 2017) or early Pliocene (4–5 Mya; Ali 2018, 2020), they gradually acquired their floras and

faunas mostly from the Eurasian mainland via exposed landbridge across the Taiwan Strait (Wang 1987; Hikida and Ota 1997) or via transoceanic dispersal (He et al. 2018). Based on the splitting time between the island species and their mainland relatives, 2 origin models were proposed for the colonization of Taiwan: the Quaternary-origin and Neogene-origin models (Lin et al. 2002; Su et al. 2016).

Members of the frog family Rhacophoridae are an example of a group whose distribution range extends across the entire region mentioned above. Rhacophorids are small-to-large frogs bearing several arboreal adaptations, including cartilaginous intercalary



**Figure 1.** Map showing the connection and isolation of Taiwan to mainland China when sea level is 0 m (A), -60 m (B), and -120 m (C). Fluctuations of sea level during the past millions years (D; Miller et al. 2005, 2011) and during the last million years (E; Bintanja et al. 2005) are presented. The map was generated using the GeoMapApp online software system (Ryan et al. 2009).

elements between the terminal and penultimate phalanges, and toe discs. This family displays a remarkable variety of reproductive modes including aquatic development, terrestrial gel-nesting, terrestrial foam-nesting, and terrestrial direct development, which facilitates less dependence on standing water bodies and may have provided new opportunities for dispersal (Meegaskumbura et al. 2015), and it is widely distributed across Asia with a disjunct occurrence in Africa (Frost 2020). Consistent with the Quaternary-origin model, rhacophorid frogs were supposed to have migrated from Chinese continent to Taiwan via landbridge during the Pleistocene glacial periods (Lue and Chen 1986), but there is no fossil record showing when the migration happened and few empirical studies have been performed to test this hypothesis (Yang et al. 1994). The genus *Kurixalus*, which is 1 of 4 rhacophorid genera occurring in Taiwan, currently comprises 18 recognized species (Frost 2020) and 5 unnamed lineages (Yu et al. 2018), distributed widely on the Asian continent and adjacent islands, including Taiwan, Ryukyu, Hainan, Sunda Islands, and Philippine archipelago. Contrary to the hypothesis of Lue and Chen (1986), Lv et al. (2018) considered that *Kurixalus* have originated in Taiwan during the Oligocene (32.8 Mya, 95% confidence interval : 25.6–39.9) and there was no exchange of *Kurixalus* between mainland China and Taiwan Island. However, these interpretations do not match with the geological

evidence that proto-Taiwan Island did not emerged from water until the Late Miocene (6.5 Mya; Huang 2017) or early Pliocene (4–5 Mya; Ali 2018, 2020), which has also been pointed out by Ali (2020). Moreover, the ancestor of *Kurixalus lenquanensis* might have come from Taiwan Island (Yu et al. 2017a). Thus, origin and colonization history of *Kurixalus* needs further examination.

Here we reconstructed the phylogenetic relationships and ancestral biogeographic areas of *Kurixalus* and estimated the lineage divergence times to investigate the evolutionary history of *Kurixalus* and to test for the hypothesis of Quaternary-origin of Taiwanese rhacophorid frogs.

## Materials and Methods

### Data preparation

The classifications of Frost (2020) and Yu et al. (2018) were followed and a total of 57 individuals belonging to 15 recognized and 6 unnamed species of *Kurixalus* were included in this study (Appendix). Sequences encoding 3 mitochondrial genes (12S rRNA, 16S rRNA, and COI) were amplified and sequenced for *K. lenquanensis* and *Kurixalus* sp6 using the primers and experiment protocols of Yu et al. (2017b), and homologous sequences of other

species were downloaded from GenBank. Eleven species representing 10 genera of Rhacophoridae were included as hierarchical outgroups according to Yu et al. (2009) and homologous sequences of them were obtained from GenBank.

### Phylogenetic analysis

Sequences were aligned using the MUSCLE option in MEGA version 7.0 (Kumar et al. 2016) with the default parameters. Fragments of the 3 mitochondrial genes were treated as 1 partition for subsequent analyses because not all homologous sequences are available for some species. The Corrected Akaike Information Criterion (AICc) was used to select the best model of nucleotide substitution in jModeltest version 2.1.10 (Darrriba et al. 2012). Bayesian phylogenetic inference and Maximum likelihood analysis were performed in MrBayes version 3.1.2 (Ronquist et al. 2012) and RAxML-HPC version 8.2.10 (Stamatakis 2014), respectively, based on the selected substitution model. For the Bayesian analysis, 2 runs were performed simultaneously with 4 Markov chains starting from random trees and the chains were run for 5,000,000 generations being sampled every 100 generations. Convergence and burn-in were checked using the program Tracer version 1.6 (Rambaut et al. 2014). Finally, the first 25% of the sampled trees were discarded as burn-in and the remaining trees were used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs). For the maximum likelihood analysis, node support was estimated by 1,000 rapid bootstrap replicates.

### Divergence dating

Lineage divergence times were estimated using an uncorrelated log-normal relaxed molecular clock model in BEAST version 1.8.0 (Drummond et al. 2012). We chose the birth-death process as the tree prior because of the mixed inter- and intraspecies sampling in the data sets (Ritchie et al. 2017). As there is no explicit fossil record for rhacophorid frogs, we adopted an inferred range of mitochondrial sequence divergence rate to calibrate the rate of divergence. Previous empirical studies of amphibians (using a variety of calibrations, taxa, and mtDNA gene fragments) have inferred model-corrected mitochondrial sequence divergence rates between 0.8% and 1.9% (after Sanguila et al. 2011). Thus, we used a mean rate of 1.35% with a normal distribution (95% CI: 0.008–0.019) as calibration following Sanguila et al. (2011) and Gonzalez et al. (2014). The estimated temporal framework for diversification in Sanguila et al. (2011) is in line with the earlier geological events of the Mindanao Islands and is generally consistent with the postulated common mechanism that may have promoted vertebrate species diversification in the Philippine archipelago (Brown and Diesmos 2009), indicating that this choice is appropriate.

Two independent runs were conducted for  $5 \times 10^7$  generations by sampling every 1,000 generations. The effective sample size ( $\geq 200$ ) values of parameters were confirmed in Tracer version 1.6 (Rambaut et al. 2014) and then trees produced by the 2 runs were combined in LogCombiner version 1.8.0 (Drummond et al. 2012) with the first 25% of trees from each run discarded as burn-in. The maximum clade credibility tree was constructed in TreeAnnotator version 1.8.0 (Drummond et al. 2012) and was visualized in FigTree version 1.4.0 (from <http://tree.bio.ed.ac.uk/software/figtree>).

### Reconstruction of ancestral area

We divided the present distribution of *Kurixalus* into 6 different biogeographic regions including South Tibet-Indian subcontinent (1),

Indochina-South China (2), Taiwan Island (3), Ryukyu Islands (4), Hainan Island (5), and Sunda Islands-Philippine archipelago (6), and then assigned each species to its own region. Combined the divergence dating analysis in BEAST version 1.8.0, we used the Bayesian stochastic search variable selection (BSSVS; Lemey et al. 2009) of the discrete phylogeographic model to reconstruct ancestral ranges of lineages within *Kurixalus* with the specification of symmetric discrete trait substitution model.

In addition to the BSSVS analysis, ancestral ranges and biogeographical history of *Kurixalus* were also estimated using BioGeoBEARS package (Matzke 2013a) implemented in RASP version 4.0 (Yu et al. 2015). This method implements 3 models including dispersal–extinction–cladogenesis (DEC; Ree and Smith 2008), the likelihood version of dispersal–vicariance (Ronquist 1997; herein DIVALIKE), and the likelihood version of BayArea model (Landis et al. 2013; herein BAYAREALIKE). In each model, an additional  $j$  parameter for founder events (jump dispersal) was added; thus, a total of 6 models resulted (Matzke 2013b). We compared the fit of each model using the AIC weighted approach (Burnham and Anderson 2002) and then ancestral ranges and biogeographical events were estimated using the best-fit model. For this analysis, the ultrametric time-calibrated trees generated from BEAST analyses were used with the same range coding as BSSVS analysis. The maximum number of individual unit areas was set to 2 because no species occupied more than 2 areas in *Kurixalus* and the number of random trees was set to 3,000.

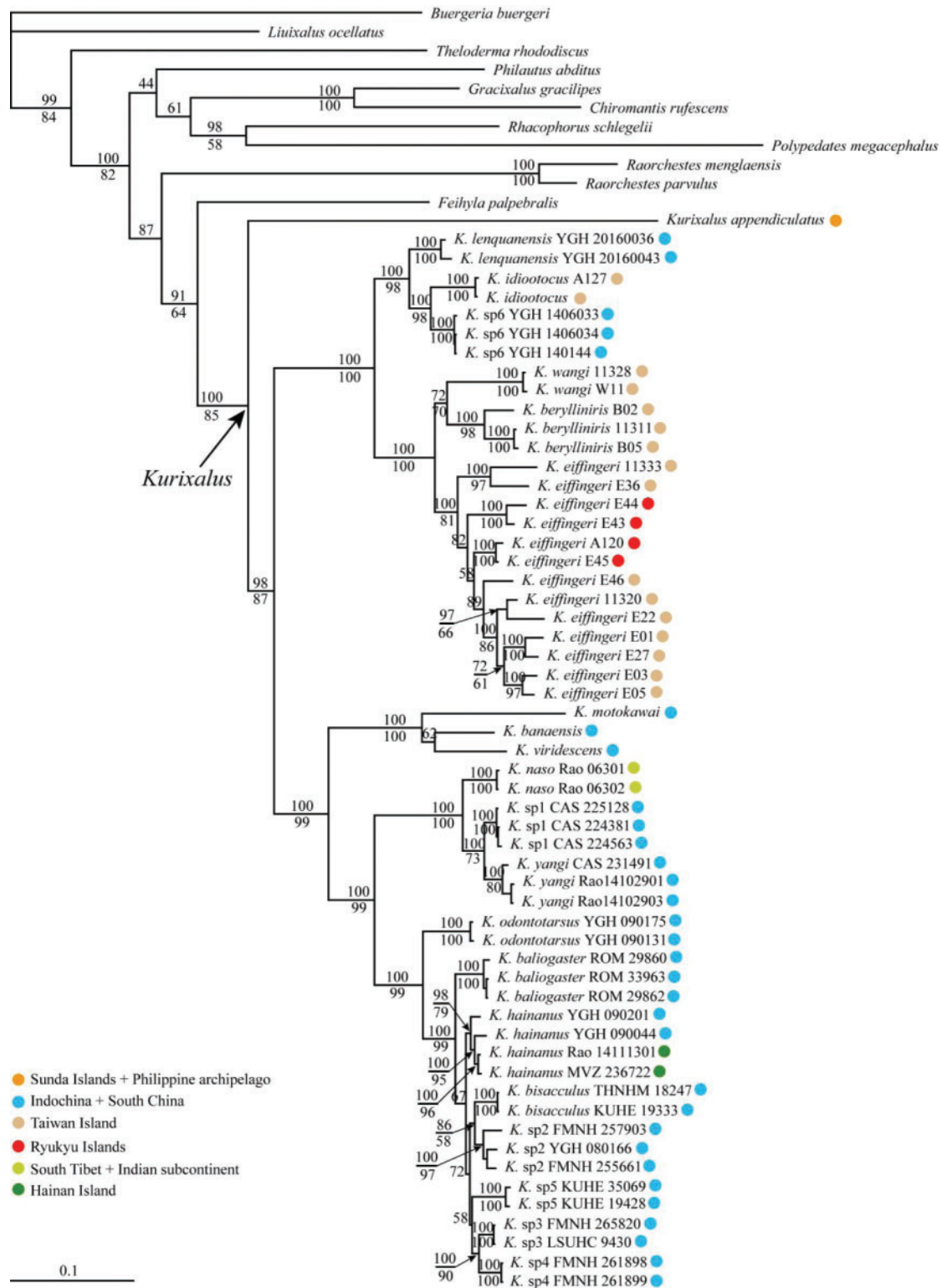
## Results

### Phylogeny

Our mitochondrial gene fragments consisted of 402 bp from 12S rRNA, 878 bp from 16S rRNA, and 807 bp from COI region. The model TIM2+I+G was selected as the best-fit model by AICc. As this model is not available in MrBayes or RAxML, we replaced it with the GTR+I+G model in the phylogenetic analyses because the TIM2 model is a special case of the GTR model. Bayesian inference and Maximum likelihood analysis yielded similar topologies (Figure 2). *Kurixalus appendiculatus* was reconstructed as the sister to the clade consisted of all other congeners. The 4 species from Taiwan and the Ryukyu islands did not form a monophyly and they were grouped in 2 sibling clades with strong support values: one consisting of 3 island species (*K. eiffingeri*, *K. berylliniris*, and *K. wangi*) and one comprising 1 island species (*K. idiototocus*) and 2 mainland species (*K. lenquanensis* and *K. sp6*). Furthermore, *K. lenquanensis* and *K. sp6* are paraphyletic with respect to *K. idiototocus*. The 4 specimens of *K. eiffingeri* from the Ryukyu Islands (A120, E43, E44, and E45) were grouped into 2 separated lineages with weak support, indicating that *K. eiffingeri* in the Ryukyu Islands probably have originated once or 2 times.

### Divergence time estimation and ancestral range estimation

The initial divergence within *Kurixalus* was dated back to ca. 10.97 Mya (95% highest posterior density [HPD]: 7.03–16.54) (Figure 3B, Table 1). The time of most recent common ancestor (TMRCA) of all Taiwanese *Kurixalus* was estimated to be 5.60 Mya (95% HPD: 3.46–8.68) and the TMRCA of *K. idiototocus* and *K. sp6* was estimated to be ca. 1.58 Mya (95% HPD: 0.76–2.77). The 2 lineages of *K. eiffingeri* from the Ryukyu Islands were grouped



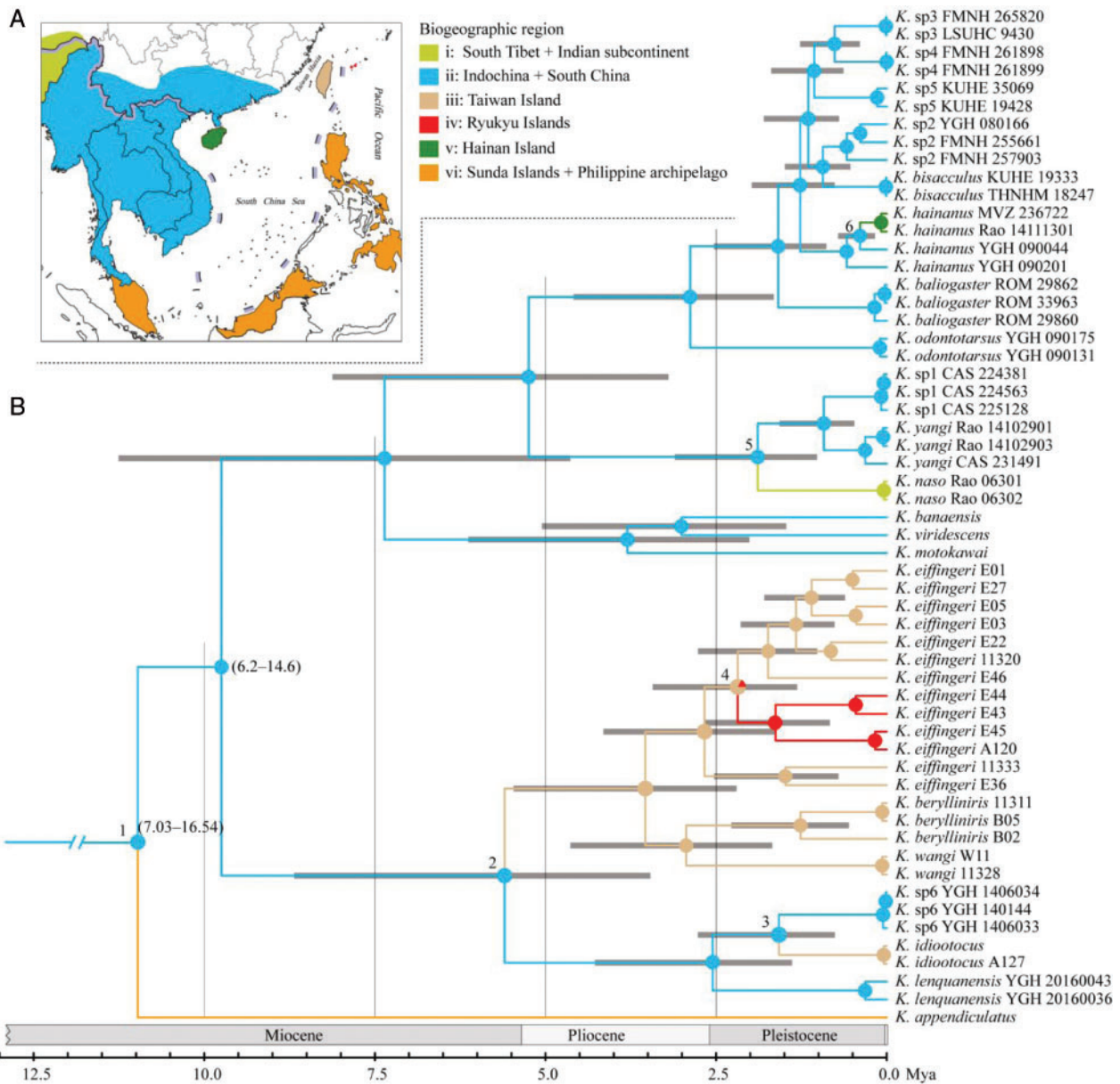
**Figure 2.** Bayesian phylogram of *Kurixalus* inferred from 2087 bp of 12S rRNA, 16S rRNA, and COI sequences. Numbers above and below branches are Bayesian posterior probabilities and ML bootstrap values (only values >50% are shown), respectively. Distribution of *Kurixalus* species are highlighted with different color.

together in the BEAST analysis (Figure 3) and they diverged from Taiwanese lineages ca. 2.18 Mya (95% HPD: 1.31–3.43).

The BioGeoBEARS analyses revealed that the model of DIVALIKE + *j* was the best-fit biogeographical model (Table 2) and the 2 methods reconstructing ancestral range (BSSVS and DIVALIKE + *j*)

obtained similar results (Figure 3B, Table 1). The ancestral range of *Kurixalus* was inferred to be Indochina-South China with single colonization of Sunda Islands-Philippine archipelago (Figure 3B). Two colonization events from mainland China to Taiwan Island were identified; 1 for the ancestor of *K. eiffingeri*, *K. berylliniris*, and *K. wangi* and 1 for





**Figure 3.** Biogeographic division of *Kurixalus* (a) and divergence dating and ancestral area reconstruction (b). Color of branch indicates the BSSVS reconstruction and color of node cycle indicates the DIVALIKE + *j* reconstruction. Detected historical colonized events are labeled with numbers at nodes. The original map (approval number: GS(2019)1652) was downloaded from the website of Ministry of Natural Resources of the People’s Republic of China.

**Table 1.** Divergence time estimates (95% HPD) and BSSVS and DIVALIKE + *j* ancestral range reconstructions for selected nodes as defined in Figure 3B (only area with highest probability is presented)

Node	Node age (95% HPD) Mya	Ancestral range reconstructions	
		BSSVS	DIVALIKE + <i>j</i>
1	10.97 (7.03–16.54)	ii, 0.974	ii, 0.987
2	5.60 (3.46–8.68)	ii, 0.794	ii, 0.962
3	1.58 (0.76–2.77)	ii, 0.891	ii, 0.976
4	2.18 (1.31–3.43)	iii, 0.909	iii, 0.829
5	1.89 (1.02–3.11)	iii, 0.959	iii, 0.988
6	0.39 (0.17–0.71)	ii, 0.991	ii, 0.988

the lineage giving rise to *K. idiootocus*. *Kurixalus eiffingeri* dispersed from Taiwan to the Ryukyu Islands. There were another 2 colonization routes including the dispersal of the ancestor of *K. naso* to southern Tibet-Indian subcontinent from Indochina-South China and the dispersal of *K. hainanus* to Hainan Island from Asian mainland.

**Discussion**

The ancestral area reconstruction indicated that originally the genus *Kurixalus* occurred in Asian mainland and subsequently it dispersed to the continental islands of East and Southeast Asia by multiple colonization events. The most ancient divergence in *Kurixalus* is the split between Bornean species and the remaining species at *ca.*

**Table 2.** Comparison of the 6 models of ancestral area estimations of *Kurixalus*

Model	Ln L	n	d	e	j	AICc_wt
DEC	-57.08	2	0.0060	0.0084	0	$1.0 \times 10^{-5}$
DEC + j	-45.84	3	$1.00 \times 10^{-12}$	$1.00 \times 10^{-12}$	0.012	0.26
DIVALIKE	-52.71	2	0.0061	$1.00 \times 10^{-12}$	0	0.0008
DIVALIKE + j	-44.86	3	$1.00 \times 10^{-12}$	$1.00 \times 10^{-12}$	0.012	0.70
BAYAREALIKE	-73.26	2	0.0087	0.060	0	$9.8 \times 10^{-13}$
BAYAREALIKE + j	-47.99	3	$1.00 \times 10^{-7}$	$1.00 \times 10^{-7}$	0.013	0.031

The model with highest value of AICc\_wt was selected as the best model. n, number of parameters; d, rate of dispersal; e, rate of extinction.

10.97 Mya (95% HPD: 7.03–16.54) (Node 1; Figure 3). A continuous land connection between Borneo and mainland Southeast Asia existed throughout much of the Neogene during times of lowered sea levels and would have allowed fairly unhindered migration of terrestrial biota (Moss and Wilson 1998). The ancestor of *Kurixalus* endemic to Borneo might have colonized the former Sundaland from mainland Southeast Asia during times of lowered sea levels. Subsequently, it might be isolated from its mainland relatives owing to the rise of the sea level during the global middle Miocene climate maximum, which resulted in extensive inundations of the former Sundaland and the loss of connection of Borneo to mainland Indochina (Hall 1998; Stelbrink 2015).

Contrary to the hypothesis that *Kurixalus* probably originated from Taiwan and there was no dispersal across the Taiwan Strait (Lv et al. 2018), we found that Taiwanese *Kurixalus* probably originated from mainland Asia via 2 colonization events (Figure 3). Initially, the ancestor of *K. eiffingeri*, *K. berylliniris*, and *K. wangi* dispersed to Taiwan and split from mainland species at 3.46–8.68 Mya (95% HPD; Figure 3, Table 1). This estimation coincides with that the Taiwan Island initially emerged from the waters at 4–5 Mya (Ali 2018, 2020) or 6.5 Mya (Huang et al. 1997; Lin et al. 2003; Huang 2017). Based on this result, we reject the hypothesis that Taiwanese rhacophorid frogs have originated from mainland China during the Pleistocene glacial periods (Quaternary-origin model; Lue and Chen 1986), instead favoring the model of Neogene-origin for Taiwanese *Kurixalus*. The late Miocene-origin or early Pliocene-origin has also been suggested for other Taiwanese animals (e.g., Creer et al. 2001; Lin et al. 2002; Shih et al. 2006; Chiang et al. 2010; Su et al. 2016). There are 2 potential mechanisms to explain the initial dispersal of *Kurixalus* to Taiwan: landbridge connection or transoceanic dispersal. The data of oxygen isotope demonstrated that the landbridge across the Taiwan Strait did not emerge until the sea level was frequently <–60 m after 2.6 Mya (Figure 1B; Miller et al. 2005, 2011). Therefore, we consider that the initial colonization of Taiwan in *Kurixalus* might be the result of transoceanic dispersal, which has been supported in other insular amphibians (e.g., Vences et al. 2003; Komaki et al. 2017) and has been proposed as the predominant pathway for the colonization of Taiwan (He et al. 2018) and Madagascar (Crottini et al. 2012).

After the initial colonization of the Taiwan Island in *Kurixalus* during Pliocene to Late Miocene, the lineage giving rise to *K. idiotoctus* also dispersed to the Taiwan Island and split from its mainland sister at 0.76–2.77 Mya (95% HPD; Figure 3, Table 1). As illustrated in Figure 1B, sea level was frequently <–60 m during the Pleistocene, which resulted in repeated landbridge connections between the Taiwan Island and mainland Asia since 2.6 Mya. Thus, we posit that the lineage of *K. idiotoctus* might have colonized the Taiwan Island by dispersal via landbridge, although it is also possible that the ancestor of *K. idiotoctus* has arrived at Taiwan via transoceanic dispersal at 2.6–2.77 Mya.

*Kurixalus eiffingeri* might have dispersed from Taiwan to the Ryukyu Islands once or 2 times (Figures 2 and 3). More data will be needed to resolve the phylogenetic relationship between the 2 Ryukyus clades and to achieve a precise estimation of number of dispersal between Taiwan and Ryukyus (Figure 2). The Taiwan-Yonaguni Strait between the Ryukyu Islands and Taiwan has a maximum sill depth of 770 m (Nakamura 2013) with average depth of >200 m (Osozawa et al. 2012), implying that a landbridge between Taiwan and Ryukyu has never occurred during past millions years, even if the sea level was below –120 m (Figure 1). Thus, we consider that the colonization of Ryukyu from Taiwan in *K. eiffingeri* was also the result of transoceanic dispersal. Similar colonization route has also been reported for the Japanese stream treefrog (Komaki et al. 2017) and the Okinawa tree lizard (Yang et al. 2018).

*Kurixalus hainanus* in Hainan originally came from Asian mainland and split from mainland populations at 0.17–0.71 Mya (Node 6; Figure 3). Although Hainan is separated from southern mainland China by the Qiongzhou Strait, they were joined together frequently when the sea level was <–40 m during the past million years (Figure 1D) because the strait is shallow with depth <40 m (Ali 2018). Thus, we consider that *K. hainanus* colonized Hainan via landbridge dispersal, which was also supported in bat (Mao et al. 2010) and lizard (Huang et al. 2013). Considering that the landbridge across the Qiongzhou Strait occurred multiple times during the past millions years (Shi et al. 2006), a phylogeographic study employing broad sampling will be necessary to investigate whether *K. hainanus* colonized Hainan once or multiple times and whether *K. hainanus* recolonized mainland Asia, which has been detected in other species (e.g., Mao et al. 2010).

The ancestral range of the lineage giving rise to *K. yangi*, *K. naso*, and *K. sp1* was reconstructed as Indochina-South China and the split between *K. naso* and the clade of *K. yangi* and *K. sp1* was estimated to be ca. 1.89 Mya (95% HPD: 1.02–3.11) (Node 5; Figure 3). This result supports the hypothesis that *Kurixalus* might have colonized the Indian subcontinent from northern Indochina (Yu et al. 2018). Lv et al. (2018) supposed that *Kurixalus* might have colonized the Indian subcontinent via a landbridge on the basis of the record of *K. appendiculatus* in India (Dinesh et al. 2013). This inference should be treated with caution because *K. appendiculatus* likely only occurs in Philippines and Sunda islands and the record of *K. appendiculatus* in India is likely a misidentification of *K. naso* or other relative according to our earlier work (Yu et al. 2017b). Geographically *K. naso* and the clade of *K. yangi* and *K. sp1* are distributed on the west and east side of Patkai Range, respectively, implying that the uplift of Patkai Range during the early Pleistocene might have driven the divergence between them.

In conclusion, the genus *Kurixalus* originally occurred in Asian mainland and subsequently it arrived at its current distribution in Borneo, Taiwan, Ryukyu, and Hainan by a series of dispersal events. Both transoceanic dispersal and landbridge dispersal have played a role in the colonization process; the former resulted in the colonization of

Taiwan and Ryukyu and the latter led to the colonization of Borneo and Hainan. In addition, *Kurixalus* might have colonized the Indian subcontinent from northern Indochina. Results in the present study favor the model of Neogene-origin rather than the model of quaternary-origin or Oligocene-origin for Taiwanese *Kurixalus*. Considering that only mitochondrial markers were used in this study owing to the absence of nuclear data for most *Kurixalus* species outside of China, which may lead to a biased understanding on the phylogeny if evolutionary factor such as mitochondrial capture, gene introgression, or incomplete lineage sorting occurred, further evidence based upon inclusion of more taxa and nuclear markers will be needed to achieve a complete understanding on the evolutionary history of *Kurixalus*.

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## Author's Contributions

G.-H.Y., J.-X.Y., and Z.-J.W. designed the study. G.-H.Y. and J.-S.W. collected samples. G.-H.Y. and L.-N.D. performed the laboratory work, analyzed the data, and wrote the manuscript. D.-Q.R., J.-X.Y., and Z.-J.W. edited the manuscript. All authors read and approved the last version of the manuscript.

## Conflict of Interest

We declare that all authors have no competing interest.

## Appendix

### Samples used in this study

Species	Locality	Voucher No	12S	16S	COI
<i>Buergeria buergeri</i>	Hiroshima, Japan	–	AB127977	AB127977	AB127977
<i>Liuxalus ocellatus</i>	Hainan, China	–	GQ285672	GQ285672	KP996840
<i>Theioderma rhododiscus</i>	Guangxi, China	–	EU215530	EU215530	KP996753
<i>Feihyla palpebralis</i>	Yunnan, China	–	EU215546	EU215546	DQ468683
<i>Gracixalus gracilipes</i>		–	GQ285668	GQ285668	KR087672
<i>Chiromantis rufescens</i>	Africa	–	AF458126	AF458126	KY079577
<i>Rhacophorus schlegelii</i>	Hiroshima, Japan	–	AB202078	AB202078	AB202078
<i>Polypedates megacephalus</i>	Guangxi, China	–	AY458598	AY458598	AY458598
<i>Philautus abditus</i>	Krong Pa, Vietnam	–	GQ285673	GQ285673	–
<i>Raorchestes menglaensis</i>	Yunnan, China	–	GQ285676	GQ285676	–
<i>Raorchestes parvulus</i>	Thailand	–	LC012865	LC012865	KR087900
<i>Kurixalus appendiculatus</i>	Bukit Sarang, Sarawak, Malaysia	FMNH 267896	JQ060948	JQ060937	KX554539
<i>Kurixalus eiffingeri</i>	Ryukyu Islands, Japan	A120	–	DQ468673	DQ468681
<i>Kurixalus eiffingeri</i>	Taiwan, China	11320	–	DQ468672	DQ468680
<i>Kurixalus eiffingeri</i>	Taiwan, China	11333	–	DQ468670	DQ468678
<i>Kurixalus eiffingeri</i>	Taiwan, China	E01	–	–	KT259075
<i>Kurixalus eiffingeri</i>	Taiwan, China	E03	–	–	KT259077
<i>Kurixalus eiffingeri</i>	Taiwan, China	E05	–	–	KT259079
<i>Kurixalus eiffingeri</i>	Taiwan, China	E22	–	–	KT259096
<i>Kurixalus eiffingeri</i>	Taiwan, China	E27	–	–	KT259101
<i>Kurixalus eiffingeri</i>	Taiwan, China	E36	–	–	KT259110
<i>Kurixalus eiffingeri</i>	Ryukyu Islands, Japan	E43	–	–	KT259117
<i>Kurixalus eiffingeri</i>	Ryukyu Islands, Japan	E44	–	–	KT259118
<i>Kurixalus eiffingeri</i>	Ryukyu Islands, Japan	E45	–	–	KT259119
<i>Kurixalus eiffingeri</i>	Taiwan, China	E46	–	–	KT259120
<i>Kurixalus idiootocus</i>	Taiwan, China	A127	–	DQ468674	DQ468682
<i>Kurixalus idiootocus</i>	Taiwan, China	–	–	AB933306	KT259131
<i>Kurixalus berylliniris</i>	Taiwan, China	11311	–	DQ468669	DQ468677
<i>Kurixalus berylliniris</i>	Taiwan, China	B02	–	–	KT259056
<i>Kurixalus berylliniris</i>	Taiwan, China	B05	–	–	KT259059
<i>Kurixalus wangi</i>	Taiwan, China	11328	–	DQ468671	DQ468679
<i>Kurixalus wangi</i>	Taiwan, China	W11	–	–	KT259074
<i>Kurixalus banaensis</i>	Krong Pa, Gia Lai, Vietnam	ROM 32986	GQ285667	GQ285667	–
<i>Kurixalus viridescens</i>	Hon Ba, Khanh Hoa, Vietnam	VNMN 03802	AB933284	AB933284	–

Species	Locality	Voucher No	12S	16S	COI
<i>Kurixalus motokawai</i>	Kon Tum, Vietnam	VNMN 03458	LC002888	LC002888	–
<i>Kurixalus bisacculus</i>	Pua, Nan, Thailand	THNHM 10051	GU227279	GU227334	KX554633
<i>Kurixalus bisacculus</i>	Phu Luanag, Loei, Thailand	KUHE 19333	KX554473	KX554535	KX554645
<i>Kurixalus baliogaster</i>	Krong Pa, Gia Lai, Vietnam	Rom 33963	KX554474	KX554536	KX554646
<i>Kurixalus baliogaster</i>	Tram Lap, Gia Lai, Vietnam	Rom 29860	KX554475	KX554537	KX554647
<i>Kurixalus baliogaster</i>	Tram Lap, Gia Lai, Vietnam	Rom 29862	KX554476	KX554538	KX554648
<i>Kurixalus naso</i>	Muotuo, Tibet, China	Rao 06301	KX554422	KX554484	KX554547
<i>Kurixalus naso</i>	Muotuo, Tibet, China	Rao 06302	KX554423	KX554485	KX554548
<i>Kurixalus yangi</i>	Mohynin, Kachin, Myanmar	CAS 231491	GU227278	GU227333	KX554556
<i>Kurixalus yangi</i>	Longdao, Ruili, Yunnan, China	Rao 14102901	KX554429	KX554491	KX554557
<i>Kurixalus yangi</i>	Longdao, Ruili, Yunnan, China	Rao 14102903	KX554431	KX554493	KX554559
<i>Kurixalus odontotarsus</i>	Mengyang, Yunnan, China	YGH 090175	GU227233	GU227282	KX554573
<i>Kurixalus odontotarsus</i>	Caiyanghe, Yunnan, China	YGH 090131	GU227240	GU227290	KX554576
<i>Kurixalus hainanus</i>	Wenshan, Yunnan, China	YGH 090044	GU227248	GU227299	KX554599
<i>Kurixalus hainanus</i>	Longmeng, Guangdong, China	YGH 090201	GU227265	GU227320	KX554616
<i>Kurixalus hainanus</i>	Diaoluo Mts, Hainan, China	Rao14111301	KX554461	KX554523	KX554620
<i>Kurixalus hainanus</i>	Mt. Wuzhi, Hainan, China	MVZ Herp 236722	JQ060939	JQ060928	KX554624
<i>Kurixalus lenquanensis</i>	Mengzi, Yunnan, China	YGH 20160036	MK348042	KY768931	MK348050
<i>Kurixalus lenquanensis</i>	Mengzi, Yunnan, China	YGH 20160043	MK348043	KY768938	MK348051
<i>Kurixalus</i> sp1	Nagmung, Kachin, Myanmar	CAS 224381	GU227274	GU227329	KX554552
<i>Kurixalus</i> sp1	Nagmung, Kachin, Myanmar	CAS 224563	GU227275	GU227330	KX554553
<i>Kurixalus</i> sp1	Nagmung, Kachin, Myanmar	CAS 225128	GU227276	GU227331	KX554554
<i>Kurixalus</i> sp2	Pingbian, Yunnan, China	YGH 080166	GU227244	GU227295	KX554582
<i>Kurixalus</i> sp2	Tuong Duong Dist, Nghe An, Vietnam	FMNH 255661	KX554450	KX554512	KX554586
<i>Kurixalus</i> sp2	Pakxong Dist, Champasak, Lao	FMNH 257903	KX554458	KX554520	KX554594
<i>Kurixalus</i> sp3	Muang Sa Kaeo, Sa Kaeo, Thailand	FMNH 265820	KX554465	KX554527	KX554635
<i>Kurixalus</i> sp3	Phnom Samkos, Cambodia	LSUHC 9430	KX554466	KX554528	KX554636
<i>Kurixalus</i> sp4	Kampot Dist, Kampot Prov, Cambodia	FMNH 261898	KX554467	KX554529	KX554637
<i>Kurixalus</i> sp4	Kampot Dist, Kampot Prov, Cambodia	FMNH 261899	KX554468	KX554530	KX554638
<i>Kurixalus</i> sp5	Pilok, Kanchanaburi, Thailand	KUHE 35069	AB933291	AB933291	KX554642
<i>Kurixalus</i> sp5	Nakhon Si Thammarat, Thailand	KUHE 19428	AB933290	AB933290	KX554643
<i>Kurixalus</i> sp6	Mainland China	YGH 1406033	MK348044	MK348047	MK348052
<i>Kurixalus</i> sp6	Mainland China	YGH 1406034	MK348045	MK348048	MK348053
<i>Kurixalus</i> sp6	Mainland China	YGH 140144	MK348046	MK348049	MK348054

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