

# A new species of green tree frog of the genus *Gracixalus* (Anura: Rhacophoridae) from the evergreen forest of Northeast India

Bitupan Boruah<sup>1</sup>, V. Deepak<sup>2,3,4</sup>, Naitik G. Patel<sup>1</sup>, Vijayan Jithin<sup>1,5</sup>, Tajum Yomcha<sup>6</sup>, Abhijit Das<sup>1</sup>

<sup>1</sup> Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand 248001, India

<sup>2</sup> Senckenberg Dresden, Königsbrücker Landstraße 159, 01109 Dresden, Germany

<sup>3</sup> University of Wolverhampton, Wulfruna St, Wolverhampton WV1 1LY, UK

<sup>4</sup> Department of Life Sciences, The Natural History Museum, London SW7 5BD, UK

<sup>5</sup> Nature Conservation Foundation, Mysore, Karnataka 570017, India

<sup>6</sup> Namdapha National Park and Tiger Reserve, Changlang, Arunachal Pradesh 792120, India

<https://zoobank.org/CB4D3007-28F7-446D-8473-D16F8A20361D>

Corresponding author: Abhijit Das ([abhijit@wii.gov.in](mailto:abhijit@wii.gov.in))

Academic editor Raffael Ernst

Received 7 December 2022

Accepted 4 May 2023

Published 26 May 2023

**Citation:** Boruah B, Deepak V, Patel NG, Jithin V, Yomcha T, Das A (2023) A new species of green tree frog of the genus *Gracixalus* (Anura: Rhacophoridae) from the evergreen forest of Northeast India. *Vertebrate Zoology* 73 557–574. <https://doi.org/10.3897/vz.73.e98444>

## Abstract

We describe a new species of rhacophorid frog of the genus *Gracixalus* from northeast India based on molecular, morphological and acoustic evidence. The new species, formally described herein as *Gracixalus patkaiensis* **sp. nov.**, is morphologically distinct from other congeners by a suite of morphological characters such as snout-vent length 23.6–26.5 mm in adult males; green dorsum with irregular brown spots; dorsal skin shagreened with numerous spinules; snout shape nearly acuminate in dorsal and ventral view; a prominent dark streak along the cranial margins; white reticulations along lateral side and ventrum distinct in life. Genetically, the new taxon is found to differ from all the recognized *Gracixalus* species by 4–14.8% divergence in the 16S mitochondrial gene. The discovery confirms the presence of genus *Gracixalus* from the Indian state of Arunachal Pradesh underlines the importance of biological exploration even in well-known protected areas of India.

## Keywords

Arunachal Pradesh, Indo-Burma Biodiversity Hotspot, Namdapha Tiger Reserve, rainforest

## Introduction

The family Rhacophoridae is represented by 14 genera in India out of the 23 genera globally recognized (Frost 2023). The genus *Gracixalus* Delorme, Dubois, Grosjean & Ohler, 2005 include small to medium-sized frogs (SVL: 20–41.6 mm) currently represented by 19 species globally. These species are distributed in Vietnam, Laos, Thailand, Southern China and Myanmar (Frost 2023).

More than half of the total known species in the genus were described in the last decade and no species of this genus have been reported from India. The majority of the species comes from Vietnam with a description of ten species. Rowley et al. (2011, 2014, 2020) described three species under this genus, and also broadly classified them into two morphological clades, a clade with species

having green dorsum and another with species having brown dorsum. However, more recent studies suggest that there are species with intermediate morphotypes which do not clearly fit into one of the two clades (Mo et al. 2013; Nguyen et al. 2013; Rowley et al. 2014; Matsui et al. 2015; Wang et al. 2018; Tran et al. 2023). Despite the inconsistency in morphology the two monophyletic clades described in Rowley et al. (2011) still remain with moderate to good support.

During our recent fieldwork in Arunachal Pradesh, we encountered a small-sized green rhacophorid frog which matches the description of the genus *Gracixalus*. This species is distinct from all known *Gracixalus* which we describe here as a new species based on morphology, molecular and acoustic data.

## Materials and Methods

### Sampling

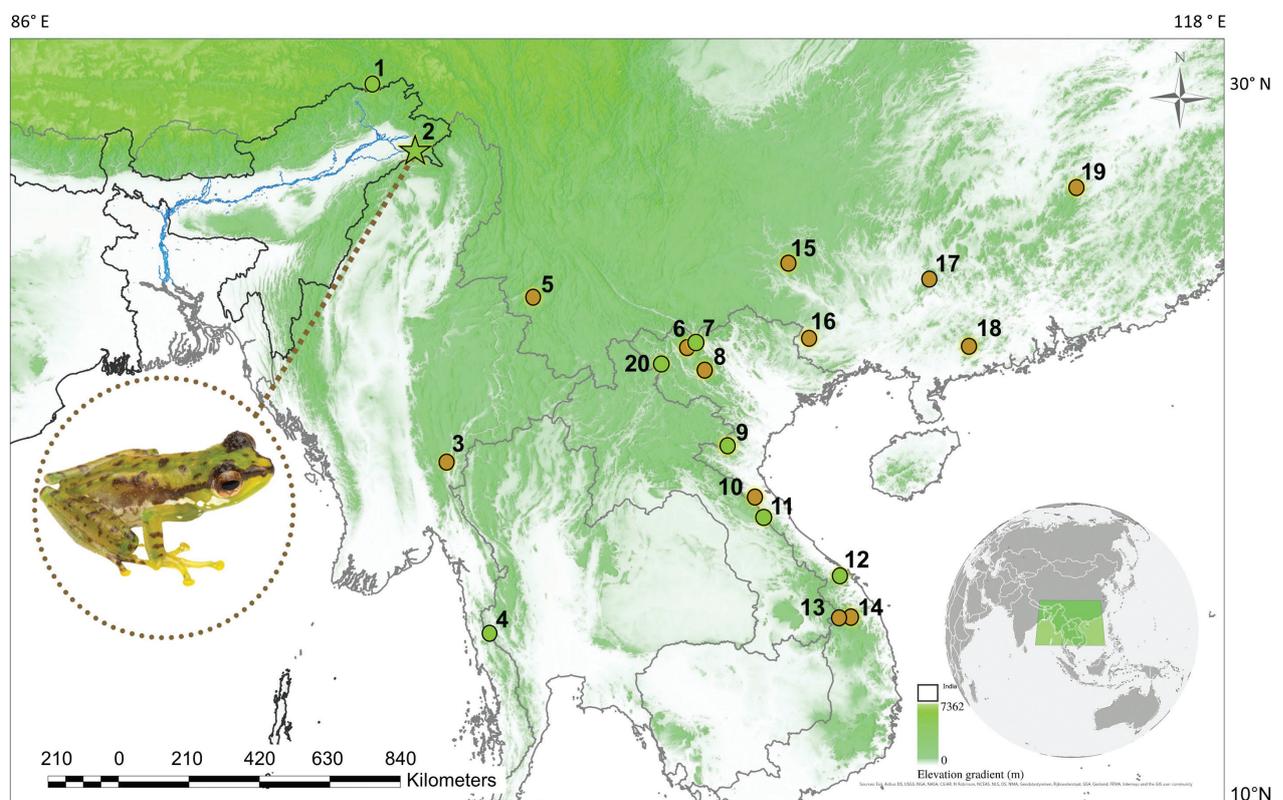
Field surveys were carried out during May, 2022 in Namdapha Tiger Reserve, Changlang district, Arunachal Pradesh, India. Nocturnal visual encounter surveys were deployed between 18:00 and 24:00 hrs to locate the frogs aided with torch lights. Collected samples were eutha-

nized using Tricaine Methanesulfonate (MS222), fixed in 4% formalin, washed and finally stored in 70% ethanol. Prior to fixation, photographs of the live frogs were taken, thigh muscle tissue was collected and kept in molecular grade ethanol for DNA extraction.

### Molecular and phylogenetic analysis

Genomic DNA was extracted from the collected tissue samples using the DNeasy (Qiagen™) blood and tissue kit following the manufacturer's protocols. We amplified and sequenced a partial fragment (570 base pairs) of the mitochondrial gene 16S using the primers 16Sar-L (Forward) and 16Sbr-H (Reverse) (Palumbi et al. 1991). Polymerase Chain Reaction (PCR) condition followed was initial denaturation at 95°C for 3 minutes, followed by 39 cycles of denaturation at 94°C for 45 seconds, annealing at 52°C for 45 seconds and extension at 72°C for 2 minutes. Final extension was at 72°C for 10 minutes. Amplified PCR products were run on a 2% agarose gel and viewed under UV transilluminator. Purified PCR product was sequenced directly in an Applied Biosystems Genetic Analyzer 3500 XL in both directions using BigDye v3.1 kits.

The newly generated sequences were aligned with 28 sequences (16S) of other *Gracixalus* species downloaded from the GenBank (Benson et al. 2007) (Appendix 1).



**Figure 1.** Map showing the type localities of *Gracixalus* species from northeast India and southeast Asia (Clade I- Green Circles, Clade II- Brown Circles): 1 *G. medogensis*; 2 *G. patkaiensis* sp. nov. (Star); 3 *G. carinensis*; 4 *G. seesom*; 5 *G. yunnanensis*; 6 *G. sapaensis*; 7 *G. gracilipes*; 8 *G. ziegleri*; 9 *G. quang*; 10 *G. ananjevae*; 11 *G. quyeti*; 12 *G. supercornutus*; 13 *G. lumarius*; 14 *G. trieng*; 15 *G. tianlinensis*; 16 *G. nonggangensis*; 17 *G. jinxiuensis*; 18 *G. guangdongensis*; 19 *G. jinggangensis*; 20 *G. truongi*. Insert image: *Gracixalus patkaiensis* sp. nov.

*Kurixalus effingeri*, *Philautus aurifasciatus* and *Rhacophorus reinwardtii* were selected as outgroups based on Rowley et al. (2020). Sequences were assembled in MEGA v7.1 (Kumar et al. 2016) and aligned with the ClustalW algorithm (Higgins et al. 1994) with default settings.

We performed a Maximum Likelihood (ML) analysis using IQ-TREE (Nguyen et al. 2015), implemented in the web server version (<http://iqtree.cibiv.univie.ac.at>) (Trifinopoulos et al. 2016). IQ-TREE server used Modelfinder (Kalyaanamoorthy et al. 2017) to find the best-fit evolutionary model for 16S gene. Support for internal branches was quantified using the bootstrap approximation (UFB 1000 pseudoreplicates) (Minh et al. 2013). We used Bayesian information criterion (BIC) as implemented in JMODELTEST v2.1.6 (Darriba et al. 2012) to select the best-fit model of nucleotide substitution, which was then used in model-based phylogenetic inference. Bayesian Inference (BI) was performed in MRBAYES v3.2 (Ronquist et al. 2012) applying the model GTR+G as best-fit model suggested by the JMODELTEST 2.1.6 (Darriba et al. 2012). Two separate runs were set up with four Markov chains, each initiated from random trees and allowed to run for 10 million generations, sampling every 1000 generations. Analyses were terminated when the standard deviation of split frequencies was less than 0.001, the first 25% of trees were discarded as “burn-in”, and trees were constructed under 50% majority consensus rule. We obtained the ESS values using the TRACER v1.6 (Rambaut et al. 2014) and confirmed values above 200 for the priors. We quantified the support for internal branches in BI tree using posterior probability. The resulting tree was edited in Figtree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>). The uncorrected pairwise distances (p distance) were calculated in MEGA v7.1 with pairwise deletion of missing data and gaps.

## Morphological characters

Metric and meristematic characters were studied using stereomicroscope (Olympus SZX10). Morphometric measurements were taken using digital slide calliper (Mitutoyo) to nearest 0.1 mm. Sex and maturity were determined by the presence of vocal sac and nuptial pad in males or by examining the gonads through a ventral incision. Measurements and associated terminologies follow Das et al. (2019), otherwise stated as follows. The following measurements were taken: SVL (snout-vent length), HW (head width, at the angle of the jaws), HL (head length, from the rear of the mandible to the tip of the snout), SL (snout length, from the tip of the snout to the anterior orbital border), EN (distance from front of the eye to the nostril), NS (distance from nostril to tip of the snout), IN (internarial distance), EL (eye length, horizontal distance between the orbital borders), IUE (inter upper eyelid width, shortest distance between the upper eyelids), UEW (maximum upper eyelid width), IFE (internal front of the eyes, shortest distance between the anterior orbital borders), IBE (internal back of the eyes,

shortest distance between the posterior orbital borders), HTYD (maximum horizontal tympanum diameter), VTYD (maximum vertical tympanum diameter), FAL (forearm length, from the flexed elbow to the base of the outer palmar tubercle), HAL (hand length, from the base of the outer palmar tubercle to the tip of the third finger), AG (distance between axilla and groin), TL (thigh length, from the vent to the knee), SHL (shank length, from knee to heel), FOL (foot length, from the base of the inner metatarsal tubercle to the tip of the fourth toe), TarL (Tarsus length, from tibio-tarsal articulation to the lower edge of the inner metatarsal tubercle), FD (disc width of finger), TD (disc width of toe), FL (finger length, from the tip of the digit to its base where it joins the adjacent digit), digit number is represented by roman numerals I–V. All measurements provided in the text are in millimetres. Webbing formulae follow Biju et al. (2014). Measurements were taken for the right side of the specimen.

Comparative morphological data of the *Gracixalus* species were obtained from the original descriptions of Boulenger (1893), Bourret (1937), Ye and Hu (1984), Matsui and Orlov (2004), Orlov et al. (2004), Nguyen et al. (2008), Rowley et al. (2011), Mo et al. (2013), Rowley et al. (2014), Matsui et al. (2015), Matsui et al. (2017), Zeng et al. (2017), Chen et al. (2018), Wang et al. (2018), Yu et al. (2019), Rowley et al. (2020), Le et al. (2021) and Tran et al. (2023), otherwise mentioned.

## Bioacoustics recording and analysis

Male advertisement calls were recorded using a Sennheiser MKH 416 unidirectional handheld microphone and Marantz PMD 620 MK-II digital audio recorder between 18:00 and 23:00 hrs. The recordings were done from a distance of approximately 10–30 cm from the calling male. Ambient temperatures were recorded using a Kestrel 5500 weather meter. Calls were visualized and analyzed in Raven Pro v1.5 (Bioacoustics Research Program 2014). Acoustic properties and terminologies follow Köhler et al. (2017).

## Institutional abbreviations

WII-ADA – Wildlife Institute of India, Dehradun, Abhijit Das Amphibian collection.

## Results

### Molecular phylogenetics

Bayesian inference (BI) and Maximum likelihood (ML) analyses showed broadly similar tree topology (Fig. 2) and *Gracixalus* species were falling into two major clades (sensu Rowley et al. 2011). Tree topologies were nearly similar to that of previously published phylogenies



Table 1. Uncorrected p distances among the *Gracixalus* species. GenBank voucher numbers are included in brackets.

Species	Voucher Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>G. patkaiensis</i> sp. nov.	WII-ADA1352, WII-ADA1353	0.01																		
<i>G. gracilipes</i>	MG520199–MG520201, KT374013	0.04–0.05	0–0.02																	
<i>G. supercornutus</i>	LC642810	0.08	0.07–0.08																	
<i>G. quangi</i>	LC642811	0.07	0.06–0.07	0.03																
<i>G. sesom</i>	LC011932, LC011935	0.05	0.06–0.07	0.08	0.07															
<i>G. quyeti</i>	EU871428, EU871429	0.07–0.08	0.07–0.08	0.06–0.07	0.07	0.08	0.01													
<i>G. sapaensis</i>	LC140969, LC140970	0.10	0.11–0.12	0.13	0.11	0.10	0.12													
<i>G. carinensis</i>	LC011938	0.11	0.11–0.12	0.13	0.11	0.10	0.12													
<i>G. jinxitensis</i>	KY624585	0.11	0.12	0.13	0.12	0.11	0.11–0.12	0.07	0.07											
<i>G. ziegleri</i>	LC642812	0.12	0.11–0.12	0.12	0.11	0.10	0.12	0.03	0.03	0.06										
<i>G. truongi</i>	OP750513, OP750514	0.11	0.12–0.14	0.12	0.11	0.12	0.12	0.07	0.07	0.08	0.06									
<i>G. yunnanensis</i>	MK234879, MK234883	0.11	0.12	0.11	0.10	0.10	0.11	0.05	0.05	0.06	0.04	0.05								
<i>G. guangdongensis</i>	MG520194	0.10	0.11–0.12	0.11	0.10	0.09	0.11	0.06	0.06	0.06	0.04	0.05	0.02							
<i>G. jinggangensis</i>	KY624586, KY624587	0.11	0.11–0.12	0.11	0.09	0.11	0.12	0.07	0.07	0.07	0.06	0.07	0.05	0.05						
<i>G. nonggangensis</i>	JX841320	0.13	0.14	0.15	0.14	0.12	0.14	0.08	0.08	0.09	0.07	0.09	0.07	0.06	0.08					
<i>G. tianlinensis</i>	MH117960, MH117961	0.10	0.11–0.12	0.13	0.12	0.09	0.11	0.03	0.03	0.06	0.02	0.06	0.04	0.04	0.07	0.07				
<i>G. ananjevae</i>	JN862546	0.12	0.12–0.14	0.13	0.12	0.13	0.12–0.13	0.06	0.07	0.08	0.06	0.07	0.04	0.05	0.08	0.08	0.07	0.07	0.07	
<i>G. trieng</i>	MT328245, MT328246	0.10	0.11	0.11	0.10	0.09	0.09–0.10	0.05	0.05	0.05	0.04	0.05	0.04	0.04	0.05	0.07	0.04	0.04	0.06	
<i>G. lumarius</i>	KF918412	0.15	0.15–0.17	0.17	0.16	0.17	0.14–0.15	0.17	0.18	0.18	0.17	0.17	0.15	0.16	0.16	0.18	0.17	0.17	0.15	0.15

and 4.0% respectively (Table 1). This therefore suggests that the newly collected specimens are separately evolving lineage and represents an undescribed species. Taking this into account and morphological difference of our newly collected materials from its congeners, we describe it as a new species herein.

## Systematics

### *Gracixalus patkaiensis* sp. nov.

<https://zoobank.org/2421026F-ED8A-4302-AA94-3C62BA9AF3BA>

**Holotype.** WII-ADA1353 (Fig. 3A, 4A–F), adult male, collected from ~300 m south of Kamala Valley Beat (27°27'34" N; 96°25'40" E; elevation 648 m a.s.l.; Fig. 1), Namdapha, Changlang district, Arunachal Pradesh on 14 May 2022 by Abhijit Das, Bitupan Boruah and Vijayan Jithin.

**Paratypes.** Adult males (WII-ADA1352, WII-ADA1354–1356) (Figs 3B–K, 4G–K) collected from the same locality (Fig. 1) and on the same date along with the holotype; WII-ADA1400, adult male collected from the same locality on 18 May 2022.

**Diagnosis.** A small sized rhacophorid frog with SVL 23.6–26.5 mm (n = 6) in adult males, body slender, snout pointed, head longer than wide, nostril closer to snout tip than eye, internasal distance shorter than inter upper eyelid distance, tympanum and supratympanic fold distinct, limbs slender, digits with large disc, circum-marginal groove on disc present, nuptial pad present on first finger in males, webbing absent between fingers, vomerine ridge absent, dorsal skin on snout, head, dorsum and limbs with spinules of different size, belly granular, dorsally chartreuse green with brown spots of irregular shape and size, upper eyelids dark brown, a broad brown stripe along canthus rostralis from anterior corner of the eye to tip of the snout covering the nostril, another brown stripe from posterior edge of the upper eyelid along supratympanic fold running dorso-laterally to one third of the trunk, white patches of irregular shape and size on the lateral side of the head below mid-eye, below tympanum in the mandibular region, continues along flank to groin, below skin semi-transparent, light green with large white patch covering

middle of throat, chest, axilla and anterior half of the belly, brown cross bars on fore-arm, thigh and tibia present.

**Description of holotype.** A small sized frog (SVL 24.5 mm) (Figs 3A & 4A–F); body slender; head longer than wide (HW/HL = 0.89); snout pointed in dorsal view and obtuse in profile, slightly projected beyond the lower jaw in ventral view; snout one third of the head length (SL/HL = 0.38) and shorter than eye length (SL/EL = 0.80); nostril small, laterally positioned, oval in shape, closer to tip of the snout than eye (NS/EN = 0.93); inter-nasal width smaller than inter-upper eyelid width (IN/IUE = 0.83) and smaller than upper eyelid width (IN/UEW = 0.89); upper eyelid width smaller than inter-upper eyelid width (UEW/IUE = 0.93); eyes comparatively large (EL/HL = 0.47); canthus rostralis distinct, sharp and vertical; loreal concave, tongue bifid and dorsal surface with tiny tubercles, vomerine teeth absent, choanae oval, tympanum distinct, oval in shape (HTYD/VTYD = 0.75), close to the eye (TE = 0.20 mm); supratympanic fold distinct; forelimbs slender, forearm shorter than hand length (FAL/HAL = 0.86); third finger is longest, relative finger length  $FIL < FIIL < FIVL < FIIL$ ; finger disc large and elliptical in shape, disc of the finger II, III and IV wider than horizontal tympanic diameter; disc with circum-marginal groove (Fig. 4H); terminal knuckle distinct; subarticular tubercles distinct, large and round, upper one on finger III and IV are larger than the lower one, subarticular tubercles FI = 1, FII = 1, FIII = 2, FIV = 2; webbing between fingers absent; hindlimbs slender; shank length more than half the snout-vent length (SHL/SVL = 0.57) and slightly longer than thigh length (TL/SHL = 0.94); foot length greater than tarsus length (TarL/FOL = 0.65); fourth toe is the longest, relative length among of the toes  $TIL < TIIL < TIIIL < TVL < TIVL$ ; toe discs relatively smaller than those of fingers, nearly round, with circum-marginal groove, subarticular tubercles large, round and distinct, the bottom subarticular tubercles on toe III, IV and V are smaller than the upper ones; webbing between toes small, I 1—1 ½ II ½ —2 III 1+—2 IV 2—½ V.

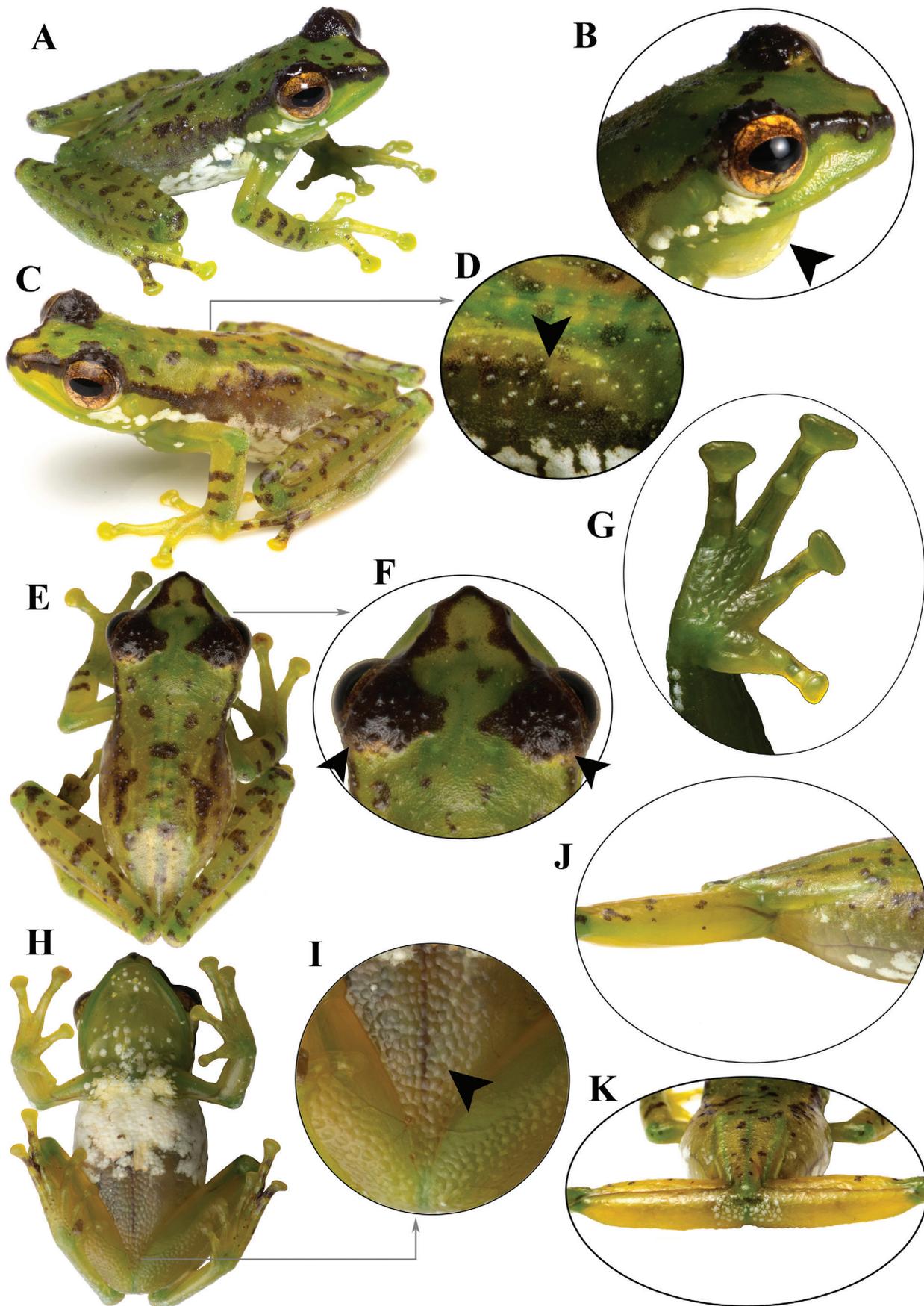
Dorsal skin on snout, head and back shagreened with dense spinules (Fig. 3D), those on upper eyelids, supratympanic fold and dorsolateral side of the back are slightly larger (Fig. 3F); lateral side of the head smooth, two flat rictal glands behind the angle of jaw in the mandibular region, tiny tubercles below supratympanic fold behind the tympanum; flank smooth; limbs dorsally smooth with scattered spinules; dermal fringe present along all fingers (Fig. 4E,G), palm with large granules, inner, middle and outer palmer tubercles present, fine granular nuptial pad present on first finger (Fig. 4I); flat granules along ventrolateral side of forearm and tarsus; ventrally throat smooth, chest nearly smooth, a few granules sparsely present; belly granular (Fig. 3H,I), those posteriorly more prominent, thighs granular at the basal half, distinct granules also present around the vent; tibia smooth; ventral side of the foot with many granules, supernumerary tubercles not visible; inner metatarsal tubercle distinct and elongated, outer metatarsal tubercle absent; dermal fringe along the toes present (Fig. 4D).

**Colouration in life.** Dorsally head, dorsum and limbs chartreuse green with brown spots of irregular shape and size (Fig. 3). Those brown spots on the forearm, thigh and tibia are in the form of short cross bars. Upper eyelids dark brown; a broad brown stripe originating from anterior corner of the eye to the snout tip, covering canthus rostralis and narial opening. Another brown stripe along the supratympanic fold which continues dorsolaterally to the middle of trunk; diffused posteriorly and broken. Head laterally green, white patches of irregular size and shape starting below mid eye to rear of mandible below the level of tympanum. Iris golden yellow with dense brown marbling. Digits slightly paler than dorsal colour or more yellowish, brown spots continue along the fourth digit of forelimb and fourth and fifth digits of hindlimb, webbing between fourth and fifth toe brown. Vent surrounded by brown marbling and large irregular white spots around it (Fig. 3K). White patches are present on the axilla, continues through flank to the groin, shape and size of the white patches are irregular on both sides. Ventrally green, similar as that of dorsal; white spots on gular region, a large white patch spreading over middle of the gular region, chest and anterior half of the belly (Fig. 3H). White marbling present along the ventrolateral side of the forearms. White marbling along the tarsus, along fifth toe and upper part of the groin. Small brown spots present on the ventral side of the fourth and fifth toe and web between them brown.

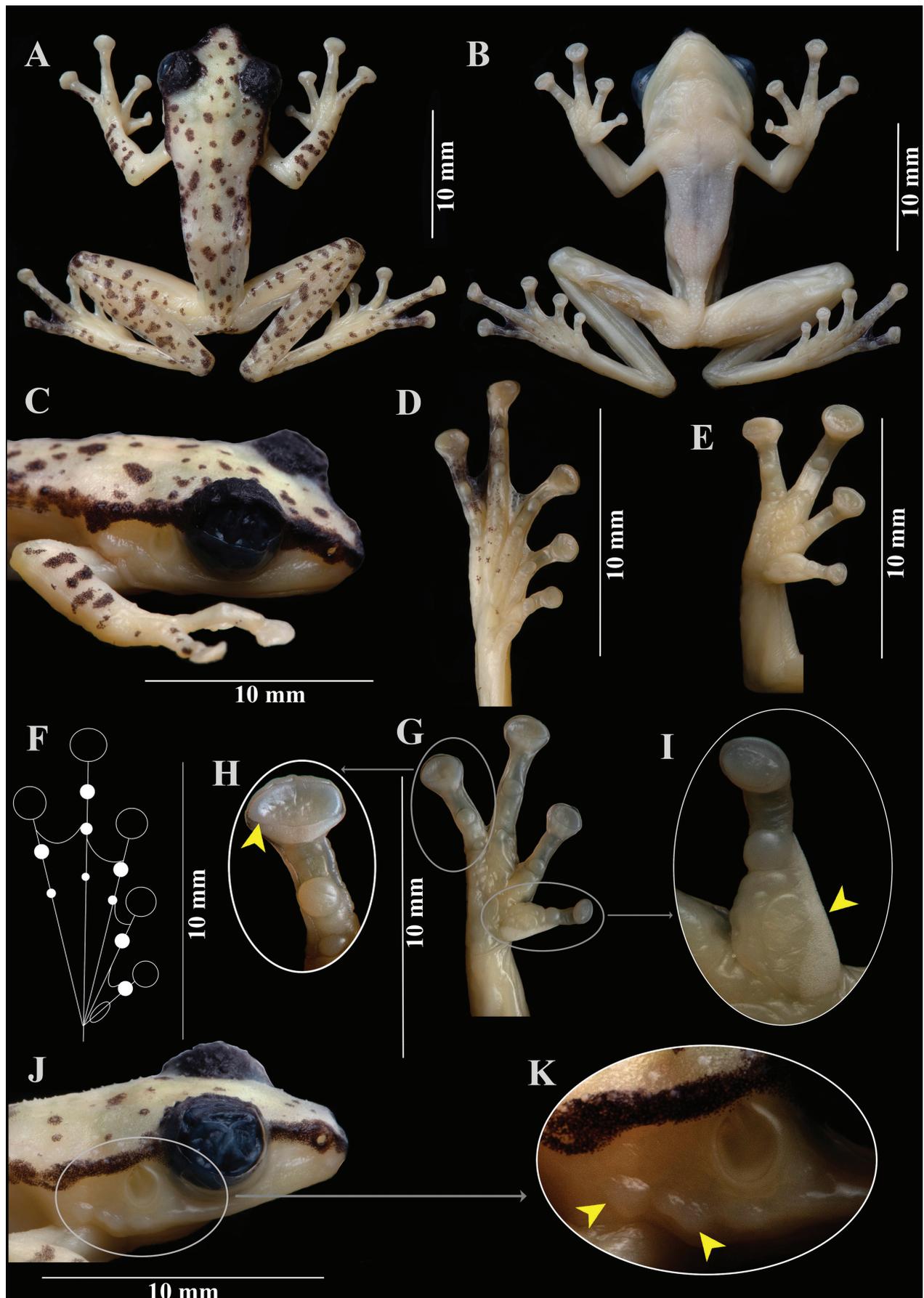
**Colouration in preservatives.** Dorsally head, dorsum and limbs creamy white with brown markings; white patches on lateral side of the head and on flank not visible; ventrally throat, chest belly and limbs pale creamy white, no white patches visible; brown markings on ventral side of the foot visible as in life (Fig. 4).

**Variation.** Measurements of the type series are given in Table 2. Dorsal colour slightly varied among the individuals. Number of brown spots and size on dorsal skin varied among the individuals (Appendix 2). Furthermore, the pattern and size of the white patches on the lateral and ventral side of head and body varied among the individuals.

**Morphological comparison.** Comparative mensural and meristic characters are given in Table 3. The new species *Gracixalus patkaiensis* sp. nov. differ from the species of the “Clade II” (*G. ananjevae* (Matsui & Orlov, 2004), *G. carinensis* (Boulenger, 1893), *G. guangdongensis* Wang, Zeng, Liu & Wang, 2018, *G. jinggangensis* Zeng et al., 2017, *G. jinxiuensis* (Hu, 1978), *G. nonggangensis* Mo et al., 2013, *G. sapaensis* Matsui, Ohler, Eto & Nguyen, 2017, *G. tianlinensis* Chen et al., 2018, *G. trieng* Rowley et al., 2020, *G. yunnanensis* Yu et al., 2019, *G. ziegleri* Le et al., 2021 by its chartreuse green dorsum and pointed snout (vs. brown dorsum and rounded snout) (sensu, Rowley et al., 2011). Furthermore, it differs by its small body size (SVL = 23.6–26.5 mm, n = 6) in adult males vs. large body size (SVL = 27.9–33.8 mm in *G. jinggangensis*, 29.9–35.3 mm in *G. nonggangensis*, 37.2–



**Figure 3.** *Gracixalus patkaiensis* sp. nov. in life. **A** Holotype (WII-ADA1353) showing dorsolateral view; **B** lateral view of the head (Paratype, WII-ADA1356) showing subgular vocal sac; **C–F** Paratype (WII-ADA1400): **C** dorsolateral view, **D** showing dorsal spinules, **E** dorsal view, **F** closeup of head showing spinules on upper eyelids; **G** ventral side of right hand (Paratype WII-ADA1352); **H** ventral side of the paratype (WII-ADA1400), **I** granular skin on belly and thigh; **J** showing groin of the paratype (WII-ADA1356); **K** showing the vent and lateral side of thigh (WII-ADA1356).



**Figure 4.** *Gracixalus patkaiensis* sp. nov. in preserved condition. **A–F** Holotype (WII-ADA1353): **A** dorsal view, **B** ventral view, **C** lateral view of the head, **D** ventral side of the right feet, **E** ventral side of the right hand, **F** schematic illustration of webbing on feet; **G** ventral view of the hand (Paratype, WII-ADA1400) showing circum-marginal groove and nuptial pad in **H** and **I** respectively; **J** lateral view of the head (Paratype, WII-ADA1352) showing the rictal glands in **K**.

**Table 2.** Morphometric measurements of *Gracixalus patkaiensis* sp. nov.

Voucher No.	WII- ADA1353	WII-ADA1356	WII-ADA1352	WII-ADA1355	WII-ADA1354	WII-ADA1400	Range	Mean ± SD
Status	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype		
Sex	Male	Male	Male	Male	Male	Male		
SVL	24.5	24	25.4	26.5	23.6	24.7	23.6–26.5	24.78 ±1.04
HW	8.3	8	8.3	8.7	7.8	8.4	7.8–8.7	8.25 ±0.31
HL	9.3	8.8	9.3	9.2	8	9.2	8.0–9.3	8.96 ±0.51
SL	3.5	3.6	3.5	3.7	3.2	3.5	3.2–3.7	3.5 ±0.17
EL	4.4	4.1	4	4	4.1	4.3	4.0–4.4	4.15 ±0.16
HTYD	1.2	1.1	1.2	1.3	1.2	1.2	1.1–1.3	1.2 ±0.06
VTYD	1.6	1.6	1.5	1.5	1.3	1.4	1.3–1.6	1.48 ±0.12
EN	1.5	1.6	1.6	1.8	1.4	1.8	1.4–1.8	1.62 ±0.16
NS	1.4	1.4	1.5	1.5	1.3	1.4	1.3–1.5	1.42 ±0.08
IUE	2.9	2.8	3.2	3.2	3.1	2.9	2.8–3.2	3.02 ±0.17
UEW	2.7	2.5	2.5	2.9	2.4	2.6	2.4–2.9	2.6 ±0.18
IN	2.4	2.6	2.5	2.6	2.4	2.4	2.4–2.6	2.48 ±0.1
MN	7.5	7.2	7.9	7.7	6.7	7.7	6.7–7.9	7.45 ±0.44
MAE	6.5	6	6.6	6	5.8	6.3	5.8–6.6	6.2 ±0.32
MPE	2.9	2.8	3	2.8	2.2	2.8	2.2–3.0	2.75 ±0.28
IFE	4.7	4.8	4.9	4.8	4.5	4.9	4.5–4.9	4.77 ±0.15
IBE	8.2	8.3	8	8.3	8	8	8.0–8.3	8.13 ±0.15
FAL	6.1	5.6	6.4	5.9	5.9	5.7	5.6–6.4	5.93 ±0.29
HAL	7.1	7.9	7.1	7.7	7.5	7.4	7.1–7.9	7.45 ±0.32
FIL	2.2	2.4	2	2.6	2.3	2.3	2.0–2.6	2.3 ±0.2
FIIL	2.9	3.1	2.7	3.2	3	2.9	2.7–3.2	2.97 ±0.18
FIIIL	4.5	5.1	4.7	5	5	4.7	4.5–5.1	4.83 ±0.23
FIVL	3.3	3.7	3.1	3.3	3.4	3.2	3.1–3.7	3.33 ±0.21
FID	0.9	0.9	0.7	0.9	0.7	0.8	0.7–0.9	0.82 ±0.1
FIID	1.5	1.5	1.2	1.6	1.1	1.2	1.1–1.6	1.35 ±0.21
FIIID	1.8	1.8	1.6	1.9	1.6	1.5	1.5–1.9	1.7 ±0.15
FIVD	1.6	1.8	1.6	1.7	1.4	1.5	1.4–1.8	1.6 ±0.14
AG	10.2	11.2	12.4	13.3	12	12	10.2–13.3	11.85 ±1.06
TL	13.1	12.4	13.6	13.8	13.2	13.4	12.4–13.8	13.25 ±0.49
SHL	14	12.8	14.1	14.6	13.4	13.9	12.8–14.6	13.8 ±0.62
Tar L	7.1	6.2	7.1	7.4	7.1	7.2	6.2–7.4	7.02 ±0.42
FOL	10.9	10.9	10.9	11.3	10.5	11	10.5–11.3	10.92 ±0.26
IMTL	1.2	0.9	1	1.3	0.8	1	0.8–1.3	1.03 ±0.19
TIL	1.7	2	1.6	1.8	1.6	1.9	1.6–2.0	1.77 ±0.16
TIIL	2.6	2.5	2.8	2.9	2	2.6	2.0–2.9	2.57 ±0.31
TIIIL	3.6	4.2	3.7	4.3	3.2	3.6	3.2–4.3	3.77 ±0.41
TIVL	6.1	6.1	5.8	7	6	5.9	5.8–7.0	6.15 ±0.43
TVL	4.3	4.3	4	4.6	3.8	3.8	3.8–4.6	4.13 ±0.32
TID	0.9	0.9	0.8	0.9	0.6	0.8	0.6–0.9	0.82 ±0.12
TIID	1.2	1	0.9	1.1	0.8	1.1	0.8–1.2	1.02 ±0.15
TIIID	1.3	1.3	1	1.4	1	1.1	1.0–1.4	1.18 ±0.17
TIVD	1.4	1.6	1.2	1.6	1.2	1.3	1.2–1.6	1.38 ±0.18
TVD	1.4	1.5	1.2	1.5	1.1	1.3	1.1–1.5	1.33 ±0.16

41.4 mm in *G. trieng*, 30.3–35.9 mm in *G. tianlinensis*, 28.1–30.5 mm in *G. ziegleri*, 32 mm in *G. ananjevae*).

Further the new species differs from *G. ananjevae* by its slender body (vs. robust body), head length greater than width (vs. head wider than its length), snout shorter than eye length (vs. snout length equal to eye length), snout pointed (vs. slightly pointed), webbing on fingers absent (vs. poorly developed web present), dorsal surface of legs with scattered spinules (vs. smooth), a large white patch on belly (vs. absent), dorsum with irregular brown

spots (vs. dorsum with a dark brown marking starting between eyes and bifurcating posteriorly).

*Gracixalus patkaiensis* sp. nov. differs from *G. carinensis* by pointed snout (vs. rounded snout), presence of spinules on dorsum (vs. absent), webbing on fingers absent (vs. rudimentary web present), white patches on lateral side of head, flank and belly present (vs. absent), x-shaped mark on dorsum absent (vs. present).

Differs from *G. guangdongensis* by its slender body (vs. robust body), spinules on eyelids present (vs. absent),

**Table 3.** Mensural and meristic information for *Gracixalus patkaiensis* **sp. nov.** and its congeners. Character keys: Adult male SVL; Dorsal color (green: 1, non-green (Brown/ yellow): 2) Snout (rounded: 0, pointed: 1); Dorsal tubercle/spinule (absent: 0, present: 1); Vocal sac (internal: 0, external: 1); Lateral marking (absent: 0, white patches present: 1, faint, large dark blotches: 2, brownish black spots present: 3, faint, small, black and white blotches scattered: 4); Skin along lateral Side (smooth: 0, smooth with sparsely distributed tubercles: 1, rough: 2, rough with tubercles: 3, large tubercles: 4, coarsely granular: 5); Skin on throat (smooth: 0, granular: 1); Finger web (absent: 0, rudimentary: 1); Tibio tarsal articulation (reaching between eye and nostril: 0, reaching eye: 1, reaching tip of snout: 2). ? indicates missing character.

Species	Adult male size (SVL)	Dorsal colour	Snout	Dorsal tubercle/spinule	Vocal sac	Lateral marking	Skin along lateral side	Skin on throat	Finger web	Tibio tarsal articulation
<i>G. patkaiensis</i> <b>sp. nov.</b>	23.6–26.5	1	1	1	1	1	0	0	0	2
<i>G. seesom</i>	21.6–23.0	2	1	0	?	1	4	0	1	0
<i>G. supercornutus</i>	22.0–24.1	1	1	1	?	1	?	1	?	?
<i>G. gracilipes</i>	20–24	1	1	1	0	1	0	0	0	0
<i>G. quangi</i>	21–24.5	1	1	1	1	3	2	0	0	?
<i>G. quyeti</i>	?	1&2	0	1	?	0	3	0	1	2
<i>G. lumarius</i>	38.9–41.6	2	0	1	1	0	?	1	1	?
<i>G. ananjevae</i>	32	?	1	1	?	0	5	0	1	1
<i>G. carinensis</i>	30.2–38.1	2	0	1	0	?	?	1	1	1
<i>G. guangdongensis</i>	26.1–34.7	2	1	1	?	4	3	1	1	1
<i>G. jinggangensis</i>	27.9–33.8	2	1	1	?	2	3	1	1	1
<i>G. jinxiuensis</i>	23.5–26.3	2	0	?	0	0	3	1	1	1
<i>G. medogensis</i>	26.5	1	0	0	0	0	?	1	0	1
<i>G. nonggangensis</i>	29.9–35.3	2	0	0	0	0	3	1	0	2
<i>G. sapaensis</i>	21–37	2	0	0	?	0	4	?	1	1
<i>G. tianlinensis</i>	30.3–35.9	2	0	1	1	0	?	1	0	?
<i>G. trieng</i>	37.2–41.4	2	0	0	1	0	1	1	?	?
<i>G. truongi</i>	32.2–33.1	1	0	0	0	3	5	0	1	0
<i>G. yunnanensis</i>	26.0–34.2	2	0	1	1	0	0	1	1	1
<i>G. zieglerei</i>	28.1–30.5	2	?	0	0	?	1	?	?	?

absence of inverse Y-shaped dark brown marking on dorsum (vs. present), lateral surface of head smooth (vs. tubercular), snout length smaller than eye length (vs. snout length greater than eye length), white patches on lateral side of head, flank and belly present (vs. absent).

Differs from *G. jinggangensis* by head length greater than width (vs. head wider than its length), presence of spinules on eyelids and dorsum (vs. absent), absence of inverse Y-shaped dark brown marking on dorsum (vs. present), nuptial pad on second finger in males absent (vs. present), white patches on lateral side of head, flank and belly present (vs. absent).

Differs from *G. jinxiuensis* by the presence of spinules on dorsum (vs. scattered tubercles present), white patches on throat, chest and belly present (vs. absent).

Differs from *G. medogensis* (Ye & Hu, 1984) by head width smaller than its length (vs. head width greater than its length), snout pointed (vs. rounded), presence of spinules on dorsum (vs. dorsum smooth), irregular brown spots present on dorsum (vs. an inverse “V” shaped mark present on dorsum), white patches on lateral side of head, flank and belly present (vs. absent).

Differs from *G. nonggangensis* by snout length smaller than eye length (vs. snout longer than eye diameter), dorsally chartreuse green (vs. yellowish-olive), dark brown spots on throat and belly absent (vs. present), white patches on lateral side of head, flank and belly present (vs. absent).

Differs from *G. sapaensis* by the presence of distinct tympanum (vs. indistinct), single external vocal sac present (vs. paired vocal sac present), dorsum with spinules (vs. nearly smooth), white patches on throat, chest and belly present (vs. absent).

Differs from *G. tianlinensis* by its slender body (vs. robust), head length greater than head width (vs. head length less than width), Y-shaped dark brown marking on dorsum absent (vs. present), nuptial pad on second finger in males absent (vs. present).

*Gracixalus patkaiensis* **sp. nov.** differ from *G. trieng* by the presence of spinules on dorsal surface of head, back and limbs (vs. absent), distinct and broad cross bars on limbs absent (vs. present), interorbital crossbar absent (vs. present), brown spots of irregular size and shape present on dorsal surface of head, back and limbs (vs. inverse Y-shaped marking present on dorsum), white patches on lateral side of head, flank and belly present (vs. absent).

Differs from *G. yunnanensis* by slender body (vs. robust), inverted Y-shaped dark brown marking on dorsum absent (vs. present), white patches on lateral side of head, flank and belly present (vs. absent).

Differs from *G. zieglerei* by head length larger than width (vs. head wider than long), spinules present on upper eyelids (vs. absent), inverse Y-shaped dark brown marking on dorsum absent (vs. present).

Differs from *G. truongi* Tran et al., 2023 by smaller body size of males, SVL = 23.6–26.5 mm (vs. SVL =

**Table 4.** Measurements of advertisement calls of *Gracixalus* species.

Species	Temp. (°C)	Call duration (ms)	Number of notes	Duration of long notes/whistle (ms)	Duration of click notes (ms)	Dominant frequency (kHz)	Reference
<i>Gracixalus patkaiensis</i> sp. nov.	22.8	—	—	45–229	7–65	3.1–4.61	This study
<i>G. guangdongensis</i>	17–21	500–650	2	360–470	40–50	2.4–4	Wang et al. 2018
<i>G. jinggangensis</i>	17.6	447–620	3–4	147–219	42–67	2.6	Zeng et al. 2017
<i>G. nonggangensis</i>	17	15060	40–72	490	120	2.2	Wang et al. 2018
<i>G. tianlinensis</i>	18	—	1	—	—	2–3	Chen et al. 2018
<i>G. gracilipes</i>	7.3–18.1	—	—	150–250	6–20	4.1–5.1	Rowley et al. 2015
<i>G. quangi</i>	24.1	—	—	370	10	4.1–4.7	Rowley et al. 2014
<i>G. supercornutus</i>	22.6–23.7	—	—	~370	6–9	3.6–4.1	Rowley et al. 2015

32.2–33.1 mm), snout pointed (vs. rounded), presence of spinules on dorsum (vs. dorsum smooth), presence of spinules on upper eyelids (vs. absent), head longer than width (vs. head wider than its length), inverse Y-shaped dark brown marking on dorsum absent (vs. present), white patches on lateral side of head and flank present (vs. absent).

The new species differs from species with greenish dorsum of the “Clade I” (sensu Rowley et al. 2011) as follows.

*Gracixalus patkaiensis* sp. nov. differs from *G. seesom* Matsui et al., 2015 by the presence of spinules on dorsal surface of head and back (vs. smooth dorsum), snout length smaller than eye length (vs. snout longer than eye diameter), upper eyelids dark brown (vs. upper eyelids without dark patch), tubercles on flank absent (vs. large tubercles present on flank), white patches on lateral side of head present (vs. absent).

Differs from *Gracixalus quangi* Rowley et al., 2011 by snout length smaller than eye length (vs. snout length larger than eye length), head length greater than width (vs. head length and width equal), the absence of tibio-tarsal projection (vs. present), serrated dermal fringe along forearm absent (vs. present), brown interorbital cross bar and X-shaped marking on dorsum absent (vs. present), brownish black spots on the flank and ventral surface of thighs absent (vs. present), white patches below the eye and mandibular region below the level of tympanum (vs. large opaque, pale turquoise patch under the supratympanic fold and eyes), webbing between the fourth and fifth toes brown (vs. all webbing, dorsal and ventral surface of the foot black).

Differs from *G. quyeti* (Nguyen et al., 2008) by snout pointed (vs. rounded), dorsal colour chartreuse green with brown spots (vs. mossy green without brown spots), white patches below the eyes, flank and belly present (vs. absent).

Differs from *G. supercornutus* (Orlov et al., 2004) by absence of large horn-like projections on upper eyelids, dorsum and along forearm and tarsus (vs. present), broad brown interorbital crossbar and dorsolateral stripes on dorsum absent (vs. present).

*Gracixalus patkaiensis* sp. nov. is morphologically more close to *G. gracilipes* (Bourret, 1937). However, it differs from *G. gracilipes* by genetic divergence of

4.0–5.0% and by the following morphological characteristics. The new species differ from *G. gracilipes* by the absence of interorbital cross bar and distinct X-shaped marking on dorsum (vs. present; Bourret 1942; Orlov et al. 2004, fig. 17; Delorme et al. 2005, fig. 7; Rowley et al. 2011, fig. 8D; Calphotos: Benjamin Tapley/ZSL), webbing between fourth and fifth toe brown (vs. all webbing on brown; Rowley et al., 2011, fig. 8D), a few scattered brown spots on fourth and fifth toe (large brown patch covering fourth and fifth toe; Rowley et al., 2011, fig. 8D), tympanic region green (vs. tympanic region including tympanum may be brown; Rowley et al., 2011, fig. 8D; Calphotos: Benjamin Tapley/ZSL).

Ecologically *G. gracilipes* is distributed in mid elevations between ~1,200–2,500 m a.s.l. (Bain & Nguyen 2004; Fellowes & Hau 1997; Orlov et al. 2004; Frost 2022) while the new species is known only from lowland evergreen forest at an elevation of 648 m a.s.l.

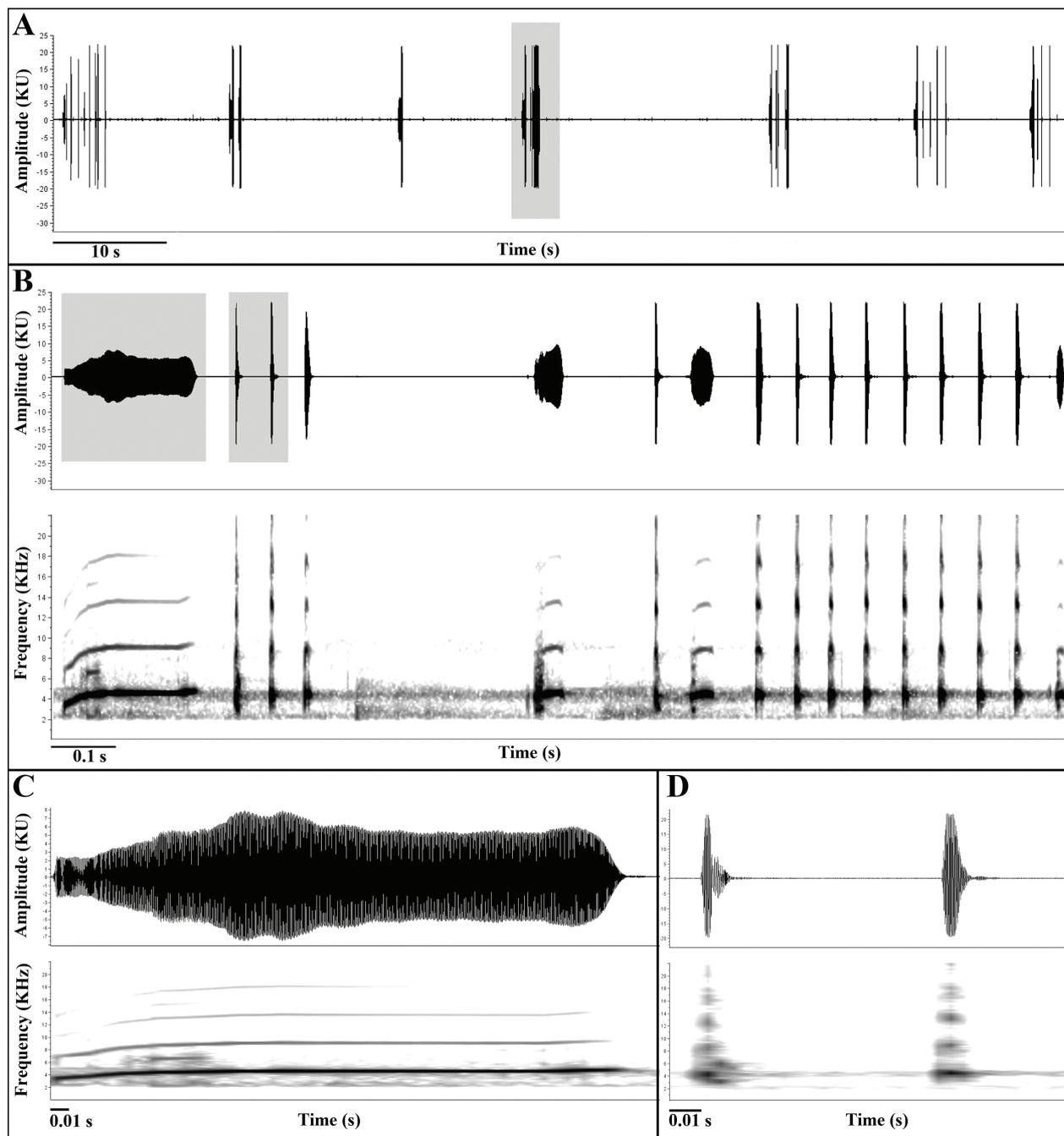
*Gracixalus patkaiensis* sp. nov. differs from *G. lumarius* Rowley et al., 2014 the only species with brown or yellow dorsum of the clade I of Rowley et al. (2011) by smaller body size, SVL = 23.6–26.5 mm (n = 6) in adult males (vs. SVL 38.9–41.6 mm), pointed snout (vs. rounded snout), dorsal surface of hindlimbs with scattered spinules (vs. smooth), spinules absent on lateral side of the head (vs. present), tympanum and supratympanic fold distinct (vs. indistinct), white patches on lateral side of head, flank and on ventrum present (vs. absent).

**Etymology.** We named the species after Patkai hills range where the type locality of the new species lies within Namdapha Tiger Reserve. The specific epithet “*patkaiensis*” is a noun in apposition.

**Suggested Common Name.** Patkai green tree frog.

**Distribution.** Currently the species is only known from a single locality in Kamala Valley Beat, popularly known as 25 mile in the Namdapha Tiger Reserve. It may occur in similar microhabitats in the forest interior of the tiger reserve.

**Acoustics.** Advertisement call of *Gracixalus patkaiensis* sp. nov. consists of highly variable non-stereotyped and non-pulsatile call types, ranging from a relatively lon-

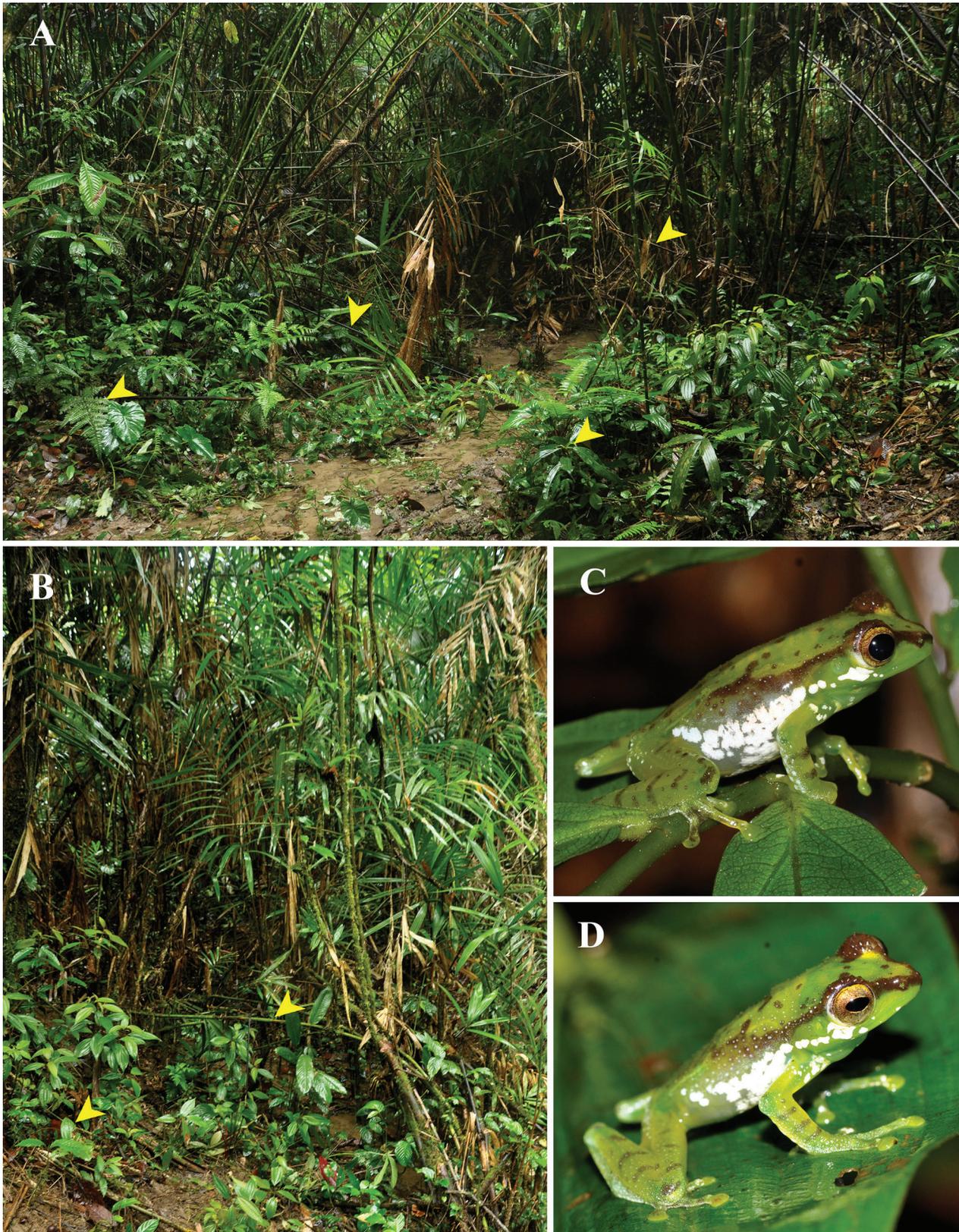


**Figure 5.** Waveforms and spectrograms of advertisement calls of paratype (WII-ADA1400) of *Gracixalus patkaiensis* sp. nov. at ambient air temperature 22.8°C. **A** waveform of relative amplitude over time of call sequence; **B** representative of calls of a call group (depicted with grey rectangle in A) shown as waveform above and spectrogram below; **C** representative of a whistle (depicted with grey rectangle in B) shown as waveform above and spectrogram below; **D** two clicks (depicted with grey rectangle in B) shown as waveform above and spectrogram below.

ger call “whistle” with narrow frequency bands to short call “click” (Fig. 5) with wide frequency band. Number of calls among the call groups varied between 3–16 ( $n = 16$ ). Whistles were variable in structure within the call group or among the call groups although clicks are broadly similar in structure. At an ambient temperature 22.8°C, duration of the whistles varied from 45–229 ms ( $123.26 \pm 67.97$  ms,  $n = 23$ ) and duration of the clicks ranged from 7–65 ms ( $16.95 \pm 10.65$  ms,  $n = 108$ ). Call repetition rate varied 1.15–18.87 calls/minute ( $8.14 \pm 6.88$  calls/minute,  $n = 17$ ). Inter-call intervals ranged from 5–1274 ms

( $160.37 \pm 231.55$  ms,  $n = 115$ ). Fundamental or dominant frequency of the whistles ranged between 4.35 kHz to 4.61 kHz ( $4.51 \pm 0.06$  kHz,  $n = 22$ ) and dominant frequency of the clicks ranged from 3.1 kHz to 4.61 kHz ( $4.22 \pm 0.27$  kHz,  $n = 108$ ). Clear harmonics were visible only in the whistles. Temporal order of the call types was not constant. A comparative account of acoustic characters of *Gracixalus* species is given in Table 4.

**Natural history.** We came across a calling aggregation of the new species on 14 May, 2022 between 17:30 hrs



**Figure 6.** Habitat of *Gracixalus patkaiensis* sp. nov. **A, B** yellow arrow marks indicate the representative perch of the species; **C** and **D** two uncollected individuals of *G. patkaiensis* sp. nov. in natural habitat.

and 19:00 hrs along a forest trail. The forest tree layer in the habitat was dominated by *Dipterocarpus retusus* and the shrub layer by *Bambusa* sp. and *Calamus* sp. (Fig. 6). The 10x10 m site in the area was characterized by a canopy cover of ~70% and clayey soil, with a first-or-

der stream associated with a marshy habitat. The area also had a few large fallen logs in an advanced decaying stage. The male individuals of the species were observed calling from leaves and twigs of shrub, fern leaf sheaths, Zingiber leaves, lamina and rachis of rattan palms (*Cal-*

amus sp.) at a height of ~ 0.5–2 m above the ground or shallow water. Other frog species present in the area were *Limnonectes* sp., *Kurixalus* sp., *Raorchestes* sp. *Rhacophorus rhodopus* Liu & Hu, 1960, *Xenophrys ancræa* (Mahony, Teeling & Biju, 2013), *Microhyla eos* Biju, Garg, Kamei & Maheswaran, 2019 and *Duttaphrynus* sp. The pool in the spot harboured the tadpoles of *Microhyla* sp. and *Rhacophorus rhodopus*. Adult frogs were active only during rainy nights. Air temperature and relative humidity were recorded as 22.7°C and 93.8% respectively at the time of observations. During our next visit to this site in September 2022 no breeding activity was observed in the area nor any individual was encountered in the spot.

## Discussion

We report the genus *Gracixalus* for the first time from India and describe a new species. Phylogenetically and morphologically *Gracixalus patkaiensis* **sp. nov.** is similar to other members in clade I (sensu Rowley et al. 2011). However, the phylogenetic relationship of the members of the genus *Gracixalus* is incomplete and not well resolved (Rowley et al. 2014; Matsui et al. 2015; Zeng et al. 2017; Yu et al. 2019). Further morphological revision of the members of this genus is required as indicated in the earlier studies by Mo et al. (2013), Nguyen et al. (2013), Rowley et al. (2014, 2020), Matsui et al. (2015), Wang et al. (2018). The generic placement of two species, *G. medogensis* and *G. carinensis* solely based on morphology remain doubtful (Matsui et al. 2015) as the genetic data of the two species from the type localities are not available. Although Che et al. (2020) showed *G. medogensis* phylogenetically as a member of the clade II (with brown dorsum and rounded snout) but the description and photographs provided in the original description is in contrast (Ye and Hu 1984). As per the original description *G. medogensis* (as *Philautus medogensis*) is green in colour which is also followed by Fei et al. (1999). However, in the description by Che et al. (2020), the dorsal colouration of *G. medogensis* is brown. Although *G. medogensis* was described from close geographical proximity to that of *G. patkaiensis* **sp. nov.**, it differs from the former species by distinct morphological characters as mentioned above.

The clade I (sensu Rowley et al. 2011) of the genus *Gracixalus*, to which the new species belongs, is a group of small rhacophorid frogs adapted to relatively low to high elevation (600–2,500 m a.s.l.), montane evergreen forests in northern and central Vietnam, southern China, Laos and Thailand (Frost 2022). *Gracixalus gracilipes* is reported at the elevation range ~1,200–2,500 m a.s.l. (Bain and Nguyen 2004; Fellowes and Hau 1997; Orlov et al. 2002; Frost 2022); *G. seesom* is known from the elevation range 942–1650 m a.s.l. (Matsui et al. 2015); *G. supercornutus* is known from central Vietnam, at elevation range 1,000–1,905 m a.s.l. (Orlov et al. 2004;

Rowley et al. 2011, Frost 2022); *G. quyeti* is known from central Vietnam at an elevation range 430–1,100 m a.s.l.; *G. quang* is reported at ~600–1,300 m (Rowley et al. 2011). The new species *Gracixalus patkaiensis* **sp. nov.** so far only known from low elevation at 648 m a.s.l.

The type locality of the new species falls within the Patkai hills of the Indo-Myanmar hill ranges extending northeast to southwest which is contiguous with the Naga hills-Chin hills in the middle and Arakan Yoma in the south (Valdiya 2015). Namdapha- Kamlang landscape is a part of the Far-Eastern Himalaya Landscape (sensu Basnet et al. 2019) and regarded as one of the most intact and biologically rich landscape (71,400 sq. km), yet poorly known to conservationists and policy makers due to low priority in research, inaccessibility and remoteness. This region has a shared faunal element and is geographically unique being northernmost limit of tropical rain forest in the world (Proctor et al. 1998). This region provides a range of interesting habitats from the lowland hollong-mekai dipterocarp forest to alpine meadows. Compared to mammals and birds, this region is least explored for herpetofauna and thus there is a huge scope for new species discovery.

With the addition of *Gracixalus patkaiensis* **sp. nov.**, six species of anurans are currently described from the Namdapha Tiger Reserve viz. *Philautus namdaphaensis* Sarkar & Sanyal, 1985, *Raorchestes sahai* Sarkar & Ray, 2006, *Rohanixalus shyamrupus* (Chanda & Ghosh, 1989), *Microhyla eos* and *Xenophrys ancræa*.

All the individuals of *Gracixalus patkaiensis* **sp. nov.** that we observed in its natural habitats have variable patterns on the flank region which is characterised by white patches of irregular shape and size. Thus, the population of this typical forest species could potentially be monitored using non-invasive mark-recapture techniques as described in Patel and Das (2020).. Although we have described the new species' advertisement call; other aspects such as breeding behaviour, oviposition site and habitat use of this interesting rhacophorid species need to be studied in future. Protected areas are the cornerstone of wildlife conservation and tiger reserves perhaps are at the top of conservation priority. However, for better management of these protected areas, one needs a detailed inventory of species. The discovery of a unique new species might serve as an example for other protected areas, especially the least explored ones along the eastern borderland of India. Apart from this such research can help in transboundary conservation initiatives and future recognition of the area as a UNESCO world heritage site.

## Acknowledgements

We thank the National Geographic Society for the award of National Geographic Explorer Grant (NGS-74044R-20) and SERB-DST (CRG/2018/000790) for financial support. We are much obliged to the Arunachal Pradesh Forest Department (APFD) for the research permit (CWL/GEN/355/2021/3178 dated 28th September 2021). Thanks, are also due to Shri Aduk Paron (Field Director), Mayur Variya (Biologist) and all the forest staff of Namdapha Tiger reserve for providing logis-

tic support. Special thanks to Shri Milo Taser of APFD for supporting our research. AD would like to thank the Director and Dean, Wildlife Institute of India, Dehradun for their constant support. Special thanks to Indraneil Das (Sarawak, Malaysia) and S. K. Dutta (Odisha, India) for their constant support and guidance. We thank Surya Narayanan (ATREE) for the help with laboratory work. VD's contribution was supported in part by the Humboldt fellowship hosted by Uwe Fritz at the Senckenberg Dresden. VD thanks David Gower, Jeff Streicher and Patrick Campbell for their support at NHM, London during his visit to examine the types of *Gracixalus carinensis*. We thank Santanu Dey and Aphu Yoha Yobin for their help during fieldwork. We thank Sandeep Das and Robin Suyesh for their comments on the previous draft of this manuscript.

## References

- Bain RH, Nguyen QT (2004) Herpetofaunal diversity of Ha Giang Province in Northeastern Vietnam, with descriptions of two new species. *American Museum Novitates* 3453: 1–42.
- Basnet D, Kandel P, Chettri N, Yang Y, Lodhi MS, Htun NZ, Uddin K, Sharma E (2019) Biodiversity research trends and gaps from the confluence of three global biodiversity hotspots in the far-eastern Himalaya. *International Journal of Ecology* 2019: 1–14.
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. *Nucleic Acids Research* 35 (Supplement1): D21–D25.
- Biju SD, Garg S, Mahony S, Wijayathilaka N, Senevirathne G, Mee-gaskumbura M (2014) DNA barcoding, phylogeny and systematics of golden-backed frogs (*Hylarana*, Ranidae) of the Western Ghats-Sri Lanka biodiversity hotspot, with the description of seven new species. *Contributions to Zoology* 83: 269–S4. <https://doi.org/10.1163/18759866-08304004>
- Biju SD, Garg S, Kamei RG, Maheswaran G (2019) A new *Microhylla* species (Anura: Microhylidae) from riparian evergreen forest in the eastern Himalayan state of Arunachal Pradesh, India. *Zootaxa* 4674: 100–116. <https://doi.org/10.11646/zootaxa.4674.1.5>
- Bioacoustics Research Program (2014) Raven Pro: Interactive sound analysis software. Version 1.5. Ithaca, New York: The Cornell Lab of Ornithology. <https://ravensoundsoftware.com/software/raven-pro> [Accessed 18 November 2022].
- Boulenger GA (1893) Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea, dealing with the collection made in Pegu and the Karin Hills in 1887–88. *Annali del Museo Civico di Storia Naturale di Genova* 13: 304–347.
- Bourret R (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze espèces ou variétés nouvelles. *Annexe au Bulletin Général de l'Instruction Publique* 1937: 5–56.
- Chanda SK, Ghosh AK (1989) A new frog of the genus *Philautus* Gistel, from the proposed Namdapha Biosphere Reserve, Arunachal Pradesh, northeast India. *Journal of the Bombay Natural History Society* 86: 215–217.
- Chen W, Bei Y, Liao X, Zhou S, Mo Y (2018) A new species of *Gracixalus* (Anura: Rhacophoridae) from West Guangxi, China. *Asian Herpetological Research* 9: 74–84. <https://doi.org/10.16373/j.cnki.ahr.170085>
- Che J, Jiang K, Yan F, Zhang Y-P (2020) *Amphibians and Reptiles of Tibet – Diversity and Evolution*. Science Press, Beijing [in Chinese].
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772–772. <https://doi.org/10.1038/nmeth.2109>
- Das A, Garg S, Hamidy A, Smith EN, Biju SD (2019) A new species of *Micryletta* frog (Microhylidae) from Northeast India. *PeerJ* 7: e7012. <https://doi.org/10.7717/peerj.7012>
- Delorme M, Dubois A, Grosjean S, Ohler A (2005) Une nouvelle classification générique et subgénérique de la tribu des Philautini (Amphibia, Anura, Rhacophoridae). *Bulletin Mensuel de la Société Linnéenne de Lyon* 74: 165–171.
- Fei L, Ye CY, Huang YZ, Liu MY (1999) *Atlas of Amphibians of China*. Henan Science and Technology Press, Henan.
- Fellowes JR, Hau CH (1997) A Faunal Survey of Nine Forest Reserves in Tropical South China, with a Review of Conservation Priorities in the Region. Kadoorie Farm and Botanic Garden, Hong Kong, 151 pp.
- Frost DR (2023) *Amphibian species of the world: An online reference*. Version 6.1. <https://amphibiansoftheworld.amnh.org> (accessed 31 March 2023)
- Haas A, Hertwig ST, Krings W, Braskamp E, Dehling JM, Min PY, Jan-kowski A, Schweizer M, Das I (2012) Description of three *Rhacophorus* tadpoles (Lissamphibia: Anura: Rhacophoridae) from Sarawak, Malaysia (Borneo). *Zootaxa* 3328: 1–19.
- Higgins D, Thompson J, Gibson T, Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>
- Hu SQ, Fei L, Ye CY (1978) Three new amphibian species in China. *Materials for Herpetological Research* 4: 20.
- Kalyanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>
- Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel M-O, Vences M (2017) The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251: 1–124. <https://doi.org/10.11646/zootaxa.4251.1.1>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Le DT, Do YT, Tran TT, Nguyen TQ, Orlov NL, Ninh HT, Nguyen TT (2021) A new species of *Gracixalus* (Anura: Rhacophoridae) from northern Vietnam. *Russian Journal of Herpetology* 28: 111–122. <https://doi.org/10.30906/1026-2296-2021-28-3-111-122>
- Liu CC, Hu SQ (1960) Preliminary report of Amphibia from southern Yunnan. *Acta Zoologica Sinica* 11: 509–533.
- Mahony S, Teeling EC, Biju SD (2013) Three new species of horned frogs, *Megophrys* (Amphibia: Megophryidae), from northeast India, with a resolution to the identity of *Megophrys boettgeri* populations reported from the region. *Zootaxa* 3722: 143–169. <http://dx.doi.org/10.11646/zootaxa.3722.2.2>
- Matsui M, Orlov NL (2004) A new species of *Chirixalus* from Vietnam (Anura: Rhacophoridae). *Zoological Science* 21: 671–676. <https://doi.org/10.2108/zsj.21.671>
- Matsui M, Khonsue W, Panha S, Eto K (2015) A new tree frog of the genus *Gracixalus* from Thailand (Amphibia: Rhacophoridae). *Zoological Science* 32: 204–210. <https://doi.org/10.2108/zs140238>

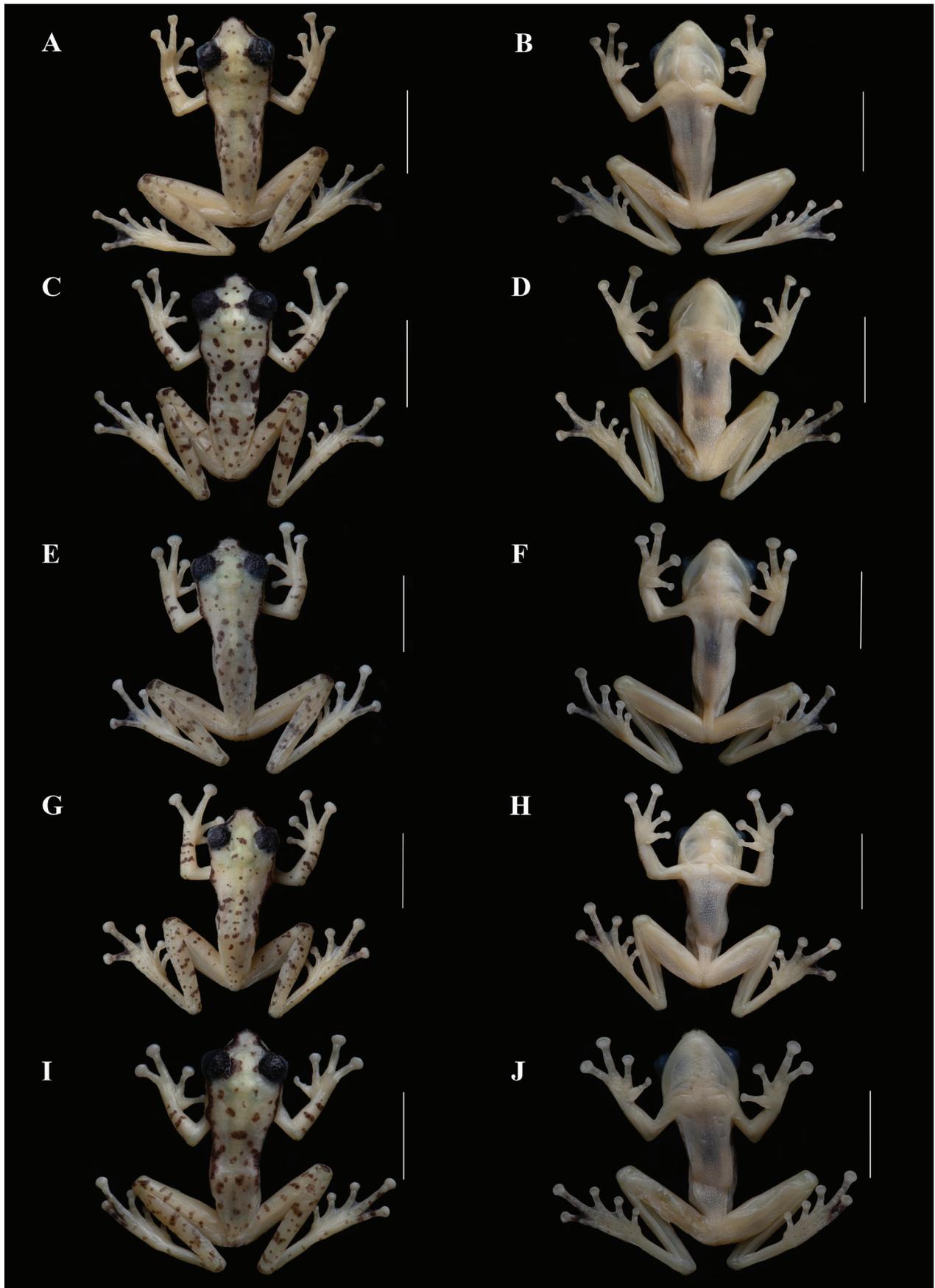
- Matsui M, Ohler A, Eto K, Tao NT (2017) Distinction of *Gracixalus carinensis* from Vietnam and Myanmar, with description of a new species. *Alytes* 33: 25–37.
- Meegaskumbura M, Bossuyt F, Pethiyagoda R, Manamendra-Arachchi K, Bahir M, Milinkovitch MC, Schneider CJ (2002) Sri Lanka: An amphibian hot spot. *Science* 298: 379–379.
- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Mo Y, Zhang W, Luo Y, Zhou S, Chen W (2013) A new species of the genus *Gracixalus* (Amphibia: Anura: Rhacophoridae) from Southern Guangxi, China. *Zootaxa* 3616: 61–72. <http://dx.doi.org/10.11646/zootaxa.3616.1.5>
- Nguyen TQ, Hendrix R, Boehme W, Thanh VN, Ziegler T (2008) A new species of the genus *Philautus* (Amphibia: Anura: Rhacophoridae) from the Truong Son Range, Quang Binh Province, central Vietnam. *Zootaxa* 1925: 1–13. <https://doi.org/10.11646/zootaxa.1925.1.1>
- Nguyen TQ, Le MD, Pham CT, Nguyen TT, Bonkowski M, Ziegler T (2013) A new species of *Gracixalus* (Amphibia: Anura: Rhacophoridae) from northern Vietnam. *Organisms Diversity & Evolution* 13: 203–214. <https://doi.org/10.1007/s13127-012-0116-0>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating Maximum-Likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274. <https://doi.org/10.1093/molbev/msu300>
- Orlov NL, Ho TC, Nguyen QT (2004) A new species of the genus *Philautus* from central Vietnam (Anura: Rhacophoridae). *Russian Journal of Herpetology* 11: 51–64. <https://doi.org/10.30906/1026-2296-2005-12-2-135-142>
- Palumbi SR, Martin AP, Romano SL, McMillan WO, Stice L, Grabowski G (1991) The Simple Fool's Guide to PCR. Version 2. University of Hawaii, Honolulu, HI, 43 pp.
- Patel NG, Das A (2020) Shot the spots: A reliable field method for individual identification of *Amolops formosus* (Anura, Ranidae). *Herpetozoa* 33: 7–15. <https://doi.org/10.3897/herpetozoa.33.e47279>
- Proctor J, Haridasan K, Smith GW (1998) How far north does lowland evergreen tropical rain forest go? *Global Ecology and Biogeography Letters* 7: 141–146.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Ronquist F, Teslenko M, Mark VDP, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rowley JLL, Dau QV, Nguyen TT, Cao TT, Nguyen SV (2011) A new species of *Gracixalus* (Anura: Rhacophoridae) with a hyperextended vocal repertoire from Vietnam. *Zootaxa* 3125: 22–38. <https://doi.org/10.11646/zootaxa.3125.1.2>
- Rowley JLL, Le DT, Dau VQ, Hoang HD, Cao TT (2014) A striking new species of phytotelm-breeding tree frog (Anura: Rhacophoridae) from central Vietnam. *Zootaxa* 3785: 25–37. <http://dx.doi.org/10.11646/zootaxa.3785.1.2>
- Rowley J, Dau VQ, Hoang HD, Nguyen TT, Le DTT, Altig R (2015) The breeding biologies of three species of treefrogs with hyperextended vocal repertoires (*Gracixalus*: Anura: Rhacophoridae). *Amphibia-Reptilia* 36: 277–285.
- Rowley JLL, Le DT, Hoang HD, Cao TT, Dau VQ (2020) A new species of phytotelm breeding frog (Anura: Rhacophoridae) from the Central Highlands of Vietnam. *Zootaxa* 4779: 341–354. <https://doi.org/10.11646/zootaxa.4779.3.3>
- Sarkar AK, Ray S (2006) Amphibia. In: Alfred JRB (Ed.) Fauna of Arunachal Pradesh, State Fauna Series, 13 (Part-1). Zoological Survey of India, Kolkata, 285–316.
- Sarkar AK, Sanyal DP (1985) Amphibia. Records of the zoological Survey of India 82: 285–295.
- Tran TT, Van Pham A, Le M D, Nguyen NH, Ziegler T (2023) A new species of *Gracixalus* (Anura, Rhacophoridae) from northwestern Vietnam. *ZooKeys* 1153: 15–35. <https://doi.org/10.3897/zookeys.1153.93566>
- Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: 232–235. <https://doi.org/10.1093/nar/gkw256>
- Valdiya KS (2015) The making of India: Geodynamic evolution. Springer, Cham, 924 pp. <https://doi.org/10.1007/978-3-319-25029-8>
- Wang J, Zeng ZC, Lyu ZT, Liu ZY, Wang YY (2018) Description of a new species of *Gracixalus* (Amphibia: Anura: Rhacophoridae) from Guangdong Province, southeastern China. *Zootaxa* 4420: 251–269. <https://doi.org/10.11646/zootaxa.4420.2.7>
- Wu SP, Huang CC, Tsai CL, Lin TE, Jhang JJ, Wu SH (2016) Systematic revision of the Taiwanese genus *Kurixalus* members with a description of two new endemic species (Anura, Rhacophoridae). *ZooKeys* 557: 121–153. <https://doi.org/10.3897/zookeys.557.6131>
- Ye C-Y, Hu S-Q (1984) A new species of *Philautus* (Anura: Rhacophoridae) from Xizang Autonomous Region. *Acta Herpetologica Sinica* 3: 67–69.
- Yu G, Hui H, Wang J, Rao D, Wu Z, Yang J (2019) A new species of *Gracixalus* (Anura, Rhacophoridae) from Yunnan, China. *ZooKeys* 851: 91–111. <https://doi.org/10.3897/zookeys.851.32157>
- Zeng ZC, Zhao J, Chen CQ, Chen GL, Zhang Z, Wang YY (2017) A new species of the genus *Gracixalus* (Amphibia: Anura: Rhacophoridae) from Mount Jinggang, southeastern China. *Zootaxa* 4250: 171–185. <https://doi.org/10.11646/zootaxa.4250.2.3>

## Appendix 1

Details of the GenBank sequences (16S rRNA) used in this study.

Species	Voucher no.	Accession no.	Locality	Reference
<i>G. cf. ananjevae</i>	VNMN 03012	JN862546	Nghe An Province, Vietnam	Rowley et al. 2011
<i>G. patkaiensis</i> sp. nov.	WII-ADA1352	OQ940026	Kamala Valley beat, Namdapha TR, Changlang dist., Arunachal Pradesh, India	<b>This study</b>
<i>G. patkaiensis</i> sp. nov.	WII-ADA1353	OQ940027	Kamala Valley beat, Namdapha TR, Changlang dist., Arunachal Pradesh, India	<b>This study</b>
<i>G. carinensis</i>	KUHE 46401	LC011938	Lao Cai, Vietnam	Matsui et al. 2015
<i>G. zieglerei</i>	MCC.2018.15	LC642812	Vietnam	Le et al. 2021
<i>G. yunnanensis</i>	KIZ:20160230	MK234883	Fazhanhe, Lancang, Yunnan, China	Yu et al. 2019
<i>G. yunnanensis</i>	KIZ:20160223	MK234879	Xuelin, Lancang, Yunnan, China	Yu et al. 2019
<i>G. guangdongensis</i>	SYS a004903	MG520194	Guangdong, Longmen County, Mt. Nankun, China	Wang et al. 2018
<i>G. jinggangensis</i>	SYS:a003186	KY624587	Juangxi Province, China	Zeng et al. 2017
<i>G. jinggangensis</i>	SYS:a003170	KY624586	Juangxi Province, China	Zeng et al. 2017
<i>G. seesom</i>	KUHE:35088	LC011935	Kanchanburi, Thailand	Matsui et al. 2015
<i>G. seesom</i>	KUHE:35084	LC011932	Kanchanburi, Thailand	Matsui et al. 2015
<i>G. nonggangensis</i>	NHMG200910010	JX841320	Longzhou, Guangxi Province, China	Wang et al. 2018
<i>G. quangi</i>	VNMN:05723	LC642811	—	Le et al. 2021
<i>G. gracilipes</i>	SYS a005003	MG520201	Dawuling forestry station, Guangdong, China	Wang et al. 2018
<i>G. gracilipes</i>	SYS a005002	MG520200	Dawuling forestry station, Guangdong, China	Wang et al. 2018
<i>G. gracilipes</i>	SYS a005001	MG520199	Dawuling forestry station, Guangdong, China	Wang et al. 2018
<i>G. gracilipes</i>	AMS R 177667	KT374013	Hoang Lien National Park, Lao Cai Province, Vietnam	Rowley et al. 2015
<i>G. tianlinensis</i>	201705016	MH117961	Guangxi Province, China	Chen et al. 2018
<i>G. tianlinensis</i>	201705015	MH117960	Guangxi Province, China	Chen et al. 2018
<i>G. supercornutus</i>	AMS R173887	JN862545	—	Le et al. 2021
<i>G. jinxiuensis</i>	SYS:a002183	KY624585	Mt. Dayao, Guangxi, Jinxiu County, China	Zeng et al. 2017
<i>G. sapaensis</i>	MNHN 1999.5964	LC140969	Lao Cai, Vietnam	Matsui et al. 2017
<i>G. sapaensis</i>	MNHN 1999.5966	LC140970	Lao Cai, Vietnam	Matsui et al. 2017
<i>G. quyeti</i>	ZFMK 82999	EU871429	Quang Binh Province, Vietnam	Nguyen et al. 2008
<i>G. quyeti</i>	VNUH 160706	EU871428	Quang Binh Province, Vietnam	Nguyen et al. 2008
<i>G. trieng</i>	AMS R176206	MT328246	Kon Tum Province, Vietnam	Rowley et al. 2020
<i>G. trieng</i>	UNS 00230/AMS R176205	MT328245	Kon Tum Province, Vietnam	Rowley et al. 2020
<i>G. truongi</i>	IEBR A.5005	OP750513	Tuan Giao District, Dien Bien Province, Vietnam	Tran et al. 2023
<i>G. truongi</i>	IEBR A.5006	OP750514	Tuan Giao District, Dien Bien Province, Vietnam	Tran et al. 2023
<i>Rhacophorus reinwardtii</i>	CAS 219931	JN377365	Sarawak, Malaysia	Haas et al. 2012
<i>Philautus aurifasciatus</i>	ZRC.1.5266	AY141850	Java, Indonesia	Meegaskumbura et al. 2002
<i>Kurixalus effingeri</i>	NTUMA 2427	DQ468673	Okinawa Islands, Japan	Wu et al. 2016

## Appendix 2



Paratypes of *Gracixalus patkaiensis* sp. nov. in preserved condition showing dorsal view on left and ventral view on right. WII-ADA1352 (A, B), WII-ADA1354 (C, D), WII-ADA1355 (E, F), WII-ADA1356 (G, H), WII-ADA1400 (I, J).