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A new species of phytotelm breeding frog (Anura: Rhacophoridae) from the Central Highlands of Vietnam

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Abstract

We describe a new species of phytotelm-breeding rhacophorid frog from central Vietnam. *Gracixalus trieng* **sp. nov.** is distinguished from all congeners by a combination of (1) body size medium (37.2–41.4 mm in five adult males), (2) snout rounded in dorsal and lateral views, (3) dorsal surface brown or yellowish with a darker brown interorbital crossbar and inverse-Y shape on the back, (4) throat and chest yellow or yellowish brown with pinkish mottling and belly and ventral surfaces of limbs including hands and feet pinkish, (5) tympanum and supratympanic fold distinct, (6) iris pale gold with darker gold radiating out from anterior and posterior edges of pupil, (7) majority of dorsal body and limb surfaces smooth in adults, with some individuals having sparsely distributed low, irregular tubercles, (8) nuptial pads on fingers I and II in adult males, and (9) eggs deposited as a tightly spaced array of non-pendent eggs on the wall of a phytotelmon. The species occurs in syntopy with *G. lumarius*. At present, *Gracixalus trieng* **sp. nov.** is known only from montane bamboo and evergreen forest (>1700 m) on Mount Ngoc Linh and adjacent peaks; and it is likely to be restricted to high-elevation forest with an estimated geographical distribution of <1000 km^2.

Key words: Anura, *Gracixalus*, Southeast Asia, Vietnam

Introduction

The family Rhacophoridae contains over 400 species of primarily arboreal frogs distributed throughout sub-saharan Africa, China, Southeast Asia, Japan, Taiwan, the Philippines, and the Greater Sunda Islands (AmphibiaWeb 2020; Frost 2020). The genus *Gracixalus* Delorme, Dubois, Grosjean & Ohler 2005 is known from southern and south-western China, Vietnam, Laos, Thailand, and Myanmar and presently contains 16 species (Frost 2020). Although evolutionary relationships in the group are not well-resolved, species in the genus generally fall within two phenotypic and phylogenetic groups; small-bodied frogs with a greenish dorsum and often a pointed snout ('Clade I' of Rowley *et al*. 2011), and medium-bodied frogs with a brownish dorsum and rounded snout ('Clade II' of Rowley *et al*. 2011). The recognized species diversity in the group has increased dramatically in recent years, with over half of the known species of *Gracixalus* described in the last 10 years (AmphibiaWeb 2020; Frost 2020).

During field work at high-elevations in central Vietnam in 2009 and 2010, we discovered two sympatric, medium-sized phytotelm-breeding rhacophorid resembling species in the *Gracixalus* 'Clade II' of Rowley *et al*. (2011).

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We were unable to assign either to known species, and the most morphologically distinct—a striking taxon with a distinctive tuberculate dorsal texture and yellow and pink nocturnal colouration—was later described as the Thorny Tree Frog (*Gracixalus lumarius*; Rowley *et al*. 2014). Here we describe the less morphologically distinct of the two forms as a new species.

Materials and methods

Specimens were deposited at the Australian Museum (AMS), the University of Science Ho Chi Minh City (UNS), and the North Carolina Museum of Natural Sciences (NCSM). We recorded morphological data from specimens fixed in 10% formalin and then stored in 70% ethanol. 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 the commissure of the jaws (HDW); snout length from tip of snout to the anterior corner of eye (SNT); diameter of the exposed portion of the eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of the eye (TEY); internarial space (IN); distance from front of eye to nostril (EN); tibia length with the 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 the inner metatarsal tubercle (PL). We use a traditional formula for finger numbering rather than one based on homology (e.g., 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 cartilage between terminal and penultimate phalanges of digits, and the shape of the distal end of the terminal phalanges. Webbing formula follows that of Myers and Duellman (1982) modified by Savage and Heyer (1997). We obtained comparative morphological data from specimens (Appendix I), photographs of these specimens in life and from the literature (original descriptions supplemented by information from Bossuyt & Dubois 2001; Rowley *et al*. 2011, 2014; Fei *et al*. 2009, 2010; Mo *et al*. 2013; Matsui *et al.* 2015, 2017; Zeng *et al*. 2017; Chen *et al*. 2018; Wang *et al*. 2018; Yu *et al*. 2019). Photographs of *G. carinensis* type material were also examined (lectotype BMNH 1947.2.6.24 and 1947.2.6.27, and MSNG 29852.A [three specimens]).

In order to support the generic placement of the new species, we obtained tissue samples from the new species, sequenced one fragments of mitochondrial DNA (see below) and compared these sequences to species currently assigned to the genus *Gracixalus* (Frost 2020) 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*), *Philautus aurifasciatus* (type species of the genus *Philautus*) and *Rhacophorus reinwardtii* were used as outgroups (sensu Rowley *et al*. 2011).

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 mitochondrial gene. PCR amplification was carried out in 25µl reactions with 100 ng of genomic DNA, 1 x Reaction Buffer (Bioline My Taq Red Reagent Buffer), 2 pmol corresponding primers and Bioline My Taq Red DNA polymerase (0.5 unit). Negative controls were included in each PCR. Thermocycling was performed on an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) under the following conditions: 16S, initial denaturation 94ºC (2 mins), 2 cycles of 94ºC (20s) denaturation, 52ºC (40s) annealing and 72ºC (60s) extension, followed by 33 cycles of 94ºC (20s) denaturation, 50ºC (40s) annealing and 72ºC (50s) extension, followed by a final extension step at 72ºC for 5 mins. PCR products were purified using ExoSap-IT (USB Corporation, OH, USA). Purified templates were sequenced directly by Macrogen (Seoul, Korea). Sequences were edited using Sequencher 4.10 (Gene Codes, Ann Arbor, MI) and aligned using MAFFT (Katoh *et al*. 2002) on the CIPRES Science Gateway (Miller *et al*. 2010). We used Akaike Information Criterion as implemented in jModelTest 2.1.6 (Darriba *et al*. 2012) and installed on the CIPRES Gateway to select the best-fit model of nucleotide substitution, which was then used in model-based phylogenetic inference. Maximum likelihood (ML) analyses were performed on the data matrix using RAxML, also installed on the CIPRES Gateway, using default settings, a GTRCAT model and 100 bootstrap replicates (Stamatakis *et al*. 2007). We consider branches receiving ≥70% bootstrap support to be well-supported following Hillis and Bull (1993). Uncorrected pairwise sequence divergence (with pairwise deletion of gaps and missing data) was calculated using MEGA 5 (Tamura *et al*. 2011).

TABLE 1. Samples and sequences used in molecular analyses.

	Locality	Voucher no.	GenBank no.
Gracixalus cf. *ananjevae*	Nghe An Province, Vietnam	VNMN 03012	JN862546
Gracixalus gracilipes	Yunnan Province, China	KIZ060821196	EF564523
	Ha Giang Province, Vietnam	AMNH A163897	DQ283051
Gracixalus guangdongensis	Hunan Province, China	CIB HN201108200	LC011936
	Guangdong Province, China	SYS a004902	MG520193
Gracixalus jingangensis	Juangxi Province, China	SYS a003186	KY624587
Gracixalus jinxiuensis	Guangxi Province, China	KIZ 060821126	EU215525
	Guangxi Province, China	KIZ060821013	EF564524
Gracixalus lumarius	Kon Tum Province, Vietnam	AMS R176202	KF918412
	Kon Tum Province, Vietnam	AMS R173838	MT328247
Gracixalus nongangensis	Guangxi Province, China	NHMG20091009	JX841319
	Guangxi Province, China	NHMG1005046	JX841320
Gracixalus quangi	Nghe An Province, Vietnam	AMS R 173410	JN862537
	Nghe An Province, Vietnam	AMS R173417	JN862539
Gracixalus quyeti	Quang Binh Province, Vietnam	VNUH160706	EU871428
	Quang Binh Province, Vietnam	ZFMK 82999	EU871429
Gracixalus sapaensis	Lai Chau Province, Vietnam	MNHN 1999.5961	AY880503
	Lao Cai Province, Vietnam	CIB XM-439	GQ285670
Gracixalus seesom	Kanchanburi, Thailand	KUHE 35084	LC011932
Gracixalus supercornutus	Kon Tum Province, Vietnam	AMS R173395	JN862542
	Kon Tum Province, Vietnam	AMS R173887	JN862545
Gracixalus tianlinensis	Guangxi Province, China	NHMG 1705015	MH117960
	Guangxi Province, China	NHMG 1705016	MH117961
Gracixalus waza	Cao Bang, Vietnam	IEBR A.2012.2	JX896681
	Cao Bang, Vietnam	VNMN A.2012.3	JX896685
Gracixalus yunnanesis	Nghe An Province, Vietnam	AMS R173454	JN862547
	Yunnan Province, China	KIZ 060821126	EF564525
Gracixalus sp.	Yunnan Province, China	03320Rao	GQ285669
Kurixalus effingeri	Japan, Okinawa Islands	A120	DQ468673
Philautus aurifasciatus	Indonesia, Java	ZRC.1.5266	AY141850
Rhacophorus reinwardtii	Malaysia, Sarawak	CAS 219931	JN377365
Gracixalus trieng **sp. nov.**	Kon Tum Province, Vietnam	AMS R176206	MT328246
	Kon Tum Province, Vietnam	UNS 00342/AMS R176205	MT328245

Gracixalus trieng sp. nov.

Holotype: AMS R176206, adult male, collected from tree hole (approx. 10 cm diameter entrance, 45 cm off ground) with eggs (AMS R176211), in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.063º N, 107.865º E, 2045 m; Fig. 1). Collected at 19:45 h on 3 April 2010 by J. J. L. Rowley, Le T. T. D., Dau Q. V. and Hoang D. H.

Paratypes: UNS 00342/AMS R176204, adult male, on dead fallen tree, 1 m from ground, in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.034º N, 107.827º E, 2055 m), collected at 19:30 h on 29 March 2010 by J. J. L. Rowley, Le T. T. D., Dau Q. V. and Hoang D. H. UNS 00343/AMS R176205, adult male, in tree hole filled with water (35 cm long, 5 cm deep, 1.5 m above ground), in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.063º N, 107.864º E, 2014 m), collected at 19:55 h on 3 April 2010 by J. J. L. Rowley, Le T. T. D., Dau Q. V. and Hoang D. H. AMS R176207 and NCSM 79748, adult males, in tree hole filled with water (approximately 1 m above ground), in montane evergreen forest in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (approximately

15.063º N, 107.860º E, 2000 m), collected at approximately 11:00 h on 3 April 2010 by local Gie-Trieng guides (A Phuoc, A Tru, A Nap, A Doi). UNS 00351/AMS R176208, juvenile, on epiphyte 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, 107.863º E, 1960 m), collected at 20:10 h on 3 April 2010 by J. J. L. Rowley, Le T. T. D., Dau Q. V. and Hoang D. H. AMS R176209, juvenile, on plant lead in stream bed in montane evergreen/bamboo forest 3 m from 3 m wide, rocky stream in Ngoc Linh Nature Reserve, Dak Glei District, Kon Tum Province, Vietnam (15.063º N, 107.859º E, 1716 m), collected at night on 4 April 2010 by J. J. L. Rowley, Le T. T. D., Dau Q. V. and Hoang D. H.

Referred Specimen: AMS R176211, eggs, collected at same time and location as holotype.

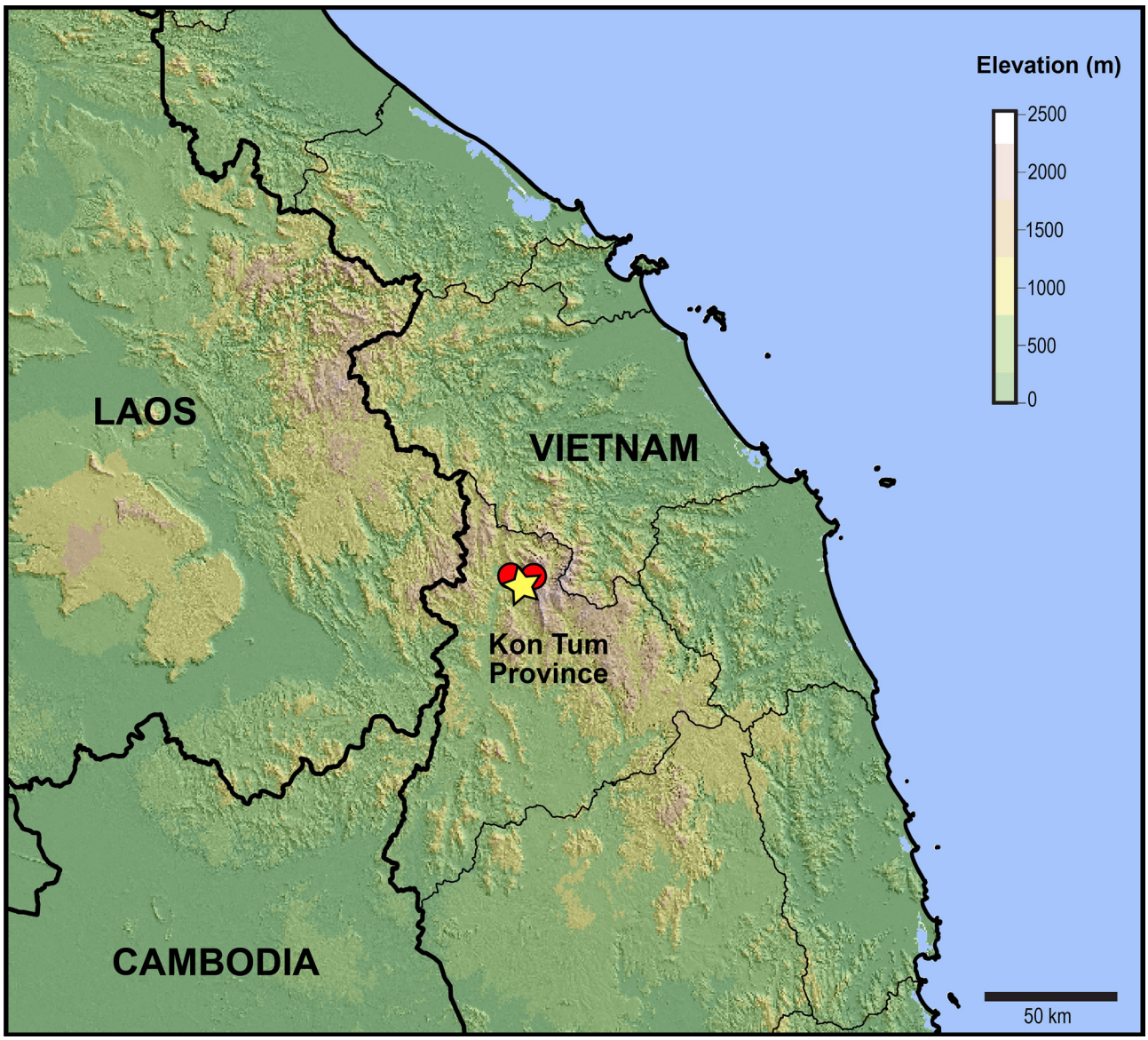

FIGURE 1. Collection sites of *Gracixalus trieng* **sp. nov.** Collection locality of holotype is marked with a yellow star. Note that the high elevation habitat of the new species is geographically isolated and of all known species in 'Clade II' (Rowley *et al.* 2011) of the genus, only *Gracixalus lumarius* occurs in the mapped area (in syntopy with *Gracixalus trieng* **sp. nov.**).

Etymology: The specific name is in reference to the Giẻ Triêng people, most of whom live in Kon Tum Province in the Central Highlands of Vietnam, and who assisted us greatly during our surveys. The species epithet is used as a noun in apposition.

Suggested Common Name: Trieng tree frog (English). Ếch cây giẻ triêng (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 discs bearing circummarginal grooves, vomerine teeth absent, pupil horizontal, large, conical tubercles/spines present on the upper eye-

lid (Liem 1970; Duellman & Trueb 1986; Brown & Alcala 1994; Delorme *et al.* 2005), and to the genus *Gracixalus* by the presence of a dark X, inverted V or inverted Y-shape marking present on the dorsal surface of the trunk (Fei *et al*. 2009). *Gracixalus trieng* **sp. nov.** is distinguished from all congeners by a combination of 1) body size medium (37.2–41.4 mm in five adult males), (2) snout rounded in dorsal and lateral views, (3) dorsal surface brown or yellowish with a darker brown interorbital crossbar and inverse-Y shape on the back, (4) throat and chest yellow or yellowish brown with pinkish mottling and belly and ventral surfaces of limbs including hands and feet pinkish, (5) tympanum and supratympanic fold distinct, (6) iris pale gold with darker gold radiating out from anterior and posterior edges of pupil, (7) majority of dorsal body and limb surfaces smooth in adults, with some individuals having sparsely distributed low, irregular tubercles, (8) nuptial pads on fingers I and II in adult males, and (9) eggs deposited as a tightly spaced array of non-pendent eggs on the wall of a phytotelmon.

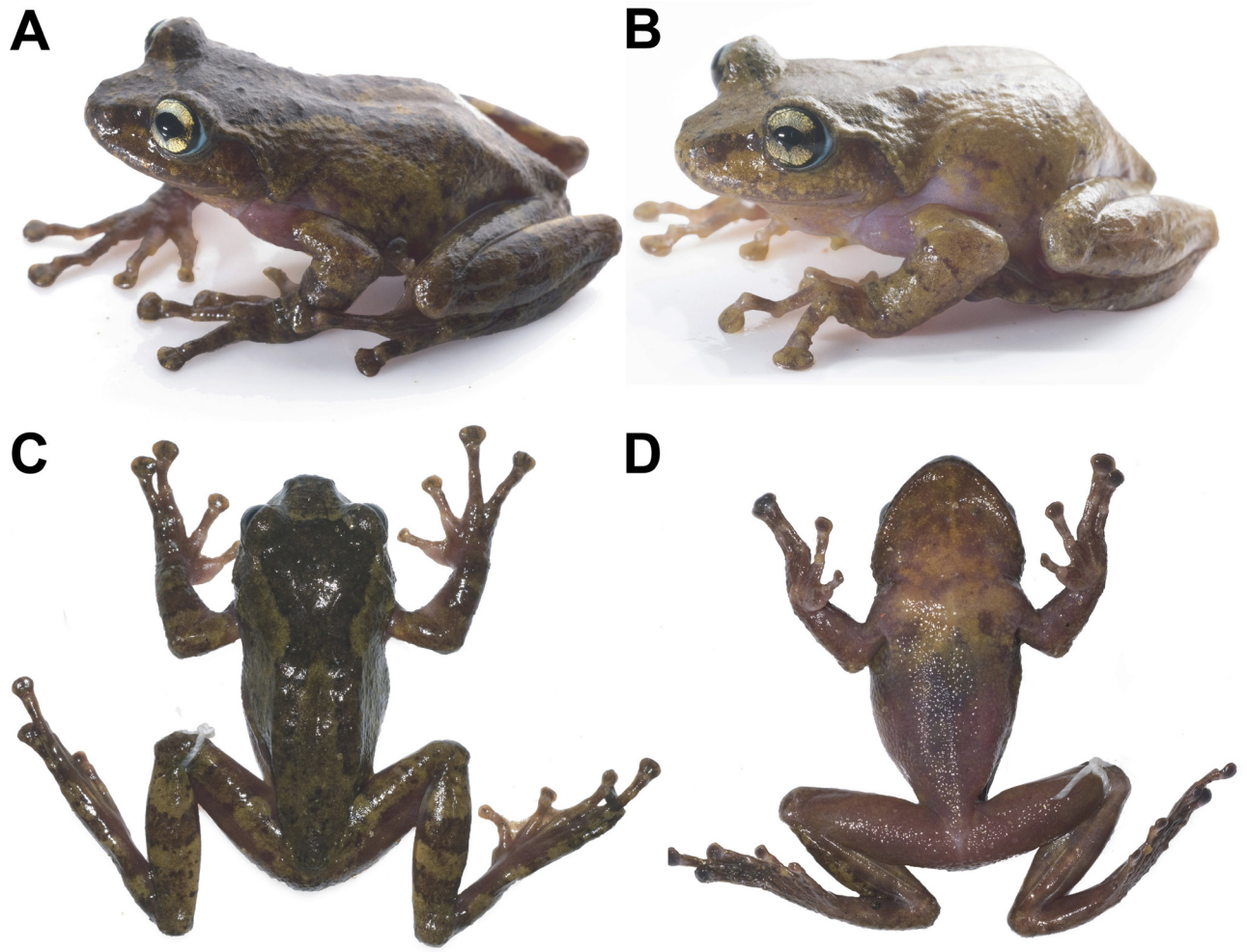

FIGURE 2. Diurnal dorsolateral view (A), nocturnal dorsolateral view (B), dorsal view (C) and ventral view (D) of the holotype of *Gracixalus trieng* **sp. nov.** (AMS R176206) in life.

Description of holotype: A medium-sized rhacophorid (37.2 mm), body robust (Figs. 2,3), head length 91% of head width, snout broadly rounded in dorsal view, rounded in profile, canthus rostralis rounded; loreal region slightly sloping, interorbital region convex, nostrils rounded, slightly protuberant, without flap of skin laterally, closer to tip of snout than eye; pupil oval, horizontal, tympanic annulus distinct, approximately one-third of eye diameter, pineal ocellus absent, skin not co-ossified to skull, vomerine teeth absent, choanae small, round, separated by about six times that of choanae diameter; tongue attached anteriorly, deeply notched posteriorly, tooth-like projections on lower jaw absent, supratympanic fold distinct, extending from eye to axilla. Vocal sac present, connected to buccal cavity by pair of distinct, oval openings at bases of jaws, only slightly baggy gular region. Forelimbs relatively robust, fingers narrow; relative length of fingers I < II < IV < III. Tips of all fingers with well-developed discs with circummarginal grooves; discs relatively wide compared to finger width (third finger disc 239% of third finger width at penultimate phalange), third finger disc width greater (131%) 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 present; supernumerary tubercles absent; palmar tubercle divided, irregular, flat; thenar tubercle indistinct; prepollex elongate, with oval tubercle; distinct (Fig. 4A), nuptial pads present on inner surface of the prepollex and dorsal and inner surfaces of fingers I and II (Fig. 4B). Relative length of toes I < II < V < III < IV, tips of toes with well-developed discs with distinct circummarginal grooves, wide when compared to toe length. Webbing present, formula I 2^{-} – 2^{+} II 1^{+} – 2^{+} III 2^{-} – 3 IV 2^{+}– 1 V, with slight dermal fringes, subarticular tubercles prominent, rounded, formula 1, 1, 2, 3, 2, inner metatarsal tubercle distinct, outer metatarsal tubercle and supernumerary tubercles absent (Fig. 4C). Dorsal surface smooth, with only sparsely distributed low, irregular, smooth tubercles. Ventral surface of thighs and abdomen coarsely granular, throat finely granular. No dermal fringes on limbs, no tibiotarsal projection, no distinct supracloacal glands.

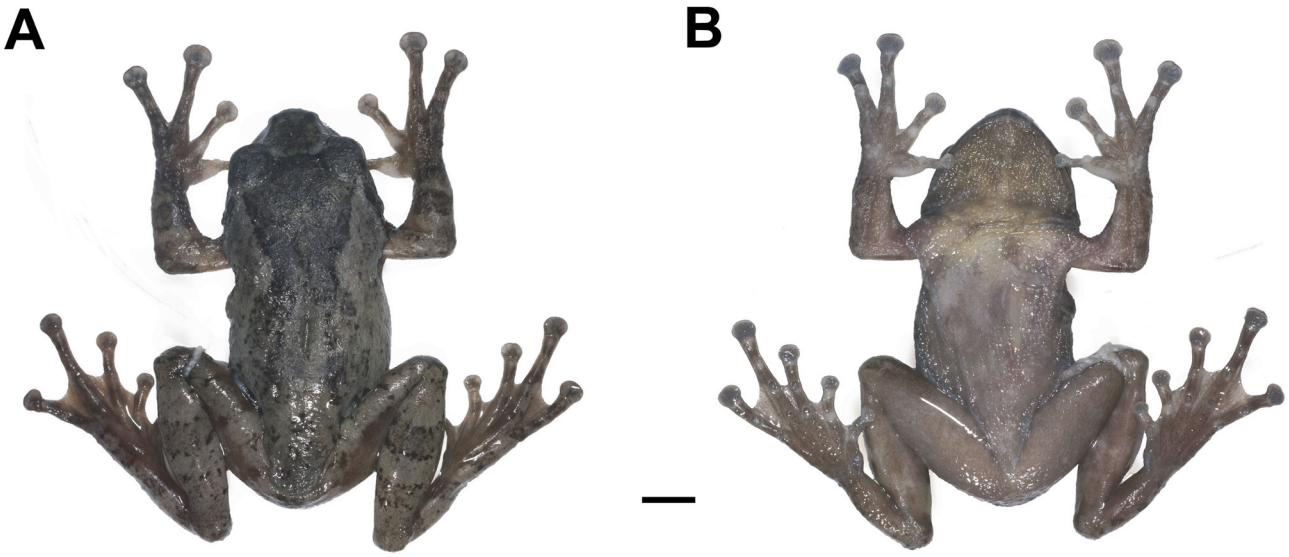

FIGURE 3. Dorsal (A) ventral (B) view of preserved male holotype of *Gracixalus trieng* **sp. nov.** (AMS R176206). Scale bar = 5 mm.

Colour of holotype in life: Dorsal surface brown (diurnally) or yellowish-tan (nocturnally) with a darker interorbital crossbar and inverted Y-shaped marking on the back starting between the eye and covering most of head; limbs with dark brown barring (Fig. 2). Patterning more distinct diurnally. Fingers brown or yellowish-tan, webbing pinkish brown. Diffuse darker brown line under canthus rostralis from eye to nostril, no obvious tympanic markings. Flanks mottled brown and dark pink (diurnally) or yellow and pink (nocturnally). Throat and chest mostly yellowish brown, with dark pinkish brown mottling (diurnally) or yellow with pink mottling (nocturnally); belly and ventral surfaces of limbs including hands and feet pinkish brown (diurnally) or pink (nocturnally). Iris pale gold with a sparse network of fine dark gold reticulations darker gold radiating out of anterior and posterior edges of pupil; iris periphery black; eye periphery pale blue posteriorly.

Colour of holotype in preservative: Dorsal surface as in life, but paler with less distinct markings (Fig. 3). Ventral surface paler, throat pale brown with pale creamy yellow mottling concentrated on chest. Belly and ventral surface of limbs pinkish grey. Dorsal and ventral surfaces of discs on fingers and toes grey.

Measurements: Holotype: SVL 37.2, HDL 13.0, HDW 14.3, SNT 5.9, EYE 4.4, IOD 4.1, TMP 2.2, TEY 1.2, IN 4.1, NS 2.6, EN 2.9, TIB 18.3. ML 12.7, PL 18.1, IMT 2.0.

Variation: Measurements of the type series are shown in Table 2. Paratypes vary in slightly in their colouration and pattern (Fig. 5). Skin texture also varies from almost completely smooth to sparsely distributed with low, irregular tubercles. Tiny, conical tubercles are present on the eyelids, under the eye and on the tympanum in some individuals (UNS 00342/AMS R176204, AMS R176207 and to a lesser extent, NCSM 79748). The tympanic annulus is less distinct in NCSM 79748, UNS 00343/AMS R176205 and AMS R176206. Considerable variation in colouration occurs in individuals over time in life, with several specimens changing from mostly pale yellow dorsally and pale pink ventrally without distinct dorsal markings (typical nocturnal coloration), to dark brown dorsally and dark pinkish brown ventrally, with distinct dark brown dorsal markings (typical diurnal coloration) over the course of 24 h (Fig. 2B; AMS R176206; UNS 00342/AMS R176204, AMS R176207). In life, juveniles of the new

species (AMS R176207, UNS 00351/AMS R176208) have a pale tan dorsum with an indistinct underlying pattern, yellowish flanks, and anterior and posterior surfaces of thighs, with dark brown blotches, and transparent creamy yellow venters (UNS 00351/AMS R176208) or transparent pale pink venters with white blotches on chest and throat (AMS R176209). In preservative, all but AMS R176207 have a distinct cross-mark on the back. In preservative, all adult paratypes are pinkish-grey ventrally, with variable amounts of indistinct, darker greyish-brown blotches concentrated anteriorly. UNS 00342/AMS R176204 and the holotype AMS R176206 have darker brown throats and juveniles of the new species (AMS R176207, UNS 00351/AMS R176208) have pale grey venters with darker blotches. All paratypes have distinct supratympanic folds and dark brown canthal and supratympanic lines. A single adult male paratype (AMS R176207) had fine, small tubercles on the dorsum in life, and the juvenile paratype UNS 00351/AMS R176208 (Fig. 5D) was slightly shagreened. All male paratypes have nuptial pads on fingers I and II.

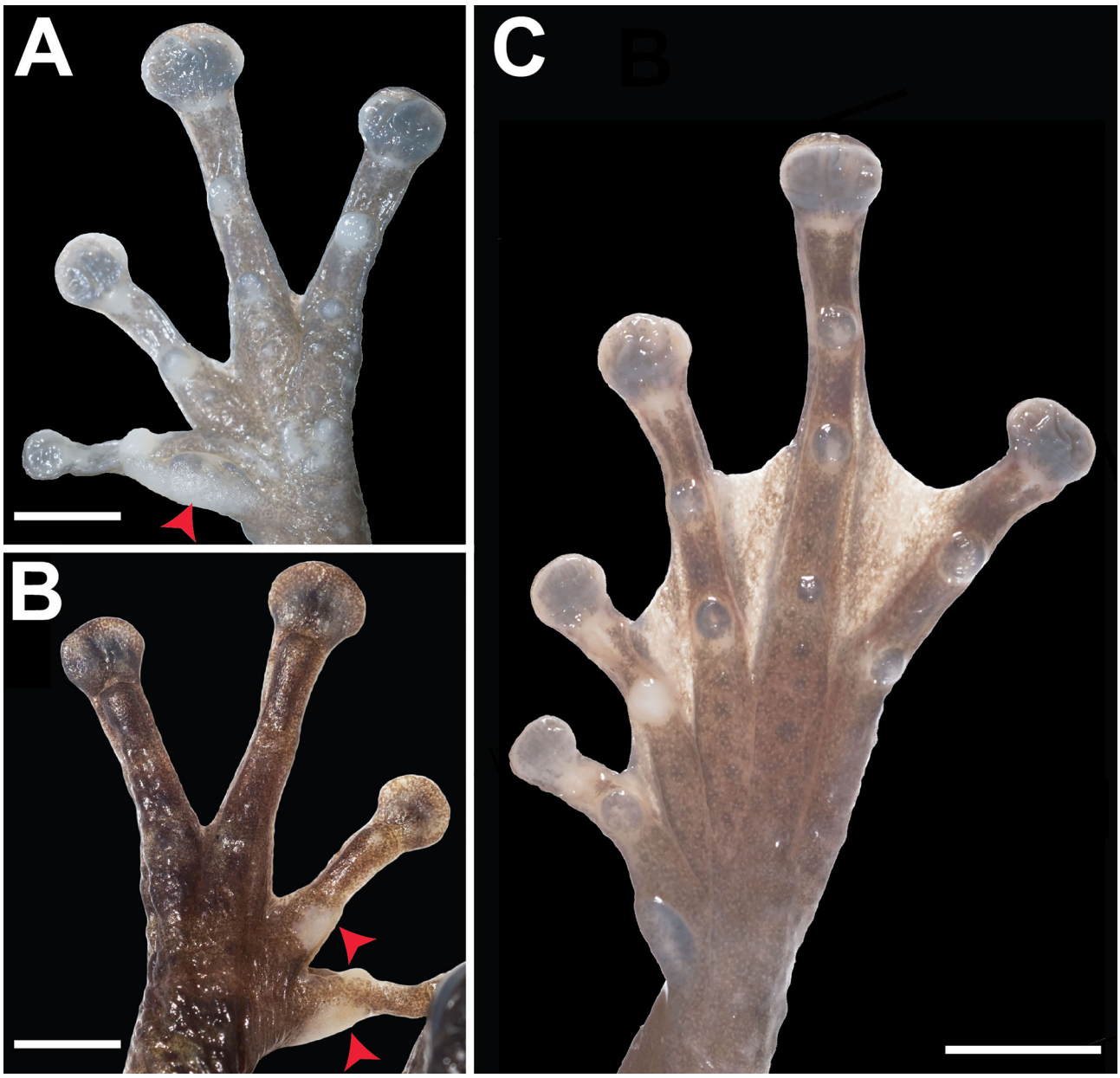

FIGURE 4. (A) Plantar surface of left hand showing nuptial pad on prepollex (marked with red arrow), (B) dorsal surface of left hand showing nuptial pads of Fingers I and II (marked with red arrows), and (C) plantar surface of left foot of the male holotype of *Gracixalus trieng* **sp. nov.** (AMS R176206). Scale bars = 2 mm.

Ecology: All specimens were found in montane evergreen forest (Fig. 6A). The holotype was found in a tree hole, with 130 eggs deposited on the trunk of the tree above the water (Fig. 6B). All other adult males in the type series were found in tree holes, and both juveniles were found on arboreal vegetation. All but juvenile AMS R176209

were found away from any streams or ponds. The species occurs in syntopy with *G. lumarius*. The females, tadpoles and advertisement call of the new species are unknown.

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

	UNS 00342/ AMS R176204	UNS 00343/ AMS R176205	**AMS R176206***	AMS R176207	NCSM 79748	UNS 00351/ AMS R176208	AMS R176209
Sex	adult male	adult male	adult male	adult male	adult male	juvenile	juvenile
SVL	40.9	37.8	37.2	38.2	41.4	24.3	19.9
HDL	12.5	12.2	13	13.4	13.8	9.4	7.5
HDW	14.9	14.2	14.3	14.5	15.1	9.4	7.5
SNT	6.1	5.3	5.9	5.6	6.0	3.5	2.9
EYE	4.4	4.0	4.4	4.7	4.8	3.0	2.9
IOD	4.6	3.9	4.1	4.0	3.9	2.9	2.7
TMP	1.8	1.7	2.2	2.1	2.0	1.3	1.1
TEY	1.1	1.5	1.2	1.6	1.7	0.8	0.7
IN	4.4	4.2	4.1	4.2	4.5	2.9	2.3
NS	2.5	2.3	2.6	2.7	2.3	1.5	0.9
EN	3.2	2.8	2.9	3.2	3.7	2.2	1.9
TIB	19.2	19.3	18.3	18.7	20.2	12.3	9.3
ML	14.0	13.3	12.7	14.0	14.0	7.6	6.4
PL	18.6	17.8	18.1	18.7	19.7	10.8	7.1
IMT	2.1	1.9	2.0	2.2	2.2	0.9	-
HW/HL	1.19	1.16	1.10	1.08	1.09	1.00	1.00
HL/HW	0.84	0.86	0.91	0.92	0.91	1.00	1.00
TIB/SVL	0.47	0.51	0.49	0.49	0.49	0.51	0.47
HDL/SVL	0.31	0.32	0.35	0.35	0.33	0.39	0.38
TMP/EYE	0.41	0.43	0.50	0.45	0.42	0.43	0.38

Distribution and conservation status: The new species is known only from between 1716–2055 m asl within Ngoc Linh Nature Reserve, Kon Tum Province, Vietnam. Mount Ngoc Linh and adjacent high-elevation peaks form an isolated area of high elevation and many faunal elements at this location are thought to be endemic (Abramov *et al*. 2006, Jenkins *et al.* 2007). The species was not located at lower elevations, despite surveys over 19 nights during March/April 2009 and July 2010 at elevations from ~930 m and above (Rowley *et al*. unpub. data). The range of the new species is not expected to extend outside of Mount Ngoc Linh and closely surrounding peaks but may occur in adjacent Quang Nam Province. Thus, we assume that the new species is restricted geographically, likely having an extent of occurrence (EOO) of <1000 km^2. The new species also most likely occurs in one threat-defined location, which has been characterized as an area with continuing decline in the quality of its habitat due to deforestation (Meyfroidt *et al*. 2013). *Gracixalus trieng* **sp. nov.** therefore likely qualifies as Endangered in accordance with the IUCN Red List of Threatened Species categories and criteria (IUCN 2012) B1ab(iii).

Comparisons: Within the genus *Gracixalus*, *Gracixalus trieng* **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 medium body size (adult males 37.2–41.4 mm SVL in five adult males) versus small body size (males < 25 mm SVL), brown or yellowish dorsum and a pink or pinkish brown venter (versus greenish dorsum and white or yellow venter), and eggs deposited on wall of a phytotelmon (versus on vegetation above pools for *G. gracilipes, G. quangi,* and *G. supercornutus*, unknown in *G. quyeti*). The round snout (versus pointed snout) further differentiates *Gracixalus trieng* **sp. nov.** from *G. gracilipes*, *G. quangi* and *G. supercornutus.*

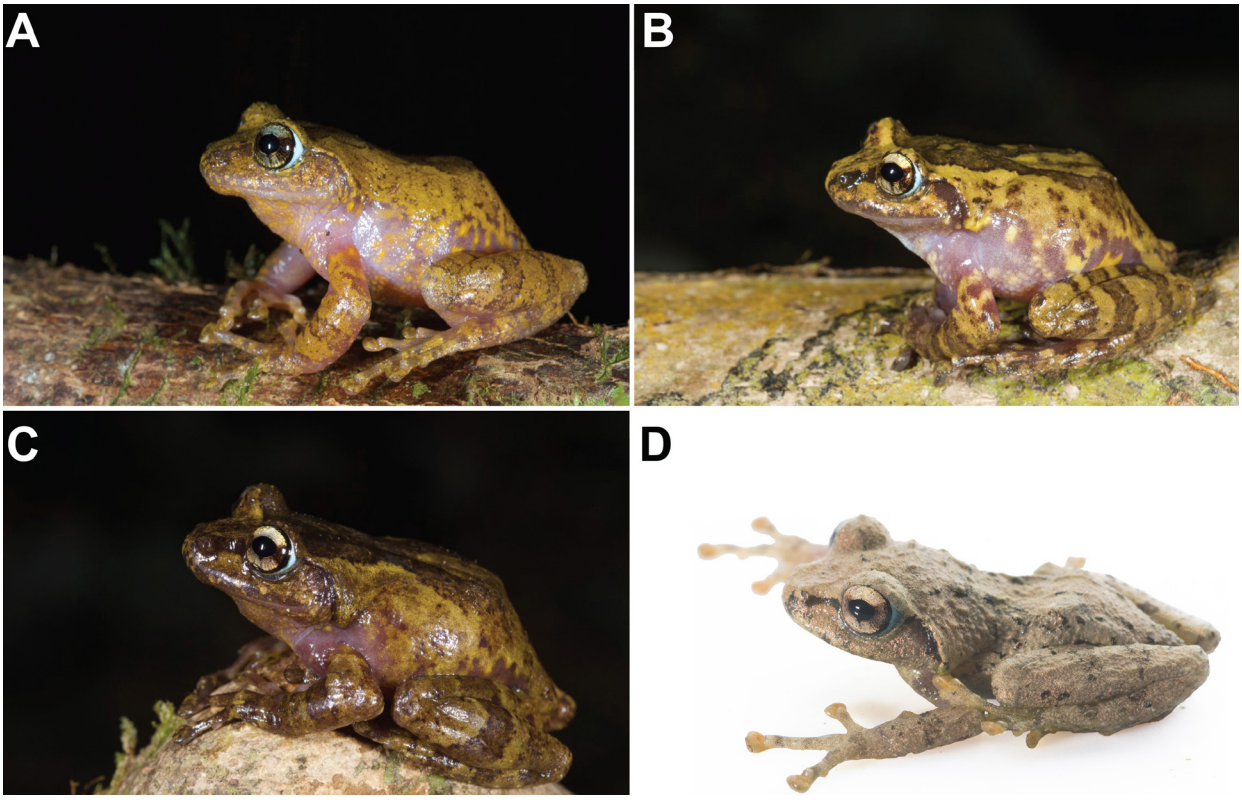

FIGURE 5. *Gracixalus trieng* **sp. nov.** (A) male paratype UNS 00342/AMS R176204, nocturnal coloration, (B) male paratype UNS 00343/AMS R176205, diurnal coloration, (C) male holotype AMS R176206, diurnal coloration, (D) juvenile paratype UNS 00351/AMS R176208, diurnal coloration.

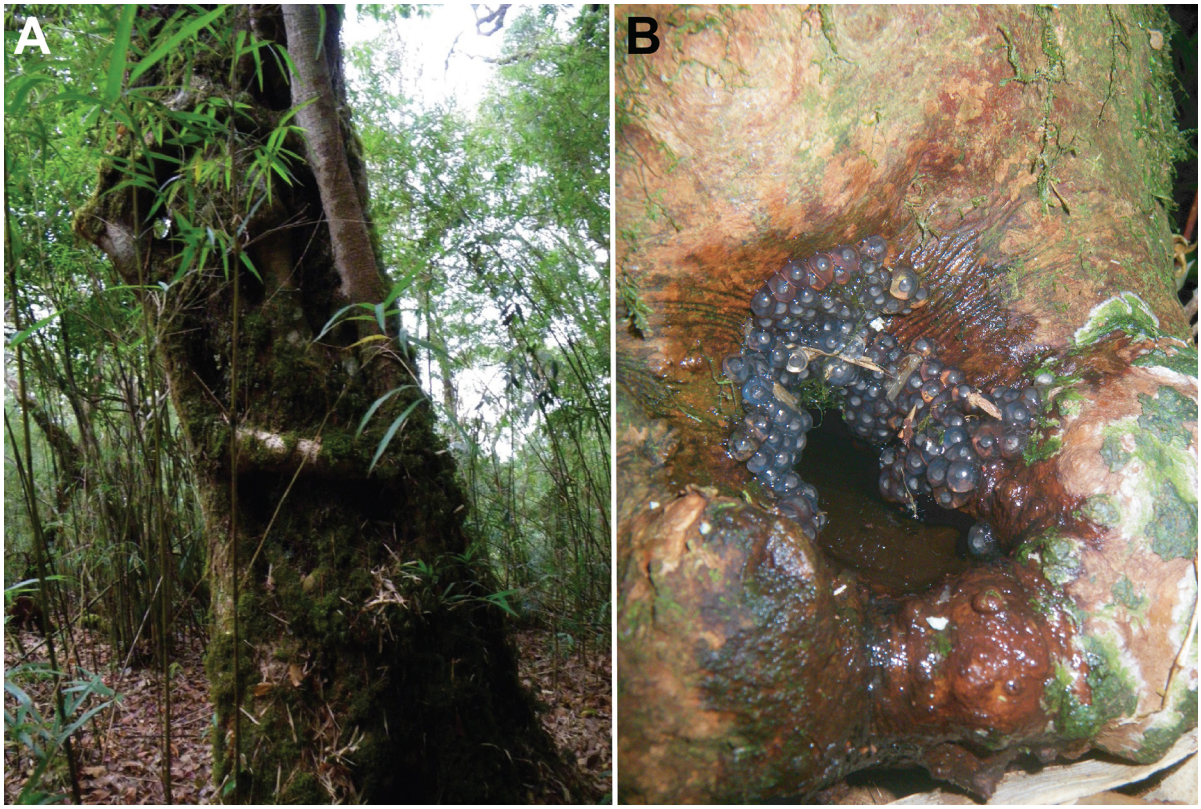

FIGURE 6. (A) Montane evergreen and bamboo forest at ~2000 m elevation, habitat of *Gracixalus trieng* **sp. nov.** and (B) tree hole with *Gracixalus trieng* **sp. nov.** eggs (AMS R176211) deposited, and where the male holotype of *G. trieng* (AMS R176206) was found.

The new species differs from *Gracixalus ananjevae* (Matsui & Orlov) by having a medium body size (adult males 37.2–41.4 mm SVL in five adult males), compared to 32.4 mm in one adult male; a yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus greyish white venter [likely in preservative] with no mottling), and adult males with nuptial pads on fingers I and II (versus finger I only).

Gracixalus trieng **sp. nov.** is distinguished from *Gracixalus carinensis* (Boulenger) by having a yellow or yellowish-brown throat and chest with pinkish mottling and pinkish belly (versus white venter with no mottling), adult males with nuptial pads on fingers I and II (versus finger I only; Bossuyt & Dubois 2001). In addition, *Gracixalus trieng* **sp. nov.** has less developed webbing (Fig. 4C) compared to *Gracixalus carinensis* (see Fig. 4B in Matsui *et al.* 2017).

From *Gracixalus guangdongensis* (Wang, Zeng, Liu & Wang), *Gracixalus trieng* **sp. nov.** differs by having a greater body size (adult males 37.2–41.4 mm SVL in five adult males, compared to SVL 26.1–34.7 mm), yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus ventral surface of throat, chest and forelimbs dirty white with small dark specks; belly white anteriorly with large dark blotches).

From *Gracixalus jinggangensis* (Zeng, Zhao, Chen, Chen, Zhang & Wang), the new species differs by having a larger body size (adult males 37.2–41.4 mm SVL in five adult males, compared to SVL 27.9–33.8 mm), and yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus ventral surface of throat, chest and forelimbs dirty white with small dark specks; belly white anteriorly with large dark blotches).

From *Gracixalus jinxiuensis* (Hu), the new species is distinguished by having a larger body size (37.2–41.4 mm SVL in five adult males compared to 23.5 mm in adult male holotype), yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus pale grey-brown with dark marbling), and adult males with nuptial pads on fingers I and II (versus finger I only).

From *Gracixalus lumarius* Rowley, Le, Dau, Hoang, & Cao, the new species differs by having large, white conical tubercles on the dorsum absent (versus at most sparsely distributed low, irregular tubercles present), iris pale gold with a sparse network of fine dark gold reticulations darker gold radiating out of anterior and posterior edges of pupil (versus a dark gold iris with a dense, relatively uniformly distributed network of black reticulations), yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus uniformly pinkish venter), and adult males with nuptial pads on fingers I and II (versus finger I only).

From *Gracixalus medogensis* (Ye & Hu), the new species is distinguished by having a larger body size (37.2–41.4 mm SVL in five adult males compared to 26.5 mm in one adult male), yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus light grey or whitish venter), adult males with nuptial pads on fingers I and II (versus finger I only), and linea masculina absent (versus present).

From *Gracixalus nonggangensis* Mo, Zhang, Luo, Zhou & Chen and *Gracixalus waza* Nguyen, Le, Pham, Nguyen, Bonkowski & Ziegler, the new species differs by having a larger body size (37.2–41.4 mm SVL in five adult males versus <36 mm in adult males), and a yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus white venter with brown spots), adult males with nuptial pads on fingers I and II (versus absent or finger I only).

From *Gracixalus sapaensis* Matsui, Ohler, Eto & Nguyen, the new species differs by having a yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus ventral surface of throat, chest, belly, and forelimb light yellow), adult males with nuptial pads on fingers I and II (versus finger I only).

From *Gracixalus seesom* Matsui, Khonsue, Panha & Eto, the new species differs by having a larger body size (37.2–41.4 mm SVL in five adult males versus 21.6–23.0 mm), round (versus pointed) snout, yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus opaque white venter), and adult males with nuptial pads on fingers I and II (versus nuptial pads absent).

From *Gracixalus tianlinensis* Chen, Bei, Liao, Zhou & Mo, the new species differs by having a larger body size (37.2–41.4 mm SVL in five adult males versus 30.3–35.9 mm), yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus ventral surface of the throat, chest grey with small dark specks and belly creamy white).

From *Gracixalus yunnanensis* Yu, Li, Wang, Rao, Wu & Yang, *Gracixalus trieng* **sp. nov.** differs by having a larger body size (37.2–41.4 mm SVL in five adult males versus 26.0–34.2 mm), yellow or yellowish brown throat and chest with pinkish mottling and pinkish belly (versus semi-transparent orangish with yellow spots), linea masculina absent (versus present), and adult males with nuptial pads on fingers I and II (versus finger I only).

Mitochondrial sequence divergence: The phylogenetic placement of the new species was not well-resolved

(Fig. 7). Uncorrected sequence divergences between *Gracixalus trieng* **sp. nov.** and all other *Gracixalus* species for which comparable molecular data are available is > 3.6% at the 16S rRNA gene fragment examined, with the least divergence between the new species and *G. guangdongensis* (3.6%) and *G. sapaensis* (3.9%). This degree of pairwise divergence in the 16S rRNA gene in frogs has been interpreted previously as indicative of differentiation at the species level (Vences *et al*. 2005).

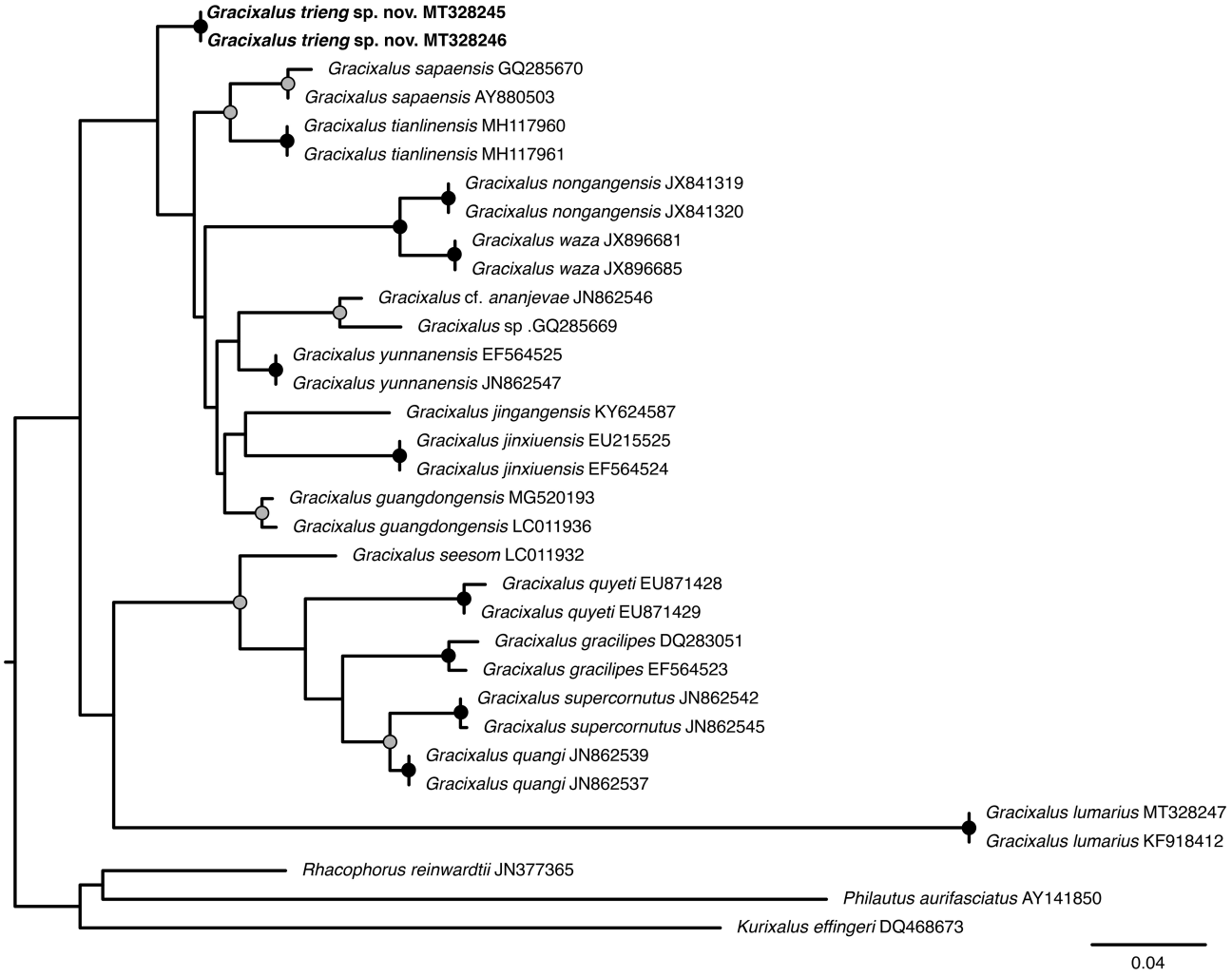

FIGURE 7. Maximum likelihood tree on ~550 bp section of 16S (mtDNA) gene for *Gracixalus trieng* **sp. nov.** and all *Gracixalus* species for which comparative sequences are available and for outgroups (*Kurixalus effingeri*, *Philautus aurifasciatus* and *Rhacophorus reinwardtii*). Node support is indicated on the branches as Maximum Likelihood bootstrap support (<70% = grey. 100% = black).

Discussion

Mount Ngoc Linh and surrounding peaks forms a relatively isolated area of high-elevation forest, in which terrestrial vertebrate fauna is characterized by a high degree of endemism (Jenkins *et al*. 2007). Three species of frog were relatively recently described from Mount Ngoc Linh and adjacent peaks, and appear to be endemic to these massifs (*Gracixalus lumarius* [Rowley, Le, Dau, Hoang, & Cao], *Leptobrachium ngoclinhense* [Orlov] and *Theloderma nebulosum* [Rowley Le, Hoang, Dau & Cao]). We likewise suspect that *Gracixalus trieng* **sp. nov.** is endemic to these isolated peaks. The scientific discovery of yet another species apparently endemic to the isolated, high-elevation (>1500m asl) habitat of Mount Ngoc Linh and adjacent peaks highlights the importance of the region in supporting a unique and highly diverse fauna.

With the exception of *G. lumarius*, which also appears restricted to Mount Ngoc Linh and adjacent peaks, the new species is geographically distant (>300 km) from all other medium-bodied species of *Gracixalus* with brownish

dorsal surfaces and rounded snouts, and much of the intervening habitat is relatively low elevation (<600 m) and very likely unsuitable for *G. trieng* **sp. nov.** Although further fauna surveys are needed throughout forested habitats in central Vietnam, we believe that the likely geographic isolation of *G. trieng* **sp. nov.** further supports its status as a distinct species.

Additional morphological, molecular and acoustic data is needed to confirm species boundaries and evolutionary relationships in the genus *Gracixalus*. This is particularly true for species the morphologically conserved 'Clade II' of Rowley *et al*. (2011), including *Gracixalus trieng* **sp. nov.** The collection of additional specimens at or near type localities and across their ranges, plus associated tissues, recordings of male advertisement calls, and clear descriptions of colour in life are needed to understand the true diversity of these forest-dependent frog species. This is increasingly important in light of ongoing deforestation in the area (Meyfroidt *et al*. 2013).

Frogs that breed in phytotelmata represent only ~2% of currently-recognized anuran taxa (Lehtinen *et al*. 2004). Although water-filled tree-holes are the most abundant source of standing water in tropical wet forests (Yanoviak 2001), and are relatively common in the study area, competition for this resource as both a shelter site and breeding habitat may occur between the phytotelma-breeding species *Theloderma nebulosum, G. lumarius* and *G. trieng* **sp. nov.** Although tadpoles were not observed, *G. trieng* **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).

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APPENDIX I. Comparative material examined.

Gracixalus cf. *ananjevae:* Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve (VNMN 03012).
Gracixalus gracilipes: Vietnam, Ha Giang Province (AMNH A163894–163898).
Gracixalus lumarius: Vietnam, Kon Tum Province, Ngoc Linh Nature Reserve (AMS R173889, 173890, 176202, UNS 00340/AMS R176203, UNS 00341/AMS R 173838).
Gracixalus supercornutus: Vietnam, Quang Nam Province, Song Thanh Nature Reserve (AMS R171537–171539). Vietnam, Kon Tum Province, Ngoc Linh Nature Reserve (AMS R173735, UNS 00353/AMS R173839, UNS 00354/AMS R173840, AMS R173886–173888, AMS R173395, AMS R173396). Vietnam, Gia Lai Province, Kon Ka Kinh National Park (AMS R176267, 176273, 176287, 176408–176428).
Gracixalus quangi: Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve (AMS R173410–173420, 173422–173423, NCSM 78277, VNMN 03000–03010).
Gracixalus yunnanensis: Vietnam, Nghe An Province, Pu Hoat Proposed Nature Reserve (AMS R173454).