

SUPPLEMENTARY MATERIALS

Materials and Methods

Taxon sampling

Our dataset included 13 species of *Polypedates* (*P. otilophus*, *P. colletti*, *P. cruciger*, *P. maculatus*, *P. pseudocruciger*, *P. macrotis*, *P. mutus*, *P. braueri*, *P. impresus*, *P. megacephalus*, *P. teraiensis*, *P. leucomystax*, and *P. discantus*). Eight additional species belonging to the genera *Feihyla*, *Ghatixalus*, and *Taruga*, which are closely related to *Polypedates* (Li et al., 2013), and two outgroup species (*Kurixalus idiootocus* and *Kurixalus banaensis*) were also sampled (Supplementary Table S1). The sampling localities covered most distribution areas, including India, Sri Lanka, South China (including Hainan and Taiwan islands), mainland Southeast Asia (Vietnam, Laos, Thailand, Myanmar, and Bangladesh), Malay Peninsula and associated islands, Sundaland (Borneo, Java, Sumatra, and Sulawesi), Philippine Archipelago, and Japan (Supplementary Figure S1).

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Sequences of mitochondrial (12S rRNA, tRNA^{Val}, and 16S rRNA) and nuclear genes (exon 1 of tyrosinase (TYR), exon 1 of rhodopsin (RHOD), proopiomelanocortin (POMC)) from previous studies (Brown et al., 2010; Grosjean et al., 2015; Haas & Das, 2008; Hasan et al., 2014; Hertwig et al., 2013; Kuraishi et al., 2013; Li et al., 2009, 2013; Matsui et al., 2014a, 2014b; Pan et al., 2013; Rujirawan et al., 2013; Yu et al., 2008) were retrieved from GenBank. Candidate DNA fragments, including three mtDNA and three nuclear DNA fragments of 15 individuals from six

species, were newly acquired in this study. Genomic DNA was extracted from either muscle or liver tissues and initially preserved in 95% ethanol. An Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech, China) was used for genomic DNA extraction, with minor modifications to the manufacturer's protocols. Double-stranded PCR amplifications were conducted in a total volume of 25 μ L, including 12.5 μ L Taq PCR Master Mix (2 X blue dye), 9.5 μ L ddH₂O, 1 μ L FS01, 1 μ L Rend (C=10 μ L/L), and 1 μ L DNA template. For mitochondrial fragments, PCR conditions included an initial denaturation at 95 °C for 3 min, followed by 94 °C denaturation for 1 min, 55 °C annealing for 1 min, and extension at 72 °C for 1 min. A final extension at 72 °C was conducted for 10 min. For TYR, RHOD, and POMC fragments, the same procedure as for mitochondrial DNA was used, but with annealing at 56 °C, 52 °C, and 52 °C, respectively.

Alignment of all gene fragments was conducted using MEGA v.7 (Kumar et al., 2016), rechecked in MAFFT v.7 (Katoh & Standley, 2013), and then adjusted manually, resulting in lengths of 923 bp for 12S rRNA, 74 bp for tRNA^{Val}, 1 468 bp for 16S rRNA, 532 bp for TYR, 316 bp for RHOD, and 571 bp for POMC. All sequences generated in this study were deposited in NCBI GenBank (accession Nos. are indicated in Supplementary Table S1).

Phylogenetic analyses

For the best partitioning scheme and substitution models, we used Bayesian Information Criterion (BIC) calculated in PartitionFinder v.2 (Lanfear et al., 2017). Based on this, our markers were divided into two partitions, i.e., a mitochondrial (12S

rRNA gene, tRNA^{Val}, 16S rRNA gene) partition and a nuclear (TYR, RHOD, POMC) partition. The best-fit substitution model was GTR+I+G for the mitochondrial partition and TRNEF+I+G for the nuclear partition. A maximum-likelihood (ML) tree was constructed using RAxML v.8.2.10 (Stamatakis, 2014) with the GTR+Gamma model for our final likelihood search with fast bootstrapping and 1 000 pseudoreplicates. Bootstrap proportions (BSP) were assessed to test node support, with $BSP \geq 70$ indicating high support. The Bayesian phylogenetic relationships were determined using MrBayes v.3.2.0 (Ronquist et al., 2012). Two independent runs of Markov chains for 5 000 000 generations were summarized and sampled every 100 generations. The first 25% of sampled trees were discarded as a conservative burn-in. The frequency of nodal resolution, i.e., Bayesian posterior probability (BPP), was determined to assess confidence in the topology. Nodes were considered strongly supported at $BPP \geq 0.95$.

Divergence time estimation

We used BEAST v.2.5 (Bouckaert et al., 2019) to determine the phylogenetic tree and divergence times. Site models were set according to the outcome of jModelTest v.2.1.6 (Darriba et al., 2012) for the concatenated mitochondrial partition and nuclear partition separately. The best-fit substitution model was GTR+I+G for the mitochondrial partition and SYM+I+G for the nuclear partition. In light of previously published data (Li et al., 2013), we implemented secondary calibration points for the time of the most recent common ancestor (tMRCA) of *Polypedates*, *Feihyla*, *Ghatixalus*, and *Taruga*, implemented as a normal-distributed calibration density

(mean=34.5; stdev=2.9; 5%–95% inter-quantile range 29.73–39.27 Ma). We applied a relaxed uncorrelated lognormal clock and a Yule tree prior. Analysis was repeated two times for 50 million generations separately, sampling every 1 000 iterations using the CIPRES Science Gateway (<https://www.phylo.org/portal2/login>). We checked for stationarity of the Markov chain and potential autocorrelation (effective sample sizes >200 for all sampled parameters) in Tracer v.1.6 (Rambaut et al., 2013). The first 25% of samples were discarded as burn-in, and the samples of both runs were combined in LogCombiner v.2.5. This file was used in TreeAnnotator v.2.5 to identify and annotate the maximum clade credibility tree. Given the inherent uncertainty of divergence time estimates, we rounded the estimates (95% HPD interval boundaries) to the full million.

Ancestral area estimation

We conducted two biogeographical analyses: (1) a likelihood method under the DIVALIKE+J model and (2) a Bayesian approach under the Bayesian Binary MCMC (BBM) method (Ronquist & Huelsenbeck, 2003) in RASP v.4 (Yu et al., 2015). We defined geographic areas based on established biogeographic and continental boundaries, such as the Red River fault between China and mainland Southeast Asia (Zhang et al., 2010), the Isthmus of Kra between mainland Southeast Asia and the Malay Peninsula (Woodruff, 2010), and the Arakan Mountains between the Indian subcontinent and mainland Southeast Asia. We identified six general areas: Sundaland and Sulawesi (area S), India (area I), mainland Southeast Asia (southwest of the Red River, including the southern part of China but excluding the part of

Vietnam northeast of the Red River) (area D), the Philippines (area P), South China (northeast of the Red River and including the part of Vietnam northeast of the Red River) (area C), and Japan (area J) (Figure 2A).

The DIVALIKE+J model approach was used with the R-package “BioGeoBEARS” (Matzke, 2013). Firstly, we compared six models adopted in this R-package based on their AIC (Akaike’s information criterion) weight. Our ancestral range evolution analyses supported DIVALIKE+J as the best fit model to our dataset (LnL=−80.48, AIC weight=167, Supplementary Table S4). For the BBM method, we set the maximum number of ancestral areas to three, used F81 as the state frequencies, and set Gamma distribution as the among-site rate variation. All other parameters were set to default.

To investigate the natural history of insular *P. leucomystax*, we pruned the BEAST maximum clade credibility tree and only left *P. leucomystax* with the R-package “Ape” (Paradis et al., 2004), applying the same DIVALIKE+J approach. The newly defined geographic areas were: S–Sumatra, M–Malay Peninsula, B–Borneo, A–Java, P–the Philippines, J–Japan, and W–Sulawesi (Figure 2B). Before analysis under the DIVALIKE+J model, we grouped all samples within every clade and separated sample 85 from other samples in the same clade as one group, and samples 102 and 103 from the other samples in the same clade as one group, and then estimated the net evolutionary divergence between each group with MEGA v.7 (Kumar et al., 2016) (Figure 2B). Results showed that the distance between groups I and V was 0.01, and between groups VI and VII was 0.004 (Supplementary Table S3);

therefore we considered the species in each clade as cryptic lineages for additional DIVALIKE+J analysis.

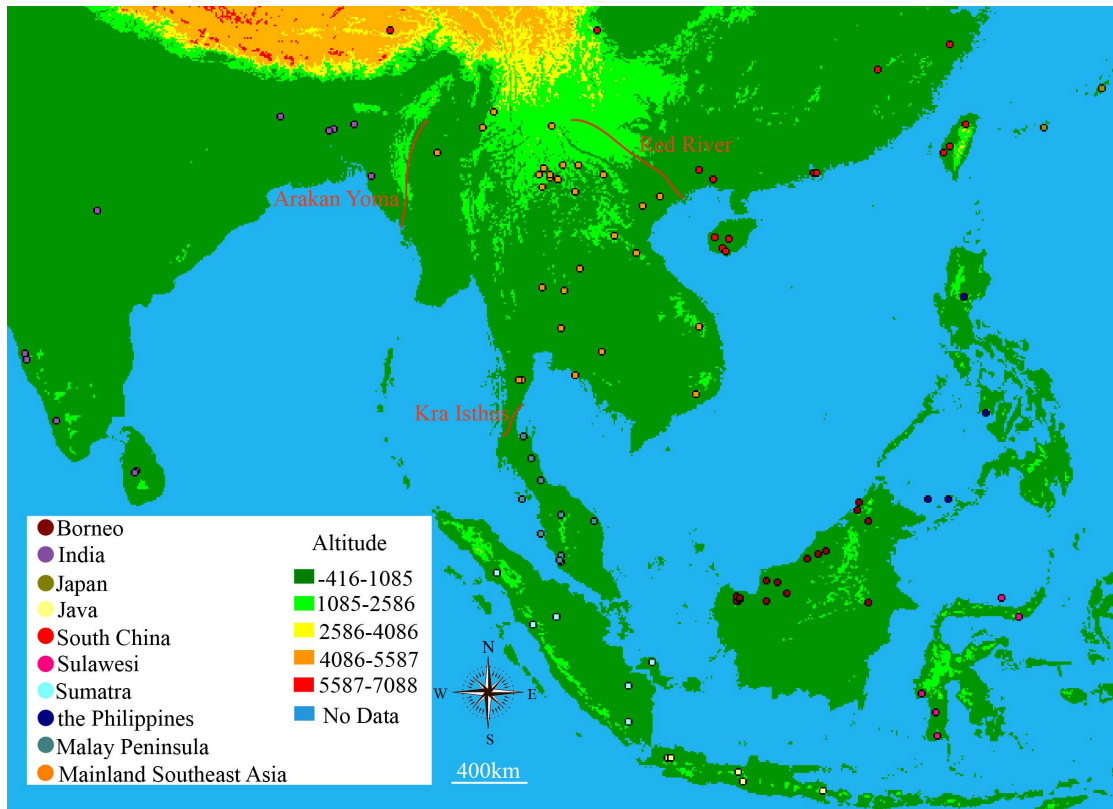
REFERENCES

- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **15**(4): e1006650.
- Brown RM, Linkem CW, Siler CD, Sukumaran J, Esselstyn JA, Diesmos AC, et al. 2010. Phylogeography and historical demography of *Polypedates leucomystax* in the islands of Indonesia and the Philippines: evidence for recent human-mediated range expansion?. *Molecular Phylogenetics and Evolution*, **57**(2): 598–619.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**(8): 772.
- Grosjean S, Ohler A, Chuaynkern Y, Cruaud C, Hassanin A. 2015. Improving biodiversity assessment of anuran amphibians using DNA barcoding of tadpoles. Case studies from Southeast Asia. *Comptes Rendus Biologies*, **338**(5): 351–361.
- Haas A, Das I. 2008. Larval identities of *Ansonia hanitschi* Inger, 1960 (Amphibia: Bufonidae) and *Polypedates colletti* (Boulenger, 1890) (Amphibia: Rhacophoridae) from East Malaysia (Borneo). *Salamandra*, **44**(2): 85–100.
- Hasan M, Islam MM, Igawa T, Kurabayashi A, Sumida M, Khan MR, et al. 2014. Genetic divergences of South and Southeast Asian frogs: a case study of several taxa based on

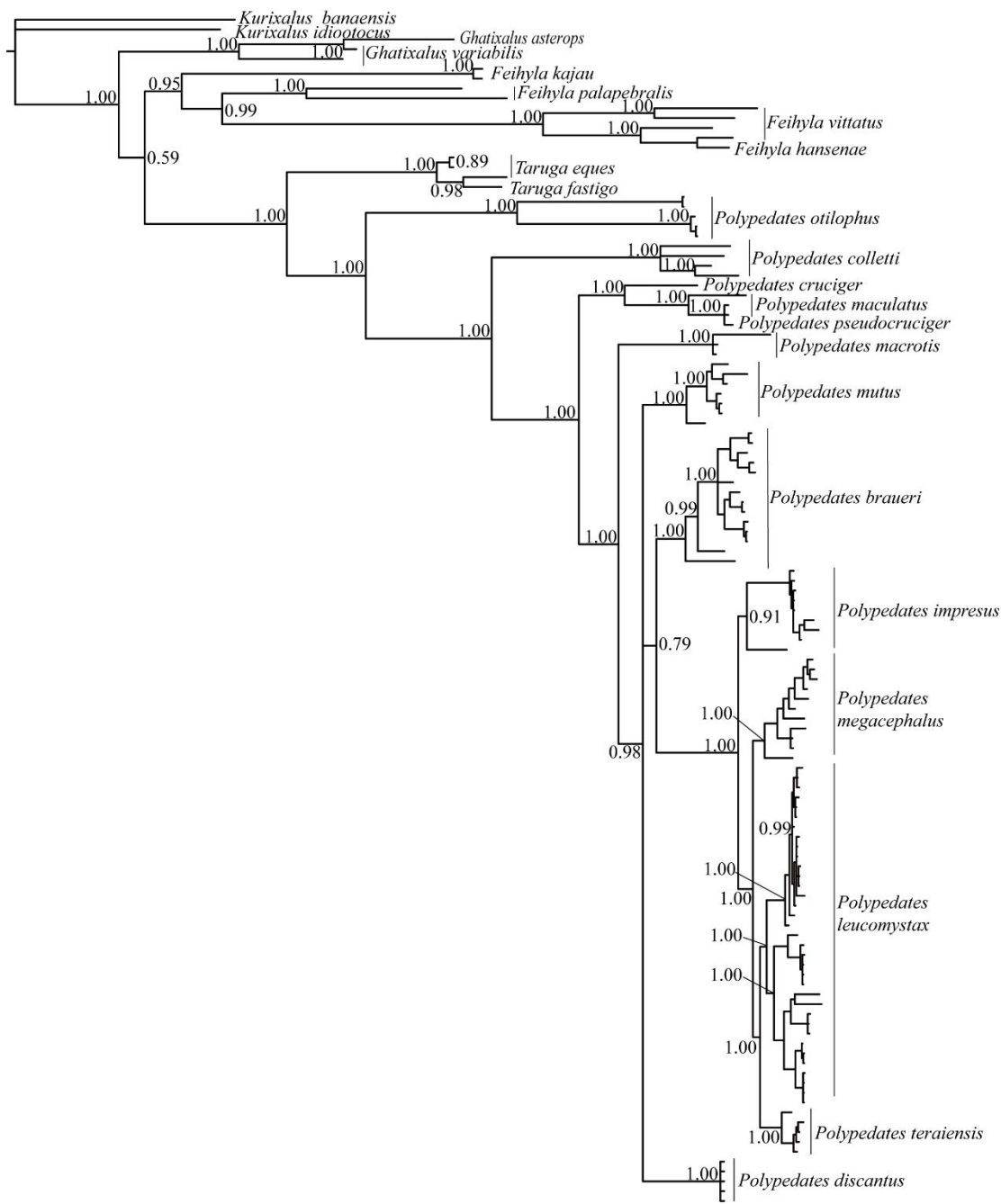
- 16S ribosomal RNA gene data with notes on the generic name *Fejervarya*. *Turkish Journal of Zoology*, **38**(4): 389–411.
- Hertwig ST, Schweizer M, Das I, Haas A. 2013. Diversification in a biodiversity hotspot—the evolution of Southeast Asian rhacophorid tree frogs on Borneo (Amphibia: Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution*, **68**(3): 567–581.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**(4): 772–780.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**(7): 1870–1874.
- Kuraishi N, Matsui M, Hamidy A, Belabut DM, Ahmad N, Panha S, et al. 2013. Phylogenetic and taxonomic relationships of the *Polypedates leucomystax* complex (Amphibia). *Zoologica Scripta*, **42**(1): 54–70.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**(3): 772–773.
- Li JT, Che J, Murphy RW, Zhao H, Zhao EM, Rao DQ, et al. 2009. New insights to the molecular phylogenetics and generic assessment in the Rhacophoridae (Amphibia: Anura) based on five nuclear and three mitochondrial genes, with comments on the evolution of reproduction. *Molecular Phylogenetics and Evolution*, **53**(2): 509–522.
- Li JT, Li Y, Klaus S, Rao DQ, Hillis DM, Zhang YP. 2013. Diversification of rhacophorid frogs provides evidence for accelerated faunal exchange between India and Eurasia

- during the Oligocene. *Proceedings of the National Academy of Sciences of the United States of America*, **110**(9): 3441–3446.
- Matsui M, Hamidy A, Kuraishi N. 2014a. A new species of *Polypedates* from Sumatra, Indonesia (Amphibia: anura). *Species Diversity*, **19**(1): 1–7.
- Matsui M, Shimada T, Sudin A. 2014b. First record of the tree-frog genus *Chiromantis* from Borneo with the description of a new species (Amphibia: Rhacophoridae). *Zoological Science*, **31**(1): 45–51.
- Matzke NJ. 2013. BioGeoBEARS: BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts. Berkeley: University of California.
- Pan SL, Dang NX, Wang JS, Zheng YT, Rao DQ, Li JT. 2013. Molecular phylogeny supports the validity of *Polypedates impresus* Yang 2008. *Asian Herpetological Research*, **4**(2): 124–133.
- Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**(2): 289–290.
- Rambaut A, Suchard MA, Xie D, Drummond AJ. 2013(2018-03-07). Tracer, version 1.6, MCMC trace analysis package. <http://tree.bio.ed.ac.uk/software/tracer/>.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**(12): 1572–1574.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**(3): 539–542.

- Rujirawan A, Stuart BL, Aowphol A. 2013. A new tree frog in the genus *Polypedates* (Anura: Rhacophoridae) from southern Thailand. *Zootaxa*, **3702**(6): 545–565.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**(9): 1312–1313.
- Woodruff DS. 2010. Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. *Biodiversity and Conservation*, **19**(4): 919–941.
- Yu GH, Rao DQ, Yang JX, Zhang MW. 2008. Phylogenetic relationships among Rhacophorinae (Rhacophoridae, Anura, Amphibia), with an emphasis on the Chinese species. *Zoological Journal of the Linnean Society*, **153**(4): 733–749.
- Yu Y, Harris AJ, Blair C, He XJ. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution*, **87**: 46–49.
- Zhang DR, Chen MY, Murphy RW, Che J, Pang JF, Hu JS, et al. 2010. Genealogy and palaeodrainage basins in Yunnan Province: phylogeography of the Yunnan spiny frog, *Nanorana yunnanensis* (Dicroglossidae). *Molecular Ecology*, **19**(16): 3406–3420.



Supplementary Figure S1 Collection sites of species in this study



0.4

Supplementary Figure S2 Bayesian phylogenetic relationships of *Polypedates* species based on mitochondrial and nuclear DNA

Numbers beside nodes are 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 new sequences in this study.

Name	Location	Voucher No.	Partial 12S and 16S and complete t-RNA Val	TYR	RHOD	POMC	Ref.
<i>Kurixalus idiootocus</i>	China: Lianhuachi, Taiwan	SCUM 061107L	EU215547	---	---	---	Li et al. 2013
<i>Kurixalus banaensis</i>	Vietnam: Krong Pa, Gia Lai	ROM32986	GQ285667	---	---	---	Li et al. 2013
1	<i>Feihyla kajau</i>	Malaysia: Kubah Natl. Park, Sarawak	NMBE 1057090	KC961234	---	KC961180	Hertwig et al.2013
			KC961088				
2	<i>Feihyla kajau</i>	Malaysia: Gunung Mulu Natl. Park, Sarawak	NMBE 1056500	KC961233	---	KC961179	Hertwig et al.2013
			KC961240				
			JN377362				
3	<i>Feihyla palpebralis</i>	China: Mt. Dawei, Yunnan	SCUM 0606132L	EU215606	EU215576	GQ285741	Li et al. 2013
4	<i>Feihyla palpebralis</i>	Viet Nam: Lam Dong	712	GQ285681	GQ285810	GQ285792	Li et al. 2009
			GQ204773				
5	<i>Feihyla vittatus</i>	Laos	FMNH255217	---	GQ204658	---	Li et al. 2013
			GQ204722				
6	<i>Feihyla vittatus</i>	China: Simao, Yunnan	KIZ 0001Rao	GQ285684	GQ285811	GQ285793	Li et al. 2013
7	<i>Feihyla vittatus</i>	Thailand:Song Khla Buri	KUHE:19441	AB813162	---	---	Matsui et al. 2014a
8	<i>Feihyla vittatus</i>	Vietnam: Ankhe District, Gia Lai	FMNH 254444	DQ283134	---	DQ283835	Li et al. 2013
9	<i>Feihyla hansena</i>	Thailand:Nam Tok Hongkaew	KUHE:34136	AB813161	---	---	Matsui et al. 2014a
10	<i>Ghatixalus asterops</i>	India	VUB 0025	EU178098	---	---	Li et al. 2013

11	<i>Ghatixalus variabilis</i>	India	VUB 0061	EU178099	---	---	---	Li et al. 2013
12	<i>Ghatixalus variabilis</i>	India	TBGRI2001.0081	AY880522	---	---	---	Li et al. 2013
13	<i>Taruga eques</i>	Sri Lanka	WHT2741	AY141847	---	---	---	Li et al. 2013
14	<i>Taruga eques</i>	Sri Lanka	WHT2741	GQ204689	---	GQ204633	---	Li et al. 2013
15	<i>Taruga eques</i>	Sri Lanka		AY920531	---	---	---	Li et al. 2013
16	<i>Taruga fastigo</i>	Sri Lanka	WHT2783	GQ204690	---	GQ204634	---	Li et al. 2013
17	<i>Polypedates otilophus</i>	Indonesia:Sumatra	MZB:Amp16303	AB907710	AB907736	AB907730	AB907724	Genbank
18	<i>Polypedates otilophus</i>	Indonesia:Sumatra	MZB:Amp16304	AB907713	AB907737	AB907731	AB907725	Matsui et al. 2014b
19	<i>Polypedates otilophus</i>	Malaysia: Bongon, N. Borneo	KUHE:17264	AB907717	---	---	---	Matsui et al. 2014b
20	<i>Polypedates otilophus</i>	Malaysia:Sarawak, Kanowit	KUHE:42555	AB728190	AB728320	AB728286	AB728247	Kuraishi et al. 2013
21	<i>Polypedates otilophus</i>	Malaysia:Borneo	BORN:08158	AB907714	AB907738	AB907732	AB907726	Genbank
22	<i>Polypedates colletti</i>	Malaysia: Kuching,Sarawak	ZMH A09362	EF566974	---	---	---	Haas et al. 2008
23	<i>Polypedates colletti</i>	Indonesia:Sumatra,Lampung		AB728189	AB728319	AB728285	AB728246	Kuraishi et al. 2013
24	<i>Polypedates colletti</i>	Indonesia: Kerangas Deer Pond	ZRC 11914	EF624066	---	---	---	Li et al. 2013
25	<i>Polypedates colletti</i>	Malaysia: Loagan Bunut NP, Sarawak	ZRC 11912	EF566973	---	---	---	Li et al. 2013
26	<i>Polypedates cruciger</i>	Sri Lanka		AF249045	AF249187	AF249124	---	Li et al. 2013
27	<i>Polypedates maculatus</i>	India:Bihar, Rajgir	KUHE:42336	AB728188	AB728318	AB728284	AB728245	Kuraishi et al. 2013
28	<i>Polypedates maculatus</i>	India: Thekkatte	PM-K.T.-62.2010	JF832394	---	---	---	Nair (Unpublished)
29	<i>Polypedates maculatus</i>	India: Western Ghats, Mangalore, Bajipe		AB167934	---	---	---	Li et al. 2013
30	<i>Polypedates pseudocruciger</i>	India:Bajipe	RBRL 070722	AB530551	---	---	---	Hasan et al. 2014
31	<i>Polypedates macrotis</i>	Malaysia:Sarawak, Kanowit	KUHE:42556	AB728187	KC961218	---	KC961185	Kuraishi et al. 2013

32	<i>Polypedates macrotis</i>	Tawi-tawi Isl.	ELR 0181	HM770139	---	---	---	Brown et al. 2010
33	<i>Polypedates macrotis</i>	Tawi-tawi Isl.	ELR 0180	HM770138	---	---	---	Brown et al. 2010
34	<i>Polypedates mutus</i>	Thailand: Prachuap Khiri Khan, Kui Buri NP	0934Y	KR828028	---	---	---	Grosjean et al. 2015
35	<i>Polypedates mutus</i>	Viet Nam: Ha Tinh, Huong Son	AMNH A161395	DQ283048	---	DQ283777	---	Li et al. 2013
36	<i>Polypedates cf. mutus 1</i>	China: Guangxi	CIB 2851K	AB728018	AB728309	AB728275	AB728236	Kuraishi et al. 2013
37	<i>Polypedates mutus</i>	Vietnam: Kon Tum, Kon PLong	VN2018037	MW266017*	---	MW265994*	MW266011*	this study
38	<i>Polypedates mutus</i>	China: Bawangling, Hainan	MVZ 236721	HM770157	---	---	---	Brown et al. 2010
39	<i>Polypedates mutus</i>	China: Mt. Wuzhi, Hainan	HN 0806105	KC465831	---	---	---	Li et al. 2013
40	<i>Polypedates cf. mutus 1</i>	China: Hainan	CIB 20081090	AB728019	AB728310	AB728276	AB728237	Kuraishi et al. 2013
41	<i>Polypedates discantus</i>	Thailand: Hat Yai District, Songkhla	ZMKU AM 00991	KF303478	---	---	---	Rujirawan et al.2013
42	<i>Polypedates discantus</i>	Thailand: Hat Yai District, Songkhla	ZMKU AM 00992	KF303479	---	---	---	Rujirawan et al.2013
43	<i>Polypedates discantus</i>	Thailand: Hat Yai District, Songkhla	ZMKU AM 01006	KF303476	---	---	---	Rujirawan et al.2013
44	<i>Polypedates discantus</i>	Thailand: Hat Yai District, Songkhla	ZMKU AM 00993	KF303480	---	---	---	Rujirawan et al.2013
45	<i>Polypedates discantus</i>	Thailand: Hat Yai District, Songkhla	ZMKU AM 01005	KF303475	---	---	---	Rujirawan et al.2013
46	<i>Polypedates braueri</i>	China: Yingjiang, Yunnan	YN2018051	MW266020*	MW269971*	MW265992*	MW266007*	this study
47	<i>Polypedates braueri</i>	China: Yingjiang, Yunnan	LABYN2018026	MW266024*	---	MW265999*	MW266013*	this study
48	<i>Polypedates braueri</i>	Thailand: Phitsanulok, Phu Hin Rong	0904Y	KR827990	---	---	---	Grosjean et al. 2015

Kla NP

49	<i>Polypedates braueri</i>	China: Fenshuiling, Luchun, Yunnan	LC 0805095	KF053211	---	---	---	Pan et al. 2013
50	<i>Polypedates braueri</i>	Viet Nam: Lao Cai, Sapa	TAD_B12	KR827989	---	---	---	Grosjean et al. 2015
51	<i>Polypedates braueri</i>	China:Sichuan, Omei		AB728015	---	---	---	Kuraishi et al. 2013
52	<i>Polypedates braueri</i>	China:Guangxi, Nanning	KUHE:33625	AB728009	---	---	---	Kuraishi et al. 2013
53	<i>Polypedates braueri</i>	China:Zhejiang, Tiantai	CIB<CHN>:ZJ20 0806091	AB728000	---	---	---	Kuraishi et al. 2013
54	<i>Polypedates braueri</i>	China:Jiangxi, Zixi	CIB<CHN>:JX20 0806183	AB728002	---	---	---	Kuraishi et al. 2013
55	<i>Polypedates braueri</i>	China: Taipei, Taiwan	KUHE:32842	AB564281	---	---	---	Kuraishi et al. 2013
56	<i>Polypedates braueri</i>	China: Nantou, Taiwan	KUHE:12973	AB727998	---	---	---	Kuraishi et al. 2013
57	<i>Polypedates braueri</i>	China: Chiaai, Taiwan	KUHE:12971	AB564278	AB728307	AB728273	AB728234	Kuraishi et al. 2013
58	<i>Polypedates braueri</i>	China: Yunnan	KUHE37040	AB728016	AB728308	AB728274	AB728235	Kuraishi et al. 2013
59	<i>Polypedates braueri</i>	China: Motuo, Xizang	KIZ 6214	KC465836	GQ285809	GQ285791	GQ285737	Li et al. 2013
60	<i>Polypedates impresus</i>	China: Puer, Yunnan	YN2018159	MW266029*	MW269969*	MW265990*	MW266005*	this study
61	<i>Polypedates impresus</i>	China: Menglun, Yunnan	YN2018206	MW266030*	MW269968*	MW265989*	MW266004*	this study
62	<i>Polypedates impresus</i>	China: Mengyang, Yunnan	YN2018355	MW266031*	MW269967*	MW265988*	MW266003*	this study
63	<i>Polypedates impresus</i>	China: Jinghong, Yunnan	BN2018016	MW266021*	MW269976*	MW266001*	---	this study
64	<i>Polypedates impresus</i>	China: Mengyang, Yunnan	BN2018130	MW266022*	MW269977*	MW266002*	MW266016*	this study
65	<i>Polypedates impresus</i>	Viet Nam: Lang So'n, Dat	K1418	KR827996	---	---	---	Grosjean et al. 2015
66	<i>Polypedates impresus</i>	China: Xishuangbanna, Yunnan	SCUM 37940C	EU215551	EU215611	EU215581	GQ285738	Li et al. 2013
67	<i>Polypedates impresus</i>	Laos: Phongsali, Long Nai Khao	2004.0366	KR827997	---	---	---	Grosjean et al. 2015
68	<i>Polypedates impresus</i>	China: Yingjiang, Yunnan	YN2018101	MW266028*	MW269970*	MW265991*	MW266006*	this study

69	<i>Polypedates megacephalus</i>	Vietnam: Kon Tum, Kon PLong	VN2018026	MW266026*	MW269978*	MW265997*	MW266009*	this study
70	<i>Polypedates megacephalus</i>	Vietnam: Kon Tum, Kon PLong	VN2018038	MW266018*	MW269973*	MW265995*	MW266010*	this study
71	<i>Polypedates megacephalus</i>	Vietnam: Kon Tum, Kon PLong	VN2018059	MW266019*	MW269972*	MW265993*	MW266008*	this study
72	<i>Polypedates megacephalus</i>	Thailand: Trat, Namtok Ko Chang	KUHE:20219	AB728091	---	---	---	Kuraishi et al. 2013
73	<i>Polypedates megacephalus</i>	Laos: Vientian, Vientian	KUHE:40655	AB728072	---	---	---	Kuraishi et al. 2013
74	<i>Polypedates megacephalus</i>	Thailand: Prachuap Khiri Khan, Kui Buri NP	0946Y	KR828024	AB728312	AB728278	AB728239	Grosjean et al. 2015
75	<i>Polypedates megacephalus</i>	Vietnam: Kon Tum, Kon PLong	VN2018036	MW266027*	MW269974*	MW265996*	MW266012*	this study
76	<i>Polypedates megacephalus</i>	China: Hong Kong	HK20181012	MW266023*	MW269979*	MW266000*	MW266015*	this study
77	<i>Polypedates megacephalus</i>	China: Hainan, Diaoluoshan	CIB<CHN>:0703 4	AB728035	---	---	---	Kuraishi et al. 2013
78	<i>Polypedates megacephalus</i>	China: Hainan Island	SN030056	EF564487 EF564559	AB728311	AB728277	AB728238	Yu et al. 2008
79	<i>Polypedates megacephalus</i>	Viet Nam: Vinh Phu, Hanoi	KUHE:38482	AB728050	---	---	---	Kuraishi et al. 2013
80	<i>Polypedates teraiensis</i>	Myanmar:Chatthin	KUHE:33880	AB728167	---	---	---	Kuraishi et al. 2013
81	<i>Polypedates teraiensis</i>	Bangladesh: Sunamganj, Bisampur	DFBGBAU Pter 179	AB530519	---	---	---	Hasan et al. 2014
82	<i>Polypedates teraiensis</i>	Bangladesh: Bandarban, Sadar Thana	DFBGBAU Pter 402	AB530521	---	---	---	Hasan et al. 2014
83	<i>Polypedates teraiensis</i>	Bangladesh: Mymensingh, Char Nilokhia	DFBGBAU Pter 202-211	AB530513	---	---	---	Hasan et al. 2014
84	<i>Polypedates teraiensis</i>	Bangladesh: Tangail, Modhupur	IABHU F4040	AB530516	---	---	---	Hasan et al. 2014
85	<i>Polypedates leucomystax</i>	Indonesia: Sumatra, Brostagi	KUHE:23556	AB728129	---	---	---	Kuraishi et al. 2013

86	<i>Polypedates leucomystax</i>	Thailand: Surat Thani, Ko Samui	KUHE:19546	AB728096	---	---	---	Kuraishi et al. 2013
87	<i>Polypedates leucomystax</i>	Malaysia	M20190701	MW266025*	MW269975*	MW265998*	MW266014*	this study
88	<i>Polypedates leucomystax</i>	Malaysia: Sabah, Tempaluri	BORN:22430	AB728144	---	---	---	Kuraishi et al. 2013
89	<i>Polypedates leucomystax</i>	Malaysia: Sabah, Imbak		AB728145	---	---	---	Kuraishi et al. 2013
90	<i>Polypedates leucomystax</i>	Malaysia: Terengganu, Lata Tembakoh	KUHE:52375	AB728110	---	---	---	Kuraishi et al. 2013
91	<i>Polypedates leucomystax</i>	Thailand: Narathiwat, Hala-Bala	KUHE:23173	AB728101	---	---	---	Kuraishi et al. 2013
92	<i>Polypedates leucomystax</i>	Malaysia: Seangor, Mimaland	KUHE:15164	AB728117	---	---	---	Kuraishi et al. 2013
93	<i>Polypedates leucomystax</i>	Malaysia: Sarawak, Kapit	KUHE:53147	AB728148	---	---	---	Kuraishi et al. 2013
94	<i>Polypedates leucomystax</i>	Malaysia: Sarawak, Matang	KUHE:12042	AB728152	---	---	---	Kuraishi et al. 2013
95	<i>Polypedates leucomystax</i>	Malaysia: Langkawi Island	IABHU 21119	AB530570	---	---	---	Hasan et al. 2014
96	<i>Polypedates leucomystax</i>	Malaysia: Sarawak, Sammunsan	KUHE:17605	AB728151	---	---	---	Kuraishi et al. 2013
97	<i>Polypedates leucomystax</i>	Malaysia: Sarawak, Kuching	KUHE:17684	AB728150	---	---	---	Kuraishi et al. 2013
98	<i>Polypedates leucomystax</i>	Malaysia: Kuala Lumpur	KUHE:15774	AB728116	---	---	---	Kuraishi et al. 2013
99	<i>Polypedates leucomystax</i>	Malaysia: Perak, Larut	KUHE:15517	AB728107	---	---	---	Kuraishi et al. 2013
100	<i>Polypedates leucomystax</i>	Malaysia: Pahang, Fraser's Hill	KUHE:15157	AB728108	---	---	---	Kuraishi et al. 2013
101	<i>Polypedates leucomystax</i>	Malaysia: Selangor, Kanching	KUHE:52576	AB728114	---	---	---	Kuraishi et al. 2013
102	<i>Polypedates leucomystax</i>	Indonesia: Java, Temanggung		AB728135	---	---	---	Kuraishi et al. 2013
103	<i>Polypedates leucomystax</i>	Indonesia: Java, Yogyakarta		AB728136	---	---	---	Kuraishi et al. 2013
104	<i>Polypedates leucomystax</i>	Philippines: Municipality of Dingasan	ACD 0890	HM770385	---	---	---	Brown et al. 2010
105	<i>Polypedates leucomystax</i>	Japan: Okinawajima, Kunigami	KUHE:22556	AB728165	---	---	---	Kuraishi et al. 2013
106	<i>Polypedates leucomystax</i>	Philippines: Panay, Iloilo	KUHE:34396	AB728164	---	---	---	Kuraishi et al. 2013
107	<i>Polypedates leucomystax</i>	Japan: Miyakojima	KUHE:37793	AB728166	---	---	---	Kuraishi et al. 2013

108	<i>Polypedates leucomystax</i>	Indonesia: Sulawesi, Banggai	MZB:Amp8026	AB728155	---	---	---	Kuraishi et al. 2013
109	<i>Polypedates leucomystax</i>	Indonesia: Sulawesi, Bolaang Mongondow	MZB:Amp8024	AB728154	---	---	---	Kuraishi et al. 2013
110	<i>Polypedates leucomystax</i>	Indonesia: Sulawesi, Polewali	MZB:Amp12444	AB728158	---	---	---	Kuraishi et al. 2013
111	<i>Polypedates leucomystax</i>	Indonesia: Sulawesi, Soppeng	MZB:Amp12884	AB728160	---	---	---	Kuraishi et al. 2013
112	<i>Polypedates leucomystax</i>	Indonesia: Sulawesi, Benteng	MZB:Amp12531	AB728163	---	---	---	Kuraishi et al. 2013
113	<i>Polypedates leucomystax</i>	Indonesia: Java, Depok	MZB:UN tissue	AB564287	---	---	---	Kuraishi et al. 2013
114	<i>Polypedates leucomystax</i>	Indonesia: Java, Bogor		AB728134	---	---	---	Kuraishi et al. 2013
115	<i>Polypedates leucomystax</i>	Indonesia: Java, Banyuwangi	MZB:UN tissue	AB564285	AB728313	AB728279	AB728240	Kuraishi et al. 2013
116	<i>Polypedates leucomystax</i>	Indonesia: Banka, Tempilang		AB728133	---	---	---	Kuraishi et al. 2013
117	<i>Polypedates leucomystax</i>	Indonesia: Sumatra, Lubuk Sikaping		AB728131	---	---	---	Kuraishi et al. 2013
118	<i>Polypedates leucomystax</i>	Indonesia: Sumatra, Palembang		AB728132	---	---	---	Kuraishi et al. 2013
119	<i>Polypedates leucomystax</i>	Indonesia: Kalimantan, Sambojo		AB728153	---	---	---	Kuraishi et al. 2013

Supplementary Table S2 Primers used in this study

Locus	Primer	Primer sequence	Size (bp)	Cited source
Partial 12S and 16S ribosomal genes and the complete valine t-RNA	FS01	5'-AAC GCT AAG ATG AAC CCT AAA AAG TTC T-3'	2465	Wilkinson et al. (2002a)
	Rend	5'-GAC CTG GAT TAC TCC GGT CTG A-3'		Wilkinson et al. (2002a)
	F0483	5'-GAA GAG GCA AGT CGT AAC ATG G -3'		Wilkinson et al. (2002a)
	F1209	5'-CTC GTA CCT TTT GCA TCA TG-3'		Yangtong (2018)
	R1209	5'-CAT GAT GCA AAA GGT ACG AG-3'		Yangtong (2018)
Exon 1 of tyrosinase	L2976	5'-TGC TGG GCR TCT CTC CAR TCC CA-3'	532	Bossuyt & Milinkovitch(2000)
	H2977	5'-AGG TCC TCY TRA GGA AGG AAT G-3'		Bossuyt & Milinkovitch(2000)
Exon 1 of rhodopsin	L2903	5'-ACC ATG AAC GGA ACA GAA GGY CC-3'	316	Bossuyt & Milinkovitch(2000)
	H2904	5'-GTA GCG AAG AAR CCT TCA AMG TA-3'		Bossuyt & Milinkovitch(2000)
Proopiomelanocortin	POMC-1	5' -GAA TGT ATY AAA GMM TGC AAG ATG GWC CT-3'	571	Wiens et al. (2005b)
	POMC-7	5' -TGG CAT TTT TGA AAA GAG TCA T-3'		Smith et al.(2005)

Supplementary Table S3 Estimates of net evolutionary divergence between groups of sequences of *Polypedates leucomystax*

	I	II	III	IV	V	VI	VII
I							
II	0.007						
III	0.021	0.020					
IV	0.024	0.022	0.030				
V	0.024	0.022	0.028	0.010			
VI	0.024	0.024	0.030	0.024	0.022		
VII	0.025	0.025	0.032	0.025	0.022	0.004	

Supplementary Table S4 Parameters from ancestral range evolution analysis of *Polypedates* conducted in “BioGeoBEARS”

Model	LnL	Parameters	d	e	j	AIC	AIC_wt
DEC	-98.78	2	0.0041	1.00E-12	0	201.6	2.20E-08
DEC+J	-81.37	3	1.00E-12	8.20E-10	0.015	168.7	0.29
DIVALIKE	-96.04	2	0.0059	1.00E-12	0	196.1	3.40E-07
DIVALIKE+J	-80.48	3	1.00E-12	1.00E-12	0.015	167	0.7
BAYAREALIKE	-152.6	2	0.0047	0.064	0	309.2	8.90E-32
BAYAREALIKE+J	-85.06	3	1.00E-07	1.00E-07	0.017	176.1	0.0072