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Citation	Current Herpetology (2014), 33(2): 101-111
Issue Date	2014-08
URL	http://hdl.handle.net/2433/216854
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Type	Journal Article
Textversion	publisher

A New Tree Frog of the Genus *Kurixalus* (Anura: Rhacophoridae) from Vietnam

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Abstract: A small rhacophorid frog from southern Vietnam is placed in the genus *Kurixalus* through molecular phylogenetic analysis. Because it is divergent genetically and morphologically from all known congeners, we describe it as a distinct species, *K. viridescens*. The species differs from the other congeners by an immaculate green dorsum, which is usually maculated gray to brown in the other species. With the addition of this new species, Vietnam now encompasses seven species of *Kurixalus*, and can be regarded as the center of speciation of this genus.

Key words: *Kurixalus viridescens* sp. nov.; MtDNA phylogeny; New species; Taxonomy; Vietnam

INTRODUCTION

A rhacophorid frog genus *Kurixalus* Ye, Fei, and Dubois In Fei, 1999 occurs in Asia from the Ryukyus of Japan, Taiwan, the Philippines, Borneo, Sumatra, the Malay Peninsula, Thailand, Laos, Cambodia, Vietnam, southern China, Myanmar, and eastern India. *Kurixalus* was originally established as a monotypic genus containing only Japanese and Taiwanese species *Chirixalus eiffingeri* (Boettger, 1895), but subsequent molecular studies have resulted in the placement in this genus of many small rhacophorids whose generic status was ambiguous.

At present, about 10 species are assigned to the genus *Kurixalus* (Yu et al., 2013), but there still remain several unnamed species from little-explored regions. During our expedition to the Central Highlands of southern Vietnam (Fig. 1), we collected several specimens of small rhacophorids that later proved to be a member of the genus *Kurixalus* by the molecular phylogenetic analysis. Because the specimens are not only genetically distinct, but also morphologically easily distinguishable from all the other congeners by their unique immaculate green dorsum, we herein describe them as a new species.

MATERIALS AND METHODS

In order to assign the generic position of

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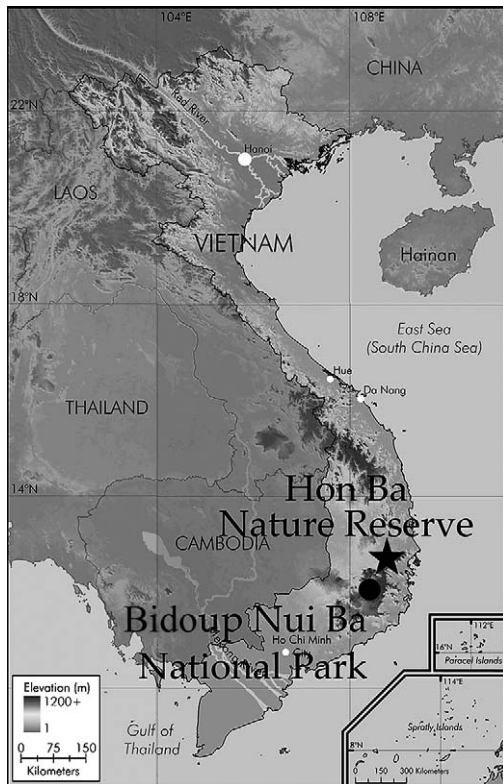


FIG. 1. Map of Vietnam showing Hon Ba Nature Reserve (filled star=type locality) and Bidoup Nui Ba National Park (filled circle), where specimens of the rhacophorid sp. was found.

the rhacophorid specimens from southern Vietnam, we compared partial sequence of 16S rRNA (ca. 1300 bp) of representing rhacophorid genera, *Chiromantis*, *Feihyla*, *Gracixalus*, *Kurixalus*, *Liuxalus*, *Nyctixalus*, *Philautus*, *Polypedates*, *Rhacophorus*, *Raorchestes*, *Theloderma*, and an outgroup *Buergeria* (Table 1). Methods for DNA extraction, and amplification and sequencing of the mtDNA fragments are the same as those reported by Kuraishi et al. (2013). The resultant new sequences were deposited in GenBank (Accession numbers AB933284–933309; Table 1). The alignment matrices with 1316 sites for 16S rRNA were subjected to estimate phylogenetic relationships using maximum likelihood (ML) and Bayesian inference (BI). Pairwise comparisons of uncor-

rected sequence divergences (p-distance) were also calculated. Details for these procedures are given in Kuraishi et al. (2013).

For morphometric comparisons, we took the following 21 body measurements to the nearest 0.1 mm with dial calipers, following Matsui (1984) and Matsui (1994): (1) snout-vent length (SVL); (2) head length (HL); (3) head width (HW); (4) internarial distance (IND); (5) interorbital distance (IOD); (6) upper eyelid width (UEW); (7) nostril-eyelid length (N-EL); (8) snout length (SL); (9) eye length (EL); (10) eye diameter (ED), diameter of the exposed portion of the eyeball; (11) tympanum diameter (TD); (12) tympanum-eye length (T-EL); (13) forelimb length (FLL); (14) lower arm and hand length (LAL); (15) first finger length (1FL); (16) inner palmar tubercle length (IPTL); (17) hindlimb length (HLL); (18) tibia length (TL); (19) foot length (FL); (20) inner metatarsal tubercle length (IMTL); and (21) first toe length (1TOEL). For finger and toe disks, measurements were taken to the nearest 0.01 mm using a binocular dissecting microscope equipped with a micrometer: (22–25) first to fourth finger disk diameter (1–4FDW); and (26–30) first to fifth toe disk diameter (1–5TDW). We followed the system of description of toe-webbing states used by Savage (1975). Specimens examined are stored in the Vietnam National Museum of Nature, Hanoi (VNMN), the Natural History Museum, London (BM), the Muséum National d’Histoire Naturelle, Paris (MNHN), and the Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

SYSTEMATICS

We obtained 1316 bp of 16S rRNA fragments of mtDNA gene for 40 samples, including outgroup (Fig. 2). Of 1316 nucleotide sites, 715 were variable, and 500 were parsimoniously informative within ingroup species. The best substitution model was J2+G with gamma shape parameter (G) of 0.280 for ML and GTR+G of 0.306 for BI. The likelihood

TABLE 1. Sample of rhacophorid sp. and other species used for DNA analysis in this study together with the information on voucher, collection locality, and GenBank accession numbers. Voucher abbreviations: AMNH=American Museum of Natural History, BORN=BORNEENSIS Collection, University Malaysia Sabah, CAS=California Academy of Science, CIB=Chengdu Institute of Biology, FMNH=Field Museum of Natural History, IABHU=Institute for Amphibian Biology, Hiroshima University, KIZ=Kunming Institute of Zoology, KUHE=Graduate School of Human and Environmental Studies, Kyoto University, RAO=field number of Ding-qi Rao, ROM=Royal Ontario Museum, VNMN=Vietnam National Museum of Nature.

Voucher	Species	GenBank	Locality	Reference
VNMN 03802	Rhacophorid sp.	AB933284	Vietnam, Khanh Hoa	This study
VNMN 03813	Rhacophorid sp.	AB933285	Vietnam, Khanh Hoa	This study
VNMN 03814	Rhacophorid sp.	AB933286	Vietnam, Khanh Hoa	This study
VNMN 01561	<i>Kurixalus bisacculus</i>	AB933287	Vietnam, Ha Giang	This study
VNMN 03805	<i>Kurixalus bisacculus</i>	AB933288	Vietnam, Cao Bang	This study
CIB K 2805	<i>Kurixalus bisacculus</i>	AB933289	China, Guangxi	This study
KUHE 19428	<i>Kurixalus bisacculus</i>	AB933290	Thailand, Nakhon Sri Tamarat	This study
KUHE 35069	<i>Kurixalus bisacculus</i>	AB933291	Thailand, Kanchanaburi	This study
VNMN 03806	<i>Kurixalus bisacculus</i>	AB933292	Vietnam, Thanh Hoa	This study
VNMN 03807	<i>Kurixalus bisacculus</i>	AB933293	Vietnam, Thanh Hoa	This study
VNMN 03808	<i>Kurixalus bisacculus</i>	AB933294	Vietnam, Thanh Hoa	This study
VNMN 03809	<i>Kurixalus bisacculus</i>	AB933295	Vietnam, Thanh Hoa	This study
VNMN 03810	<i>Kurixalus bisacculus</i>	AB933296	Vietnam, Thanh Hoa	This study
VNMN 03811	<i>Kurixalus baliogaster</i>	AB933297	Vietnam, Gia Lai	This study
VNMN 03812	<i>Kurixalus baliogaster</i>	AB933298	Vietnam, Gia Lai	This study
VNMN 03652	<i>Kurixalus baliogaster</i>	AB933299	Vietnam, Kon Tum	This study
VNMN 03618	<i>Kurixalus baliogaster</i>	AB933300	Vietnam, Kon Tum	This study
VNMN 03636	<i>Kurixalus baliogaster</i>	AB933301	Vietnam, Kon Tum	This study
CIB 201307012	<i>Kurixalus odontotarsus</i>	AB933302	China, Yunnan	This study
CIB 201307071	<i>Kurixalus odontotarsus</i>	AB933303	China, Yunnan	This study
CAS 231489	<i>Kurixalus verrucosus</i>	KC465823	Myanmar, Kachin	Li et al. (2013)
RAO 6305	<i>Kurixalus verrucosus</i>	KC465825	China, Xizang	Li et al. (2013)
ROM 32986	<i>Kurixalus banaensis</i>	GQ285667	Vietnam, Gia Lai	Li et al. (2009)
VNMN JJ07(NK)	<i>Kurixalus banaensis</i>	AB933304	Vietnam, Lam Dong	This study
KIZ 359	<i>Kurixalus banaensis</i>	KC465795	Vietnam, Lam Dong	Li et al. (2013)
KUHE 12910	<i>Kurixalus effingeri</i>	AB933305	Japan, Iriomote Is.	This study
KUHE 12979	<i>Kurixalus idiootocus</i>	AB933306	Taiwan, Jiayi	This study
KUHE 53614	<i>Kurixalus appendiculatus</i>	AB847125	Borneo, Sarawak	Matsui et al. (2014)
KUHE 52141	<i>Kurixalus appendiculatus</i>	AB933307	Malaysia, Johor	This study
KUHE 46345	<i>Chiromantis xerampelina</i>	AB813157	pet trade	Matsui et al. (2014)
KUHE 53591	<i>Feihyla kajau</i>	AB847122	Borneo, Sarawak	Matsui et al. (2014)
AMNH A163897	<i>Gracixalus gracilipes</i>	DQ283051	Vietnam, Ha Giang	Frost et al. (2006)
CIB 20080048	<i>Liuxalus romeri</i>	AB871412	China, Hong Kong	Nguyen et al. (2014)
FMNH 231095	<i>Nyctixalus pictus</i>	DQ283133	Borneo, Sabah	Frost et al. (2006)
VNMN 03461	<i>Philautus abditus</i>	AB933308	Vietnam, Kon Tum	This study
BORN 12420	<i>Polypedates leucomystax</i>	AB728138	Borneo, Sabah	Kuraishi et al. (2013)
KUHE 55165	<i>Rhacophorus kio</i>	AB781695	Vietnam, Thanh Hoa	Matsui et al. (2013)
KUHE 55238	<i>Raorchestes gryllus</i>	AB933309	Vietnam, Vinh Phuc	This study
KUHE 52581	<i>Theloderma leporosum</i>	AB847128	Malaysia, Kenaboi	Matsui et al. (2014)
IABHU 41011	<i>Buergeria buergeri</i>	AB127977	Japan, Hiroshima	Sano et al. (2004)

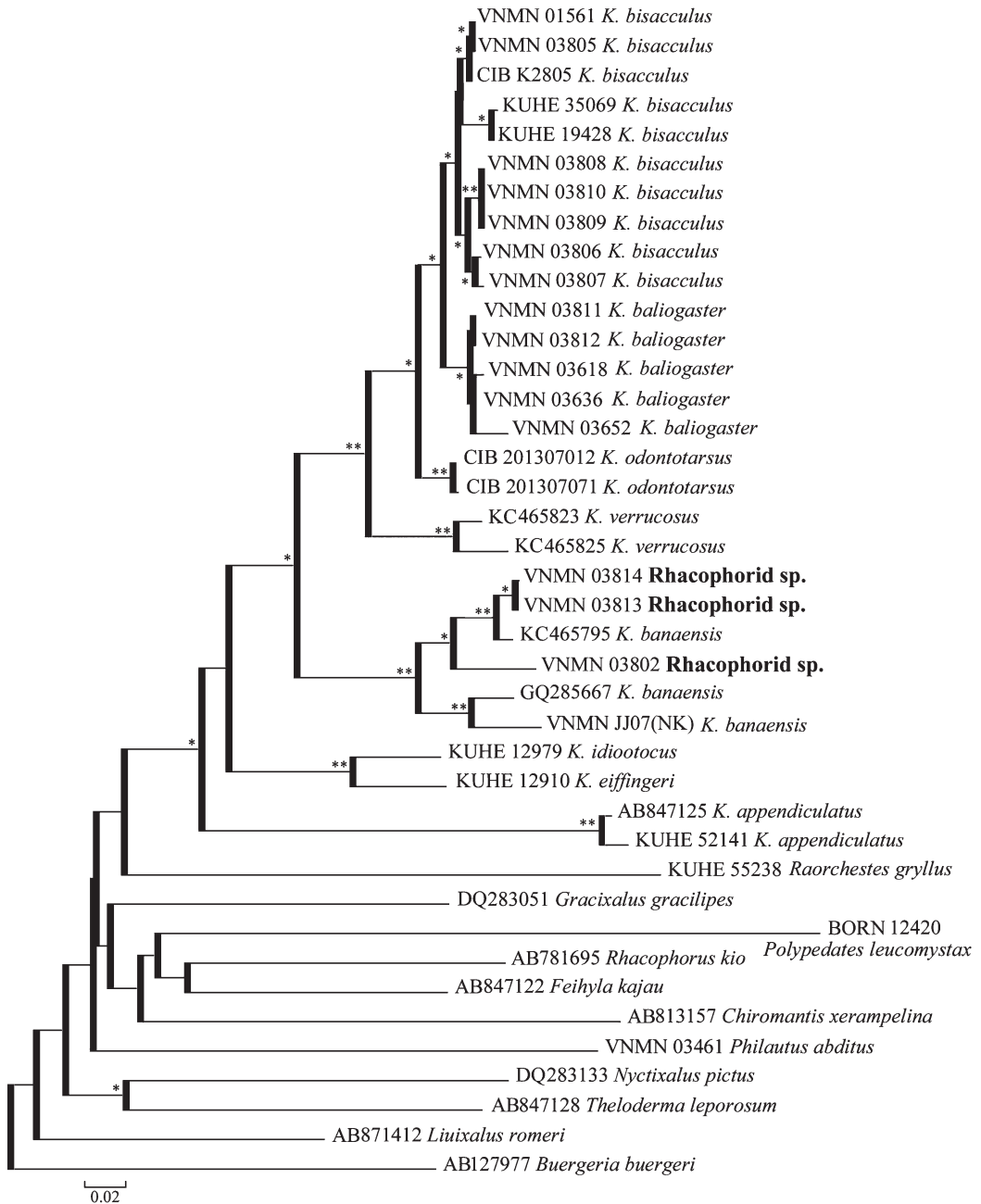


FIG. 2. ML tree from a 1316 bp sequence of mitochondrial 16S rRNA gene for a rhacophorid sp. from southern Vietnam and representative rhacophorid members. *: ML inferences (ML-BS) between 70% and 95%, and Bayesian posterior probabilities (BPP) between 95% and 100%. **: ML-BS \geq 95% and BPP = 100%.

values (-lnLs) of the ML and BI trees were 20861.279 and 20915.613, respectively.

The specimens of rhacophorid species from

southern Vietnam examined here showed some variations from each other (uncorrected p-distance of 0.4–5.4%), but formed a fully-

supported clade to be regarded as a species. The clade of this species is a sister clade to *K. banaensis* (Bourret, 1939), and the species was confirmed to be a member of *Kurixalus*. Although relationships within the genus were not fully resolved (Fig. 2), the clade containing *Kurixalus* sp. from southern Vietnam and *K. banaensis* was sister to the clade including all the other congeners except for *K. eiffingeri*, *K. idiootocus* (Kuramoto and Wang, 1987), and *K. appendiculatus* (Günther, 1858). From the eight species of *Kurixalus* examined, *Kurixalus* sp. from southern Vietnam exhibited substantially large genetic distances (uncorrected p-distance of 6.9–18.1%), values larger than the distance between specimens of *K. bisacculus* (Taylor, 1962) and *K. baliogaster* (Inger, Orlov, and Darevsky, 1999) (3.0–5.2%). Furthermore, *Kurixalus* sp. from southern Vietnam is also clearly separated morphologically from all the other congeners, including its sister species, in congruence with genetic separation. Thus, we describe *Kurixalus* sp. from southern Vietnam as follows:

***Kurixalus viridescens* sp. nov.**

Figs. 3–6

Etymology

The specific name is a Latin adjective, referring to the uniformly greenish dorsal color of the new species.



FIG. 3. Female paratype (VNMN 03814) of *Kurixalus viridescens* sp. nov. in life.

Holotype

Adult female VNMN 03802 (field number KH2011.04), collected by Duc Minh Hoang on 29 December 2011 from Hon Ba Nature Reserve (12°07'04" N, 108°56'46" E, 1540 m asl), Khanh Hoa Province, southern Vietnam (Fig. 1).

Paratype

Nine females: VNMN 03813–03816 (field number KHA 003–006) data same as the holotype; VNMN 03803 (field number TA0968), VNMN 03804 (field number TA0971) collected by Tao Thien Nguyen in November 2011 from the same locality as the holotype; VNMN 2013.25, 2013.72, 2013.74 (field number all TN-3-BQ) collected by Cuong The Pham in June 2013 from Bidoup of Bidoup Nui Ba National Park (12°10'49" N, 108°40'24" E; 1590 m asl), Lam Dong Province, southern Vietnam.

Diagnosis

The new species is assigned to the genus *Kurixalus* only by molecular phylogenetic evidence, but has the characteristics common to the genus: intercalary cartilage present between terminal and penultimate phalanges of digits; tips of digits expanded into large discs bearing circummarginal grooves; snout tip pointed; fingers poorly webbed; dermal fringes on forearm and tarsus present. *Kurixalus viridescens* can be distinguished from all other species of *Kurixalus* by the following combination of characteristics: female SVL 28.7–36.6 mm; snout tip pointed but not forming a corn; no dermal ridge around cloaca; vomerine teeth absent; solid green dorsum without any dark spots or markings; a lemon-yellowish venter without markings.

Description of holotype

SVL 36.3 mm; body robust; head (HL 13.4, 36.9%SVL) slightly shorter than wide (HW 13.8, 38.0%SVL); snout (SL 5.2, 14.3%SVL) subequal to eye (EL 5.3, 14.6%SVL), dorsally pointed at tip (Fig. 4), sloping anteroventrally in profile, and projecting over lower jaw; can-

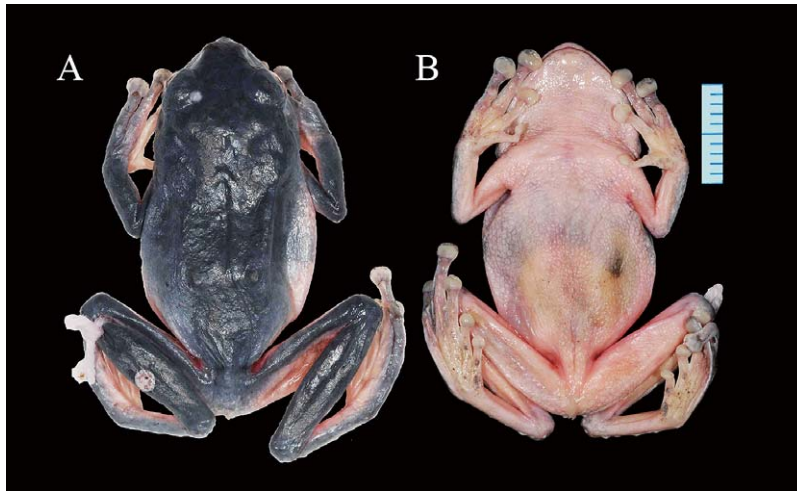


FIG. 4. Dorsal (A) and ventral (B) views of female holotype (VNMN 03802) of *Kurixalus viridescens* sp. nov. after preservation. Scale bar=10 mm.

thus blunt; lore oblique, slightly concave; nostril slightly protuberant, nearer to tip of snout than to eye; internarial distance (IND 3.3, 9.1%SVL) narrower than interorbital (IOD 4.3, 11.8%SVL); latter wider than upper eyelid (UEW 3.3, 9.1%SVL); pineal spot absent; eye large, protuberant, diameter (ED 4.2, 11.6%SVL) much larger than eye-nostril (N-EL 2.2, 6.1%SVL); pupil horizontal; tympanum distinct, subcircular, length (1.7, 4.7%SVL) less than half eye diameter and separated from eye by one-third of tympanum diameter (T-EL 0.7, 1.9%SVL); vomerine teeth absent; tongue deeply notched posteriorly.

Forelimb long (FLL 24.1, 66.4%SVL); relative finger length I<II<IV<III; length of first finger (1FL 4.3, 11.8%SVL measured from distal edge of inner palmar tubercle) subequal to diameter of eye; tips of all fingers dilated into horizontally elongate large disks with circummarginal and transverse ventral grooves; disk of third (3FDW 2.5, 6.8%SVL) and fourth fingers (4FDW 2.3, 6.5%SVL) wider than tympanum; webs between fingers poorly developed, finger webbing formula I 2-2¹/₄ II 2-3 III 2¹/₂-2¹/₂ IV (Fig. 5A); fringe of skin on edge of fingers; subarticular tubercles distinct, rounded, formula 1, 1, 2, 2; no

supernumerary tubercles on metacarpal; prepollex not prominent, oval; distinct inner (IPTL 1.7, 4.7%SVL) and two indistinct outer palmar tubercles.

Hindlimb long (HLL 56.8, 156.5%SVL), about 2.4 times length of forelimb; tibia not long (TL 18.1, 49.9%SVL), heels overlapping when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching middle of eye; foot (FL 16.2, 44.6%SVL) shorter than tibia; relative length of toes I<II<III<V<IV; tips of toes expanded into round disks with distinct circummarginal grooves, smaller than those of fingers (4TDW 2.1, 5.7%SVL; 5TDW 2.1, 5.7%SVL); webs between toes moderately developed, formula I 2-2³/₄ II 1¹/₂-2³/₄ III 1¹/₂-3 IV 2¹/₂-1³/₄ V (Fig. 5B); two outer metatarsals separated with webbing; subarticular tubercles partially distinct, rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles absent; a small, oval inner metatarsal tubercle, length (IMTL 1.8, 5.0%SVL) about half length of first toe (1TOEL 3.7, 10.2%SVL), but no outer metatarsal tubercle.

Dorsum nearly smooth, with few sparsely distributed small tubercles; skin free of skull; a distinct, oblique supratympanic fold from eye above tympanum, ending at above arm inser-

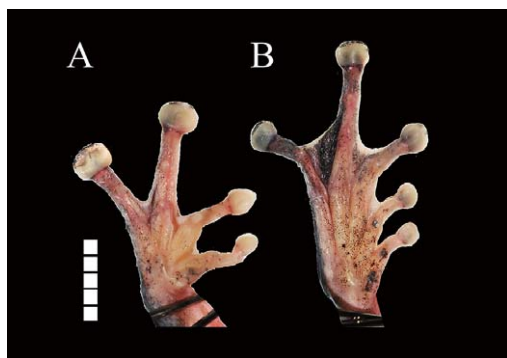


FIG. 5. Dorsal views of right hand (A) and foot (B) of female holotype (VNMN 03802) of *Kurixalus viridescens* sp. nov. after preservation. Scale bar = 5 mm.

tion; skin of sides and abdomen areolate; weak ridge of skin on outer edge of forearm forming weak serration; hindlimb smooth, except for coarsely granular ventral side of thigh, weak serration along tarsus, and asperities on base of tarsus; no dermal appendage at vent.

Color

In preservative, dorsal color of body slate blue, extending laterally on flanks and limbs, to lateral half of third and fourth fingers on forelimb, and to lateral half of fourth and fifth toes; serrations at lateral margins of forearm and tarsus light cream; groin to anterior surface of thigh cream; ventrally pinkish cream without marking; distal half of posterior thigh pinkish cream finely dotted with pale brown; ventral sides of hand and foot sparsely dotted with dark brown; webbing between third to fifth toes black, margined with cream, but cream between first to third toes. Color in life, based on a color transparency (Fig. 3), head and body dorsally light green without markings, fading to lemon yellow on sides to ventrum; limbs dorsally with very faint dark crossbars; iris gold with some black reticulations.

Variation

Morphometric data are summarized in Table 2. Because the holotype and paratypes

of the new species are all female, sexual dimorphism could not be determined. The paratypes are similar to the holotype in the body proportion except for the following: SVL varies from 28.7–36.6 mm, with the mean of 32.8 ± 2.8 mm. Relative sizes of characters on the head are larger in the paratypes. A few individuals have IOD smaller than UEW, and SL larger than EL. Some individuals have vaguely defined tympanum or indistinct supratympanic fold on its posterior half. The tibiotarsal articulation reaches the center of the eye, except for one specimen, in which the joint reaches the anterior end of the eye. Some individuals have conical dermal appendage at the heel (Fig. 3), which is lacking in the holotype. One specimen has trace of dark brown markings on interorbital, sacrum, and tibial regions. All the other specimens are nearly uniform in coloration and pattern.

Comparisons

By its unique green and immaculate, and nearly smooth dorsum, *K. viridescens* sp. nov. can be easily distinguished from all the other congeners that have dorsum basically brown in color with darker spots, and covered by tubercles. In addition, the new species is differentiated from the other congeners in the following way.

From *K. ananjevae* (Matsui and Orlov, 2004), the new species differs by the size and color of body and size of eggs (female SVL 28.7–36.6 mm and ova large and unpigmented vs. female 43 mm, eggs bicolored and small, 1.3–1.5 mm in diameter in *K. ananjevae*). *Kurixalus viridescens* sp. nov. differs from *K. appendiculatus* in smaller body size, skin structure, and vomerine teeth (female SVL 28.7–36.6 mm, dermal appendage at cloaca or vomerine teeth absent vs. females 42–50 mm, conspicuous transverse, infra-cloacal dermal flap and vomerine teeth present in *K. appendiculatus*). From *K. baliogaster*, the new species differs by the body size, ventral color, and shape of snout tip (female SVL 28.7–36.6 mm, ventral surface immaculate, and lacking a rostral cone vs. females 35–42 mm, ventral

TABLE 2. Measurements of female *Kurixalus viridescens* sp. nov. (in mm). *Holotype.

	VNMN 03804	VNMN 03803	VNMN 2013.25	VNMN 03815	VNMN 03814	VNMN 2013.72	VNMN 2013.74	VNMN 03816	VNMN 03802*	VNMN 03813
SVL	28.7	28.8	30.9	31.0	32.8	34.0	34.1	34.5	36.3	36.6
HL	11.7	11.8	12.6	12.7	12.9	14.9	14.3	13.2	13.4	14.6
HW	11.2	12.2	12.6	13.4	13.0	13.6	14.1	13.9	13.8	14.2
IND	3.1	3.1	2.8	3.2	3.3	3.2	3.5	3.5	3.3	3.2
IOD	3.3	3.6	3.4	3.8	3.7	4.0	3.8	4.2	4.3	3.8
UEW	3.2	3.3	3.5	4.0	3.6	3.7	3.3	3.6	3.3	3.6
N-EL	2.7	2.5	2.8	2.5	2.5	3.0	2.7	3.3	2.2	2.8
SL	4.7	5.0	5.0	4.6	4.9	5.0	5.3	5.1	5.2	5.6
EL	4.5	4.6	5.9	5.2	5.3	6.0	5.6	5.3	5.3	6.5
ED	4.0	3.9	4.8	4.5	4.6	4.9	4.2	4.4	4.2	5.0
TD	1.4	1.7	2.1	1.7	1.9	1.7	2.1	2.0	1.7	2.0
T-EL	0.5	0.5	0.6	0.6	0.5	0.5	1.0	0.6	0.7	0.4
FLL	17.9	18.7	20.5	20.6	22.5	23.4	22.8	22.4	24.1	23.5
LAL	14.8	15.5	16.6	16.4	17.8	18.6	18.0	17.8	18.9	18.4
1FL	3.0	3.0	3.5	3.1	3.0	3.7	3.5	3.1	4.3	3.3
IPTL	1.5	1.7	1.9	1.6	1.7	2.3	1.9	1.9	1.7	1.9
HLL	45.2	47.6	48.5	50.3	52.4	51.7	54.2	55.4	56.8	53.2
TL	15.5	15.7	15.1	16.3	17.0	16.6	16.5	17.2	18.1	17.0
FL	12.4	12.7	13.7	13.8	13.7	15.5	15.7	14.0	16.2	15.0
IMTL	1.5	1.2	1.7	1.4	1.1	1.8	1.8	1.5	1.8	1.9
1TOEL	3.1	3.0	3.2	3.7	3.3	3.8	3.5	3.7	3.7	3.0
1FDW	1.02	0.83	1.40	1.27	0.93	—	—	1.43	1.26	1.00
2FDW	1.02	0.92	1.96	1.53	1.24	—	1.70	1.76	1.87	1.91
3FDW	1.09	1.38	1.91	1.89	1.32	—	1.77	2.01	2.46	1.88
4FDW	0.97	1.06	1.89	1.95	1.43	—	1.86	1.82	2.34	2.01
1TDW	0.71	0.83	0.90	1.16	0.92	—	0.65	1.29	1.27	1.16
2TDW	0.77	.	1.12	1.25	1.06	—	1.20	1.41	1.56	1.44
3TDW	0.88	1.11	1.52	1.31	1.29	—	1.16	1.43	1.80	1.51
4TDW	1.03	1.28	1.58	1.41	1.44	—	1.37	1.60	2.08	1.52
5TDW	0.69	1.00	1.49	1.56	1.56	—	1.64	1.49	2.06	1.61

surfaces of head and trunk with black spots, females with a strong rostral cone in *K. balio-gaster*). The new species is similar to *K. banaensis* in body size (female SVL 28.7–36.6 mm vs. 34.2 mm in *K. banaensis*) and having large, unpigmented ova, but differs from it by the shape of loreal region and snout tip, and the dermal appendage at cloaca (loreal region normal, snout tip moderately pointed, and dermal appendage absent at cloaca vs. loreal region sloping and deeply concave, snout tip markedly pointed, and fringes present below cloaca in *K. banaensis*). *Kurixalus viridescens* sp. nov. overlaps in

body size with *K. bisacculus* with the female SVL of 29 mm, but differs from it by the ventral color and vomerine teeth (ventrum immaculate and lacking vomerine teeth vs. ventral surface with black spots and vomerine teeth present in *K. bisacculus*). The new species also overlaps in body size with *K. eiffingeri* with female SVL of 32–44 mm, but differs from it in skin structure and color of eggs (a skin flap above cloaca present in females and eggs bicolored, 1.6–1.8 mm in diameter in *K. eiffingeri*). With *K. idiootocus* the new species again overlaps in body size but differs from it in skin structure (females

33–39 mm, granules around anus and on limbs, and dark markings on sides of body present in *K. idiootocus*). *Kurixalus viridescens* sp. nov. differs from *K. naso* (Annandale, 1912) by smaller body size, skin structure, poorly developed toe webbing, and ventral color (SVL 43 mm, snout with a cone, upper arm and tarsus fringed, toe webbing well-developed, and chin and breast marked with dark reticulation in *K. naso*). From *K. odontotarsus* (Ye and Fei, 1993), the new species differs by smaller body size and different skin structure and ventral color (female SVL 43 mm, snout pointed and ventrum with dark markings in *K. odontotarsus*). The new species is similar to *K. verrucosus* (Boulenger, 1893) in body size and absence of vomerine teeth, but differs from it in skin structure and ventral color (females 30–36 mm, dark ventral spots, especially on throat, and several whitish tubercles below cloaca present, and toe webbing much better developed in *K. verrucosus*).

Range

Known only from the type locality, Hon Ba Nature Reserve, Khanh Hoa Province, and Bidoup Nui Ba National Park, Lam Dong Province, southern Vietnam. At present, the species is known only from near the peak (>1500 m) of mountains and is likely to be restricted to high-elevation forests.

Natural history

The type locality in the Hon Ba Nature Reserve is in the montane evergreen forest dominated by species of the Fagaceae, Theaceae, and Lauraceae. The holotype was collected on leaf of a shrub approximately 1 m above ground, away from ponds or streams. No tadpoles or eggs were found in ponds and calling males were absent from November to late December, but a paratype had large ovarian eggs about 3.8 mm in diameter and cream in color. Otherwise, no information for breeding is available. Associated species observed near the type locality (altitudes 1494–1557 m asl) were *Ingerophrynus galeatus* (Günther, 1864), *Theloderma truongsongense* (Orlov

and Ho, 2005), *Rhacophorus calcaneus* Smith, 1924, *Rh. vampyrus* Rowley, Le, Thi, Stuart, and Hoang, 2010, *Polypedates megacephalus* Hallowell, 1861, and *Hylarana nigrovittata* (Blyth, 1856). Besides, *Raorchestes gryllus* (Smith, 1924) and *Microhyla butleri* Boulenger, 1900 were found at lower elevations (1350–1363 m asl).

DISCUSSION

Unfortunately, no reliable morphological synapomorphies have been established to define the genus *Kurixalus* until now and taxonomic assignment is made solely on molecular bases (Yu et al., 2013). The new species, with monotonous green dorsum and without distinct dark patterns, is unusual in this genus and can be misidentified at a glance as some species of *Rhacophorus*. Because presence of many frogs, including rhacophorids, was noticed by the calls of males, it is strange that all specimens of the new species are females and no male specimens have so far been collected. However, since no remarkable sexual dimorphism has been reported in species of *Kurixalus*, the characteristics described on the basis of females would hold for males.

Due to limited data available at present, phylogenetic relationships with the genus *Kurixalus* and the other rhacophorid genera were poorly resolved in the tree constructed. However, this preliminary result suggested some interesting phylogenetic problems within the genus *Kurixalus*. Remote relationships of *K. appendiculatus*, *K. eiffingeri*, and *K. idiootocus* from the other species, suggested by Yu et al. (2013), were supported in our tree, but at the same time we confirmed a clade of the remaining species, which Yu et al. (2013) could not resolve. Two large clades were recognized in this major clade, and one of these is composed of *K. banaensis* and the new species. In this clade, the sequence of *K. banaensis* from Bi Doup (Bidoup Nui Ba) National Park (KC465795; Li et al. [2013]) kept in the Kunming Institute of Zoology

(KIZ 359) was embedded in a clade with the new species. The genetic distances between this sequence and those of *K. viridescens* (1.8–5.2%) were within the range of *K. viridescens* (0.4–5.4%), and the fact that two specimens of *K. viridescens* were collected in Bidoup, one of two known localities of the new species, strongly indicates their conspecific status. Future analyses including enigmatic *K. ananjevae* and *K. naso* are badly needed to understand more concrete relationships with the genus.

Vietnam encompasses the largest number of *Kurixalus* species, and six species (*K. ananjevae*, *K. appendiculatus*, *K. baliogaster*, *K. banaensis*, *K. odontotarsus*, and *K. verrucosus*) have been recorded from the country (Orlov et al., 2008: under various generic names). Of these, *K. appendiculatus* has been recorded outside Vietnam from a very wide range including the Philippines, Borneo, Sumatra, the Malay, Thailand, Myanmar, northern India, and Cambodia. From the pattern of its distribution, it is probable that the records from Vietnam, as well as Cambodia, are based on misidentification. In this study we confirmed the presence in this country of *K. bisacculus*, sequence of which is very similar to Thai samples of the species (p-distances of 2.1–2.8%). We suspect the previous record of *K. appendiculatus* in Vietnam may be based on misidentification of *K. bisacculus*.

Some of other Vietnamese species also occur in neighboring Laos (*K. baliogaster*, *K. bisacculus*, and *K. odontotarsus*), and China (*K. bisacculus*, *K. odontotarsus*, and *K. verrucosus*), as well as in Thailand (*K. bisacculus* and *K. verrucosus*) and Myanmar (*K. verrucosus*), and only three species (*K. naso* from northern India, Myanmar, and southern China; *K. eiffingeri* from Ryukyu Is. of Japan and Taiwan; and *K. idiootocus* from Taiwan) are absent from the country. Thus, Vietnam can be regarded as the center of speciation of the genus *Kurixalus*, and the present addition of a new species, *K. viridescens*, further strengthens such an idea.

ACKNOWLEDGMENTS

We thank the directorates of the Hon Ba Nature Reserve and Bidoup Nui Ba National Park for issuing relevant permits and support of our fieldwork, and D. M. Hoang and C. T. Pham for providing valuable specimens. We also thank K. Eto, Y. Kawahara, and N. Kuraishi for laboratory assistance. J.-P. Jiang kindly allowed us to use Chinese materials. M. Matsui thanks C. McCarthy and B. T. Clarke (BM), and A. Dubois and A. Ohler (MNHNP) for allowing him to examine specimens under their care. Duc M. Hoang thanks M.V. Thang, T.T. Gai and N.T. Hong for their kind support. Fieldwork by T.T. Nguyen was partly supported by a grant from the Japan Society for the Promotion of Science (JSPS AA Science platform Program, FY2011–2013) to M. Motokawa, and his research in Japan is funded by the JSPS RONPAKU Program (VAST-11224). Fieldwork by D.M. Hoang was conducted within the project “Building a database of forest biodiversity in Khanh Hoa Province” funded by Khanh Hoa Provincial Department of Natural Resource and Environment to SIE and managed by Khanh Hoa Environmental Protection Agency. This research was partly supported by Grants-in Aid from the Monbusho through the Japan Society for the Promotion of Science (Field Research, Nos. 15370038, 20405013 and 23405014) to M. Matsui and the Project TN3/T13 of the National Program Tay Nguyen III to T.T. Nguyen.

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Accepted: 7 May 2014