Zootaxa 3785 (1): 025–037
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3785.1.2
http://zoobank.org/urn:lsid:zoobank.org:pub:E515AAC3-811B-48BA-A680-E4851887633F

A striking new species of phytotelm-breeding tree frog (Anura: Rhacophoridae) from central Vietnam

JODI J. L. ROWLEY[1,5], DUONG THI THUY LE[2], VINH QUANG DAU[3], HUY DUC HOANG[2] & TRUNG TIEN CAO[4]

[1]*Australian Museum Research Institute, Australian Museum, 6 College St, Sydney, NSW, 2010, Australia*
[2]*Faculty of Biology, University of Science- Ho Chi Minh City, 227 Nguyen Van Cu, District 5, Ho Chi Minh City, Vietnam*
[3]*Institute of Ecology and Biological Resources, 18 Hoang Quoc Viet, Nghia Do, Tu Liem, Hanoi, Vietnam*
[4]*Biology Faculty, Vinh University, 182 Le Duan St, Vinh City, Vietnam*
[5]*Corresponding author. E-mail: Jodi.Rowley@austmus.gov.au*

Abstract

We describe a distinctive new species of phytotelm-breeding rhacophorid frog from central Vietnam. *Gracixalus lumarius* **sp. nov.** is distinguished from all other rhacophorids in Indochina by a combination of (1) medium body size (adult males 38.9–41.6 mm; adult female 36.3 mm), (2) dorsum brown diurnally and yellow nocturnally, (3) venter pink, (4) tympanum and supratympanic fold indistinct (5) iris dark gold with a dense, relatively uniformly distributed network of black reticulations, (6) dorsum with distinctive white conical tubercles in males, and (7) eggs deposited on wall of a phytotelm. The new species is known from montane bamboo and montane evergreen forest in Ngoc Linh Nature Reserve in Kon Tum Province, between ~1845–2160 m elevation.

Key words: Amphibian, montane, Southeast Asia

Introduction

The family Rhacophoridae contains over 300 species of mostly arboreal frogs distributed throughout Sub-Saharan Africa, China, Southeast Asia, Japan, Taiwan, the Philippines, and the Greater Sunda Islands (Frost 2014). At present, ten genera of rhacophorids occur in Indochina; *Chiromantis* Peters, *Feihyla* Frost, *Gracixalus* Delorme, *Kurixalus* Yet *et al*. *Nytixalus* Boulenger, *Philautus* Gistel, *Polypedates* Tschudi, *Pseudophilautus* Laurent, *Rhacophorus* Kuhl & van Hasselt, and *Theloderma* Tschudi. Due to the high species diversity and morphological conservatism, infrageneric classification has been controversial. The validity, species composition, and evolutionary relationships within and among genera remain unclear.

During field work at high-elevations in central Vietnam, we discovered a medium-sized phytotelm-breeding rhacophorid superficially resembling *Gracixalus* 'Clade II' of Rowey *et al*. (2011), but with a distinctive tuberculate dorsal texture and striking yellow dorsal and pink ventral nocturnal colouration. Here we describe this charismatic species as new.

Materials and methods

Specimens were deposited at the Australian Museum (AMS). Some specimens currently at the AMS will be deposited at the University of Science, Ho Chi Minh City (UNS) and have been cross-catalogued at both institutions. In these instances, voucher numbers are reported as UNS/AMS. We recorded morphological data from adult specimens fixed in 10% formalin and then stored in 70% ethanol and eggs fixed in 10% formalin and stored in 5% formalin. Morphometric data were taken (to the nearest 0.1 mm) with digital callipers. Measurements include snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); head width at commissure of

Accepted by M. Vences: 25 Feb. 2014; published: 2 Apr. 2014

jaws (HDW); snout length from tip of snout to anterior corner of eye (SNT); diameter of exposed portion of eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of eye (TEY); internarial space (IN); distance from front of eye to nostril (EN); tibia length with hindlimb flexed (TIB); manus length from tip of third digit to base of tubercle on prepollex (ML); and pes length from tip of fourth toe to base of inner metatarsal tubercle (PL). We use a traditional formula for finger numbering rather than one based on homology (eg. Alberch & Gale 1985). Sex was determined by the presence of nuptial pads, vocal sacs and/or gonadal inspection. Mass was recorded in life (to the nearest 0.1 g), using Pesola scales. Radiographs of the holotype were prepared to examine osteological features including the presence of intercalary discs and the shape of the distal end of the terminal phalanges. Vocal sac terminology follows Liu (1935). Terminology for describing eye colouration in life follows Glaw and Vences (1997). Webbing formula follows that of Savage and Heyer (1997). We compared the new species to species in the genus *Gracixalus*, and all other medium-sized (35–45 mm adult SVL) rhacophorids in Indochina. We obtained comparative morphological data from specimens of *Gracixalus, Philautus* and *Kurixalus* from AMS, Vietnam National Museum of Nature (VNMN), North Carolina Museum of Natural Sciences (NCSM) and the American Museum of Natural History (AMNH), and photographs of these specimens in life (Appendix 1), and from the literature (original descriptions supplemented by information from Bossuyt & Dubois 2001; Rowley *et al*. 2011; Fei *et al*. 2009, 2010). Photographs of *G. carinensis* type material were also examined (lectotype BMNH 1947.2.6.24 and 1947.2.6.27).

In order to support the generic placement of the new species, we obtained tissue samples from the new species, and compared them to species currently assigned to the genus *Gracixalus* (Frost 2014) and for which GenBank data (trimmed to match the length of the fragment obtained here) were available (Table 1). Specimens of *Kurixalus effingeri* (type species of the genus *Kurixalus*), *Kurixalus odontarsus*, and *Philautus aurifasciatus* (type species of the genus *Philautus*) were used as outgroups (sensu Rowley *et al*. 2011). We also included type species of other rhacophorid genera in the region containing medium-sized (35–45 mm adult SVL) species; *Polypedates leucomystax*, and *Rhacophorus reinwardtii.*

TABLE 1. Sequences used in this study. *generated as part of this study.

Species	Locality	Voucher no.	GenBank no.
Gracixalus cf. *ananjevae*	Vietnam, Nghe An Province	VNMN 03012	JN862546
Gracixalus ("Kurixalus") carinensis	Vietnam, Lai Chau Province	MNHN 1999.5961	AY880503
Gracixalus gracilipes	China, Yunnan Province	KIZ060821196	EF564523
Gracixalus cf. *jinxiuensis*	Vietnam, Nghe An Province	AMS R 173454	JN862547
Gracixalus jinxiuensis	China, Guangxi Province	KIZ060821013	EF564524
Gracixalus lumarius **sp. nov.**	Vietnam, Kon Tum Province	AMS R 176202	KF918412*
Gracixalus nongangensis	China , Guangxi Province	NHMG20091009	JX841319
Gracixalus quangi	Vietnam, Thanh Hoa Province	IEBR A.2012.5	JX896683
Gracixalus quyeti	Vietnam, Quang Binh Province	ZFMK 82999	EU871429
Gracixalus supercornutus	Vietnam, Kon Tum Province	AMS R 173428	JN862544
Gracixalus waza	Vietnam, Cao Bang Province	IEBR A.2012.2	JX896681
Kurixalus odontotarsus	China, Yunnan Province	KIZ060821122	GU227288
Kurixalus eiffingeri	Japan, Okinawa Islands	A120	DQ468673
Philautus aurifasciatus	Indonesia, Java	ZRC.1.5266	AY141850
Polypedates leucomystax	Philippines, Quezon Province	CAS 219931	AF458140
Rhacophorus reinwardtii	Malaysia, Sarawak	NMBE 1056518	JN377365

Total genomic DNA was extracted from tissues using DNeasy tissue extraction kits (Qiagen). We used the primers 16SAR and 16SBR of Palumbi *et al.* (1991) to amplify ~550 base pairs of the 16S rRNA gene for the new species. Standard PCR protocols were used and PCR products were purified using ExoSap-IT (USB Corporation, OH, USA). Purified templates were sequenced directly by Macrogen (Seoul, Korea). Sequences were validated using Sequencher 4.10 (Gene Codes, Ann Arbor, MI), aligned using the Clustal option in MEGA 5. Uncorrected

pairwise sequence divergence was calculated using MEGA 5. The three sequences obtained for the new species, including the holotype, generated a single haplotype of 551 bp in length, submitted to GenBank with the accession number KF918412.We used Akaike Information Criterion as implemented in jModelTest 2.1.4 (Darriba *et al*. 2012) to select the best-fit model of nucleotide substitution, which was then used in model-based phylogenetic inference.

FIGURE 1. Collection sites of *Gracixalus lumarius* **sp. nov.** (star = type locality).

Bayesian phylogenetic analyses were performed in MrBayes 3.2 (Ronquist *et al*. 2012) under a GTR+I +Γ (general time reversible model with Gamma distributed substitution rates and a proportion of the sites invariable; model parameters estimated during the search). Four independent Markov Chain Monte Carlo searches were run for 2 million generations, sampled every 2000 generations, each with four chains, and default priors. Output files were examined visually in Tracer v1.5 (Rambaut & Drummond 2007), to assess stationarity and determine the number of generations to remove as burn-in. To be conservative, we considered 200,000 generations from each run as burn-in, and removed 100 trees before summarizing topology and posterior probabilities. Trees were visualized

using the FigTree v1.3.1 program, available at http://tree.bio.ed.ac.uk/software/figtree/. We consider branches receiving ≥0.95 posterior probabilities to be well-supported (Wilcox *et al.* 2002). We carried out this analysis both including and excluding pairwise comparisons for gapped sites.

***Gracixalus lumarius* sp. nov.**

Holotype: AMS R 176202, adult male, in tree hole (approx. 6 cm diameter entrance, 20 cm deep) with eggs, in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.063º N, 107.786º E, 1845 m; Fig. 1). Collected at 20:55 h on 3 April 2010 by J.J.L. Rowley, D.T.T. Le, V.Q. Dau and H.D. Hoang.

Paratypes: UNS 00341/AMS R 173838, female, collected on a tree branch approximately 2 m above ground in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.0785º N, 107.971º E, 2160 m; Fig. 1), collected at 21:30 h on 16 July 2009 by D.T.T. Le and V.Q. Dau. AMS R 173889 and AMS R 173890, adult males collected on tree branch in close proximity, in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.077958º N, 107.960757º E, 1990 m; Fig. 1), collected at night on 17 July 2009 by D.T.T. Le and V.Q. Dau. UNS 00340/AMS R 176203, juvenile, on plant leaf 1 m above ground in montane evergreen/bamboo forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.063º N, 107.862º E, 2035 m; Fig. 1), collected at 20:35 h on 3 April 2010 by J.J.L. Rowley, D.T.T. Le, V.Q. Dau and H.D. Hoang.

Other material. Eggs collected from same tree hole and at the same time and date as the holotype, AMS R 176213 (Fig. 7B).

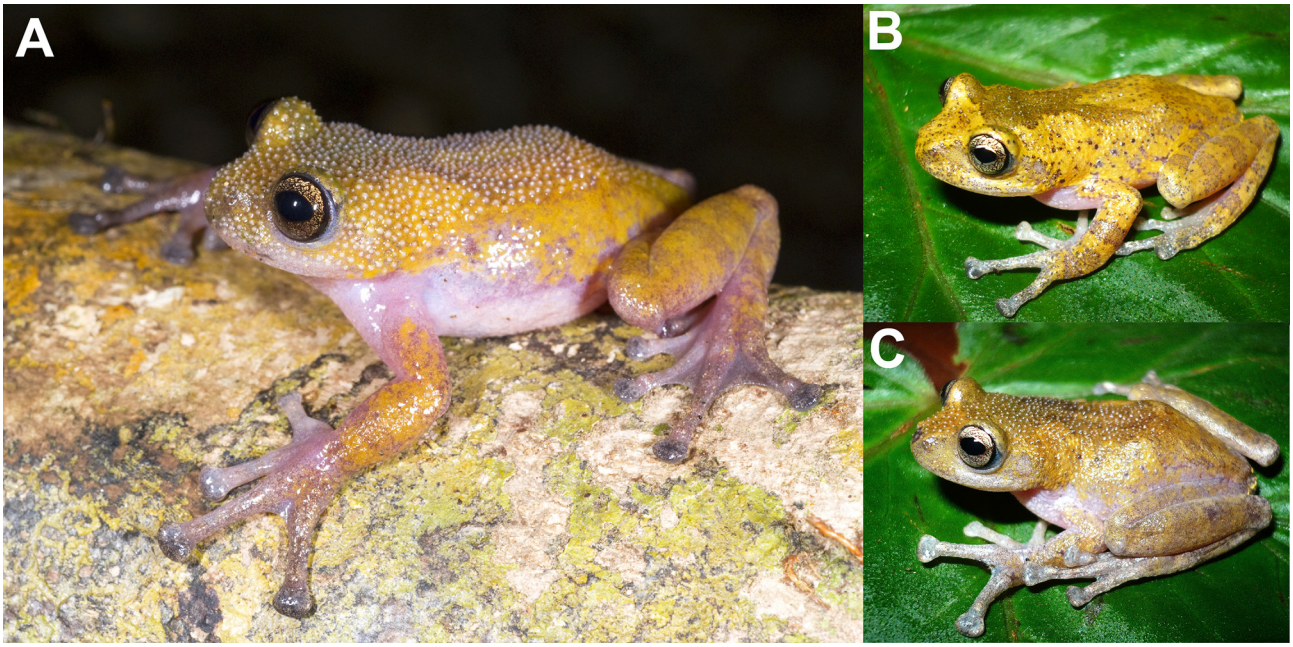

FIGURE 2. Dorsolateral view of *Gracixalus lumarius* **sp. nov.** in life showing variation in skin texture (A) male holotype AMS R 176202, (B) male paratype AMS R 173890, (C) male paratype AMS R 173889.

Etymology. Specific name from *lumarius* L., meaning of thorns, in reference to the extensive conical tubercles over the dorsal surface of the new species.

Suggested common name. Thorny tree frog (English), Ếch cây gai (Vietnamese).

Diagnosis. The new species is assigned to the family Rhacophoridae by the presence of intercalary cartilage between the terminal and penultimate phalanges of digits, tips of digits expanded into large disks bearing circummarginal grooves, vomerine teeth absent and horizontal pupil (Liem 1970; Duellman & Trueb 1986; Brown & Alcala 1994). Although morphological characters for the genus require revision, the species is allocated to the genus *Gracixalus* by the presence of spines on the upper eyelid, distance between nostrils less than between eyes,

rictal gland connected to the mouth, tibia length greater than four times width (Delorme *et al.* 2005), back bearing dark X-or inverted V-shape (Fei *et al*. 2009) and by molecular data (see below). *Gracixalus lumarius* **sp. nov.** is distinguished from other rhacophorids in Indochina by a combination of (1) medium body size (adult males 38.9–41.6 mm; adult female 36.3 mm), (2) dorsum brown diurnally and yellow nocturnally, (3) venter pink, (4) tympanum and supratympanic fold indistinct (5) iris dark gold with a dense, relatively uniformly distributed network of black reticulations, (6) dorsum with distinctive white conical tubercles in adult males, and (7) eggs deposited on wall of a phyptotelm.

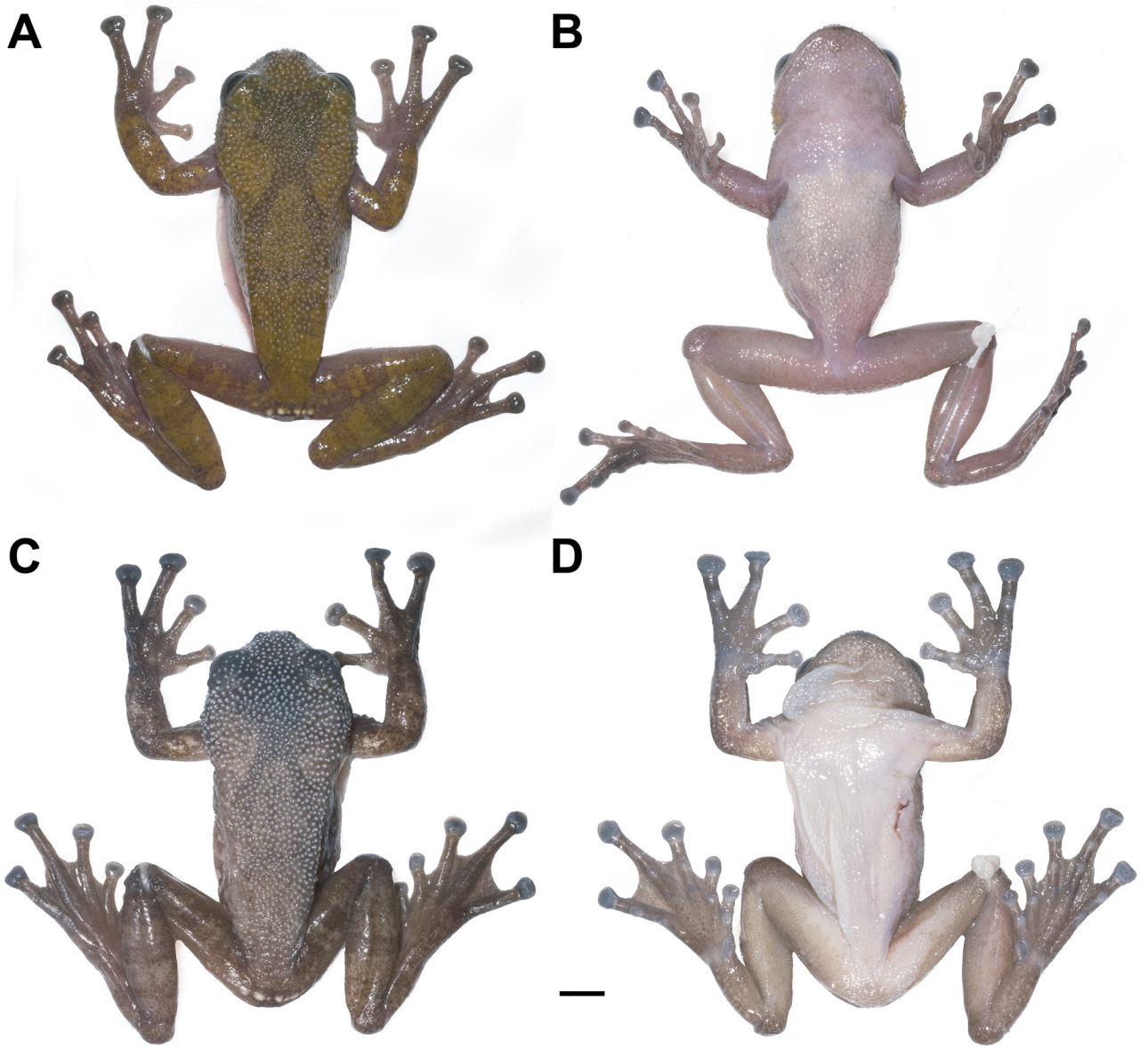

FIGURE 3. Male holotype of *Gracixalus lumarius* **sp. nov.** (AMS R 176202) in life in dorsal (A) and ventral (B) view, and same individual preserved in dorsal (C) and ventral (D) view. Scale bar = 5 mm.

Description of holotype. Medium-sized rhacophorid (41.6 mm), body robust, head length 89% of head width, snout rounded in dorsal view, gently truncate in profile, projecting slightly beyond margin of lower jaw, canthus rostralis indistinct, rounded, loreal region straight, interorbital region convex, nostrils oval, slightly protuberant, without flap of skin laterally, slightly closer to tip of snout than eye, pupil oval, horizontal, tympanum indistinct, approximately one third of eye diameter, pineal ocellus absent, skin not co-ossified to forehead, vomerine teeth absent, choanae oval, at margins of roof of mouth, tongue attached anteriorly, deeply notched posteriorly, tooth-like projections on lower jaw absent, supratympanic fold indistinct. Weakly visible rictal gland connected to the mouth. Vocal sac present, pair of distinct, oval openings at base of jaw, baggy gular region. Forelimbs relatively robust,

relative length of fingers I < II < IV < III. Tips of all fingers with well-developed disks with circummarginal grooves; disks relatively wide compared to finger width (third finger disk 204% third finger width), third finger disk width greater (144%) than tympanum diameter; slight dermal fringes on fingers, basal webbing at base of fingers II–IV. Subarticular tubercles prominent, rounded, formula 1, 1, 2, 2. Accessory palmar tubercles distinct; palmar tubercle irregular, flat; thenar tubercle indistinct; prepollex elongate, with low, oval tubercle; distinct, nuptial pads present on inner surface of the prepollex (Fig. 4A). Relative length of toes I < II < V < III < IV, tips of toes with well-developed disks with distinct circummarginal grooves, wide when compared to toe width. Webbing present, formula I 1 ½ – 2 II 1 ⅓ – 2^{+} III 1 ½ – 2^{+} IV 2^{+}– 1^{+} V, with dermal fringes, subarticular tubercles prominent, rounded, formula 1, 1, 2, 3, 2, inner metatarsal tubercle distinct, outer metatarsal tubercle and supernumary tubercles absent (Fig. 4B). Dorsal surface of head and back, including snout, eyelids and tympanum covered in dense network of conical tubercles; except for several tubercles scattered on the dorsal surface of arms, the dorsal surface of forelimbs and hindlimbs are smooth. Ventral surface of thighs and abdomen coarsely granular, throat finely granular. No dermal fringes on limbs, no tibiotarsal projection, supracloacal glands.

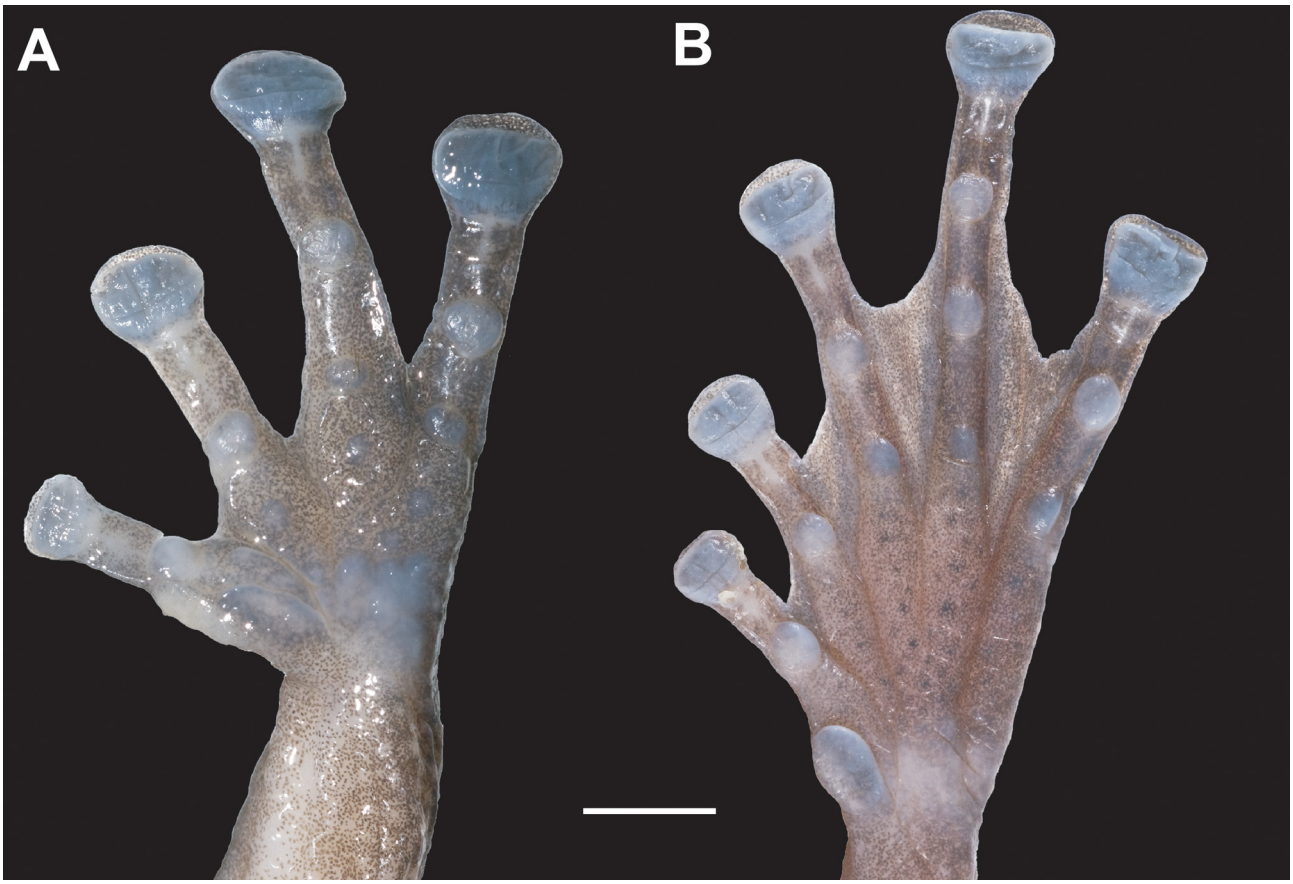

FIGURE 4. Palmar surfaces of left hand (A) and plantar surface of left foot (B) of the male holotype of *Gracixalus lumarius* **sp. nov.** (AMS R 176202). Scale bar = 2 mm.

Colour of holotype in life. Dorsal surface yellowish brown with a darker brown interorbital crossbar and cross-mark on the back starting between the eye; limbs with faint darker barring. Tips of toes and webbing pinkish grey. Loreal and tympanic markings absent. Flanks bright yellow with pink mottling dorsally, changing to pink with yellow mottling ventrally. Throat, chest, belly and ventral surfaces of limbs pink, slightly paler pink on chest. Small glands around vent creamy whitish. Iris dark gold with a dense, relatively uniformly distributed network of fine black reticulations; iris periphery black; eye periphery grey posteriorly. Dorsal colouration varied in brightness over time.

Colour of holotype in preservative. Dorsal surface as in life, but conical tubercles paler, white, and more distinct. Bright yellow and pink colours faded. Chest and belly pinkish white, margins of throat, ventral surfaces of limbs, hands, feet, including webbing, pale brown.

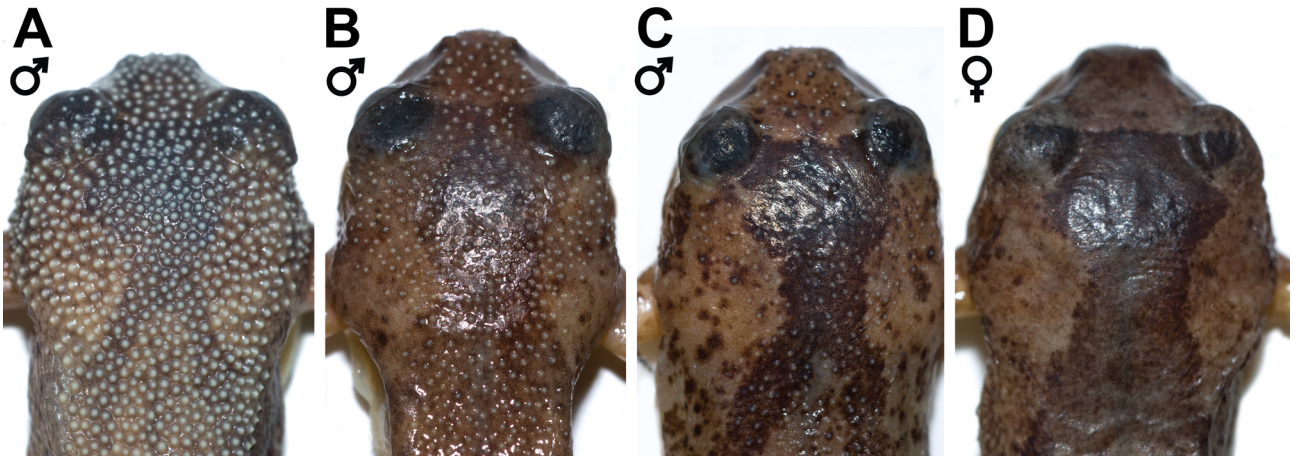

FIGURE 5. Dorsal surfaces of preserved *Gracixalus lumarius* **sp. nov.** specimens (A) male holotype AMS R 176202, (B) male paratype AMS R 173889, (C) male paratype AMS R 173890, (D) female paratype UNS 00341/AMS R 173838.

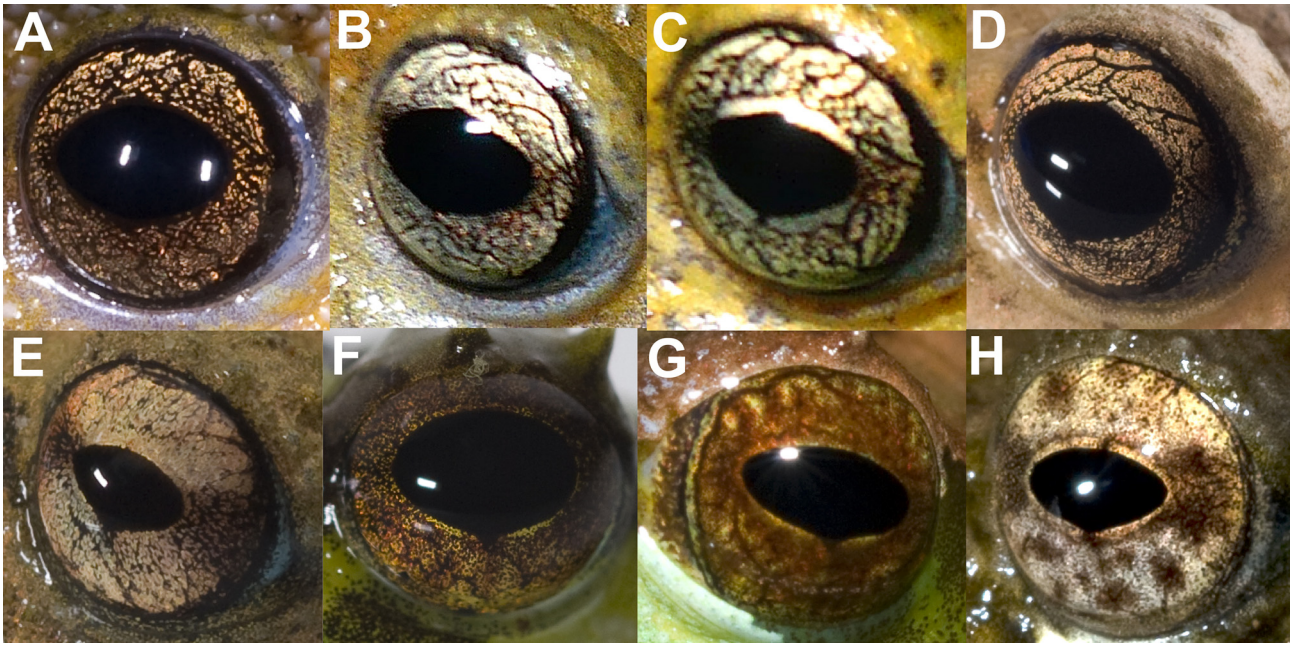

FIGURE 6. Iris colouration in life of (top row) *Gracixalus lumarius* **sp. nov.** (A) AMS R 176202, (B) AMS R 173889, (C) AMS R 173890, (D) UNS 00340/AMS R 176203, and (bottom row) representative iris coloration in (E) Clade II *Gracixalus* of Rowley *et al*. 2011 (*G.* cf. *ananjevae,* Nghe An Province, Vietnam), (F–G) Clade I of Rowley *et al*. 2011 (*G. supercornutus* and *G. quangi*), and (H) *Kurixalus baliogaster* (Lam Dong Province, Vietnam).

Measurements. Holotype: SVL 41.6, HDL 14.3, HDW 16.1, SNT 5.4, EYE 5.8, IOD 4.3, TMP 2.0, TEY 0.8, IN 3.9, NS 2.5, EN 2.6, TIB 19.6. ML 14.8, PL 18.8, IMT 2.1.

Variation. Paratypes do not vary considerably in their colouration. In preservative, AMS R 176202 is slightly darker than other specimens. AMS R 173890 has higher contrast patterning. All adult males have a dorsum with distinctive, white conical asperities, but these vary in extent among individuals. Compared to the holotype, AMS R 173889–1738890 have smaller, less raised tubercles covering anterior two-thirds of dorsum, and not extending on to dorsal surface of arms. The single female (UNS 00341/AMS R 173838) and single juvenile (UNS 00340/AMS R 176203) specimens have less obvious raised tubercles in place of asperites (Fig. 5). The single juvenile (UNS 00340/AMS R 176203) is uniformly pale tan without dorsal markings or barring on limbs, but with a slightly darker canthal stripe. Male paratypes lack nuptial pads. Measurements of the type series are shown in Table 2.

TABLE 2. Measurements (mm) of *Gracixalus lumarius*, **sp. nov**. Abbreviations defined in text.

	AMS R 176202*	AMS R 173889	AMS R 173890	UNS 00341/AMS R 173838	UNS 00340/AMS R 176203
Sex	male	male	male	female	juvenile
SVL	41.6	39.5	38.9	36.3	25.5
HDL	14.3	14.3	13.4	12.7	8.9
HDW	16.1	14.8	15.0	14.1	10.0
SNT	5.4	5.8	6.6	5.6	3.8
EYE	5.8	4.7	4.4	3.9	3.5
IOD	4.3	4.6	5.1	4.5	3.7
TMP	2.0	1.7	1.7	1.7	1.5
TEY	0.8	1.1	1.3	1.2	0.7
IN	3.9	4.1	4.0	3.6	2.8
NS	2.5	2.4	1.9	2.3	1.3
EN	2.6	2.9	3.2	3.0	1.9
TIB	19.6	20.2	19.7	18.4	13.0
ML	14.8	14.5	13.2	12.5	8.7
PL	18.8	19.5	18.6	17.0	11.5
IMT	2.1	2.1	1.9	2.2	1.2
HW:HL	1.13	1.03	1.12	1.11	1.12
HL:HW	0.89	0.97	0.89	0.90	0.89
TIB:SVL	0.47	0.51	0.51	0.51	0.51
HDL:SVL	0.34	0.36	0.34	0.35	0.35
TMP:EYE	0.34	0.36	0.39	0.44	0.43

*holotype

Eggs. Approximately 130 eggs were deposited as a tightly spaced array of nonpendent eggs on the wall of a phytotelm above the water (AMS R 176213; Fig. 7B). The eggs are approximately Gosner (1960) stage 12, contain two jelly layers and measure an average of 2.75 mm (n=3) in diameter in preservative. Ova, which measure ~1.3 mm diameter, were coloured bluish grey in life and creamy grey in preservative. The eggs were identical in the mtDNA fragment of sequenced adults.

Ecology. The holotype was found underwater in a tree hole with conspecific eggs on the wall of the phytotelm (Fig 7B). All other specimens were found on arboreal vegetation, away from streams or ponds in montane evergreen or bamboo forest (eg. Fig 7A). The advertisement call and larvae of the new species are unknown. At present, the species is only known from montane bamboo and evergreen forest (>1800 m) on Mount Ngoc Linh and adjacent peaks, with less than 20 km between the farthest localities. The distribution of the species is unknown, but is likely to be restricted to high-elevation forest on Mount Ngoc Linh and adjacent peaks.

Comparisons. *Gracixalus lumarius* **sp. nov.** is distinguished from all other species of *Gracixalus*, and all other medium-sized (35–45 mm adult SVL) rhacophorids with brownish dorsum in Indochina by a combination of the following characters: dorsum brown diurnally and yellowish brown nocturnally; venter pink; tympanum and supratympanic fold indistinct; vomerine teeth absent; iris dark gold with a dense, relatively uniformly distributed network of black reticulations; dorsum with distinctive, white conical asperities in males; and eggs deposited as a tightly spaced array of nonpendent eggs on the wall of a phytotelm.

Within the genus *Gracixalus*, *Gracixalus lumarius* **sp. nov.** is distinguished from Clade I of Rowley *et al*. (2011), *G. gracilipes* (Bourret), *G. quangi* Rowley, Dau, Nguyen, Cao & Nguyen, *G. quyeti* (Nguyen, Hendrix, Böhme, Vu & Ziegler), and *G. supercornutus* (Orlov, Ho, & Nguyen) by having a dorsum brown diurnally and yellow nocturnally and a pink venter (versus greenish dorsum and white or yellow venter), an indistinct (versus distinct) tympanum and supratympanic fold, a dark gold iris with a dense, relatively uniformly distributed network

of black reticulations (versus bright copper or olive-gold iris with limited, non-uniform black reticulations), distinctive white, conical asperities on the dorsum (versus dorsum mostly smooth or slightly tuberculate) in males, and eggs deposited on wall of a phytotelm (versus on vegetation above pools for *G. gracilipes, G. quangi,* and *G. supercornutus*, Rowley pers. obs.; unknown in *G. quyeti*). *G. lumarius* **sp. nov.** also has a larger body size (38.9–41.6 mm in three adult males) compared to *G. gracilipes, G. quangi,* and *G. supercornutus* (all with male SVL <25 mm).

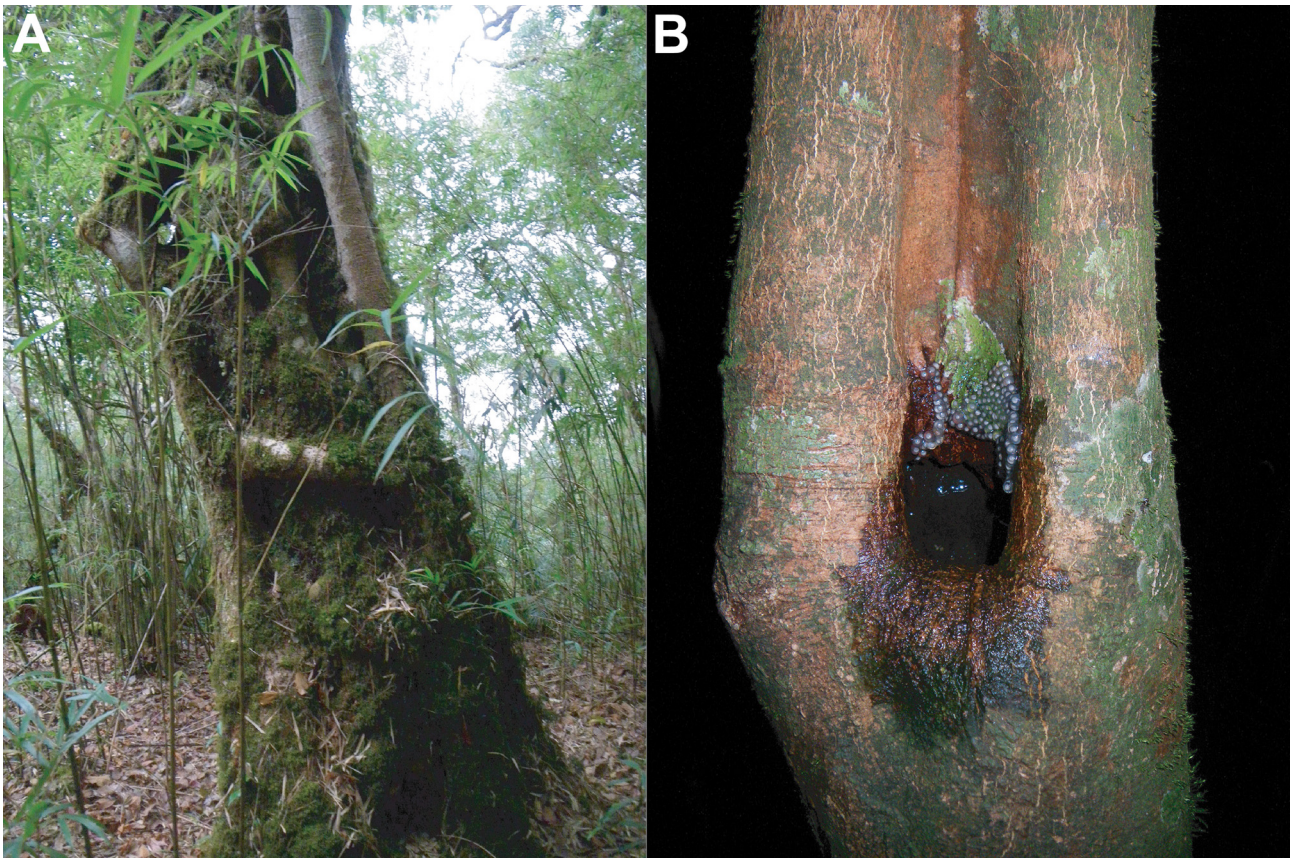

FIGURE 7. (A) High elevation bamboo forest at type locality of *Gracixalus lumarius* **sp. nov**. and (B) phytotelm in which holotype of *G. lumarius* **sp. nov.** (AMS R 176202) and eggs (AMS R 176213) were collected.

Gracixalus lumarius **sp. nov.** is distinguished from *G. carinensis* (Boulenger) by having a pink (versus white) venter, an indistinct (versus distinct) tympanum and supratympanic fold, dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold iris with limited, non-uniform black reticulations), dorsum with distinctive, white conical asperities (versus dorsum smooth or granulated with small warts on head and anterior part of back only) in males. From *G. jinxiuensis* (Hu), the new species is distinguished by having a larger body size (38.9–41.6 mm in three adult males versus 23.5 mm in adult male holotype), indistinct (versus distinct) tympanum and supratympanic fold, dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold iris with limited, non-uniform black reticulations), and distinctive, white conical asperities (versus low, scattered tubercles on dorsum) in males. From *G. medogensis* (Ye & Hu), the new species is distinguished by having a larger body size (38.9–41.6 mm in three adult males compared to 26.5 mm in one adult male), pink (versus light grey or whitish) venter, indistinct (versus distinct) tympanum and supratympanic fold, and distinctive, white conical asperities (versus smooth) in males. From the recently described *G. nonggangensis* Mo, Zhang, Luo & Chen and *G. waza* Nguyen, Le, Pham, Nguyen, Bonkowski & Ziegler, the new species differs by having a larger body size (38.9–41.6 mm in three adult males versus <36 mm in adult males), a pink venter (versus white venter with brown spots), indistinct (versus distinct) tympanum and supratympanic fold, dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus olive iris with limited, non-uniform brown reticulations), and dorsum with distinctive, white conical asperities (versus dorsum smooth or with some small tubercles) in males.

The new species differs from *Kurixalus ananjevae* (Matsui & Orlov), which is morphologically and likely molecularly similar to *Gracixalus* Clade II (Rowley *et al*. 2011), by having a larger body size (38.9–41.6 mm in three adult males compared to 32.4 mm in one adult male), pink (versus greyish white) venter, indistinct (versus distinct) tympanum and supratympanic fold, and dorsum with distinctive, white conical asperities (versus dorsum with irregularly distributed, small tubercles) in males.

Gracixalus lumarius **sp. nov.** also differs from all other medium-sized (35–45 mm adult SVL) rhacophorids from Indochina. From *Kurixalus banaensis* (Bourret), the new species is distinguished by having a pink (versus white) venter, indistinct (versus distinct) tympanum and supratympanic fold, dorsum with distinctive, white conical asperities (versus dorsum granular) in males, no dermal fringing along limbs (versus marked fringing along forearm and tarsus). From *Kurixalus odontotarsus* (Ye & Fei), the new species differs by having a pink venter (versus white venter with black or grey marbling), indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), and dorsum with distinctive, white conical asperities (versus dorsum rugose) in males. From *Polypedates leucomystax* (Gravenhorst) and *P. megacephalus* Hallowell, the new species differs by having a pink (versus white) venter, indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold iris lacking distinct black reticulations), dorsum with distinctive, white conical asperities (versus dorsum smooth) in males and eggs deposited as a tightly spaced array of nonpendent eggs on the wall of a phyptotelm (versus eggs in foam nest). From *Rhacophorus bipunctatus* Ahl the new species differs by having a pink (versus yellow) venter, indistinct (versus distinct) supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold iris with limited, non-uniform black reticulations), dorsum with distinctive, white conical asperities (versus dorsum smooth) in males and eggs deposited as a tightly spaced array of nonpendent eggs on the wall of a phyptotelm (versus eggs in foam nest). From *R. calcaneus* Smith, the new species differs by having a brown dorsum diurnally and yellow nocturnally (versus green or deep reddish-brown dorsum), pink (versus yellow) venter, indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus iris with a bright red periphery), and dorsum with distinctive, white conical asperities (versus dorsum smooth) in males. From *R. orlovi* Ziegler & Köhler, the new species differs by having a brown dorsum diurnally and yellow nocturnally (versus a reddish brown dorsum with dark brown markings), pink (versus white to light brown) venter, indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold iris with limited, non-uniform black reticulations), dorsum with distinctive, white conical asperities (versus dorsum smooth) in males and eggs deposited as a tightly spaced array of nonpendent eggs on the wall of a phyptotelm (versus eggs in foam nest). From *R. rhodopus* Liu and Hu the new species differs by having a pink (versus yellowish) venter, indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold iris with limited, non-uniform black reticulations), dorsum with distinctive, white conical asperities (versus dorsum smooth) in males and eggs deposited as a tightly spaced array of nonpendent eggs on the wall of a phyptotelm (versus eggs in foam nest). From *R. spelaeus* Orlov, Gnophanxay, Phimminith, & Phomphoumy, the new species differs by having a pink venter (versus light gray venter covered by dark specks), indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold or yellowish cream iris with limited, non-uniform black reticulations), and dorsum with distinctive, white conical asperities (versus dorsum smooth) in males. From *R. robertingeri* Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Nguyen, & Geissler, *Gracixalus lumarius* **sp. nov.** differs by having a pink (versus white, grey or cream) venter, indistinct (versus distinct) tympanum and supratympanic fold, vomerine teeth absent (versus present), dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus bright gold or yellowish cream iris with limited, non-uniform black reticulations), dorsum with distinctive, white conical asperities (versus dorsum smooth) in males and eggs deposited as a tightly spaced array of nonpendent eggs on the wall of a phyptotelm (versus eggs in foam nest). Lastly, from *Theloderma ryabovi* Orlov, Dutta, Ghate, & Kent, the new species differs by having a brown dorsum diurnally and yellow nocturnally (versus light beige with lilac patterns and black spots), pink (versus dark grey) venter, dark gold iris with a dense, relatively uniformly distributed network of black reticulations (versus dark brown iris), and dorsum with distinctive, white conical asperities (versus dorsum with only small dorsal asperities) in males.

Molecular data. Our preliminary molecular analysis supports our placement of *G. lumarius* **sp. nov.** in the genus *Gracixalus* (1.00 Bayesian posterior probability; Fig. 8). *G. lumarius* **sp. nov.** falls within "Clade I" of *Gracixalus* (Rowley *et al*. 2011), which also includes the type specimen of the genus, *G. gracilipes* (1.00 Bayesian posterior probability). Tree toplogy was near-identical when we excluded pairwise comparisons for gapped sites.

Molecular differentiation among *G. lumarius* **sp. nov**. and all other *Gracixalus* species for which comparable molecular data is available, is high, with 13.3–15.91% uncorrected sequence divergence at the 16S rRNA gene fragment examined. This degree of pairwise divergence in the 16S rRNA gene in frogs is strongly indicative of differentiation at the species level (Vences *et al*. 2005). Our preliminary molecular analysis recovers the same relationships within the genus *Gracixalus* as Rowley *et al*. 2011 (eg. 'Clade I' and 'Clade II'), but more extensive molecular analysis will be required to resolve the evolutionary relationships within the genus and among the family Rhacophoridae.

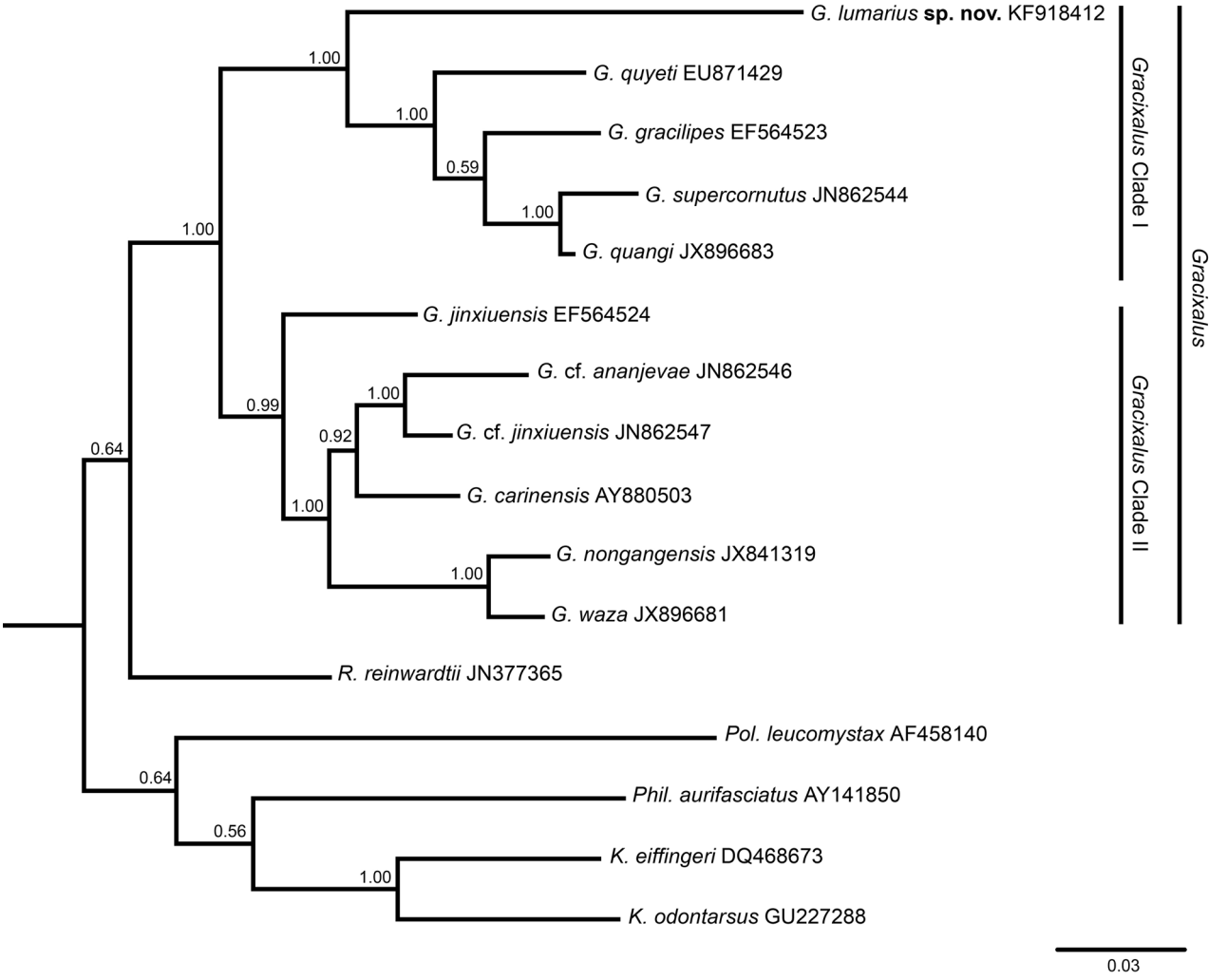

FIGURE 8. Bayesian inference tree based on 16S ribosomal RNA mitochondrial gene sequences for species currently assigned to *Gracixalus* (Frost 2014) and for outgroups (*Kurixalus eiffingeri*, *Kurixalus odontarsus*, *Philautus aurifasciatus*, *Polypedates leucomystax*, and *Rhacophorus reinwardtii*). Numbers on branches are Bayesian posterior probabilities.

Discussion

The fauna of Mount Ngoc Linh and surrounding peaks is characterized by high endemism (Jenkins *et al*. 2007), and *Gracixalus lumarius* **sp. nov.** is among several high-elevation (>1700 m) frog species known only from this area. Both *Leptobrachium ngoclinhense* (Orlov) and *Theloderma nebulosum* Rowley Le, Hoang, Dau & Cao were described from Ngoc Linh and adjacent peaks, and currently appear to be endemic to them. This is perhaps not surprising given the isolation of this area from other high elevation (>1800 m) forest.

Frogs that breed in phytotelmata represent only ~2% of currently recognized anuran taxa (Lehtinen *et al*. 2004). Within the family Rhacophoridae, at least nine species of obligate phytotelm breeders with free living larvae are known (Lehtinen *et al*. 2004). These species currently fall within the genera *Chiromantis, Kurixalus, Nyctixalus* and *Theloderma*. Although tadpoles were not observed, *Gracixalus lumarius* **sp. nov.** is almost certainly an obligate phytotelm breeder and has free-living tadpoles, given the observed oviposition above water, and relatively small, pigmented ova (R. Altig pers. comm). Phyptotelm breeding is generally interpreted as a strategy to avoid predation, competition, or unfavourable abiotic conditions in the ancestral water body (Crump 1982; Duellman & Trueb 1986). However, the relatively high availability of water-filled tree-holes and rarity of streams or pools above 1800 m in the study area may indicate that the species is simply taking advantage of the most available water source.

The distinctive, white conical asperities on the dorsum of the new species are unique among known species in the family Rhacophoridae, however similar conspicuous asperities on the dorsum of males are known in some species of *Afrixalus* (Hyperoliidae), *Boophis* (Mantellidae), and *Osteocephalus* (Hylidae). Previous authors have speculated that sexual dimorphism in skin texture may facilitate sex recognition in frogs (Jungfer & Hoedl 2002), and at least in *Osteocephalus,* males only display tuberculate dorsal skin with keratinized spicules during the breeding season, while females have a more or less smooth dorsum (Jungfer *et al*. 2013). Based on the type series, it appears that only male *Gracixalus lumarius* **sp. nov.** have a tuberculate dorsum with distinctive, white conical asperities, and that the size of asperities may be associated with breeding readiness. The male holotype collected in the dry season (April) with distinct nuptial pads and found with conspecific eggs also had the most distinctive asperities. In contrast, the two adult males collected in the wet season (July), had no distinct nuptial pads and less obvious (but still very distinct) white conical asperities, suggesting that the asperities remain present but are less distinct in the non-breeding season.

Acknowledgements

The Vietnamese Ministry of Agriculture and Rural Development, Dinh Quoc Thang and staff at Ngoc Linh Nature Reserve kindly facilitated surveys and issued permission to collect (Permit numbers776/BNN-KL, 748/BNN-KL). The People's Committee of Kon Tum Province issued permit number 548/UBND-DN for Rowley for work in the Province. This work was supported by grants from Ocean Park Conservation Foundation Hong Kong and ADM Capital Foundation to the Australian Museum and The John D. and Catherine T. MacArthur Foundation to the North Carolina Museum of Natural Sciences. Le Van Khanh and Nguyen Quoc Hung assisted with field work. Glenn Shea and Bryan Stuart provided images of *Gracixalus carinensis* types. Ronald Altig provided valuable advice on egg morphology. For all this assistance we are most grateful.

References

Alberch, P. & Gale, E.A. (1985) A developmental analysis of evolutionary trend: Digital reduction in Amphibians. *Evolution*, 39, 8–23.
http://dx.doi.org/10.2307/2408513

Brown, W.C. & Alcala, A.C. (1994) Philippine frogs of the family Rhacophoridae. *Proceedings of the Californian Academy of Sciences,* 48, 185–220.

Bossuyt, F. & Dubois, A. (2001) A review of the frog genus *Philautus* Gistel, 1848 (Amphibia, Anura, Ranidae, Rhacophorinae). *Zeylanica*, 6, 1–112.

Crump, M.L. (1982) Amphibian reproductive ecology on the community level. Herpetological communities. *Wildlife Research Report*, 13, 21–36.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
http://dx.doi.org/10.1038/nmeth.2109

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.

Duellman, W.E. & Trueb, L. (1986) *Biology of Amphibians*. The John Hopkins University Press, Baltimore and London, 670 pp.

Fei, L., Hu, S., Ye, C. & Huang, Y. (2009) *Fauna Sinica. Amphibia Vol. 2 Anura.* Science Press, Beijing, 957 pp.

Fei, L., Ye, C. & Jiang, J. (2010) *Colored Atlas of Chinese Amphibians*. Sichuan Publishing Group, Sichuan Publishing House of Science & Technology, Sichuan, 519 pp.

Frost, D.R. (2014) *Amphibian Species of the World: an online reference. Version 6.0.* American Museum of Natural History, New York, USA. Available from: http://research.amnh.org/vz/herpetology/amphibia/ (accessed 21 February 2014)

Glaw, F. & Vences, M. (1997) Anuran eye colouration: definitions, variation, taxonomic implications and possible functions. *In*: Böhme, W., Bischoff, W. & Ziegler, T. (Eds.), *Herpetologia Bonnensis*. *SEH Proceedings*, Bonn, pp. 125–138.

Gosner, K.L. (1960) A simple table for staging anuran embryos and larvae with notes on identification. *Herpetologica,* 16, 183–190.

Jenkins, P.D., Abramov, A.V., Rozhnov, V.V. & Makarova, O.V. (2007) Description of two new species of white-toothed shrews belonging to the genus *Crocidura* (Soricomorpha: Soricidae) from Ngoc Linh Mountain, Vietnam. *Zootaxa*, 1589, 57–68.

Jungfer, K.-H. & Hödl, W. (2002) A new species of *Osteocephalus* from Ecuador and a redescription of *O. leprieurii* (Duméril & Bibron, 1841) (Anura: Hylidae). *Amphibia-Reptilia*, 23, 21–46.
http://dx.doi.org/10.1163/156853802320877609

Jungfer K.-H., Faivovich J., Padial J.M., Castroviejo-Fisher S., Lyra M., Von Muller Berneck, B., Iglesias, P., Kok, P.J.R., MacCulloch, R.D., Rodrigues, M.T., Verdade, V.K., Torres Gastello, C.P., Chaparro, J.C., Valdujo, P.H., Reichle, S., Moravec, J., Gvozdík, V., Gagliardi-Urrutia, G., Ernst, R., De La Riva, I., Means, D.B., Lima, A.P., Señaris, J.C., Wheeler, W. & Haddad, C.F.B. (2013) Systematics of spiny-backed treefrogs (Hylidae: *Osteocephalus*): an Amazonian puzzle. *Zoologica Scripta,* 42, 351–380.
http://dx.doi.org/10.1111/zsc.12015

Lehtinen, R.M., Lannoo, M.J. & Wassersug, R.J. (2004) Phytotelm-breeding anurans: past, present and future research. *Special Publications of Museum of Zoology, University of Michigan*, 193, 1–9.

Liem, S.S. (1970) The morphology, systematics, and evolution of the old world treefrogs (Rhacophoridae and Hyperoliidae). *Fieldiana Zoology*, 57, 1–145.

Liu, C.-C. (1935) Types of vocal sac in the Salentia. *Proceedings of the Boston Society of Natural History*, 41, 19–40.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) *The simple fool's guide to PCR.* Department of Zoology, University of Hawaii, Honolulu, 47 pp.

Rambaut, A, & Drummond, A.J. (2007) Tracer v1.4, Available from: http://beast.bio.ed.ac.uk/Tracer (accessed 11 March 2014)

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.

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

Savage, J.M. & Heyer, W.R. (1997) Digital webbing formulae for anurans: a refinement. *Herpetological Review*, 28, 131.

Vences, M., Thomas, M., Bonett, R.M. & Vieites, D.R. (2005) Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society London, Ser. B*, 360, 1859–1868.
http://dx.doi.org/10.1098/rstb.2005.1717

Wilcox, T.P., Zwickl, D.J., Heath, T.A. & Hillis, D.M. (2002) Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution*, 25, 361–371.
http://dx.doi.org/10.1016/s1055-7903(02)00244-0

APPENDIX. Comparative material examined.

Gracixalus cf. *ananjevae:* Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve (VNMN 03012).

Gracixalus cf. *jinxiuensis:* Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve (AMS R 173454).

Gracixalus gracilipes: Vietnam, Ha Giang Province (AMNH A163894–163898, 5 adult males).

Gracixalus supercornutus: Vietnam, Quang Nam Province, Song Thanh Nature Reserve (AMS R 171537–171539). Vietnam, Kon Tum Province, Ngoc Linh Nature Reserve (AMS R 173735, UNS 00353/AMS R 173839, UNS 00354/AMS R 173840, AMS R 173886–173888, AMS R 173395, AMS R 173396).

Gracixalus quangi: Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve (AMS R 173410–173420, 173422–173423, NCSM 78277, VNMN 03000–03010).

Rhacophorus orlovi: Vietnam, Nghe An Province, Pu Mat National Park (AMS R 171731–171735).

Rhacophorus robertingeri: Vietnam, Kon Tum Province, Ngoc Linh Nature Reserve (AMS R 173193, 173609-173611, 173614, 173641).

Rhacophorus rhodopus: Vietnam, Binh Thuan Province, Nui Ong Nature Reserve (AMS R 173327, AMS R 173328, UNS 00419/AMS R 173329).