

Supplementary Materials

Supplementary Methods

Molecular analyses: Total genomic DNA was extracted from liver tissues stored in 99% ethanol. Sequences encoding three mitochondrial genes (12S rRNA, 16S rRNA, and COI) were amplified and sequenced using the primers and experiment protocols of Yu et al. (2020). Homologous sequences of other *Kurixalus* species were obtained from GenBank and all new sequences have been deposited in GenBank under Accession Nos. MW345613–MW345628 and MW346332–MW346339 (Table S1). *Buergeria buergeri* (Temminck & Schlegel) was included in the data as outgroup.

Sequences were aligned using MUSCLE with default parameters in MEGA 7 (Kumar et al., 2016). Uncorrected pairwise distances between species were calculated in MEGA 7. The best substitution model of the combined data was selected using the corrected Akaike Information Criterion (AICc) in jMODELTEST v2.1.10 (Darriba et al., 2012). Bayesian inference was performed in MRBAYES v3.2.6 (Ronquist et al., 2012) under the selected substitution model (GTR + I + G). Two runs were performed simultaneously with four Markov chains starting from random tree. The chains were run for 3 000 000 generations and sampled every 100 generations. The first 25% of the sampled tree was discarded as burn-in after the standard deviation of split frequencies of the two runs was less than 0.01. The remaining trees were then used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs).

Morphology: Morphometric data were taken using digital calipers to the nearest 0.1 mm. Morphological terminology follows Fei et al. (2017). Measurements included: snout-vent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to rear of jaws); head width (HW, width of head at its widest point); snout length (SL, from tip of snout to anterior corner of eye); internarial distance (IND, distance between nares); interorbital distance (IOD, minimum distance between upper eyelids); upper eyelid width (UEW, maximum width of upper eyelid); eye diameter (ED, diameter of exposed portion of eyeball); distance between nostril and eye (DNE, from nostril to anterior border of eye); tympanum diameter (TD, the greater of tympanum vertical and horizontal diameters); forearm and hand length (FHL, from elbow to tip

of third finger); tibia length (TL, distance from knee to heel); foot length (FL, from proximal end of inner metatarsal tubercle to tip of fourth toe); and length of foot and tarsus (TFL, from tibiotarsal joint to tip of fourth toe). Webbing formula followed Myers & Duellman (1982).

REFERENCES

- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**: 772–772.
- Fei L, Ye CY, Wang YF, Jiang K. 2017. A new species of the genus *Amolops* (Anura: Ranidae) from high-altitude Sichuan, southwestern China, with a discussion on the taxonomic status of *Amolops kangtingensis*. *Zoological Research*, **38**: 138–145.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**: 870–1874.
- Myers CW, Duellman WE. 1982. A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. *American Museum Novitates*, 2752: 1–32.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**: 539–542.
- Yu GH, Du LN, Wang JS, Rao DQ, Wu ZJ, Yang JX. 2020. From mainland to islands: colonization history in the tree frog *Kurixalus* (Anura: Rhacophoridae). *Current Zoology*, doi: 10.1093/cz/zoaa023.

Supplementary Tables

Supplementary Table S1 Species used in this study (*B.* = *Buergeria*, *K.* = *Kurixalus*)

Species	Voucher number	Locality	12S	16S	COI
<i>B. buergeri</i>	-	Hiroshima, Japan	AB127977	AB127977	AB127977
<i>K. absconditus</i>	MZB 21862	Borneo, Indonesia	-	MN727052	-
<i>K. appendiculatus</i>	KU 324192	Bohol, Philippines	KF933206	-	-
<i>K. baliogaster</i>	ROM 29860	Gia Lai, Vietnam	KX554475	KX554537	KX554647
<i>K. banaensis</i>	ROM 32986	Gia Lai, Vietnam	GQ285667	GQ285667	-
<i>K. berylliniris</i>	11311 (CE01X)	Taiwan, China	-	DQ468669	DQ468677
<i>K. bisacculus</i>	THNHM 10051	Nan, Thailand	GU227279	GU227334	KX554633
<i>K. chaseni</i>	MZB 30584	Sumatera, Indonesia	-	MN727053	-
<i>K. chaseni</i>	FMNH 267896	Sarawak, Malaysia	JQ060948	JQ060937	KX554539
<i>K. eiffingeri</i>	11333	Taiwan, China	-	DQ468670	DQ468678
<i>K. gracilloides</i>	SIEZC 30188	Nghe An, Vietnam	-	MN510864	-
<i>K. gracilloides</i>	SIEZC 30189	Nghe An, Vietnam	-	MN510865	-
<i>K. hainanus</i>	Rao14111301	Hainan, China	KX554461	KX554523	KX554620
<i>K. idiootocus</i>	UMFS 5702	Taiwan, China	DQ283054	DQ283054	-
<i>K. idiootocus</i>	KUHE 12979	Taiwan, China	AB933306	AB933306	-
<i>K. idiootocus</i>	SCUM 061107L	Taiwan, China	EU215547	EU215547	-
<i>K. idiootocus</i>	ZRC1.1.5276	Taiwan, China	GQ204744	GQ204686	-
<i>K. idiootocus</i>	CAS 211366	Taiwan, China	AF458129	AF458129	-
<i>K. idiootocus</i>	UMMZ 190578	Taiwan, China	AF026347	AF026364	-
<i>K. idiootocus</i>	A127	Taiwan, China	-	DQ468674	DQ468682
<i>K. idiootocus</i>	JL-17-11	Taiwan, China	-	-	MH034257
<i>K. idiootocus</i>	JL74-1-1	Taiwan, China	-	-	MH034256
<i>K. idiootocus</i>	17759	Taiwan, China	-	-	MH034255
<i>K. idiootocus</i>	17496	Taiwan, China	-	-	MH034254
<i>K. idiootocus</i>	16581	Taiwan, China	-	-	MH034253
<i>K. idiootocus</i>	16580	Taiwan, China	-	-	MH034252
<i>K. idiootocus</i>	15887	Taiwan, China	-	-	MH034251
<i>K. idiootocus</i>	15886	Taiwan, China	-	-	MH034250
<i>K. idiootocus</i>	15885	Taiwan, China	-	-	MH034249
<i>K. idiootocus</i>	18241	Taiwan, China	-	-	MH034248
<i>K. lenquanensis</i>	KIZ170175Y	Yunnan, China	MK348042	KY768931	MK348050
<i>K. lenquanensis</i>	KIZ170182Y	Yunnan, China	MK348043	KY768938	MK348051
<i>K. motokawai</i>	VNMN 03458	Kon Tum, Vietnam	LC002888	LC002888	-
<i>K. naso</i>	Rao 06301	Tibet, China	KX554422	KX554484	KX554547
<i>K. odontotarsus</i>	YGH 090131	Yunnan, China	GU227240	GU227290	KX554576
<i>K. viridescens</i>	VNMN 03802	Khanh Hoa, Vietnam	AB933284	AB933284	-
<i>K. wangi</i>	11328 (CE06)	Taiwan, China	-	DQ468671	DQ468679
<i>K. yangi</i>	Rao 14102901	Yunnan, China	KX554429	KX554491	KX554557
<i>K. raoi sp. nov.</i>	GXNU YU140141	Xingyi, Guizhou, China	MW345613	MW345621	MW346332
<i>K. raoi sp. nov.</i>	GXNU YU140143	Xingyi, Guizhou, China	MW345614	MW345622	MW346333
<i>K. raoi sp. nov.</i>	GXNU YU140144	Xingyi, Guizhou, China	MW345615	MW345623	MW346334

Table S1 (*continued*)

Species	Voucher number	Locality	12S	16S	COI
<i>K. raoi</i> sp. nov.	GXNU YU140145	Xingyi, Guizhou, China	MW345616	MW345624	MW346335
<i>K. raoi</i> sp. nov.	GXNU YU140146	Xingyi, Guizhou, China	MW345617	MW345625	MW346336
<i>K. raoi</i> sp. nov.	GXNU YU140147	Xingyi, Guizhou, China	MW345618	MW345626	MW346337
<i>K. raoi</i> sp. nov.	GXNU YU140148	Xingyi, Guizhou, China	MW345619	MW345627	MW346338
<i>K. raoi</i> sp. nov.	GXNU YU20160078	Xingyi, Guizhou, China	MW345620	MW345628	MW346339
<i>K. raoi</i> sp. nov.	GXNU YU1406033	Xingyi, Guizhou, China	MK348044	MK348047	MK348052

Supplementary Table S2 Uncorrected pairwise distances (%) between members of *Kurixalus* estimated from 16S rRNA (lower triangle) and COI sequences (upper triangle). NA: not available.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 <i>K. raoi</i> sp. nov.		5.4	5.6	11.7	12.3	13.8	NA	NA	NA	NA	15.9	16.9	15.6	15.0	15.3	16.1	NA	18.7
2 <i>K. idiootocus</i>	2.9		7.7	12.8	12.9	12.8	NA	NA	NA	NA	16.4	18.3	17.1	17.1	16.6	17.3	NA	19.5
3 <i>K. lenquanensis</i>	4.4	4.8		13.2	12.8	14.1	NA	NA	NA	NA	17.5	17.8	16.9	15.1	15.3	16.3	NA	20.2
4 <i>K. berylliniris</i>	5.1	6.1	5.5		9.9	9.7	NA	NA	NA	NA	15.3	15.7	16.9	16.4	16.9	17.9	NA	20.8
5 <i>K. eiffingeri</i>	5.3	5.9	6.1	4.9		11.7	NA	NA	NA	NA	17.2	18.4	16.0	16.9	17.3	17.3	NA	19.3
6 <i>K. wangi</i>	5.9	6.5	5.9	4.0	4.4		NA	NA	NA	NA	16.3	17.0	16.6	17.6	17.5	18.1	NA	20.2
7 <i>K. gracilloides</i>	5.9	5.4	6.6	6.1	7.0	6.6		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8 <i>K. viridescens</i>	12.4	12.5	12.7	11.2	12.2	11.0	10.8		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9 <i>K. banaensis</i>	12.5	12.6	12.4	10.8	11.8	11.0	10.8	6.4		NA	NA	NA	NA	NA	NA	NA	NA	NA
10 <i>K. motokawai</i>	13.4	13.8	13.7	12.0	12.7	12.5	11.3	9.2	8.6		NA	NA	NA	NA	NA	NA	NA	NA
11 <i>K. naso</i>	10.9	11.3	10.6	9.5	10.4	8.9	10.5	10.7	10.9	12.5		6.3	12.6	13.1	13.0	12.6	NA	19.1
12 <i>K. yangi</i>	10.9	10.7	10.6	10.2	9.9	9.1	11.2	10.3	9.7	12.1	4.5		12.9	12.9	13.3	13.6	NA	19.7
13 <i>K. odontotarsus</i>	11.8	11.8	11.0	10.6	11.0	10.4	11.2	9.7	9.3	11.7	8.4	7.1		9.2	8.8	9.3	NA	20.1
14 <i>K. bisacculus</i>	12.2	12.2	11.3	11.6	11.0	10.6	11.3	9.7	9.9	11.9	8.7	7.9	3.1		3.6	5.0	NA	18.0
15 <i>K. hainanus</i>	12.2	12.3	11.2	11.6	11.4	11.0	11.9	9.9	10.0	11.8	8.1	7.5	2.9	1.4		3.6	NA	19.6
16 <i>K. baliogaster</i>	11.6	12.2	11.2	11.8	11.0	10.6	11.9	9.7	10.0	11.9	8.4	7.6	3.5	3.4	2.9		NA	19.1
17 <i>K. absconditus</i>	15.4	15.9	15.3	13.3	14.0	12.7	14.1	16.5	15.1	16.2	15.1	16.0	16.4	16.0	16.1	16.3		NA
18 <i>K. chaseni</i>	16.9	17.2	17.0	16.1	18.0	16.9	16.6	18.2	16.5	17.1	16.1	16.6	17.6	17.6	17.9	17.8	11.1	

Supplementary Table S3 Morphological comparison between species of the genus *Kurixalus*.

Species	Adult SVL (in mm)		Snout shape	Vocal sac	Iris
	Male	Female			
<i>K. raoi</i> sp. nov.	28.2–32.2 (mean 30.3);	38.6	Round with no projection on tip	Single internal (two vocal slit)	Golden brown
<i>K. absconditus</i>	27.3	?	Pointed with projection on tip	? (single vocal slit)	Golden
<i>K. appendiculatus</i>	29.3–35.4	41.1–51.5	Pointed with projection on tip	Single internal	Brown
<i>K. baliogaster</i>	33–33.3	35.8–41.5	Pointed with projection on tip	Single internal	Golden brown
<i>K. banaensis</i>	26.2–33.2	30.5–37	Pointed with projection on tip	Single internal	?
<i>K. berylliniris</i>	29–42.3	35.8–41.5	Pointed with projection on tip	Single internal	Emerald to light green
<i>K. bisacculus</i>	30–31.5	?	Pointed with projection on tip	Paired external	?
<i>K. chaseni</i>	30.1–33.4	30.6–44.3	Pointed with projection on tip	Single internal	Golden
<i>K. eiffingeri</i>	31.1±2.3	33.7±2.9	Pointed with projection on tip	Single internal	Golden
<i>K. gracilloides</i>	27.9–31.2	?	Obtusely pointed with no projection on tip	Single internal	Golden brown
<i>K. hainanus</i>	30–39.1	40.6–47.8	Pointed with projection on tip	Single internal	Golden
<i>K. idiootocus</i>	24.9–29.3	37.5	Pointed with projection on tip	Single external	Golden brown
<i>K. lenquanensis</i>	25–28.9 (mean 27)	?	Obtuse with no projection on tip	Single internal	Golden brown
<i>K. motokawai</i>	23.2–28.4 (mean 26.1)	25.1	Pointed with projection on tip	Single internal	Golden brown
<i>K. naso</i>	29.3–32.5	?	Pointed with projection on tip	Single internal	Golden brown
<i>K. odontotarsus</i>	32.1–34.3	?	Pointed with projection on tip	Single internal	Golden brown
<i>K. verrucosus</i>	?	43–45	Round with no projection on tip	?	?
<i>K. viridescens</i>	?	28.7–36.6	Pointed with projection on tip	?	Gold
<i>K. wangi</i>	28.6–31.6	30.8–37.1	Pointed with projection on tip	Single internal	Golden-yellow
<i>K. yangi</i>	31.6–34.7	?	Pointed with projection on tip	Single internal	Golden brown

Supplementary Table S3 (continued)

Species	Dorsal skin texture	Dorsal ground color	SL vs. ED	Vomerine teeth
<i>K. raoi</i> sp. nov.	Scattered with a few small tubercles	Light brown or dark brown	SL greater than ED	Present
<i>K. absconditus</i>	Rough with small tubercles	Brown	SL greater than ED	Present
<i>K. appendiculatus</i>	Rough with small tubercles	Brown	SL greater than ED	Present
<i>K. baliogaster</i>	Smooth	Dark brown	SL equal to or less than ED	Present
<i>K. banaensis</i>	Scattered with tubercles	Brown	SL greater than ED	Absent
<i>K. berylliniris</i>	Rough with small tubercles	Dark green to deep tan	?	Present
<i>K. bisacculus</i>	Scattered with tubercles	Dark brown	SL as long as ED	Present
<i>K. chaseni</i>	Scattered with tubercles	Brown	?	Present
<i>K. eiffingeri</i>	Rough with tubercles	Brown	?	Present
<i>K. gracilloides</i>	Rough with small tubercles	Golden-brown	SL less than ED	Present
<i>K. hainanus</i>	Rough with tubercles	Brown	SL less or greater than ED	Present
<i>K. idiootocus</i>	Rough with numerous small tubercles	grayish brown, yellowish brown, or dark brown	SL greater than ED	Present
<i>K. lenquanensis</i>	Rough with numerous small tubercles	Grayish brown	SL less than ED	Present
<i>K. motokawai</i>	Scattered with sparse small tubercles	Brown	SL greater than ED	Absent
<i>K. naso</i>	Rough with many tubercles	Purplish brown	SL greater than ED	Present
<i>K. odontotarsus</i>	Rough with tubercles	Grayish brown or dark brown	SL equal to or less than ED	Present
<i>K. verrucosus</i>	Rough with small warts	Grey or brown	SL equal to ED	Present
<i>K. viridescens</i>	Nearly smooth with few small tubercles	Solid green	SL greater than ED	Absent
<i>K. wangi</i>	Scattered with small tubercles	Dark brownish-green	?	Present
<i>K. yangi</i>	Scattered with numerous small tubercles	Brown	SL greater than ED	Present

Supplementary Table S3 (continued)

Species	Nuptial pad	Tibiotarsal articulation	Mandibular symphysis	Throat skin	Color pattern on chin
<i>K. raoi</i> sp. nov.	Present, slight	Reaching the center of eye	Weak	Granular	Clouded with dark
<i>K. absconditus</i>	Absent	Reaching the center of eye	Prominent	Smooth	Scattered with small dark speckles
<i>K. appendiculatus</i>	?	?	?	Smooth	Nearly immaculate
<i>K. baliogaster</i>	Present, slight	?	Weak	Smooth	Scattered with large dark speckles
<i>K. banaensis</i>	?	?	?	Smooth	?
<i>K. berylliniris</i>	Present, greatly expanded	?	Weak	Smooth	Faintly maculated with black spots
<i>K. bisacculus</i>	Present, slight	Reaching between eye and nostril	Weak	Finely granular	Clouded blackish
<i>K. chaseni</i>	?	Reaching the tip of snout	Weak	Granular	Scattered with small dark speckles
<i>K. eiffingeri</i>	Present, greatly expanded	?	?	?	?
<i>K. gracilloides</i>	Present, slight	Reaching between eye and nostril	?	Finely granular	Scattered with dense dark spots
<i>K. hainanus</i>	Present, slight	Reaching between eye and nostril	Weak	Finely granular	Scattered with large dark spots
<i>K. idiootocus</i>	Present, slight	Reaching the center of eye	Weak	Finely granular	Clouded blackish
<i>K. lenquanensis</i>	Present, slight	Reaching the center of eye	Weak	Finely granular	Clouded with dark
<i>K. motokawai</i>	?	Reaching the center of eye	?	Granular	Scattered with dark spots
<i>K. naso</i>	Present, slight	Reaching the eye	Weak	Granular	Scattered with dark spots
<i>K. odontotarsus</i>	Present, slight	Reaching the center of eye	Weak	Finely granular	Scattered with black spots
<i>K. verrucosus</i>	?	Reaching between eye and nostril	Weak	Smooth	Scattered with black spots
<i>K. viridescens</i>	?	Reaching the center of eye	?	Finely granular	Immaculate
<i>K. wangi</i>	Present, greatly expanded	?	Weak	Slightly granular	Speckled with dark spots
<i>K. yangi</i>	Present, slight	?	Weak	Finely granular	Clouded with dark

Supplementary Table S3 (continued)

Species	Outer metacarpal tubercle	Flank	Paired symmetric large dark blotches on chest	Toe webbing formula	Source
<i>K. raoi</i> sp. nov.	Present	Rough	Present	I2–2II1.5–3III2–3IV3–2V	1
<i>K. absconditus</i>	Absent	Rough	Absent	I1–2II1.5–2III1–2IV2–1V	2
<i>K. appendiculatus</i>	Absent	Rough	Absent	?	2, 3
<i>K. baliogaster</i>	Present	Smooth	Absent	I1.5–2II1–2.5III1–2.5IV2.5–1V	4
<i>K. banaensis</i>	Present	Smooth	Absent	?	5, 6
<i>K. berylliniris</i>	Present	Smooth or slightly shagreened	Absent	I1.5–2II1.5–2.5III2–3IV3–1.5V	7
<i>K. bisacculus</i>	Present	Rough	Absent	I1.25–2II1–2III1–2IV2–1V	8
<i>K. chaseni</i>	?	?	Absent	I1–1.5II1–1.5III1–(1–1.5)IV1–(1.5–1)V	2, 9
<i>K. eiffingeri</i>	Present	?	Absent	?	7
<i>K. gracilloides</i>	Present	Rough	Absent	I2–2½II1½–3III1¾–3½IV3–1½V	10
<i>K. hainanus</i>	Present	Rough	Absent	I1.5–2II1–2III1–2IV1.5–1V	11, 12
<i>K. idiootocus</i>	Present	Rough with small tubercles	Present	I2–2II1.5–2.5III1.5–3IV2.5–1.5V	1, 13
<i>K. lenquanensis</i>	Present	Rough	Absent	I2–2.5II1.5–3III1.5–3IV2.75–1.5V	14
<i>K. motokawai</i>	Present	Areolate	Absent	I2–2II1/3–2III1–2.5IV2.5–1.5V	6
<i>K. naso</i>	Present	Rough	Absent	I1–2II1–2III1–2IV2–1V	12, 15
<i>K. odontotarsus</i>	Present	Rough	Absent	I2–2II1.25–2.5III1.5–2.5IV2–1.5V	14
<i>K. verrucosus</i>	Present	Rough	Absent	I1–2II1–2III1–2IV2–1V	16
<i>K. viridescens</i>	Present	Areolate	Absent	I2–2.75II1.5–2.75III1.5–3IV2.5–1.75V	17
<i>K. wangi</i>	Present	Smooth	Absent	I2–2II1.5–2.5III2–3IV2.5–1V	7
<i>K. yangi</i>	Present	Rough	Absent	I1.5–2II1–2III1–2IV2–1V	12

Note: 1, this study; 2, Mediyansyah et al., 2019; 3, Günther, 1858; 4, Inger et al., 1999; 5, Bossuyt and Dubois, 2001; 6, Nguyen et al., 2014a; 7, Wu et al., 2016; 8, Taylor, 1962; 9, Smith, 1924; 10, Nguyen et al., 2020; 11, Zhao et al., 2005; 12, Yu et al., 2018; 13, Kuramoto & Wang, 1987; 14, Yu et al., 2017; 15, Annandale, 1912; 16, Boulenger, 1893; 17, Nguyen et al., 2014b;

