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## A NEW CRYPTIC SPECIES OF BUSH FROG (AMPHIBIA: ANURA: *Raorchestes*) FROM THE SOUTHERN EASTERN GHATS, INDIA

Section Editor: Suranjan Karunaratna

Submitted: 30 April 2020, Accepted: 31 October 2020

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### Abstract

A new cryptic lineage of bush frogs of the genus *Raorchestes* from the understudied Eastern Ghats of India is described. Molecular phylogenetic reconstructions using mitochondrial 16S rRNA gene sequences suggest that the new species belongs to the *Raorchestes charius* clade, is sister to the allopatric *R. charius* of the Western Ghats and support the species-level distinctiveness of the lineage. The new species morphologically resembles *R. charius* from which it can be distinguished using the following characters: narrower head (HW/SVL 0.31 vs 0.34–0.36), wider inter upper eyelid (IUE/SVL 0.14–0.16 vs 0.11–0.13), greater maximum upper eyelid width (UEW/SVL 0.10–0.12 vs 0.06–0.09) and shorter thigh (TL/SVL 0.32–0.43 vs 0.44–0.51). Acoustic observations from the breeding males further corroborate the distinct specific status of the new species. The discovery of this new species of bush frog from an understudied landscape suggests that additional extensive surveys in the Eastern Ghats would yield several more amphibian lineages yet unknown to science.

**Key words:** arboreal frog, cryptic species, Kollimalai Massif, *Philautus*, Rhachophoridae, systematics

### Introduction

Bush Frogs, genus *Raorchestes* Biju, Shouche, Dubois *et al.*, 2010, belong to the arboreal frog family Rhachophoridae. These frogs were earlier placed in the genus *Philautus* Gistel, 1848, however molecular phylogenetic reconstruction revealed the polyphyly in the genus (Biju *et al.* 2010, Vijayakumar *et al.* 2014). The Peninsular Indian and Sri Lankan representatives were consequently shifted to the genera *Raorchestes* and *Pseudophilautus* Laurent, 1943 (Biju *et al.*

2010). Currently, more than 50 species of *Raorchestes* are known from India, primarily from the Western Ghats and north-east India. On the other hand, bush frogs of the drier Eastern Ghats have received comparatively little taxonomic attention. About five species of bush frogs were previously described from the Eastern Ghats: *Philautus sanctisilvaticus* Das & Chanda, 1998; *P. terebrans* Das & Chanda, 1998; *P. similipalensis* Dutta, 2003; *R. cf. leucolatus* Vijayakumar, Dinesh, Prabhu *et al.*,

2014 (Ganesh & Arumugam 2016) and *Pseudophilautus* cf. *wynaadensis* (Jerdon, 1853). However, Mirza *et al.* (2019) showed that the first three species also belong to the genus *Raorchestes* and represent not three, but a single species, *R. sanctisilvaticus*. The taxonomic status of the population inhabiting the Kolli Hills of *R. cf. leucolatus* and *P. cf. wynaadensis* in the Eastern Ghats remains unstudied. During a recent survey of the herpetofauna in the Kollimalai Massif, southern Eastern Ghats, we encountered and collected bush frog specimens of the genus *Raorchestes*. We tentatively identified the specimens as *R. charius*, due to the overall morphological similarity. Ganesh & Arumugam (2016) identified this population as *Raorchestes cf. leucolatus*. However, detailed morphological, molecular and acoustic analyses revealed that these specimens represent an undescribed, cryptic species of the *R. charius* clade. Here we describe and present a detailed morphological, molecular and acoustic account to diagnose the new species and distinguish it from its congeners.

#### Material and Methods

**Fieldwork and specimen acquisition:** One of us (SRG) undertook fieldwork throughout the Kolli Hills during 2011–2012 and observations on this species from those surveys are summarized in Ganesh & Arumugam (2016). However, no specimens were collected during this period. In October 2018, GG & ZM conducted a short survey during which *Raorchestes* sp. specimens were located in the field by the calls of the males at night. Specimens were collected by hand and subsequently euthanized with Tricaine Methanesulfonate (MS 222) following standard euthanasia protocols for amphibians. Thigh muscle tissue was collected and preserved in >95% ethanol for DNA extraction purposes. The specimens were subsequently fixed in 4% formaldehyde buffer for 24 hours, after which they were washed in water for five hours and transferred to 70% ethanol solution for long-term preservation. Photographs were taken in preservative (70% ethanol) with a Canon 70D illuminated with a Canon MT-24EX flash.

The specimens have been deposited at the Bombay Natural History Society, Mumbai (BNHS) and the collection facility of the National Centre for Biological Sciences, Bangalore (NCBS). Comparison of the new species was largely based on the topotypes of *R. charius* BNHS 4420 and BNHS 4421, two adult

males, from Chikmagalur, Karnataka.

**Acoustic data and analysis:** Calls of a breeding male were recorded in the field using the voice recorders loaded on an android mobile phone, and were analyzed later using RavenPro v1.5. Range, mean and standard deviation were calculated from five individual notes of a call. Calls were compared with the calls of breeding males of other species that showed genetic affinity. Published acoustic data of *R. charius* were used to make these comparisons (Priti *et al.* 2016).

**Molecular analysis:** Complete genomic DNA was isolated from the preserved tissues using Qiagen DNAeasy kits following protocols directed by the manufacturer. A fragment of the mitochondrial 16S rRNA gene was amplified using the primers 16SarL and 16Sbr (Yu *et al.* 2009). A 12µl reaction was set containing 5µl of QiagenTaq PCR Master Mix, 4µl of water, 0.5µl of each primer and 2µl template DNA, carried out with an Eppendorf Mastercycler Nexus GSX1. Thermo-cycle profile used for amplification were as follows: 94°C for 15 minutes, (denaturation temperature 94°C for 50 seconds, annealing temperature 45°C for 50 seconds, elongation temperature 72°C for 2 minutes) x 30 cycles, 72°C for 15 minutes, hold at 4°C. PCR product was cleaned using QIAquick PCR Purification Kit and sequenced with an AB 3730 DNA Analyzer. Sequences for 16s rRNA gene of selected bush frog species were downloaded following (Vijayakumar *et al.* 2016). Downloaded sequences were aligned in MegaX (Kumar *et al.* 2018) using ClustalW (Thompson *et al.* 2003) with default settings. Maximum Likelihood method for describing the phylogeny was implemented using W-IQ-TREE (Trifinopoulos *et al.* 2016), the web implementation of IQ-TREE (Nguyen *et al.* 2015). Branch support was tested using ultrafast bootstrap (UFboot) analysis (Minh *et al.* 2013), performing 1000 bootstrap alignments. TIM2e+I model of sequence evolution was used for ML analysis, which was selected following the model test run using ModelFinder (Kalyanamoorthy *et al.* 2017). For optimal partitioning strategy and evolutionary substitution model for BI analysis, aligned data were analyzed using PartitionFinder v.1.1.1. (Lanfear *et al.* 2012). Bayesian Inference (Appendix III) was implemented in MyBayes 3.2.2 (Ronquist & Huelsenbeck 2003) and was run for 10 million generations and sampled every 1000 generations, under GTR model of

sequence evolution. BI run included five parallel chains, three hot and two cold chains. The standard deviation of split frequencies of the analysis reached were below 0.01, and the analysis was not continued further. Twenty-five percent of trees generated were discarded as burn-in. Un-corrected p-distance (divergence) was calculated in MegaX and a pairwise deletion was adopted to deal with missing data and gaps. Data for NADH dehydrogenase 1 (*NDI*) generated by Vijaykumar *et al.* (2014) and p-distance for the same is also presented (Table 1).

**Morphological analysis:** The new species was compared with other members of the *R. charius* clade using morphometric data from Biju & Bossuyt (2009) and Bossuyt & Dubois (2001) for *R. charius*, and from Priti *et al.* (2016) for *R. honnametti* Gururaja, Priti, Roshmi *et al.*, 2016.

The following morphometric data were collected using digital calipers, to the nearest 0.01 mm: snout–vent length (SVL), head width (HW), head length (HL), inner upper eyelid width (IUE), maximum upper eyelid width (UEW), snout length (SL), eye length (EL), internarial distance (IN), nostril–snout distance (NS), eye–nostril distance (EN), tympanum–eye distance (TYE), distance from rear of the mandible to the nostril (MN), distance from rear of the mandible to the anterior orbital border of the eye (MFE), distance from rear of the mandible to the posterior orbital border of the eye (MBE), distance between the anterior corner of the eyes (IFE), distance between the posterior corner of the eyes (IBE), largest tympanum diameter (TYD), forelimb length (FLL), hand length (HAL), thigh length (TL), shank length (ShL), foot length (FOL), distance from the heel to the tip of the fourth toe (TFOL), disc width on fingers I, II, III, IV (FD I, II, III, IV), width of fingers I, II, III, IV (FW I, II, III, IV), length of fingers I, II, III, IV (FL I, II, III, IV), tibia width (TW), disc width on toes I, II, III, IV (TD I, II, III, IV), width of toes I, II, III, IV (TW I, II, III, IV), length of inner metatarsal tubercle (IMT), distance from distal edge of metatarsal tubercle to maximum incurvature of web between fourth and fifth toe (MTFF).

A total of 31 characters for all the specimens were recorded. Measurements and acronyms follow Gururaja *et al.* (2007), Biju & Bossuyt (2009) and Priti *et al.* (2016). In the morphological comparisons section, the values in parenthesis follow (new species vs compared species) format.

## Results

Molecular phylogenetic reconstruction based on the ~515bp long fragment of 16S rRNA mitochondrial gene (Fig. 1) suggests that the new species belongs to the *R. charius* clade (Vijayakumar *et al.* 2014, Priti *et al.* 2016) and is a sister to *R. charius* with high support (ML 100, BI 1.0). The clade containing the new species and *R. charius* is a sister to *R. honnametti* with high support (ML 100, BI 1.0). The intra-specific genetic p-distance is 0–0.02% ( $n=4$ ), whereas the species is 1.2–1.9% divergent from *R. charius*; for 580bp of mitochondrial *NDI* the new species shows a divergence of 5% from *R. charius* (Tables 1, 2). The interspecific p-distance obtained between the new species and *R. charius* is reasonably shallow for its recognition as a distinct species. Nonetheless, morphology and acoustics, in addition with geography, establish its distinctness, which is here described as a cryptic species of the *R. charius* clade. A sequence from unknown locality generated by Vijayakumar *et al.* (2014), labelled as an unidentified species of bush frogs was found to be conspecific with the new species. This sequence was recovered as a member of the *R. charius* clade, sister to *R. charius* by Vijayakumar *et al.* (2014, 2016).

## Systematics

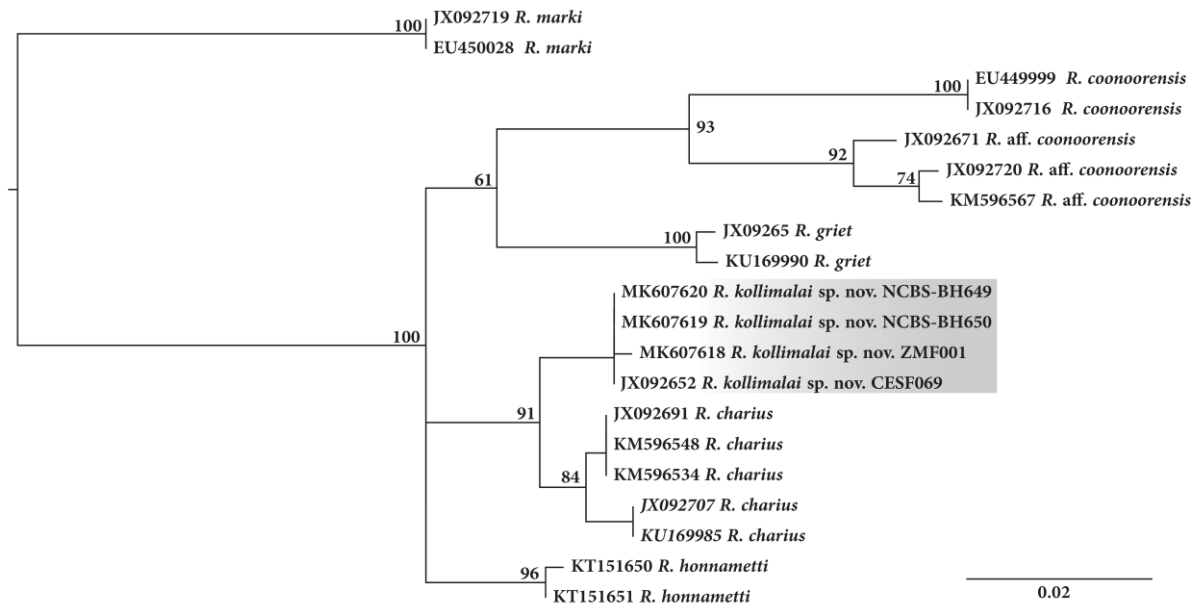
The new species is identical in general appearance to *R. charius*. However, it can be distinguished from the latter by a few morphometric variables, by its call and by its allopatric distribution in lowland dry forest. The combination of low genetic as well as morphological divergence hints at a recent diversification, a case common with most cryptic species.

**Table 1.** Un-corrected p-distance calculated for the mitochondrial *NDI* gene for *Raorchestes* species.

Genbank AN & Species	1	2	3	4	5	6
EU450060 <i>R. marki</i>						
EU450043 <i>R. charius</i>	0.21					
JX092870 <i>R. charius</i>	0.21	0.00				
EU450033 <i>R. griet</i>	0.19	0.14	0.14			
EU450035 <i>R. coonoorensis</i>	0.20	0.19	0.19	0.15		
JX092910 <i>R. aff. coonoorensis</i>	0.20	0.19	0.19	0.15	0.13	
JX092893 <i>R. kollimalai</i> sp.nov.	0.19	0.05	0.05	0.13	0.17	0.18

**Table 2.** Un-corrected p-distance calculated for the mitochondrial 16S rRNA gene for *Raorchestes* species; H, holotype, P, paratype

Genbank AN & Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 JX092719 <i>R. marki</i>																			
2 EU449999 <i>R. coonoorensis</i>	0.07																		
3 MK607620 <i>R. kollimalai</i> sp. nov. <sup>H</sup>	0.06	0.05																	
4 MK60719 <i>R. kollimalai</i> sp. nov. <sup>P</sup>	0.06	0.05	0.00																
5 MK60718 <i>R. kollimalai</i> sp. nov.	0.06	0.05	0.00	0.00															
6 JX092652 <i>R. kollimalai</i> sp. nov.	0.06	0.05	0.00	0.00	0.00														
7 JX092654 <i>R. griet</i>	0.07	0.05	0.04	0.04	0.04	0.04													
8 JX092671 <i>R. aff. coonoorensis</i>	0.07	0.05	0.06	0.06	0.06	0.05	0.04												
9 JX092691 <i>R. charius</i>	0.06	0.05	0.01	0.01	0.01	0.01	0.04	0.05											
10 JX092707 <i>R. charius</i>	0.05	0.05	0.01	0.01	0.01	0.01	0.04	0.05	0.01										
11 JX092716 <i>R. coonoorensis</i>	0.07	0.00	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05									
12 JX092720 <i>R. aff. coonoorensis</i>	0.08	0.04	0.05	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.04								
13 KM596534 <i>R. charius</i>	0.06	0.05	0.01	0.01	0.01	0.01	0.04	0.05	0.00	0.01	0.05	0.05							
14 KM596548 <i>R. charius</i>	0.06	0.05	0.01	0.01	0.01	0.01	0.04	0.05	0.00	0.01	0.05	0.05	0.00						
15 KM596567 <i>R. aff. coonoorensis</i>	0.08	0.05	0.06	0.06	0.06	0.05	0.05	0.01	0.05	0.05	0.04	0.00	0.05	0.05					
16 KU169985 <i>R. charius</i>	0.06	0.06	0.02	0.02	0.02	0.01	0.04	0.06	0.01	0.00	0.05	0.05	0.01	0.01	0.06				
17 EU450028 <i>R. marki</i>	0.00	0.08	0.08	0.08	0.08	0.06	0.07	0.08	0.06	0.06	0.07	0.08	0.06	0.06	0.09	0.07			
18 KU169990 <i>R. griet</i>	0.07	0.06	0.04	0.04	0.04	0.04	0.00	0.05	0.04	0.04	0.05	0.05	0.04	0.04	0.06	0.04	0.09		
19 KT151650 <i>R. hommametti</i>	0.06	0.06	0.03	0.03	0.04	0.03	0.04	0.05	0.03	0.03	0.05	0.05	0.03	0.03	0.06	0.04	0.07	0.04	
20 KT151651 <i>R. hommametti</i>	0.06	0.06	0.03	0.03	0.03	0.02	0.04	0.05	0.02	0.03	0.05	0.05	0.02	0.02	0.05	0.03	0.07	0.04	0.00



**Figure 1.** Maximum likelihood phylogeny of the members of the “*Raorchestes charius*” clade based on 16S rRNA gene reconstructed through 1000 non-parametric bootstrap replicates. Numbers at nodes represent bootstrap support

***Raorchestes kollimalai* sp. nov.**

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(Figs. 1–5, Tables 3, 4)

*Raorchestes* cf. *leucolatus* — Ganesh & Arumugam 2016: 10

**Holotype.** Adult male, NCBS-BH649, collected from low-lying shrubs at Kollimalai Massif (11.245840 °N, 78.335365 °E; alt. 1,100 m a.s.l.), Namakkal District, Tamil Nadu, India, by A. Gawai, P. Phansalkar, G. Gowande, and Z. Mirza on 26<sup>th</sup> October 2018 at 22:00 h.

**Paratypes** ( $n=2$ ). Males, NCBS-BH650 and BNHS 6068, collection details same as the holotype.

**Diagnosis.** *Raorchestes kollimalai* sp. nov. is allocated to the genus *Raorchestes* based on the following combination of diagnostic characters: relatively small adult body size (SVL 15–45 mm), absence of vomerine teeth, presence of vocal sacs, which appear transparent/translucent while calling, and nocturnal habits. Dorsum light brown with dark brown spots. Canthus rostralis dark brown, a dark brown streak from posterior of the eye through the tympanic region terminating at the shoulder. Legs banded with dark brown. Pupil black with gold specks around it. A medium-sized bush frog (SVL 25.8–29.7 mm,  $n=3$ ), characterized by wider than long head (HW/SVL 0.31, HL/SVL 0.26–0.30), short or subequal snout in comparison to the eye

(SL/SVL 0.12–0.14 vs EL/SVL 0.14–0.15) and by relatively shorter hind limbs (ShL/SVL < 0.50).

**Description of holotype.** A male (NCBS-BH649), medium-sized (SVL 29.6 mm). Head slightly wider than long (HW 9.4, HL 9.1); snout rounded in dorsal and ventral view, as long as the eye (SL 4.2, EL 4.2, MFE 5.7, MBE 1.7), shorter than the distance from the rear of the mandible to the nostril (MN 7.8); canthus rostralis rounded; distance between the posterior corner of the eye 1.8 times greater than the distance between the anterior corner of the eye (IBE 8.2, IFE 4.6); tympanum indistinct (TYD 1.2), supratympanic fold distinct, from the posterior border of the eye to the shoulder; nostril distinct, rounded; loreal region slightly concave. Nostrils without flap, closer to the tip of the snout (NS 1.5, EN 1.9). Eye large (EL 4.2), protruding, pupil horizontal. Interorbital region flat, distance between the upper eyelids (IUE 4.7) greater than the maximum upper eyelid width (UEW 3.2) and the internarial distance (IN 2.7). Symphyseal knob not protruding the maxilla, pineal ocellus absent. Tongue bifurcated, smooth lacking lingual papilla. Forelimb nearly as long as the hand (FLL 8.2 vs HAL 8.1), 3<sup>rd</sup> finger longest (FL III 5.1), 1<sup>st</sup> shortest (FL I 2.6), relative length of the fingers: III (5.1) > IV (3.9) > II (3.2) > I (2.6), all fingers with very well-developed discs (FD I = 0.8, FD II = 1.2, FD III = 1.5, FD IV = 1.4);

discs wider than the fingers (FD III 1.5 vs FW III 1.0) with circum-marginal grooves, intercalary ossification present between penultimate and distal phalanges. Dermal fringe present only on the outer aspect of hind-limb. Hindlimbs short, do not touch or overlap when folded at right angles to the body, shank more than 5 times longer than wide (ShL 13.2 vs TW 2.6), longer than thigh (TL 10.3) and the foot (FOL 11.6), the foot being longer than the distance from the heel to the tip of the fourth toe (TFOL 10.9). Relative toe lengths IV>III>V>II>I (ToL I= 2.6, ToL II= 2.9, ToL III= 4.8, ToL IV= 6.9, ToL V= 4.6). Toe with well-developed discs at its distal tip (TD I= 0.7, TD II= 0.9, TD III= 1, TD IV= 1.3, TD V= 1.4,); (TW I= 0.6, TW II= 0.5, TW III= 0.7, TW IV= 0.9, TW V= 0.9,). Webbing moderate (MTTF= 5.2, MTF= 6.5, TTF= 5.7, FTF=5.5). First toe (ToL I= 2.6) two times the length of the inner metatarsal tubercle (IMT= 1.3). Outer metatarsal tubercle absent, supernumerary and tarsal tubercle present on all toes.

**Colouration.** A morphologically variable species, with light or dark brown phase morphs where its dorsal colour varies from ashy yellow to blackish brown, with either bold or feeble dorsal patterning (Fig. 3). In life, dorsum light brown with dark brown spots; canthus rostralis dark brown, a dark brown streak from posterior of the eye through the tympanic region terminating at the shoulder; legs banded with dark brown; pupil black with gold specks around it. In preservative, dorsum grey with paired symmetrical dark markings. A pair of large spots between the occipital region followed by a broad irregular longitudinal stripe running from the temporal region to the posterior half of the body along the dorso-lateral aspect of the body. A single large spot on the flank. Limbs cream to off-white with dark grey bands. Finger in a lighter shade, toes of the same colour as the grey bands. Ventrally cream colored with heavy brown mottling on the gular region and light mottling on the limbs.

**Etymology.** The specific epithet refers to the type locality, Kollimalai, as a noun in apposition and hence invariable. Suggested common name: Kollimalai bush frog.

**Comparison.** *Raorchestes kollimalai* sp. nov. most closely resembles the allopatric *R. charius* of the Western Ghats, in external appearance, from which it is difficult to diagnose. However, it can be differentiated by

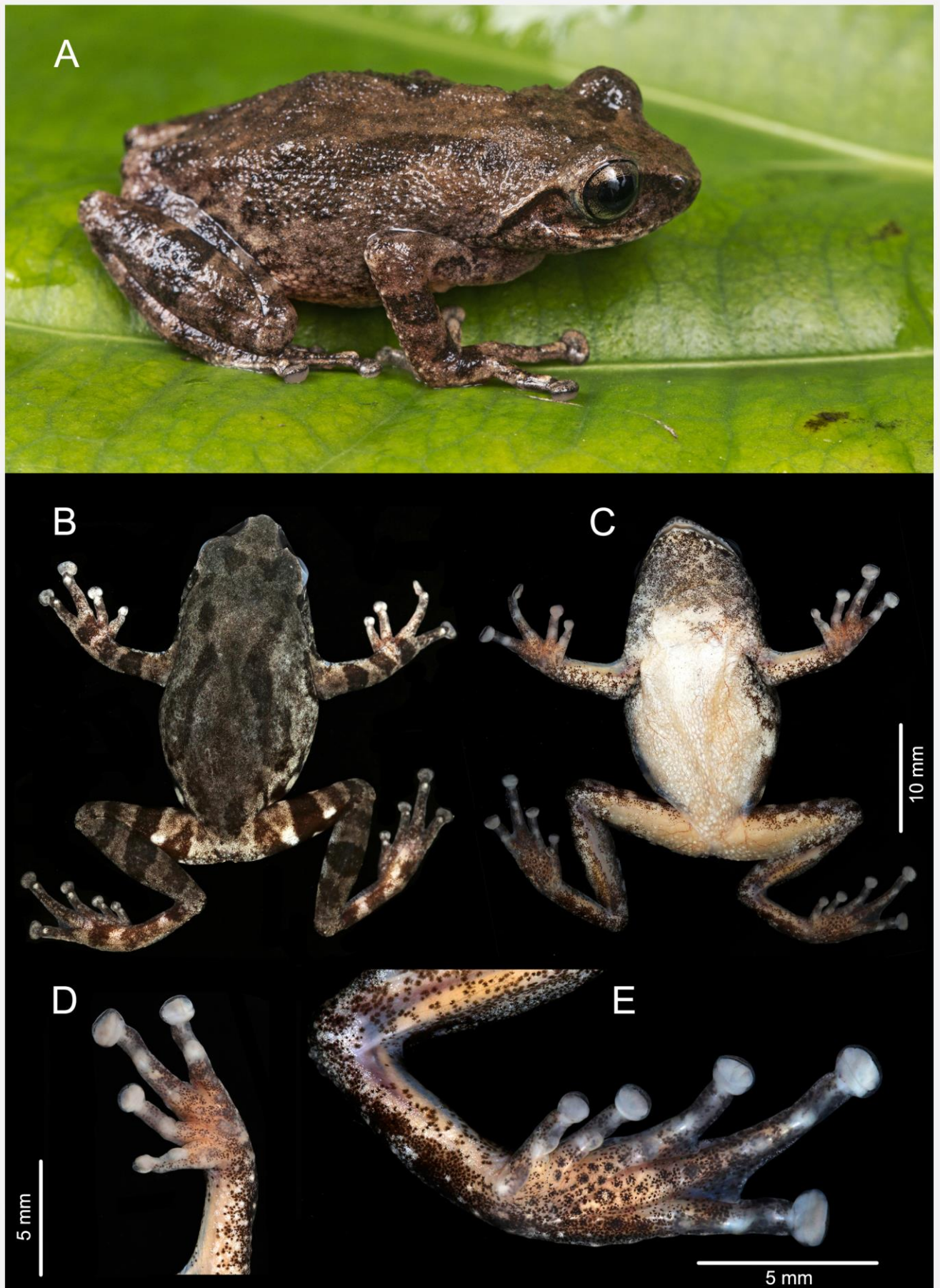
**Table 3.** Morphometric data of *Raorchestes kollimalai* sp. nov. holotype and paratypes (males).

Character	<i>Raorchestes kollimalai</i> sp. nov.		
	Holotype	Paratype	Paratype
	NCBS- BH649	NCBS- BH650	BNHS 6068
SVL	29.6	25.8	29.7
HW	9.4	9.3	9.4
HL	9.1	7.7	8.9
IUE	4.7	4.3	4.4
UEW	3.2	3.3	3.0
SL	4.2	3.8	3.6
EL	4.2	4.0	4.2
IN	2.7	2.3	2.9
NS	1.5	1.3	1.4
EN	1.9	1.8	1.9
TYE	0.5	0.6	0.6
MN	7.8	5.3	6.8
MFE	5.7	4.4	5.2
MBE	1.7	1.2	1.1
IFE	4.6	3.6	4.5
IBE	8.2	8.3	7.0
TYD	1.2	0.7	1.1
FLL	8.2	6.9	7.8
HAL	8.1.0	8.0	8.0
TL	10.3	11.1	9.8
ShL	13.2	11.8	13.3
FOL	11.6	11.1	11
TFOL	10.9	10.9	10.7
TW	2.6	2.3	2.3
IMT	1.3	0.8	0.9
MTFF	5.8	5.9	5.6
FD 1–4	0.8, 1.2, 1.5, 1.4	0.8, 1.1, 1.7, 1.7	0.8, 1.3, 1.6, 1.7
F I–IV L	2.6, 3.2, 5.1, 3.9	3.0, 3.9, 5.7, 3.4	3.1, 4.0, 6.0, 3.7
FW 1–4	0.6, 0.7, 1.0, 0.9	0.5, 0.7, 0.9, 0.9	0.6, 0.7, 0.9, 0.8
TD 1–5	0.7, 0.9, 1.0, 1.3, 1.4	0.8, 0.9, 0.9, 1.3, 1.4	0.7, 0.9, 1.1, 1.2, 1.3
TW 1–5	0.6, 0.5, 0.7, 0.9, 0.9	0.5, 0.6, 0.7, 0.8, 0.9	0.5, 0.6, 0.7, 0.8, 0.8

having narrower head, HW/SVL 0.31 (vs 0.34–0.36), wider inter upper eyelid, IUE/SVL 0.14–0.16 (vs 0.11–0.13), greater upper eyelid width, UEW/SVL 0.10–0.12 (vs 0.06–0.09), and shorter thigh, TL/SVL 0.32–0.43 (vs 0.44–0.51); from the allopatric *R. honnametti* of Bilgiri Massif, by having larger adult male size, SVL 25.8–29.7 (vs 21.7–25.0), wider head, HL/SVL 0.31 (vs 0.33–0.40), much shorter mandible–eye distance, MBE/SVL 0.03–0.05 (vs 0.08–0.1), shorter eye–nostril distance, EN/SVL 0.063–0.069 (vs 0.072–0.096), longer forelimb,



# Plate 49



**Figure 2.** Adult male holotype (NCBS-BH 649) of *Raorchestes kollimalai* sp. nov. (A) dorsolateral view of the body in life; (B) dorsal and (C) ventral views of the body (preserved); and ventral view of the (D) right forelimb, (E) left hindlimb.

FLL/SVL 0.26–0.27 (*vs* 0.20–0.24), and shorter foot, TFOL/SVL 0.36–0.42 (*vs* 0.44–0.74); from the allopatric *R. griet* Bossuyt, 2002 of the Southern Western Ghats, by having larger adult male body size, SVL 25.8–29.7 (*vs* 19.7–22.4), greater distance between upper eyelids, IUE/SVL 0.14–0.16 (*vs* 0.11), greater upper eyelid width, 0.10–0.12 (*vs* 0.05–0.09). Based on acoustic data, the new species differs from *R. charius* in its call duration, 0.305–0.34s (*vs* 0.06–0.14s) and number of pulses per call, 21–24 (*vs* 13–18).

**Call description.** Advertisement call of a single male (paratype NCBS-BH650) was recorded and ranges provided here (Fig. 4). Call syllable “*treek treek treek*” with varying call duration and frequency and is quite similar to that of *R. charius*. The highest frequency recorded was 22050 Hz. Call duration was between 0.305–0.34s  $\pm$  0.012; inter-call duration was 1.764–2.1s  $\pm$  0.128 and number of pulse per call ranges from 21–24  $\pm$  1.51 (Table 4).

**Table 4.** Comparison of acoustic data for *R. kollimalai* sp. nov. and *R. charius*

Feature	<i>R.</i>	<i>R.</i>
	<i>kollimalai</i>	<i>charius</i>
Dominant frequency	22050	2346–2541
Call duration (s)	0.305–0.34	0.06–0.14
Number of pulses	21–24	13–18
Inter-call duration (s)	1.764–2.1	1.88–3.47

**Natural history.** The new species is common at the type locality above 500 m a.s.l. It is found in sympatry with an unidentified *Pseudophilautus* species. Currently, the new species is known only from the type locality. However, this or similar species is likely to be found on adjacent massifs that exceed 500 m in elevation. The type specimens were collected from low bushes along a road in Thinnanur Nadu atop Jambuthu Slopes, in the southern part of the Kolli Hills. Calls of the species were heard from plantations, gardens, evergreen forest and mid-elevation semi-evergreen forest. The type locality is a tourist hotspot and habitat is being altered there, although patches of forest currently remain in the ghats leading to the hill top (Jayakumar *et al.* 2002). During surveys in 2011–12 in Kolli Hills, this species was sighted extensively and the following natural history details were gathered. Number of sightings: 173 out of 500 hr fieldwork; encounter rate estimate: 2.8 hr per sighting; microhabitat associations:

plants ( $n=100$ ) > tree trunk ( $n=24$ ) > leaf-litter ( $n=22$ ) > building walls ( $n=10$ ) > bare ground ( $n=9$ ) > fallen log ( $n=4$ ) > tar road ( $n=2$ ) and rocks ( $n=2$ ). Tallest perching height of a sighting was 4.2 m up a tree (*Terminalia* sp.). The new species was sighted from Sengarai-Navakkadu in the north, through Kuzhi Valavu-Ariyur Shola regions; through Solakkadu-Semmedu areas southwards to Selur Nadu-Thinnanur Nadu (type locality). Given the current geographic range of the new species is entirely outside any Protected Area and the anthropogenic onslaught its range faces, *R. kollimalai* sp. nov. is likely a threatened species. This species is mainly nocturnal; dormant individuals were observed resting under leaf litter during day. Calling individuals were observed from as early as 18:00 h to up to 00:04 h, mainly during May to September. When it rains, this species was seen calling even during broad daylight, at 12:00 noon. Heavily gravid females with apparently distended belly and developing ova faintly visible through the see-through venter, were seen during June. During late monsoon (third week of August), tiny froglets of the new species, measuring ~12 mm in SVL (Fig. 3) were seen in dense leaf litter during the daytime.

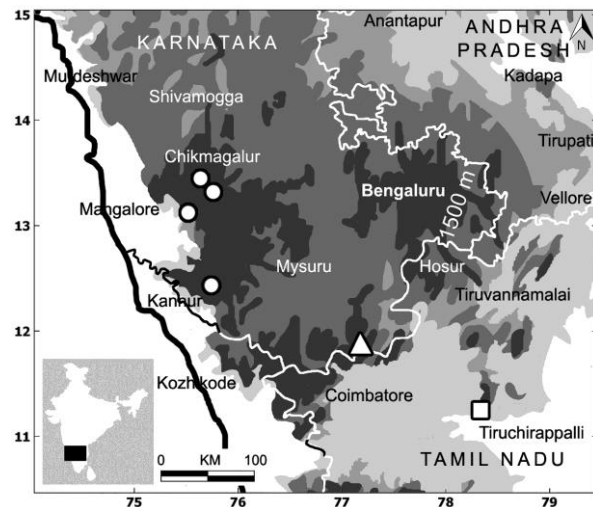
## Discussion

*Raorchestes kollimalai* sp. nov. shows limited diagnostic morphological characters like most cryptic species, however with geographic, molecular and acoustic data, it can be readily distinguished from other members of the clade. *R. charius* is distributed from Bababudangiri to Brahmagiri Hill range in central Western Ghats, which is at least 230 km a.s.l. from the type locality of *R. kollimalai* sp. nov. (Priti *et al.* 2016). Similarly, the intervening dry plains (< 300 m a.s.l.) are unsuitable for the survival of wet zone species such as bush frogs; this dry area effectively acts as a biogeographic barrier for *Raorchestes*. The new species currently is known only from the type locality, however, it might be found in neighboring hills of southern Eastern Ghats which share similar biotopes. Ganesh & Arumugam (2016) reported this species (as *R. cf. leucolatus*) from Kolli Hills and Shevaroy Hills which is ~40 km north of the type locality of *R. kollimalai* (Fig. 5). The low genetic divergence between *R. charius* and the new species is likely due to recent diversification, a scenario observed in several species of the genus *Raorchestes* (Vijayakumar



*et al.* 2014, 2016). Such shallow divergences have been previously reported in bush frog species pairs, such as *R. bombayensis* and *R. sanctisilvaticus* (1.0% to 1.9%), *R. bombayensis* and *R. tuberothumerus* (1.7 to 1.9%) (Mirza *et al.* 2019). The new species is phylogenetically sister to *R. charius*, despite the geographical vicinity of the type locality of the new species to that of *R. honnametti*. Sequences of the new species were included in the phylogenetic analyses by Vijayakumar *et al.* (2014, 2016), and the species was classified as being unidentified, nonetheless, distinct from *R. charius*. Although the precise locality of the samples sequenced by Vijayakumar *et al.* (2016) is unknown, we hypothesize that the samples originated in the Eastern Ghats. We further hypothesize that the River Cauvery acts as the biogeographic barrier for members of the clade, in addition to the intervening lowlands separating the new species and *R. charius*; however, testing this hypothesis would require investigations into the population genetics of the species of the *R. charius* clade. The Indian peninsula is flanked by two major series of mountain ranges, the Western Ghats, and the Eastern Ghats (Mani 1974). The Western Ghats are typically known for the high proportions of herpetofaunal species richness and endemism (Myers *et al.* 2000). The high species diversity and endemism are, primarily attributed to the elevation gradient, discontinuity due to gaps in elevation and speciation on the sky islands, and their complex geological history (Robin *et al.* 2010, 2017, Vijayakumar *et al.* 2016). As a consequence, the Western Ghats have always been at the center of attention for herpetologists, which is evident from the discovery of many new reptile and amphibian genera and species in recent years (Giri 2008, Biju *et al.* 2014, Mirza & Sanap 2014, Mirza *et al.* 2014, Vijayakumar *et al.* 2014, Dinesh *et al.* 2015, Giri *et al.* 2017, Sadasivan *et al.* 2018, Pal *et al.* 2018, Vijayakumar *et al.* 2019). On the other hand, the Eastern Ghats have been thought of as comparatively faunally impoverished and degenerate (Mani 1974, Dinesh *et al.* 2009, Aengals *et al.* 2018, Ganesh & Arumugam 2016), with their faunal assemblage composed of derivatives from the Western Ghats (Ganesh & Arumugam 2015). This, in turn, has resulted in comparatively fewer herpetofaunal expeditions in the Eastern Ghats. However, recent efforts to document the herpetofauna of the regions have led to the discovery of notable species of reptiles and

amphibians from of the Eastern Ghats (e.g. Ganesh & Arumugam 2016), and have also resulted in the description of new species (Aengals & Ganesh 2013, Vogel & Ganesh 2013, Giri *et al.* 2017, Mirza *et al.* 2017, Agarwal *et al.* 2019, Ganesh & Srikanthan 2020). It is only recently that biologists have realized that, in contrast to what was previously thought, the Eastern Ghats harbor their own set of herpetofaunal assemblages, including endemics like the monotypic skink genus *Sepsophis* Beddome, 1870, and gecko species such as *Cyrtodactylus jeyporensis* (Beddome, 1878); *Cnemaspis yercaudensis* Das & Bauer, 2000; *C. otai* Das & Bauer, 2000; *Hemidactylus kangerensis* Mirza, Bhosale & Patil, 2017; *H. sushilduttai* Giri, Bauer, Mohapatra *et al.*, 2017; *H. kolliensis* Agarwal, Bauer, Giri *et al.*, 2019; *Hemiphyllodactylus kolliensis* Agarwal, Khandekar, Giri *et al.*, 2019, a caecilian, *Gegeneophis orientalis* Agarwal, Wilkinson, Mohapatra *et al.*, 2013 (Mirza *et al.* 2019). Observations suggest that further species of lizards remain to be described from this region (Z.A. Mirza pers. observ.).



**Figure 5.** The distribution of the members of the *Raorchestes charius* clade in southern Indian peninsula; *R. charius* (circles), *R. honnametti* (triangle), and *R. kollimalai* sp. nov. (square). Map © A.A.T. Amarasinghe

The description of a new cryptic species of bush frogs from the Eastern Ghats adds to our growing knowledge of the genus *Raorchestes* and the biodiversity of the under-surveyed Eastern Ghats. The genus certainly contains several undescribed species across the Western Ghats, and perhaps also the Eastern Ghats. Evidence for multiple undescribed lineages is presented in the recent phylogeny of the genus



from India (Vijayakumar *et al.* 2014, 2016). Morphology alone cannot be used to delimit species and additional studies incorporating molecular and acoustic data will help elucidate exact diversity of many anuran genera across India (Vijayakumar *et al.* 2014). A second new species of bush frog of the genus *Pseudophilautus* that occurs in sympatry with *R. kollimalai* sp. nov. and work in progress.

### Acknowledgements

ZM thanks Singinawa Conservation Foundation and Rufford Small Grant for funding the fieldwork and the Forest Department, Tamil Nadu for permission. GG thanks A. Patwardhan (Principal), A. Kulkarni (Head, Department of Biodiversity), and D. Paranjpe (advisor) at Abasaheb Garware College, Pune; and Principal and Head (Department of Biotechnology) of Fergusson College, Pune. ZM thanks K. Vijay Raghavan for hosting at NCBS, and all the lab members for the support. SRG thanks to the Chairman and Trustees of the Chennai Snake Park. We thank Pushkar Phansalkar and Atish Gawai for the support during fieldwork. Rahul Khot and Saunak Pal helped registering of types at BNHS and taking images of *R. charius*; Vivek R. helped registering of types at NCBS. Finally, Nikolay Poyarkov (Lomonosov Moscow State University, Russia) and Thasun Amarasinghe (University of Indonesia) are acknowledged for reviewing the manuscript.

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Published date: 28 November 2020