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A new species of *Gracixalus* (Amphibia: Anura: Rhacophoridae) from northern Vietnam

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Abstract We describe a new species of small tree frog from northern Vietnam based on morphological differences and molecular divergence. *Gracixalus waza* sp. nov. is distinguishable from its congeners and other small rhacophorid species on the basis of a combination of the following characters: (1) size small (snout-vent length of males 27.1–32.9 mm, of females 37.6 mm); (2) head as wide as or wider than long; (3) vomerine teeth absent; (4) snout rounded and long (16–18 % of the snout-vent length); (5) spines on upper eyelid absent; (6) tibiotarsal projection absent; (7) dorsal skin smooth; (8) dermal fringes on forearm and tarsus absent; (9) dorsal surface of head and body greyish-green to moss-green with dark brown pattern forming an inverse Y marking; and (10) throat and chest with dark marbling. Our molecular data showed that the new species is nested in the same group with *Gracixalus jinxiuensis* sensu lato.

Keywords Treefrog · Taxonomy · Phylogeny · Cao Bang Province · Ha Lang forest

Introduction

The subgenus *Gracixalus* Delorme et al. 2005 was recently upgraded to a distinct genus based on phylogenetic data of Li et al. (2008) and Yu et al. (2009). This genus currently contains six species with a range restricted to China, Vietnam, and Thailand (Frost 2011; Rowley et al. 2011). Rowley et al. (2011) suggested that the members of *Gracixalus* are clustered into two clades: Clade I comprising *G. gracilipes* (Bourret, 1937), *G. superconutus* (Orlov, Ho and Nguyen, 2004), *G. quyeti* (Nguyen, Hendrix, Böhme, Vu and Ziegler, 2008), and *G. quangi* Rowley, Dau, Nguyen, Cao and Nguyen, 2011; and

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Clade II consisting of *G. jinxiuensis* (Hu, 1978), *G.* cf. *jinxiuensis*, *G.* cf. *ananjevae* (Matsui and Orlov, 2004), and another unidentified species from Hoang Lien Range in Lao Cai and Lai Chau provinces, northern Vietnam. Three *Gracixalus* species are currently known only from Vietnam, viz., *G. quangi*, *G. quyeti*, and *G. supercornutus* (Orlov et al. 2004; Nguyen et al. 2008, 2009; Rowley et al. 2011).

During our recent field work in northern Vietnam, specimens of a small treefrog species were collected in the karst forest of Cao Bang Province. This treefrog taxon appears to be a member of the genus *Gracixalus* due to its small size (SVL < 40 mm), the presence of an intercalary cartilage between the terminal and penultimate phalanges of digits, tips of digits expanded into large discs bearing circummarginal grooves, the absence of vomerine teeth, horizontal pupil, tibia about four to five times longer than wide, and translucent skin (Delorme et al. 2005; Rowley et al. 2011). Closer examination showed that this taxon could be distinguished clearly from other known members of the genus by a combination of several features of the adult morphology. In phylogenetic analyses, this taxon is separated distinctly from its congeners and clustered within the *Gracixalus jinxiuensis* species group with a high support level. Owing to these distinctions, we describe it herein as a new species.

Materials and methods

Sampling

Field surveys were conducted in October 2011, and in April and May 2012 in Ha Lang District, Cao Bang Province, northern Vietnam. Tissue samples were preserved separately in 95 % ethanol and voucher specimens were fixed in approximately 80 % ethanol, then later transferred to 70 % ethanol for permanent storage. Specimens referred to in this paper are deposited in the collections of the Chengdu Institute of Biology (CIB), Chinese Academy of Sciences, Sichuan, China; Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam; University of Science, Vietnam National University, Hanoi (VNUH), Vietnam; University of Science, Vietnam National University, Ho Chi Minh City (UNS), Vietnam; Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam; and Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany.

Molecular data and phylogenetic analyses

We used the protocols of Le et al. (2006) for DNA extraction, amplification, and sequencing. A fragment of the mitochondrial gene 16S was amplified using the primer pair 16Sar + 16Sbr (Palumbi et al. 1991). After sequences were aligned by Clustal X v2 (Thompson et al. 1997), data were analyzed using maximum parsimony (MP) and Bayesian analysis (BA), as implemented in PAUP*4.0b10 (Swofford 2001) and MrBayes v3.2 (Huelsenbeck and Ronquist 2001), respectively. Settings for these analyses followed Le et al. (2006). The optimal model for nucleotide evolution was set to GTR + I + Γ as selected by Modeltest v3.7 (Posada and Crandall 1998). Nodal support was evaluated using bootstrap replication (BP) as calculated in PAUP and posterior probability (PP) in MrBayes v3.2. Uncorrected pairwise divergences were calculated in PAUP*4.0b10. Based on Rowley et al. (2011), we selected three outgroups—*Kurixalus eiffingeri*, *K. odontotarsus*, and *Philautus aurifasciatus*—for our phylogenetic analyses (Table 1).

Morphological characters

Measurements were taken with digital callipers to the nearest 0.1 mm. The following abbreviations were used: SVL: snout-vent length, HL: head length (from the back of mandible to the tip of snout), HW: maximum head width (across angle of jaws), SNL: snout length (from anterior corner of eye to the tip of snout), NS: distance from nostril to the tip of snout, EN: distance from anterior corner of the eye to the nostril, IN: internarial distance, IOD: interorbital distance, ED: eye diameter, UEW: maximum width of upper eyelid, DAE: distance between anterior corner of eyes, DPE: distance between posterior corner of eyes, MAE: distance between angle of jaws and anterior corner of the eye, MPE: distance between angle of jaws and posterior corner of the eye, MN: distance from the back of mandible to the nostril, TYD: tympanum diameter, TYE: distance from anterior margin of tympanum to posterior corner of the eye, FLL: forelimb length (from axilla to elbow), HAL: hand length (from elbow to the tip of third finger), fd1-4: width of discs of fingers I-IV, fw1-4: width of fingers I-IV, TFL: third finger length, OPT: outer palmar tubercle length, FeL: femur length (from vent to knee), TbL: tibia length (from knee to tarsus), TbW: tibia width, FoL: foot length (from tarsus to the tip of fourth toe), FTL: fourth toe length, IMT: inner metatarsal tubercle length, OMT: outer metatarsal tubercle length, td1-4: width of discs of toes I-IV, tw1-4: width of toes I–IV. For webbing formula we followed Glaw and Vences (2007).

Results

Phylogenetic analyses

The combined matrix contained 533 aligned characters. MP analysis of the dataset recovered the five most parsimonious trees with 330 steps (CI=0.68; RI=0.86). One of the five trees is shown in Fig. 1. Sixty-seven percent of the major

Table 1 Samples used in molecular analyses (Names in bold represent newly collected samples)

Species	GenBank no.	Locality	Voucher information
Gracixalus gracilipes	AY880504	Vietnam, Lao Cai Province	MNHN 1999.592
Gracixalus gracilipes	DQ283051	Vietnam, Ha Giang Province	AMNH A163897
Gracixalus jinxiuensis	EF564524	China, Guangxi Province	KIZ 060821013
	EU215525	China, Guangxi Province	KIZ061210YP
G. cf. "*jinxiuensis*"	EU871425−7	Vietnam, Lai Chau Province	IEBR 2351−2353
	JN862547	Vietnam, Nghe An Province	AMS R173454
Gracixalus quangi	JN862537−41	Vietnam, Nghe An Province	AMS R173410−173411, 173417, 173423, 173426
Gracixalus quangi	JX896683	Vietnam, Thanh Hoa Province	IEBR A.2012.5
Gracixalus quyeti	EU871428−9	Vietnam, Quang Binh Province	ZFMK 82999, VNUH 160706
Gracixalus supercornutus	JN862542−5	Vietnam, Kon Tum Province	AMS R173395−173396, 173428, 173887
***Gracixalus* sp. nov.**	JX896681−82, 84−85	Vietnam, Cao Bang Province	IEBR A.2012.2−A.2012.3, VNMN A.2012.2−A.2012.3
Kurixalus cf. *ananjevae*	JN862546	Vietnam, Nghe An Province	VNMN 03012
Kurixalus carinensis	GQ285670	Vietnam, Lao Cai Province	CIB XM-439
Kurixalus eiffingeri	DQ468673	Japan, Okinawa Islands	A120
Kurixalus "*odontotarsus*"	AY880507	Vietnam, Lai Chau Province	MNHN 1999.5942
Philautus aurifasciatus	AY141850	Indonesia, Java	ZRC 1.5266

nodes in the parsimonious tree (Fig. 1) received strong support (Bootstrap value≥70 %) (Hillis and Bull 1993) and the new species is placed with strong support in the clade containing mainly taxa named *G. jinxiuensis* (bootstrap value=81). In the Bayesian analysis, –ln*L* scores reached stationarity after 9,000 generations in both runs. The Bayesian topology is almost identical to the tree topology shown in Fig. 1, but there are a couple of minor

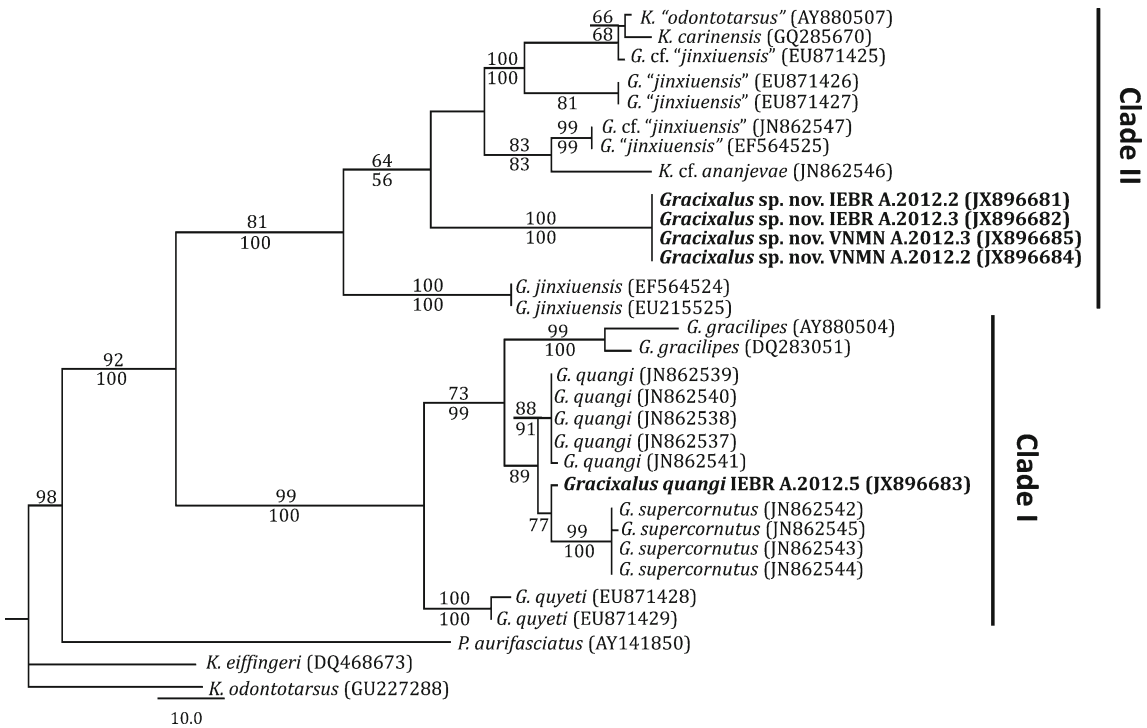

Fig. 1 One of the five most parsimonious maximum parsimony trees based on the partial 16S gene (TL=330; CI=0.68; RI=0.86). The dataset included 533 aligned characters of which 134 were potentially parsimony informative. Numbers above and under branches are bootstrap (>50%) values and Bayesian posterior probabilities, respectively

differences, including *G. jinxiuensis* (EU871425) being grouped with *G. jinxiuensis* (EU871426-871427) with weak support (PP=55), and the basal node of the clade with BP< 50 % becoming unresolved. In addition, two nodes strongly supported in the parsimony analysis receive low PP values in the Bayesian analysis, i.e., the node consisting of JN862546 + JN862547 and JN862537-862541 of *G. quangi* (Fig. 1). Both MP and BA analyses strongly support the monophyly of the clade II (sensu Rowley et al. 2011) including the new species (BP=81; PP=100, see Fig. 1). This species is significantly divergent from others within the clade II in terms of genetic distance with the minimum pairwise divergence of approximate 6.6% in the mitochondrial fragment of 16S (Table 2).

Gracixalus waza sp. nov. (Figs. 2 and 3)

Holotype. IEBR A.2012.2, adult male, collected by T.Q. Nguyen on 13 October 2011 in the karst forest near Ban Coong Village (22°43.216′N, 106°39.437′E), Duc Quang Commune, Ha Lang District, Cao Bang Province, Vietnam, at an elevation of ca. 480 m.

Paratypes. IEBR A.2012.3, adult female, the same collection data as the holotype; IEBR A.2012.4, adult male, VNMN A.2012.2, adult female, VNMN A.2012.3, adult male, ZFMK 93666-93667, adult males collected on 18 October 2011 by T.Q. Nguyen, C.T. Pham and D.T. Le in the karst forest near Lung Tung Village (22°43.765′N, 106° 35.377′E), Kim Loan Commune, Ha Lang District, Cao Bang Province, Vietnam, at elevations between 620 m and 650 m.

Diagnosis. The new species is distinguished from its congeners and other small rhacophorid species by a combination of the following characters: (1) size small (SVL 27.1–32.9 mm in males, 37.6 mm in females); (2) head as wide as or wider than long; (3) vomerine teeth absent; (4) snout rounded and long (SNL/SVL 0.16–0.18); (5) spines on upper eyelid absent; (6) tibiotarsal projection absent; (7) dorsal skin smooth; (8) dermal fringe on forearm and tarsus absent; (9) dorsal surface of head and body greyish green to moss-green with dark brown pattern forming an inverse Y marking; and (10) throat and chest with dark marbling.

Description of holotype. Small rhacophorid (SVL 32.8 mm), body robust, dorsoventrally compressed.

Head. Head as long as wide (HL 12.1 mm, HW 12.0 mm), convex above; snout rounded anteriorly from dorsal view, slightly protruding, its length (SNL 5.8 mm) longer than horizontal diameter of eye (ED 4.1 mm); canthus rostralis rounded, loreal region oblique, concave; interorbital region flat, broader than upper eyelid (IOD 3.8 mm, UEW 3.1 mm), as broad as internarial distance (IN 3.8 mm); distance between anterior corner of eyes (DAE 7.3 mm) about 68 % distance between posterior

Table 2 Uncorrected ("p") distance matrix showing pairwise genetic divergence (16S) between species in the genus *Gracixalus*

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Kurixalus "odontotarsus"* (AY880507)	-													
2. *K. carinensis* (GQ285670)	0.2	-												
3. *Gracixalus "jinxiuensis"* (EU871425)	0.2	0.4	-											
4. *G. "jinxiuensis"* (EU871426-7)	0.4	0.6	0.2	-										
5. *G.* cf. *"jinxiuensis"* (JN862547)[a]	4.7	5.1	4.5	4.7	-									
6. *K.* cf. *ananjevae* (JN862546)	5.7	6.3	5.5	5.7	4.0	-								
7. *G. jinxiuensis* (EF564524)[b]	6.3	6.8	6.4	6.2	5.9	7.4	-							
8. *Gracixalus* sp. nov. (JX896681-82, 84-85)	6.9	7.8	7.0	6.8	6.6	8.7	8.2	-						
9. *G. gracilipes* (AY880504)	10.9	11.1	11.1	11.3	11.5	12.2	11.7	12.7	-					
10. *G. gracilipes* (DQ283051)	9.7	10.5	9.9	10.0	10.6	10.8	10.8	13.3	3.0	-				
11. *G. quangi* (JN862537-41)	8.5-8.7	9.0-9.2	8.7-8.8	8.8-9	7.9-8.0	9.4-9.6	9.6-9.8	11.5	6.1-6.3	5.0-5.1	-			
12. *G. quangi* (JX896683)	8.6	9.0	8.7	8.9	7.5	9.4	9.6	11.7	6.0	4.8	1.0-1.1	-		
13. *G. supercornutus* (JN862542-5)	9.7-9.9	9.9-10.1	9.0-10.0	10.0-10.2	8.2-8.4	10.2-10.4	10.5-10.7	12.5-12.7	7.3-7.5	6.3-6.5	2.5-2.9	1.9-2.1	-	
14. *G. quyeti* (EU871428-9)	9.1-9.7	9.3-9.9	9.2-9.8	9.4-10.0	9.1-9.3	10.5-10.7	9.3-9.5	11.5-12.0	8.1-8.7	7.3-7.5	5.3-6.2	5.5-5.7	5.7-6.1	-

[a] JN862547 also includes EF564525

[b] EF564524 also includes EU215525

Fig. 2 Dorsal and ventral views of the holotype (IEBR A.2012.2, adult male) of *Gracixalus waza* sp. nov. from Cao Bang Province, northern Vietnam

Fig. 3 Dorsal and ventral views of a paratype (IEBR A.2012.3, adult female) of *Gracixalus waza* sp. nov. from Cao Bang Province, northern Vietnam

corner of eyes (DPE 10.7 mm); nostril rounded, without a lateral flap of skin, closer to tip of snout than to the eye (NS 2.6 mm, EN 3.6 mm); pupil oval, horizontal; tympanum distinct (TYD 2.0 mm), rounded, half of the eye diameter but greater than tympanum-eye distance (TYE 1.8 mm); pineal ocellus absent; spinules on upper eyelid absent; vomerine teeth absent; choanae small, oval; tongue cordate, deeply notched posteriorly; a pair of vocal sac openings present at base of jaw; supratympanic fold distinct, extending from behind the eye to beyond level of axilla.

Forelimbs. Arm short, about half of hand length (FLL 8.4 mm, HAL 15.9 mm), dermal fringe along outer side of forearm absent; relative length of fingers: I < II < IV < III; tips of all fingers with well developed discs with distinct circum-marginal grooves, discs relatively wide compared to width of finger (fd3/fw3 1.5/0.9 mm), disc of finger III smaller than tympanum diameter; finger webbing absent; subarticular tubercles distinct, blunt, rounded, one on finger I and II, two on fingers III and IV; nuptial pads prominent, oval; outer palmar tubercle divided into two.

Hindlimbs. Heels overlapping when held at right angles to the body; tibia length (TbL 18.1 mm) about five times greater than tibia width (TbW 3.5 mm), longer than thigh (FeL 15.6 mm) but shorter than foot length (FoL 22.3 mm); relative length of toes: I < II < III ≤ V < IV; tips of all toes with well developed discs with distinct circum-marginal grooves, discs slightly smaller than those of fingers; webbing formula Ii(1)(11/3)iIIe(1/2)(2)iIIIe(1)(2)iIV(2)(1)iV; subarticular tubercles distinct, blunt, rounded: one on toes I and II, two on toes III and V, and three on toe IV; inner metatarsal tubercle small (IMT 1.3 mm); dermal ridge along outer side of tibia and tarsal fold absent; outer metatarsal and supernumerary tubercles absent; pointed projection at tibiotarsal articulation absent; intercalary cartilage present in between the terminal and penultimate phalanges of digits.

Skin. Dorsal surface of head and body smooth; posterior part of tympanum, flank and lateral sides of limbs with small, flattened granulars; dorsolateral folds absent; throat and chest smooth, belly and ventral surface of thigh granular; dermal appendage at vent absent.

Coloration in alcohol. Snout and dorsum grey with a dark brown pattern forming an inverse Y marking, notably a triangular pattern between eyes bifurcating into two bands continuing posteriorly; a dark pattern running from above cloaca forward to the middle of the back; lateral side of head and flank grey with dark spots; lips with white narrow bars; tympanum light brown; forelimb, dorsal surface of thigh, tibia and foot grey with some darker bands, posterior part of thigh below the vent yellowish brown with small white spots; throat and chest with dark brown marbling; belly immaculate cream to white; ventral part of forelimbs white; ventral surface of thighs white to grey; webbing grey.

Coloration in life. Background of dorsal surface of head, body and limbs greyish green to moss-green; a dark brown, blotched pattern between eyes bifurcating into two bands continuing posteriorly on the back; a dark stripe present in the middle of posterior part of dorsum. Forelimb, dorsal parts of thigh, tibia, and foot moss-green with some dark brown bands; throat and chest white with dark brown marbling; belly immaculate white.

Variation. Measurements and morphological characters of the type series are given in Table 3. Males are smaller than the females in size (SVL 27.1–32.9 mm, n=5 vs 37.6 mm, n=2, respectively), have smaller average ratio of TYE/TYD (0.87 versus 0.98), and have developed nuptial pads on finger I. Dorsal coloration in life varied among individuals from light greenish brown to moss-green. Dark marbling on throat and chest are present in all preserved specimens but more bright in the female paratype IEBR A.2012.3.

Etymology. The new species is named in honour of the World Association of Zoos and Aquariums (WAZA), for the support of amphibian research and conservation in Vietnam. As common names, we suggest Waza Treefrog (English name), Waza Ruderfrosch (German name), and Nhái cây wa-za (Vietnamese name).

Distribution. *Gracixalus waza* sp. nov. is currently known only from the type locality in Cao Bang Province, northern Vietnam (Fig. 4).

Natural history. Gracixalus waza sp. nov. seems to be closely associated with the karst environment. Specimens were found at night between 1900 and 2300 hours near the entrance of caves and in valleys surrounded by limestone cliffs, and far from water sources. The nearest distance to the water sources recorded by us was about 200 m, from the entrance to the underground stream inside a cave near Ban Coong Village, Duc Quang Commune. The main habitat at the type locality is secondary karst forest of medium and small hardwoods mixed with shrubs and vines. Most of specimens were found on trees, about 0.2–0.5 m above the ground, but two specimens were collected on a limestone cliff inside a cave near Ban Lung Tung Village, Kim Loan Commune, on 8 April 2012 (Fig. 5). The call of the new species was not heard during our surveys in October 2011 or in April and May 2012. Several reptile species, known as karst forest inhabitants, were found at the site, including Lui's Leopard Gecko *Goniurosaurus luii* Grismer, Viets and Boyle, 1999 and Moellendorf's Rat Snake *Orthriophis moellendorffii* (Boettger, 1886).

Comparisons. Based on data obtained from the literature (Boulenger 1893; Bourret 1937, 1942; Hu 1978; Hu et al. 1981; Ye and Hu 1984; Ye et al. 1993; Inger et al. 1999; Ohler et al. 2002; Bain and Nguyen 2004; Matsui and Orlov 2004; Orlov et al. 2004; 2009; 2012; Nguyen et al. 2008; Fei et al. 2010; Li et al. 2011; Rowley et al. 2011) and specimens examined (see Appendix) we compared the new species with its congeners and other small treefrogs from Vietnam, Cambodia, Laos and China which have a SVL less than 40 mm and a dorsum with a dark inverse Y marking or similar color pattern.

Gracixalus waza sp. nov. is most similar to *G. quyeti* (Nguyen, Hendrix, Böhme, Vu and Ziegler, 2008) in terms of color pattern, but it differs from the latter species by having a head as wide as or wider than long (vs longer than wide in *G. quyeti*), a greater distance from tympanum to posterior corner of the eye (ratio of TYE/TYD 0.88–0.96 vs 0.40–0.44 in *G. quyeti*), and small tubercles on dorsum absent (vs present in *G. quyeti*). The new species differs from *G. gracilipes* (Bourret, 1937), *G. quangi* Rowley, Dau, Nguyen, Cao and Nguyen, 2011, and *G. supercornutus* (Orlov, Ho and Nguyen, 2004) by having a round snout (vs triangular pointed snout in *G. gracilipes*, *G. quangi* and *G. supercornutus*) and spines on upper eyelid absent (vs present in all latter species). It further differs from *G. supercornutus* and *G. quangi* by having a tibiotarsal projection absent (vs present in *G. supercornutus* and *G. quangi*), from *G. gracilipes* and *G. quangi* by having a dark inverse Y marking (vs X marking in latter species) on the dorsum; from *G. jinxiuensis* (Hu, 1978) by having a larger size (SVL of males 27.1–32.9 mm vs 23.5 mm, of females 37.6 mm vs 28.7–30.0 mm), a higher ratio of TYE/TYD (0.88-0.96 versus 0.78 in *G. jinxiuensis*), and ground color of dorsum greyish green to moss-green (dorsum brown in *G. jinxiuensis*). The new species further differs from "*G.* cf. *jinxiuensis*" from Ha Giang and Lai Chau by the absence of tubercles on dorsum. *Gracixalus waza* sp. nov. can be distinguished from *G. medogensis* (Ye and Hu, 1984) by having a longer snout (ratio of SNL/SVL 0.16–0.18 vs 0.13 in *G. medogensis*), lower ratio of ED/SNL (0.71–0.82 vs

Table 3 Measurements (in mm) of *Gracixalus waza* sp. nov. (abbreviations defined in text)

	IEBR A.2012.3	VNMN A.2012.2	Range	IEBR A.2012.2	IEBR A.2012.4	ZFMK 93666	ZFMK 93667	VNMN A.2012.3	Range Mean±SD
Sex	F	F	n=2	M	M	M	M	M	n=5
SVL	37.6	37.6	37.6	32.8	32.9	27.1	32.5	32.6	27.1–32.9 31.6±2.51
HW	14.0	13.3	13.3–14.0	12.0	12.3	10.0	12.5	12.1	10.0–12.5 11.8±1.01
HL	13.8	13.1	13.1–13.8	12.1	11.8	9.9	12.2	12.1	9.9–12.2 11.6±0.97
MN	12.3	11.5	11.5–12.3	11.3	10.2	9.1	10.7	11.1	9.1–11.3 10.5±0.87
MFE	9.3	8.6	8.6–9.3	8.0	7.6	6.7	7.5	8.1	6.7–8.1 7.6±0.55
MBE	5.8	5.2	5.2–5.8	4.7	5.2	4.4	4.8	4.8	4.4–5.2 4.8±0.28
SNL	6.5	6.0	6.0–6.5	5.8	5.8	4.9	5.8	5.6	4.9–5.8 5.6±0.38
ED	5.3	4.5	4.5–5.3	4.1	4.1	4.0	4.1	4.2	4.0–4.2 4.1±0.07
UEW	3.7	3.2	3.2–3.7	3.1	3.3	2.8	2.9	3.0	2.8–3.1 3.0±0.19
IN	4.0	3.8	3.8–4.0	3.8	3.7	3.4	3.7	3.6	3.4–3.8 3.6±0.15
IOD	4.1	3.9	3.9–4.1	3.8	3.8	3.3	3.3	3.9	3.3–3.9 3.6±0.29
DAE	7.5	7.5	7.5	7.3	7.2	6.0	7.0	7.1	6.0–7.3 6.9±0.53
DPE	11.7	11.6	11.6–11.7	10.7	10.0	8.9	10.5	10.6	8.9–10.7 10.1±0.74
NS	2.8	2.9	2.8–2.9	2.6	2.3	2.3	2.7	2.6	2.3–2.7 2.5±0.19
EN	4.0	4.0	4.0	3.6	3.5	3.2	4.0	3.6	3.2–4.0 3.6±0.29
TYD	2.5	2.4	2.4–2.5	2.0	2.0	1.7	1.9	2.0	1.7–2.0 1.9±0.13
TYE	2.5	2.3	2.3–2.5	1.8	1.8	1.5	1.7	1.7	1.5–1.8 1.7±0.12
FLL	9.1	9.2	9.1–9.2	8.4	6.9	6.1	7.3	7.3	6.1–8.4 7.2±0.83
HAL	17.7	17.5	17.5–17.7	15.9	16.2	12.9	16.2	16.8	12.9–16.8 15.6±1.54
TFL	10.0	10.6	10.0–10.6	9.6	9.3	8.2	9.6	9.2	8.2–9.6 9.2±0.58
NPL	1.9	1.8	1.8–1.9	1.7	2.1	1.5	2.1	2.1	1.5–2.1 1.9±0.28
fd3	2.0	1.8	1.8–2.0	1.5	1.4	1.2	1.5	1.6	1.2–1.5 1.4±0.15
FeL	17.6	17.6	17.6	15.6	16.9	13.1	16.0	15.8	13.1–16.9 15.5±1.42
TbL	19.5	19.7	19.5–19.7	18.1	18.3	15.4	18.8	18.6	15.4–18.8 17.8±1.39
TbW	4.3	4.0	4.0–4.3	3.5	3.1	3.6	3.7	3.8	3.1–3.8 3.5±0.27
FoL	25.7	25.1	25.1–25.7	22.3	22.9	19.3	24.1	24.1	19.3–24.1

Table 3 (continued)

	IEBR A.2012.3	VNMN A.2012.2	Range	IEBR A.2012.2	IEBR A.2012.4	ZFMK 93666	ZFMK 93667	VNMN A.2012.3	Range Mean±SD
									22.5±1.97
FTL	15.6	14.7	14.7–15.6	13.9	13.7	11.2	13.9	14.1	11.2–14.1
									13.4±1.22
SNL/SVL	0.17	0.16	0.16–0.17	0.18	0.18	0.18	0.18	0.17	0.17–0.18
									0.18±0.01
ED/SNL	0.81	0.75	0.75–0.81	0.71	0.71	0.82	0.71	0.75	0.71–0.82
									0.74±0.05
TYE/TYD	1.00	0.96	0.96–1.00	0.90	0.90	0.88	0.89	0.85	0.85–0.90
			0.98						0.87±0.17
fd3/TYD	0.80	0.75	0.75–0.80	0.75	0.70	0.71	0.79	0.80	0.70–0.80
			0.78						0.75±0.45

0.88 in *G. medogensis*), and lower ratio of fd3/TYD in males (0.75–0.80 vs 0.83 in *G. medogensis*).

The new species differs from *Buergeria japonica* (Hallowell, 1861) by having hindlimbs with less developed webbing and the absence of tubercles and ridges on dorsum; from *Feihyla palpebralis* (Smith, 1924) by the presence of a dark inverse Y marking on dorsum (vs. absent in *F. palpebralis*) and a white spot on upper lip absent (vs present in *F. palpebralis*); from *Kurixalus ananjevae* (Matsui and Orlov, 2004) by having snout length greater than the diameter of eye (vs equal in latter species), less developed toe webbing, and ventral surface with dark marbling (vs without marking in *K. ananjevae*); from *K. baliogaster* (Inger, Orlov and Darevsky, 1999) by the absence of vomerine teeth and white pattern on flanks; from *K. carinensis* (Boulenger, 1893) by having a snout longer than diameter of eye (vs shorter in *K. carinensis*), less developed webbing on toes (less than 1/2 vs 3/4 webbed in *K. carinensis*), and ventral surface with dark marbling (vs immaculate white in *K. carinensis*); from *K. idiootocus* (Kuramoto and Wang, 1987) by lacking a pointed snout, the absence of vomerine teeth and dermal fringe on forearm and tarsus; from *K. banaensis* (Bourret, 1939), *K. odontotarsus* (Ye, and Fei, 1993), and *K. verrucosus* (Boulenger, 1893) by lacking serrated dermal fringes on forearm and tarsus. The new species further differs from *K. odontotarsus* by the absence of vomerine teeth (present in latter species). *Gracixalus waza* sp. nov. can be separated from *Liuixalus romeri* (Smith, 1953) by having a larger size (SVL of males 27.1–32.9 mm vs 15–18 mm, of females 37.6 mm vs 18–23 mm), presence of a dark inverse Y marking (vs X marking in *L. romeri*) on back, and the absence of dermal fringes on forearm and tarsus; from *Philautus abditus* Inger, Orlov and Darevsky, 1999 by having tympanum distinct (vs hidden in *P. abditus*) and large black spots on anterior and posterior surfaces of thigh absent (vs present in *P. abditus*); from *Philautus cardamonus* Ohler, Swan and Daltry, 2002 by having a larger size of adult males (SVL 27.1–32.9 mm vs 19.3 mm) and a snout longer than diameter of eye (vs equal in *P. cardamonus*); from *P. maosonensis* Bourret, 1937 by having a head as wide as or wider than long (vs longer than wide in *P. maosonensis*), webbing on toes less developed (3/4 webbed in *P. maosonensis*), belly immaculate cream to white (vs mottled white on black in *P. maosonensis*), and white a spot on flank absent (vs present in *P. maosonensis*). The new species differs from *Raorchestes gryllus* (Smith, 1924) by having a rounded snout (vs pointed snout in *R. gryllus*), small tubercles on dorsum and dermal fringes on forearm and tarsus absent (vs present in *R. gryllus*); from *R. menglaensis* (Kou, 1990) by having a larger size (SVL of males 27.1–32.9 mm vs 15–18 mm, of females 37.6 mm vs 20 mm), the presence of a dark inverse Y marking (vs X marking) on back, and the absence of tubercles on dorsum (vs present in *R. menglaensis*); from *R. parvulus* (Boulenger, 1893) by having a large size (SVL of males 27.1–32.9 mm vs 18.3–21.1 mm in *R. parvulus*), a distinct tympanum (vs indistinct in latter species), and black bars in posterior region of flank absent (vs present in *R. parvulus*). *Gracixalus waza* sp. nov. also differs from *Rhacophorus spelaeus* Orlov, Gnophanxay, Phimminith and Phomphoumy, 2009, another species associated with the limestone cave habitat, by the absence of vomerine teeth and dermal fringe along outer side of forearm (vs present in *R. spelaeus*).

Discussion

Our phylogenetic analyses strongly support clades I and II of Rowley et al. (2011), regardless of the methods used for analyzing data. However, the resolution within each clade is unclear. In clade I, the molecular monophyly of *G. quangi* is supported strongly by both maximum likelihood and

Fig. 4 Type locality (*red circle*) of *Gracixalus waza* sp. nov. in Cao Bang Province, northern Vietnam

maximum parsimony, but weakly corroborated by Bayesian analysis. In particular, a specimen (IEBR A.2012.5) collected from Xuan Lien Nature Reserve (Thanh Hoa Province) suggests that this species is paraphyletic to the type specimens (single collection locality in Nghe An Province) in the 16S gene fragment analyzed. Sequencing additional genes and including samples from *G. quangi* from localities throughout its range will be required in order to help clarifying this issue. In clade II, species identification is confusing, with most

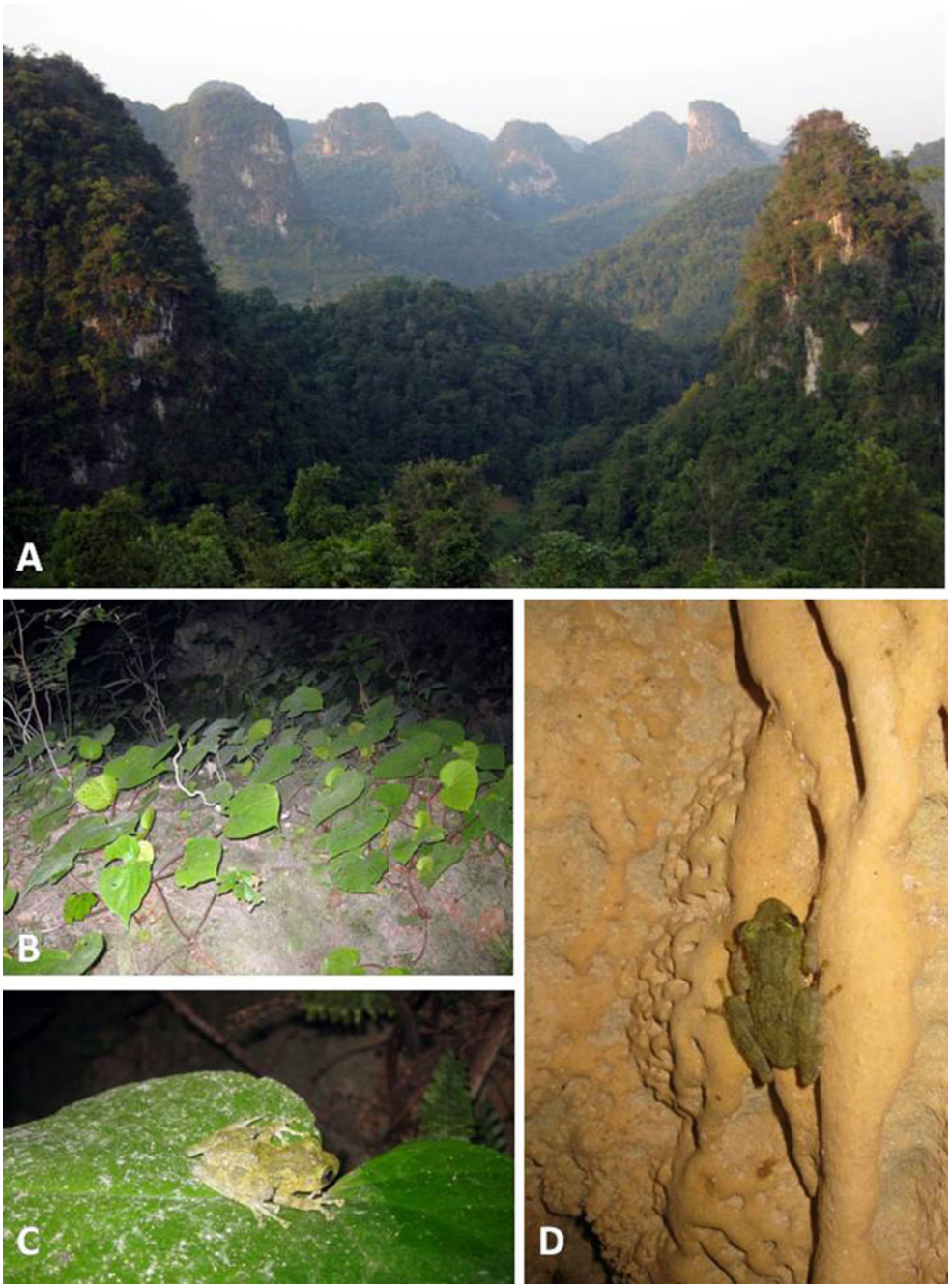

Fig. 5 **a** General vegetation type of the karst forest in Ha Lang, Cao Bang Province. **b** Biotope of *Gracixalus waza* sp. nov. **c** *Gracixalus waza* sp. nov. *in situ* on vegetation. **d** *Gracixalus waza* sp. nov *in situ* on limestone cave wall

samples being named *G. jinxiuensis*, and the Vietnamese *K. odontotarsus* from Lai Chau Province (MNHN 1999.5942) also needs to be re-determined. Careful examination of morphology and additional data sets, such as bioacoustics, may help to resolve this confusion. Finally, although *Gracixalus waza* sp. nov. and *G. quyeti* are clustered in different clades, they show a strikingly similar color pattern in life. The greenish-brown or moss-green coloration on the dorsal surface of both species, which can blend remarkably well into the background of stones covered with lichens or tree leaves, seems to be an adaptation to the life mode associated with the karst environment.

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Appendix Examined specimens

G. jinxiuensis (1): CIB 58795 (holotype, formerly CIB 660386): China: Guangxi Province: Jinxiu.

*G.*cf. "*jinxiuensis*" (7): IEBR 2351−2353: Vietnam: Lai Chau Province: Phong Tho; ZFMK 88046−88047: Vietnam: Lai Chau Province: Tam Duong; ZFMK 93668−93669: Vietnam: Ha Giang Province: Quan Ba.

G. gracilipes (1): ZFMK 93670: Vietnam: Ha Giang Province: Quan Ba.

G. quangi (1): IEBR A.2012.5: Vietnam: Thanh Hoa Province: Xuan Lien Nature Reserve.

G. quyeti (2): ZFMK 82999 (holotype): Vietnam: Quang Binh Province: Minh Hoa: Dan Hoa and VNUH 160706 (paratype): Vietnam: Quang Binh Province: Phong Nha - Ke Bang National Park.

G. supercornutus (2): IEBR 332−333 (paratypes): Vietnam: Kon Tum Province: Kon Plong: Mang Canh.

Feihyla palpebralis (4): IEBR A.2012.9−A.2012.11, ZFMK 93671: Vietnam: Gia Lai Province: Kon Ka Kinh National Park.

Raorchestes gryllus (8): UNS DT.0108, DT.0146, DT.0215, 0229, DT.0241, DT.0300, DT.0357, DT.0417: Vietnam: Lam Dong Province: Bi Doup - Nui Ba National Park.

Raorchestes parvulus (1): IEBR 2323: Vietnam: Lai Chau Province: Phong Tho.

Rhacophorus spelaeus (2): IEBR A.2012.14−A.2012.15: Laos: Khammuane Province: Ban Konglor.

References

Bain, R. H., & Nguyen, Q. T. (2004). Herpetofaunal diversity of Ha Giang Province in northeastern Vietnam, with description of two new species. *American Mususeum Novitates, 3453*, 1–42.

Boettger, O. (1886). Diagnoses reptilium novorum ab ill. viris O. Herz et Consule Dr. O. Fr. de Moellendorff in Sina meridionali repertorum. *Zoologischer Anzeiger, 9*, 519–520.

Boulenger, G. A. (1893). Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea dealing with the collection made in Pegu and the Karin Hills in 1887−88. *Annali del Museo Civico di Storia Naturale di Genov, 13*, 304–347.

Bourret, R. (1937). Notes herpétologiques sur lIndochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de lUniversité. Descriptions de quinze especes ou variétés nouvelles. *Annexe au Bulletin Général de l'Instruction Publique, 1937*, 5–56.

Bourret, R. (1939). Notes herpétologiques sur lIndochine française. XVII. Reptiles et batraciens reçus au Laboratoire des Sciences Naturelles de lUniversité au cors de lannée 1938. Descriptions de trois espèces nouvelles. *Annexe au Bulletin Général de l'Instruction Publique.* Hanoi, 1939, 13−34.

Bourret, R. (1942). *Les Batraciens de l'Indochine*. Hanoi: Institut Océanographique de l'Indochine.

Delorme, M., Dubois, A., Grosjean, S., & Ohler, A. (2005). Une nouvelle classification générique et subgénérique de la tribu des *Philautini* (Amphibia, Anura, Rhacophorinae). *Bulletin Mensuel de la Société Linnéenne de Lyon, 74*, 165–171.

Fei, L., Ye, C. Y., & Jiang, J. P. (2010). *Colored atlas of Chinese amphibians*. Sichuan Publishing House of Science and Technology.

Frost, D. R. (2011). Amphibian species of the world: an online reference. Version 5.5 (31 January 2011). Electronic Database accessible at http://research.amnh.org/vz/herpetology/amphibia/ American Museum of Natural History, New York, USA. Accessed 10 August 2012.

Glaw, F., & Vences, M. (2007). *A field guide to the amphibians and reptiles of Madagascar*. Third Edition. Frosch Verlag.

Grismer, L. L., Viets, B. E., & Boyle, L. J. (1999). Two new continental species of *Goniurosaurus* (Squamata: Eublepharidae) with a phylogeny and evolutionary classification of the genus. *Journal of Herpetology, 33*, 382–393.

Hallowell, E. (1861 "1860"). Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S. N. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 12, 480−510.

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology, 42*, 182–192.

Hu, S. Q. (1978). Three new amphibian species in China. In: S. Q. Hu, L. Fei, & C. Y. Ye (Eds), *Materials for Herpetological Research*, 4, p. 20.

Hu, S. C., Tian, W. S., & Wu, G. F. (1981). Three new species of amphibians from Guangxi. *Acta Herpetologica Sinica, 5*, 111–120.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics, 17*, 754–755.

Inger, R. F., Orlov, N. L., & Darevsky, I. (1999). Frogs of Vietnam: A report on new collections. *Fieldiana: Zoology, 92*, 1–46.

Kou, Z. T. (1990). A new species of genus *Philautus* (Amphibia: Rhacophoridae) from Yunnan, China. In: Zhao, E. M. (Ed.), *From Water onto Land*. A volume Issued to Commemorate the Ninetieth Birthday of the Late Professor Liu Cheng-zhao. Forestry, Beijing, pp. 210−212.

Kuramoto, M., & Wang, C. S. (1987). A new rhacophorid treefrog from Taiwan, with comparisons to *Chirixalus eiffingeri* (Anura, Rhacophoridae). *Copeia, 1987*, 931–942.

Le, M., Raxworthy, C. J., McCord, W. P., & Mertz, L. (2006). A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetic and Evolution, 40*, 517–531.

Li, J. T., Che, J., Bain, R. H., Zhao, E. M., & Zhang, Y. P. (2008). Molecular phylogeny of Rhacophoridae (Anura): A framework of taxonomic reassignment of species within the genera *Aquixalus, Chiromantis, Rhacophorus*, and *Philautus*. *Molecular Phylogenetics and Evolution, 48*, 302–312.

Li, J. T., Chen, Y. Y., Li SQ, L. V. K., & Wang, Y. Z. (2011). Catalogue of the type specimens of amphibians and reptiles in the Herpetological Museum of Chengdu Institute of Biology, Chinese Academy of Sciences: I. Rhacophoridae (Anura, Amphibia). *Asian Herpetological Research, 2*, 129–141.

Matsui, M., & Orlov, N. L. (2004). A new species of *Chirixalus* from Vietnam (Anura: Rhacophoridae). *Zoological Science, 21*, 671–676.

Nguyen, T. Q., Hendrix, R., Böhme, W., Vu, N. T., & Ziegler, T. (2008). A new species of the genus *Philautus* (Amphibia: Anura: Rhacophoridae) from the Truong Son Range, Quang Binh Province, central Vietnam. *Zootaxa, 1925*, 1–13.

Nguyen, S. V., Ho, C. T., & Nguyen, T. Q. (2009). *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt am Main.

Ohler, A., Swan, S. R., & Daltry, J. C. (2002). A recent survey of the amphibian fauna of the Cardamom Mountains, Southwest Cambodia with description of three new species. *Raffles Bulletin of Zoology, 50*, 465–481.

Orlov, N. L., Ho, C. T., & Nguyen, T. Q. (2004). A new species of the genus *Philautus* from Central Vietnam (Anura: Rhacophoridae). *Russian Journal of Herpetology, 11*, 51–64.

Orlov, N. L., Gnophanxay, S., Phimminith, T., & Phomphoumy, K. (2009). A new species of *Rhacophorus* genus (Amphibia: Anura: Rhacophoridae: Rhacophorinae) from Khammouan Province, Lao PDR. *Russian Journal of Herpetology, 16*, 295–303.

Orlov, N. L., Poyarkov, N. A., Vassilieva, A. B., Ananjeva, N. B., Nguyen, T. T., Nguyen, N. S., & 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. *Russian Journal of Herpetology, 19*, 23–64.

Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). *The simple fool's guide to PCR*. Hawai: Department of Zoology and Kewalo Marine Laboratory.

Posada, D., & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics, 14*, 817–818.

Rowley, J. J. L., Dau, V. Q., Nguyen, T. T., Cao, T. T., & Nguyen, S. V. (2011). A new species of *Gracixalus* (Anura: Rhacophoridae) with a hyperextended vocal repertoire from Vietnam. *Zootaxa, 3125*, 22–38.

Smith, M. A. (1924). New tree-frogs from Indo-China and the Malay Peninsula. *Proceedings of the Zoological Society of London, 1924*, 225–234.

Smith, M. A. (1953). Description of a new species of frog of the genus Philautus. *Annals and Magazine of Natural History*, Series 12, 6, 477−478.

Swofford, D. L. (2001). *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), version 4*. Sinauer, Sunderland, MA.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The ClustalX windows interface: Xexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*, 4876–4882.

Ye, C. Y., & Hu, S. Q. (1984). A new species of *Philautus* (Anura: Rhacophoridae) from Xizang Autonomou Region. *Acta Herpetologica Sinica, 3*, 67–69.

Ye, C. Y., Fei, L., & Hu, S. Q. (1993). *Rare and economic amphibians of China*. Chengdu, China: Sichuan Publishing House of Science and Technology.

Yu, G. H., Rao, D. Q., Zhang, M. W., & Yang, J. X. (2009). Re-examination of the phylogeny of Rhacophoridae (Anura) based on mitochondrial and nuclear DNA. *Molecular Phylogenetics and Evolution, 50*, 571–579.