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continued on the back inside cover

Cover: Common Silverline *Spindasis vulcanus vulcanus* in poster colours adapted from photograph by Kalpesh Tayade. © Pooja R. Patil.



## Systematics of the enigmatic and narrowly endemic toad genus *Bufoides* Pillai & Yazdani, 1973: rediscovery of *Bufoides kemp* (Boulenger, 1919) and expanded description of *Bufoides meghalayanus* (Yazdani & Chanda, 1971) (Amphibia: Anura: Bufonidae) with notes on natural history and distribution

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**Abstract:** *Bufoides kemp* (Boulenger, 1919) known only from the two historical syntype specimens until now was rediscovered after more than a century from near its type locality in the Garo Hills, Meghalaya, northeastern India. Analysis of mitochondrial 16S rRNA gene reveals congenericity between *B. kemp* and *B. meghalayanus* with an inter-specific genetic divergence of 4.67%. Description of *B. kemp* is expanded based on the six male and two female specimens collected during this study. We provide the first description of calls for this genus, notes on their breeding biology and larval morphology. Additional specimens of *B. meghalayanus* collected during this study are described to supplement its characterization.

**Keywords:** Amphibians, breeding biology, calls, Garo Hills, Khasi Hills, larval morphology, new records, syntype specimens.

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**Author contributions:** SRC and SB conceived and designed the study, RSN & GK conducted field studies, SRC, RSN, & SB generated and analysed the data, SRC, RSN, & SB wrote the manuscript, SB, PVK, & HNK procured funds, SB, PVK, HNK, & NP supervised the study, reviewed, & edited the manuscript draft, and all authors approved the final draft.

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## INTRODUCTION

The cosmopolitan anuran family Bufonidae Gray, 1825 is represented in India by nine genera comprising 33 species spread across several biogeographic regions (Frost 2022). One of the most poorly-known among them is the genus *Bufoides* Pillai & Yazdani (1973) which comprises two species namely *B. meghalayanus* (Yazdani & Chanda 1971) and *B. kempfi* (Boulenger 1919) (after Chandramouli & Amarasinghe 2016). Among them, the type species, *B. meghalayanus* is fairly better-known in terms of its distribution, biology, ecology, and natural history (Yazdani & Chanda 1971; Pillai & Yazdani 1973; Das et al. 2009; Deuti et al. 2012). On the other hand, there has been no information on any of the above aspects for the previously-described species *B. kempfi*. *B. kempfi* was originally described as *Nectophryne kempfi* from 'above Tura, 2,500 ft' based on two specimens, an adult and a subadult (ZSI 18481a,b), from which the species is known till date (Boulenger 1919). It was later transferred to the genus *Pedostibes* Günther, 1875 by Barbour (1938) and subsequently transferred to *Bufoides* by Chandramouli & Amarasinghe (2016) based on morphological characters. As a part of an on-going project documenting faunal diversity in community reserves of Meghalaya, we rediscovered *B. kempfi* from near its type locality and located additional specimens of *B. meghalayanus* whose descriptions are expanded based on new data.

## MATERIALS AND METHODS

The study was conducted in the northeastern Indian state of Meghalaya. Surveys were conducted in ten different locations spread across the Garo, Khasi, and Jaintia Hills of Meghalaya. Specifically, the type localities of the two known species viz. "above Tura, Garo Hills" (Boulenger 1919) for *B. kempfi* and "Mawblang plateau" (Yazdani & Chanda 1971) for *B. meghalayanus* and the vicinity of these localities were surveyed intensively, in addition to the other sites to locate the target species. Additional sites within Meghalaya (Deuti et al. 2012), apart from these two localities from where *Bufoides* is known until now were also surveyed. Field sampling was carried out from March–May & October 2021. A total of seven specimens of *B. kempfi*, comprising six adult males and one adult female along with a subadult female were collected from Eman Asakgre (25.37° N, 90.55° E, 100–250 m). Likewise, three adult specimens of *Bufoides meghalayanus* were collected from a hill stream in the

Khasi Hills (25.23° N, 91.73° E, 1,100–1,250 m). Other locations of *B. kempfi* and *B. meghalayanus* were marked with a GPS. Encounter rates of these species, expressed as the number of individuals encountered over an hour's duration of field sampling, is presented as an index of their abundance.

The following measurements were recorded to the nearest 0.02 mm from the specimens with a dial caliper: snout–vent length (SVL, from the tip of the snout to the anterior margin of the cloaca), axilla–groin distance (AG, from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body), head length (HL, from the posterior edge of the mandible to the tip of the snout), head width (HW, the maximum width of the head at the angle of the jaws), head depth (HD, the maximum depth of the head), body width (BW, the maximum width of the body at the trunk), eye diameter (ED, the greatest horizontal diameter of the orbit), eye–nostril distance (EN, from the anterior border of the orbit to the middle of the nostril), eye–snout distance (ES, from the anterior border of the orbit to the tip of the snout), upper eyelid width (UEW, the maximum width of the upper eyelid), interorbital distance (IO, distance between the upper eyelids), internarial distance (IN, distance between the nostrils), upper arm length (UAL, from the axilla to elbow), lower arm length (LAL, from the posterior margin of the elbow to the base of the outer metacarpal tubercle), palm length (PAL, from the posterior border of the outer metacarpal tubercle to tip of the 3<sup>rd</sup> finger), femur length (FEL, from the cloaca to the knee), tibia length (TBL, from knee to heel), foot length (FOL, from inner metatarsal tubercle to the tip of the 4<sup>th</sup> toe). Webbing formulae follows Savage & Heyer (1997). A principal component analysis was conducted based on 18 morphometric measurements (standardised to their SVL; Table 1) of the two *Bufoides* species to examine their morphometric distinction from each other. Calls were recorded with the camera as videos and the audio (as .mp4 at an audio sampling rate of 48 kHz) was extracted and analysed with Adobe Audition 6 and Adobe Soundbooth CS3. Two specimens, SACON VA 157 (female) and VA 159 (male) were radiographed to study osteological characters of *B. kempfi*. A brief description of its osteology is provided following the terminologies of Noble (1931).

Eggs of *B. kempfi* observed in tree holes were collected and reared for 11 days, and the growth of the larvae was monitored with preservation of samples across various developmental stages. The following measurements of the tadpoles: HBL— head-body length; HBW— head-



body width; HBD— head-body depth; TOT— total length; TAIL— tail length; IO— inter-orbital distance; and TH— tail-fin height were recorded with a stereo microscope following Chandramouli & Kalaimani (2014). Staging of tadpoles follow Gosner (1960) and terminologies follow McDiarmid & Altig (1999). Labial tooth-row formula for the larvae follow Rödel (2000).

Total genomic DNA was extracted from one specimen of *B. kempfi* (SACON VA 180) with a DNA extraction and purification kit, following the manufacturer's protocols. 16S rRNA gene was amplified using the primers 16sAR-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16sB R-H (5'-CCGGTCTGAACTCAGATCACGT 3'), respectively (Kocher et al. 1989). Amplifications were performed in a Applied Bio Systems Veriti 96 well thermal cyclers: 20 µl reactions with 4 µl of 5X Phusion HF buffer, 0.4 µl of 10 mM dNTP, 0.2 µl of Phusion DNA Polymerase, 0.1 µl each of forward and reverse primers, 2.0 µl of DNA template and 13.2 µl of nuclease free water with the following procedure: initial denaturation of DNA at 95 °C for 5 min, 35 cycles of: denaturation at 95 °C for 1 m, annealing at 55 °C for 1 min, extension at 72 °C for 1 m and at last, final extension at 72 °C for 10 min. The amplicon was checked by running it through an agarose gel electrophoresis for a clear band of the desired region in the amplified PCR product. The amplified PCR product was purified and sequenced commercially (National Centre for Biological Sciences, Bengaluru). The sequence thus obtained (NCBI voucher no: OP920605) was aligned along with ten other taxa from Bufonidae, comprising the genera *Adenomus*, *Beduka*, *Blythophryne*, *Bufoides*, *Bufotes*, *Duttaphrynus*, and *Pedostibes* with *Hyla arborea* as the outgroup taxon. The sequences were aligned with MUSCLE (Edgar 2004) in MEGA 6.0 (Tamura et al. 2013). This alignment of 491 bp was exported in FASTA and MEGA formats, and was then used to determine uncorrected pairwise genetic distances between the samples with MEGA 6. The FASTA alignment was converted to PHYLIP format in the Alignment Transformation Environment (ALTER) website ([www.sing.ei.uvigo.es/ALTER](http://www.sing.ei.uvigo.es/ALTER)) and was subjected to a maximum likelihood (ML) analysis in RAxML GUI v. 1.3 (Stamatakis 2006) using the general time reversible model, GTR GAMMA, (as RAxML uses only the general time reversible (GTR) model of sequence evolution) with 500 bootstrap replicates. Likewise, for the Bayesian analysis, the FASTA alignment was converted to NEXUS format and analysed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) by running it for three million MCMC iterations initially until the standard deviation of the split frequencies reached a value of  $\leq 0.001$ . Else, the analysis was continued for another 10000–100000 generations

until the standard deviation of  $\leq 0.001$  was obtained for the split frequencies. Initial 20% of the trees were discarded as 'burn-in'. The tree files generated were then visualized using Fig Tree v. 1.4.0.

## RESULTS

Our analyses of molecular data (both maximum likelihood - ML and Bayesian - BI) recovered the two species allocated to the genus *Bufoides* to form a monophyletic group; with the two species *B. meghalayanus* and *B. kempfi* showing a congeneric, sister relationship to each other with high support (87 & 1.0 in ML and BI, respectively). The ML and BI analyses recovered the genera *Blythophryne* Chandramouli et al., 2016 & *Beduka* Dubois et al., 2021 to be close to the genus *Bufoides*, as assessed earlier (Chandramouli et al. 2016) although with low support (36 & 0.63 in ML & BI, respectively). Pairwise genetic divergence between *B. meghalayanus* and *B. kempfi* was found to be moderate (4.67 % at 16s rRNA) supporting their specific distinction from each other (Figures 1a,b). The PCA conducted based on 18 morphometric variables clearly separates the two species into two discrete clusters (Figure 2, Table 1).

## SYSTEMATICS

### *Bufoides kempfi* (Boulenger, 1919)

*Nectophryne kempfi* Boulenger, 1919

*Pedostibes kempfi* – Barbour, 1938

*Bufoides kempfi* – Chandramouli & Amarasinghe, 2016

Syntypes: Two specimens; an adult (29.8 mm SVL) and a subadult (17.4 mm) (ZSI 18481 a&b, respectively)

Other material studied: SACON VA 157 (an adult female) and, VA 181(a subadult female), and SACON VA156; VA 158 –160; VA 164 & VA 180 six adult males collected from Eman Asakgre (25.37°N, 90.54°E, 200 m asl.), Garo Hills, Meghalaya (Image 1).

Diagnosis: (after Chandramouli & Amarasinghe 2016)

A semi-arboreal to rupicolous *Bufoides* from the Garo Hills diagnosed by: small to medium body size (SVL 24.1–32.36 mm); presence of irregular, non-keratinized cranial ridges (pre and post orbital); short, ovoid parotoid glands; absence of an externally visible tympanum; moderate degree of webbing between toes (two phalanges of toe IV free); partial webbing between fingers, and the presence of small, slightly dilated, rounded terminal digital discs at the tips of both fingers and toes. Dorsum black with mossy green shade along the flanks in males, females predominantly green with black reticulations; a

**Table 1. Eigenvalues and the proportion of variance explained by each of the principal component.**

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
AG	0.13	-0.05	0.66	-0.25	-0.26	-0.16	0.19	0.13
BW	0.73	-0.38	-0.01	0.25	-0.25	0.11	-0.02	0.09
HL	0.24	0.32	-0.13	-0.46	-0.29	-0.10	0.05	-0.07
HW	0.51	0.20	-0.02	-0.02	0.31	0.01	0.04	0.10
HD	0.11	0.09	-0.19	0.08	0.02	-0.24	-0.15	-0.25
ED	0.16	0.08	0.06	0.10	-0.02	0.01	-0.36	0.23
EN	0.08	0.26	0.09	0.13	-0.16	0.22	-0.38	-0.49
ES	0.04	0.20	-0.22	0.16	-0.20	0.32	0.39	-0.18
UEW	0.04	0.08	-0.12	-0.09	0.16	0.13	-0.22	0.14
IO	-0.06	0.22	-0.12	0.16	-0.05	0.19	-0.03	0.33
IN	-0.07	0.27	0.03	0.08	-0.17	0.34	-0.15	0.54
UAL	-0.06	0.18	0.53	0.54	0.05	-0.08	-0.14	-0.14
LAL	-0.01	0.14	0.01	0.22	-0.13	-0.29	0.48	0.12
PAL	0.11	0.06	-0.17	0.38	0.35	-0.13	0.26	0.01
FEL	0.14	0.54	0.01	-0.03	-0.15	-0.10	0.09	-0.17
TBL	-0.03	0.29	-0.01	0.04	0.09	-0.40	-0.10	0.31
TAR	0.06	0.14	0.33	-0.18	0.45	0.51	0.28	-0.07
FOL	0.19	0.07	0.05	-0.22	0.44	-0.21	-0.14	-0.04
Eigenvalue	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
% variance	46.40	24.50	11.91	6.90	5.37	2.64	1.47	0.81

pale white venter; eggs partially pigmented and laid in strings within water-filled tree holes (phytotelmata).

Description and variation: (based on the newly collected material) Table 2

Female (SVL 32.36 mm) slightly larger than males (mean SVL 26.38 mm  $\pm$  0.88, n = 6). Head flat, fairly large, and distinct, (HL:SVL 0.31), broader than long, slightly more wider in the female (HL:HW 0.82) than in males (HL:HW 0.91), with an obtusely pointed to rounded snout tip. Trunk short (AG:SVL 0.4) and slightly gracile in males (AG:BW1.45) than in females (AG:BW 1.81). Eyes fairly large (ED:HL 0.33) their diameter shorter than the snout length (ED:ES 0.71). Nostrils situated closer to the snout tip than to the eyes (EN:ES 0.74). Upper eyelids wide, (mean UEW 2.92  $\pm$  0.12) rugose with keratinized pustules, narrower than the interorbital space (IO:UEW 1.79). Inter-orbital space broader than inter-narial space (IO:IN 1.59). Upper arms short (UAL:SVL 0.23), nearly as long as the lower arms (UAL:LAL 1.02); palm slightly shorter than the upper arms (UAL:PAL 0.88). Fingers partially webbed, webbing formula  $I_{0-1} II_{2-3} III_{3-2} IV$ ; relative length of fingers  $III > IV > II > I$ . Outer metacarpal tubercle large and evident. Finger tips with slightly expanded rounded discs. Femur relatively short (FEL:SVL 0.38), tibia slightly longer than femur (FEL:TBL 0.91); foot about as long as the femur

(FEL:FOL 0.99). Toes moderately webbed, webbing formula:  $I_{0-0} II_{0-0.5} III_{0.5-2} IV_{2-1} V$ , a relatively large inner and a slightly smaller ovoid metatarsal tubercle at the base of the foot. Toe tips with discs as broad as the toes; tarsal ridge not discernible. Vocal sac not discernibly distinct in males. Skin rugose in texture with keratinized granules.

#### Colouration in life

Males were generally dark grey in colour with traces of mossy green along the flanks and yellow patches near the axilla, belly and groin on the ventro-lateral region. Females are predominantly mossy green with an irregular black hour-glass pattern on the dorsum. Limbs visibly barred with black. Venter pale and much lighter than the dorsum (Image 2).

#### Osteology

Skull large and triangular, with an obtusely pointed snout tip. Pre and post-orbital ridges discernible. Frontoparietals fairly broad and hexagonal in shape. Nasal bones of the skull short, nearly as long as broad. Vertebral column with eight procoelous presacral vertebrae; the first four relatively larger than the following. Sacral diapophyses broad, flattened, and expanded laterally. Urostyle cylindrical, about half the length of the presacral



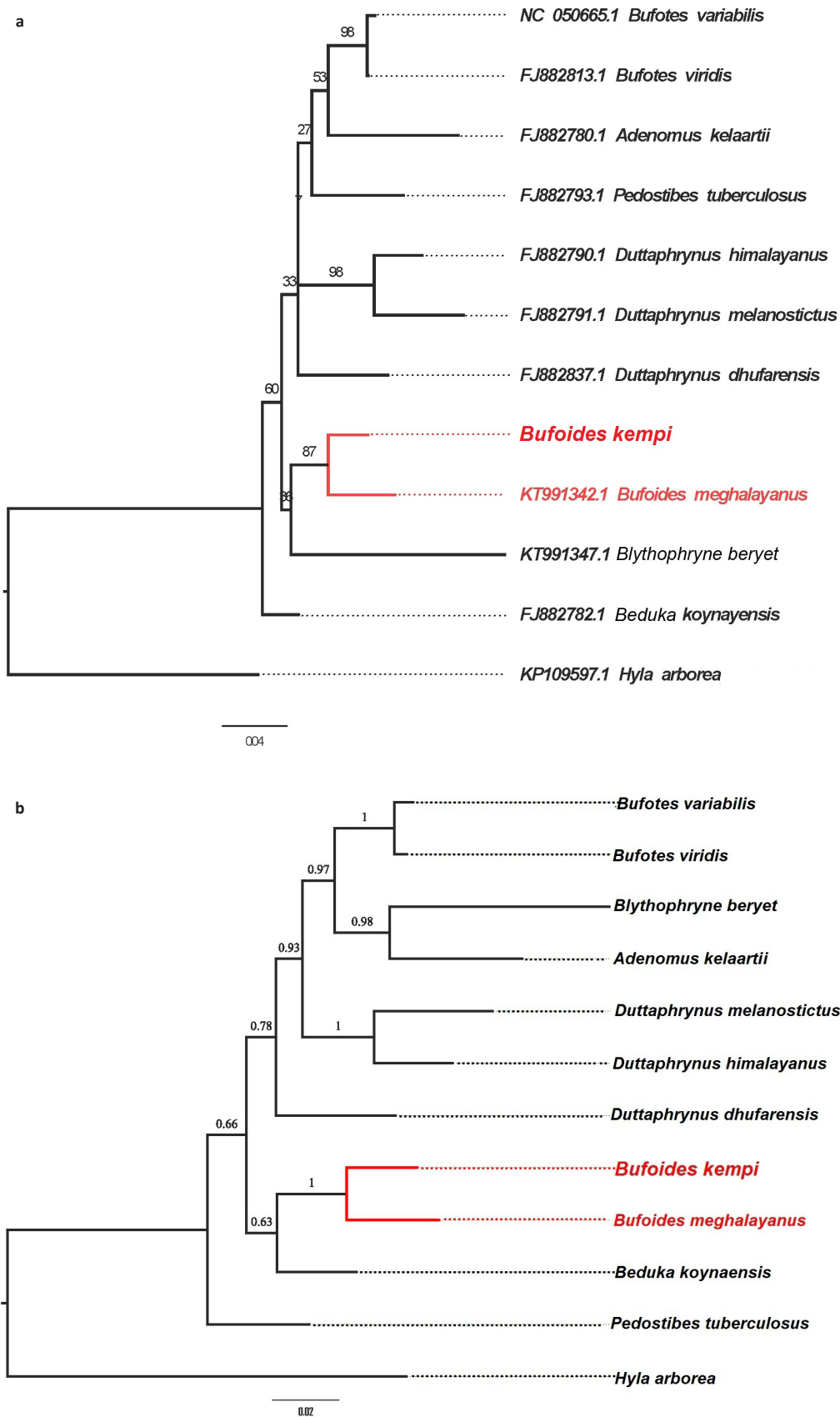


Figure 1. a— Maximum likelihood | b— Bayesian phylogenetic trees of Bufonids, showing the distinction of *Bufo*ides from other genera and sister relationship between *B. meghalayanus* and *B. kemp*i.

**Table 2. Morphometric measurements of *Bufoides kempfi* and *B. meghalayanus* at SACON.**

Species	<i>Bufoides kempfi</i>								
Voucher no:	VA 164	VA 159	VA 158	VA 160	VA 180	VA 156	MEAN	±SD	VA 157
Sex	M	M	M	M	M	M			F
SVL	25.3	29.3	26.8	24.1	32.7	26.3	27.4	3.1	32.4
AG	8.5	11.3	10.9	7.8	13.9	10.6	10.5	2.2	13.8
BW	6.2	9.5	7.3	5.3	12.5	5.5	7.7	2.8	7.6
HL	7.7	9.5	8.06	8.7	10.4	8.4	8.8	1.0	9.9
HW	8.8	10.6	8.8	9.2	13.9	9.2	10.1	2.0	12.1
HD	4.1	4.0	4	4.2	5.1	3.6	4.2	0.5	5.5
ED	2.8	3.1	2.9	2.3	3.8	2.5	2.9	0.5	3.3
EN	2.6	2.9	3.4	3.1	3.3	2.2	2.9	0.4	3.5
ES	4.1	4.7	3.7	4.6	4.9	2.9	4.1	0.8	4.5
UEW	2.6	2.9	2.2	2.7	2.8	2.7	2.7	0.3	3.2
IO	4.9	4.9	4.3	4.7	4.9	4.0	4.6	0.4	5.9
IN	2.9	2.9	2.4	2.6	2.2	2.2	2.5	0.3	4.1
UAL	6.5	6.2	7.7	5.4	8.9	6.2	6.8	1.3	7.2
LAL	6.3	6.2	6.1	5.8	8.1	5.6	6.4	0.9	7.4
PAL	7.2	6.9	6.3	6.7	9.4	6.2	7.1	1.2	8.9
FEL	9.6	10.1	9.7	10.2	12.2	9.4	10.2	1.0	12.3
TBL	11.2	11.1	10.9	10.6	13.3	11.2	11.4	1.0	13.1
TAR	5.4	7.6	6.2	6.2	8.9	7.3	6.9	1.3	7.5
FOL	8.8	10.2	9.3	9	12.7	10.4	10.1	1.4	13.0
F1	1.7	2.3	1.2	1.4	2.3	1.6	1.8	0.5	2.5
F2	2.8	2.6	2.4	1.7	3.1	2.4	2.5	0.5	4.9
F3	5.4	4.4	4.2	3.6	5.9	3.5	4.5	1.0	7.1
F4	3.7	4.0	2.9	2.6	4.9	2.8	3.5	0.9	5.5
T1	1.3	1.4	1.2	0.9	1.5	1.3	1.3	0.2	2.1
T2	2.5	4.1	1.8	2.7	2.7	2.1	2.7	0.8	2.9
T3	4.9	3.8	2.6	4.1	4.0	3.3	3.8	0.8	3.9
T4	7.4	6.0	4.7	5.9	6.6	4.8	5.9	1.0	7.7
T5	4.0	2.4	2.7	4.2	3.9	2.9	3.3	0.8	4.9

Species	<i>Bufoides meghalayanus</i>				
Voucher no:	VA 215	VA 251	VA 252	MEAN	±SD
Sex	M	M	M		
SVL	31.3	33.5	31.2	31.9	1.3
AG	10.7	13.2	12.1	12.3	1.4
BW	12.0	14.1	10.9	12.3	1.6
HL	9.7	12.9	12.7	11.8	1.8
HW	12.0	16.8	12.9	13.9	2.5
HD	5.4	6.2	5.4	5.7	0.5
ED	3.2	5.3	3.8	4.1	1.1
EN	2.1	4.9	3.6	3.5	1.4
ES	4.2	5.4	5.1	4.9	0.6
UEW	3.2	4.1	3.1	3.5	0.6



Species	<i>Bufoides meghalayanus</i>					
	Voucher no:	VA 215	VA 251	VA 252	MEAN	±SD
IO		4.3	5.7	5.1	5.0	0.7
IN		1.1	3.2	2.9	2.4	1.2
UAL		5.3	8.2	6.4	6.6	1.5
LAL		6.5	7.4	7.6	7.1	0.6
PAL		8.6	9.7	7.5	8.6	1.1
FEL		9.6	14.4	13.2	12.4	2.5
TBL		11.9	14.4	13.4	13.2	1.3
TAR		6.4	9.3	6.9	7.6	1.5
FOL		11.9	14.3	11.8	12.7	1.4
F1		2.1	2.0	1.8	1.9	0.2
F2		3.2	3.2	3.1	3.2	0.1
F3		5.4	4.7	4.4	4.8	0.5
F4		4.3	4.4	3.9	4.2	0.3
T1		2.1	2.4	1.6	2.0	0.4
T2		3.2	2.9	3.2	3.1	0.2
T3		5.4	4.5	3.7	4.5	0.8
T4		9.7	6.6	5.9	7.4	2.0
T5		6.5	5.0	3.8	5.1	1.3

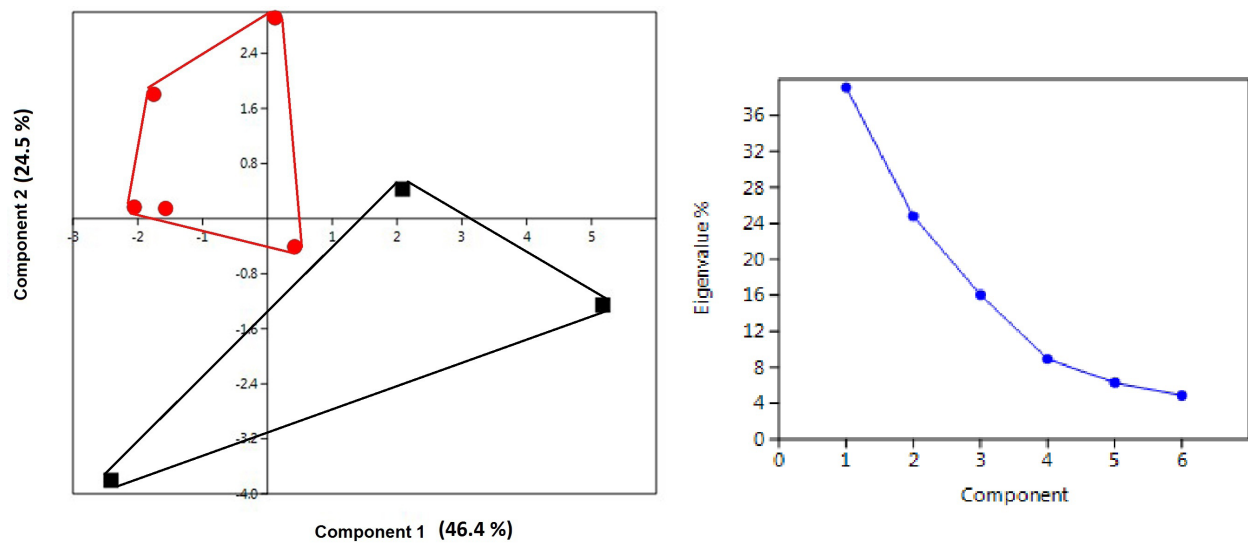


Figure 2. Left— Plot of PCA showing morphometric distinction between *B. meghalayanus* (black squares) and *B. kempi* (red dots) | Right— Scree plot showing the eigenvalues of each principal components.

vertebral column, lacking lateral expansions. Iliac curved laterally, as long as the urostyle. Ischium protruding posteriorly. Pectoral girdle arciferous. Humerus longer than radio-ulna. Phalangeal formula of the fingers: 2-2-3-3. Femur long, nearly as long as the tibiofibula; tarsus about 3/4<sup>th</sup> the length of tibiofibula. Phalangeal formula of the toes 2-2-3-4-3 (Image 3).

**Breeding biology and natural history**

A total of 17 individuals were seen in the following precise locations surrounding Eman Asakgre community reserve, South Garo Hills, the details of which are mentioned below (Image 4).

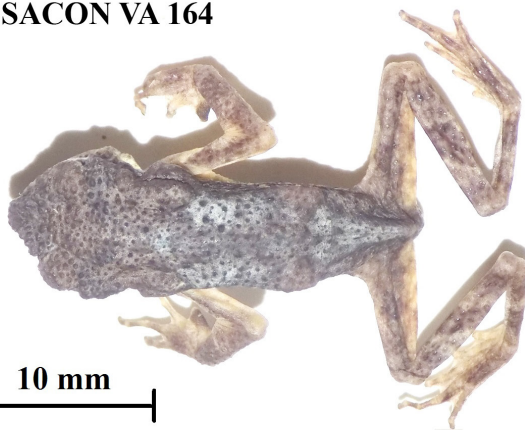
10 mm  
SACON VA 157



10 mm



SACON VA 164



10 mm

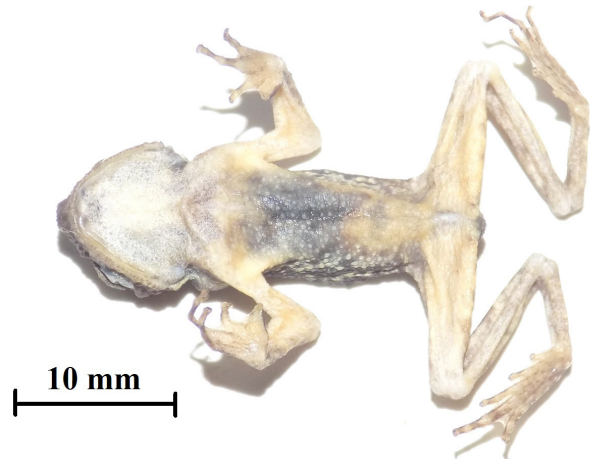


Image 1. *Bufoides kempí* in preservation—adult female (above: SACON VA 157) and an adult male (below: SACON VA 164) collected during this study.

#### Chibanda cave (25.36° N, 90.53° E, 122 m)

The cave was about 6 m below the ground level, surrounded by moist evergreen forest. A small creek was flowing into the cave and the surface of the rocks and boulders in the area was covered with moss and were wet. The canopy cover provided about 70% shade to the ground. The first individual, a subadult male was found under a boulder near the mouth of the cave. Second individual was seen inside the cave in a deep narrow Horizontal crevice of a limestone rock. *Odorrana chloronota*, *Amolops assamensis*, *Limnonectes khasianus*, and *Ingerana borealis* were some of the anuran species that were observed in sympatry with *B. kempí* at this location. During the night surveys in the subsequent months, seven more individuals were spotted in total. Some individuals were observed on leaves of shrubs without exhibiting any specific

behaviour between 1900–2200 h. Later in October, an adult male was sighted in a tree hole filled with rain water at a height of about 2 m above the ground and a subadult female was recorded on low lying shrubs at about one foot above the ground.

#### Dhangit cave (25.36° N, 90.52° E, 220 m)

This cave, surveyed in May was at a depth of about 12 m, surrounded by moist evergreen forest; the terrain was rocky and filled with boulders covered with moss. No individuals were found inside the cave, however two males were observed near the cave about 150 m away which were calling actively from a cavity filled with rain water (5 cm deep, water temperature 21° C) measuring 10 cm in diameter at about 1.2 m off the ground at around 2200 h possibly trying to attract the attention of a receptive female nearby. Two males were observed





Image 2. *Bufoides kempii* (male—left & middle and female—right) in life from Eman Asakge, Garo Hills. © left and middle: S.R. Chandramouli, right: R.S. Naveen

Table 3. Larval measurements of *Bufoides kempii*.

Stage	20	20	20	Mean	±SD	21	30	31	31	Mean
HBL	5.4	6.14	5.8	5.77	0.37	5.3	5.34	5.28	5.5	5.39
TOT	10.1	9.56	9.58	9.75	0.31	10.6	11.64	12.2	14.36	13.28
TAIL	4.7	3.42	3.78	3.98	0.66	5.3	6.3	6.92	8.86	7.89
HBW	1.7	2.3	1.74	1.91	0.34	2.76	2.36	2.72	2.8	2.76
TH	1.6	2.44	1.7	1.91	0.46	2.34	1.88	2.44	2.2	2.32
IO	0.3	0.3	0.3	0.00	0.00	0.4	1.46	2.64	2.6	2.62
HBH	2.82	2.6	1.9	2.44	0.48	2.0	2.24	2.24	2.42	2.33

to show aggression by kicking each other while calling and one of them was seen kicking the other with its hind feet repeatedly. Upon further search in the region, a tree cavity with about 30–40 eggs laid in strings was found. Eggs from this cavity were collected and maintained in a plastic jar with water from the same cavity for the next 11 days.

#### Cehise Stream (25.34° N, 90.51° E, 250 m)

The area surrounding a small stream flowing near the village of Eman Dura Banda was surveyed in May. This area had a rocky terrain and was covered with moist evergreen forest, with a tall canopy. An adult male was found resting under a boulder.

#### Eman Asakge Community Reserve (25.37° N, 90.54° E, 108 m)

Congregation of four males was seen along with a female in a buttress root cavity at a height of 0.6 m above the ground, measuring about 15 cm in diameter, filled with 10 cm of rain water. The water temperature here was 23.5° C, the humidity of the location was 80%. The group was first spotted at about 1730 h in amplexus with four males and a female. One of the four males was seen mounted ventrally while the other three

males were mounted dorsally and laterally. Amplexus was axillary. The episode lasted till about 2100 h by the time the female laid egg strings with 30–40 partially pigmented eggs that measured about 2 mm diameter. Once the eggs were laid both males and the female started leaving the cavity and no further attendance was observed.

#### Description of calls

The call of *B. kempii* described here was composed of syllables of 'treak.. treak...' that lasted for a duration of 2.1 s and was composed of three distinct notes, each of which comprise seven–ten pulses. The mean duration of each note was 6.33 ms, with a mean interval of 1.05 s in between. Maximum amplitude of the call was -1 dB with a dominant frequency of 2.5 kHz (Image 5).

#### Larval description: (Table 3)

On day three, 39 larvae emerged which were reared subsequently for the next eight days during which their development was documented. Stage-wise descriptions of the larvae are presented in detail below.

#### Stage 20: (n = 3)

The larvae reached stage 20 on day two after

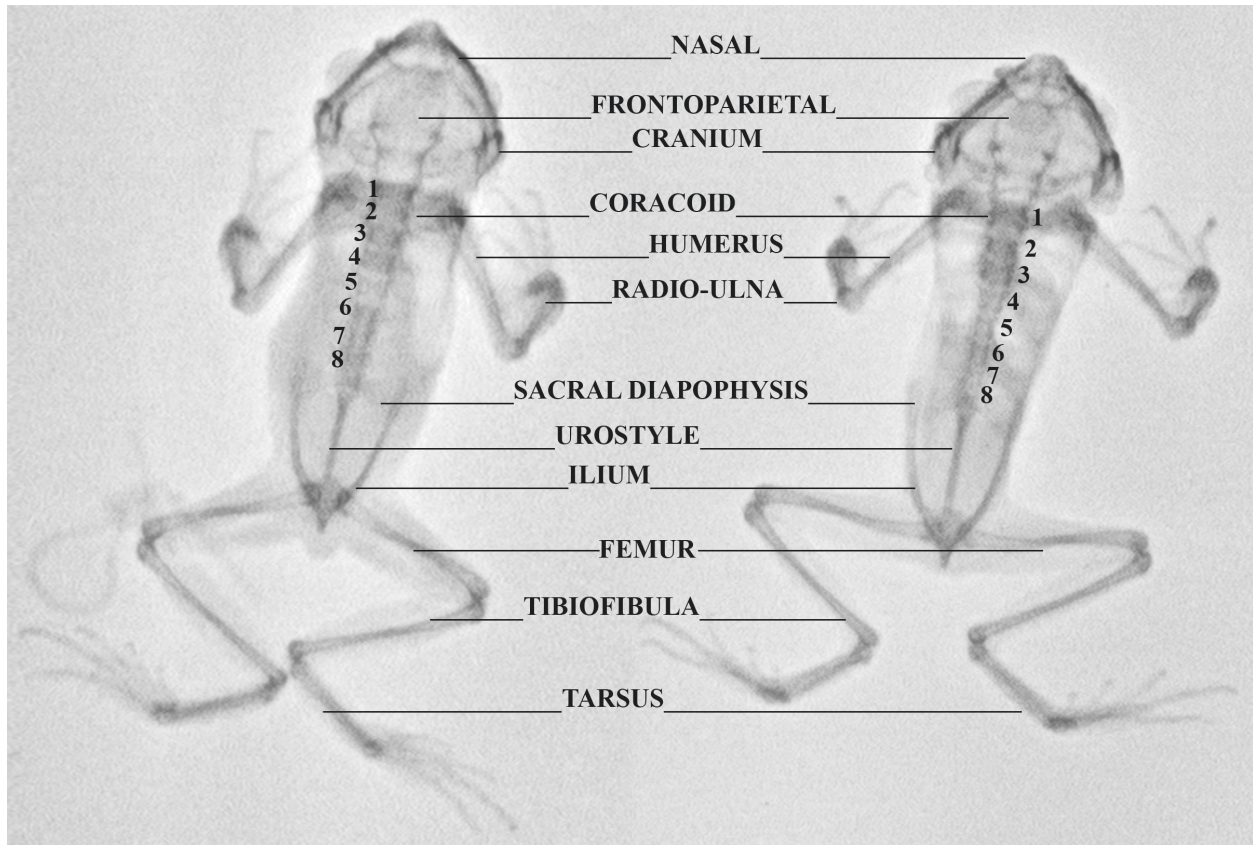


Image 3. Osteology of *B. kempfi* based on an adult male and female vouchers SACON VA 157 and VA 159, respectively.



Image 4. Axillary amplexus between one female and three males of *B. kempfi* observed in a phytotelmata at Eman Asakgre, Garo Hills.

emerging from the eggs. At this stage, they measured  $9.75 \text{ mm} \pm 0.31$  in total length, with a head-body length of  $5.77 \text{ mm} \pm 0.37$ ; streamlined and narrow in form (HBW  $1.91 \text{ mm} \pm 0.3$ ); slightly higher than broad (HBH  $2.44 \text{ mm} \pm 0.48$ ); tail a little shorter than the head-body ( $3.98 \text{ mm} \pm 0.66$ ); and with an average tail height of  $1.91 \text{ mm} \pm 0.46$ . Eyes and mouthparts not discernible at this stage.

**Stage 22: (n = 1)**

The larvae reached stage 22 on day four after emergence from the eggs. At this stage, the body & tail elongated a little more, with the larva measuring  $10.6 \text{ mm}$  in total length, with a head-body length of  $5.3 \text{ mm}$  and a relatively longer tail ( $5.3 \text{ mm}$ ) which equalled the HBL. Head-body oval & narrow (HBW  $2.76 \text{ mm}$ ; HBH  $2.0 \text{ mm}$ ). Tail-fin relatively well developed than in the earlier stage, longer and broader, with a height of  $2.34 \text{ mm}$ . Eyes dorsal in position and traces of gills discernible at this stage.

**Stage 30: (n = 2)**

At this stage, the larvae grew a little longer, measuring



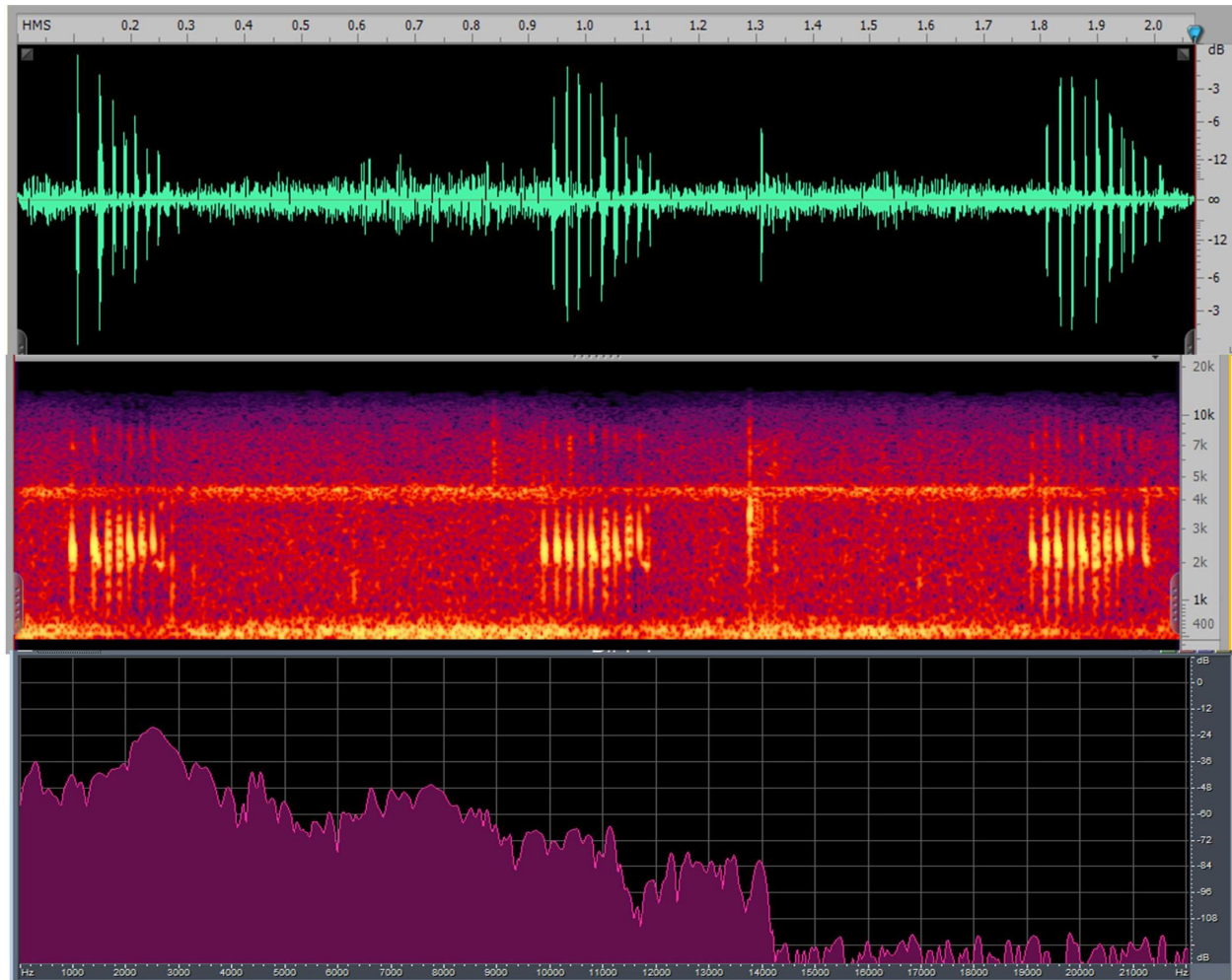


Image 5. Oscillogram, spectrogram and power spectrum of the call of *B. kemp*i.

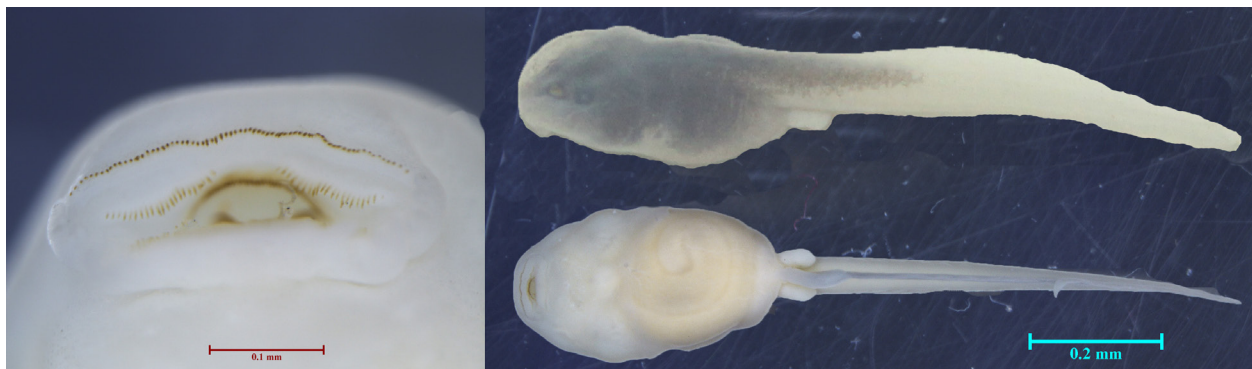


Image 6. Stage 30 larva of *B. kemp*i: Mouthpart and ventral view.

13.28 mm in length, with a 7.9 mm long tail and 5.39 mm long head-body. Head-body ovoid, broader (HBW 2.76 mm) than deep (HBH 2.33 mm). Oral disc discernible at this stage with keratodont and keratinized jaw sheaths; oral tooth-row formula 1/1+1//1/1. Tail fins transparent

and high 2.32 mm. Rudimentary hindlimb buds visible at the posterior end of the head-body. Eyes well developed than in the earlier stage and in lateral position, with an inter-orbital space of 2.62 mm (Image 6).



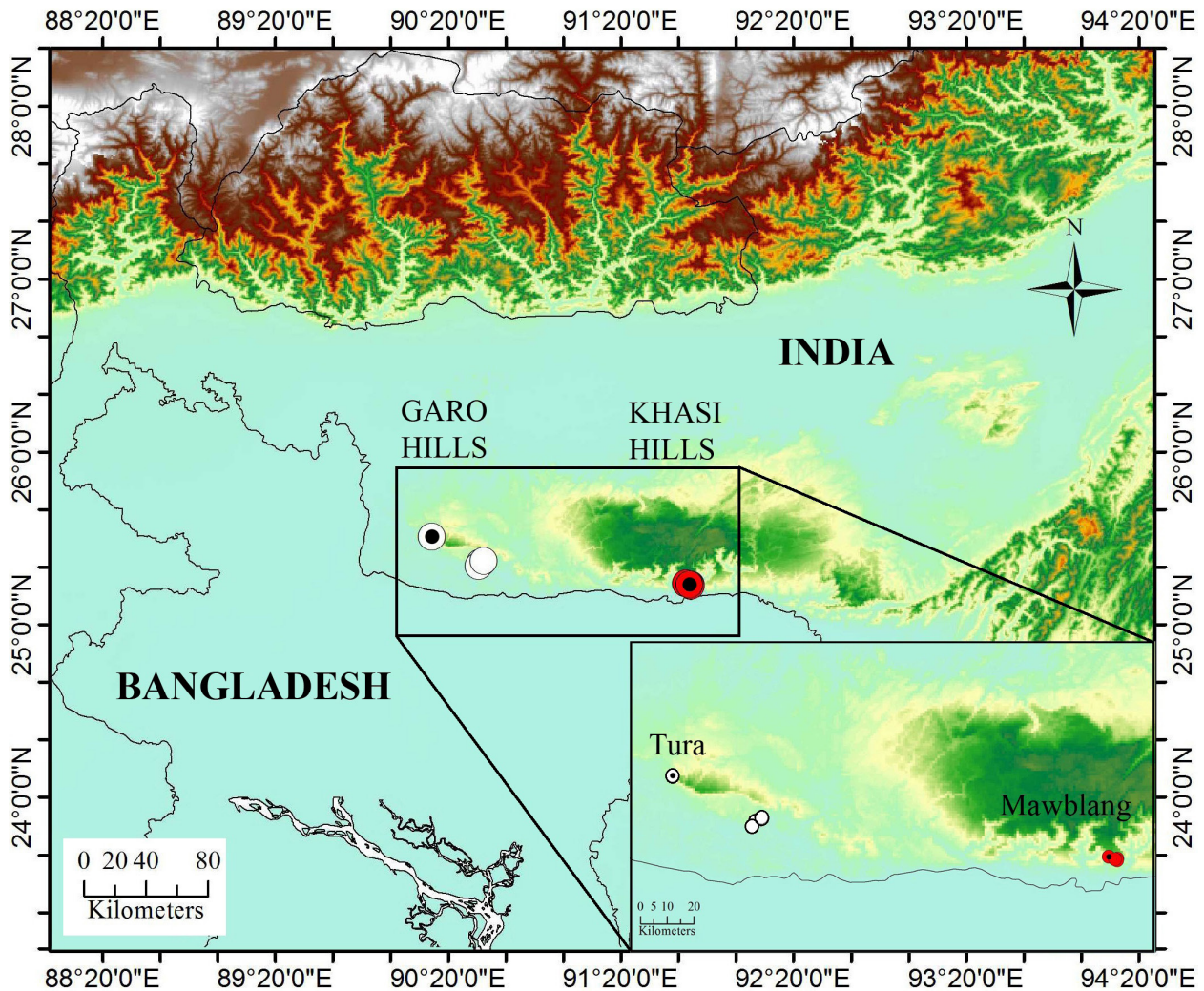


Figure 3. Map showing the distribution of *B. kemp*i (white circles; Garo Hills) and *B. meghalayanus* (red circles; Khasi Hills; type localities with a dot in the middle).

### Distribution

During the present study, *B. kemp*i was recorded from the above four locations, of which, two are quite close-by and the type locality, Tura, lies at about 64 km northwest of the present study sites. However, our surveys at locations north of the Garo ridge at a higher elevation such as Mandalgre (25.50 °N, 90.37 °E, 1,019 m) and Daribokgre (25.48 °N, 90.31 °E, 1,123 m) could not locate this species. Further surveys at the vicinity of the current study sites and locations to the north of the Garo ridge in the lower reaches are necessary to determine whether the species occurs in those areas as well (Figure 3).

### Abundance

The rate of encounter of *B. kemp*i was 0.53/hour, or two hours of effort to locate one individual in this region.

The encounter rate across the survey duration of 57 hours ranged from 0.17–2.0 (Table 4).

### *Bufo*ides *meghalayanus* (Yazdani & Chanda, 1971)

*Ansonia meghalayana* Yazdani & Chanda, 1971

Holotype: ZSIC A 6969, an adult from Mawblang, Cherrapunji, Khasi Hills, Meghalaya.

Material studied: SACON VA 215, SACON VA 251 and SACON VA 252 three adult males from a hill stream in the Khasi Hills, Meghalaya (Image 7).

Diagnosis and comparison: A semi-arboreal to rupicolous *Bufo*ides from the Khasi Hills diagnosed by: small–medium body size (SVL 31. –33.5 mm); presence of irregular, non-keratinized cranial ridges (pre and post orbital); short, elongated parotoid glands (vs. ovoid in *B. kemp*i); absence of an externally visible tympanum; well-developed webbing between toes, with only half a

phalange free of webbing on toe IV (vs. relatively poor, with two phalanges free of webbing on toe IV in *B. kemp*i); partial, but better developed webbing between fingers than *B. kemp*i and the presence of small, slightly dilated, rounded terminal digital discs at the tips of both fingers and toes. Dorsum black with mossy green along the flanks in males, females predominantly green with black reticulations; a dark grey venter with small white spots (vs. pale white in *B. kemp*i).

Description and Variation: (based on the newly collected material) Table 2

Body small (31.99 mm  $\pm$  0.76), trunk relatively short (AG:SVL 0.38) and stout (AG:BW 1.0). Head large (HL:SVL 0.37); slightly broader than long (HL:HW 0.85); and half as deep as long (HL:HD 2.08); snout tip obtusely pointed in dorsal view. Eyes large (ED:HL 0.35); snout slightly longer than eye (ED:ES 0.84); interorbital space about one and half times the width of the upper eyelid (IO:UEW 1.46) and nearly twice the internarial distance (IO:IN 2.09). Tympanum absent. Upper arm short (UAL:SVL 0.21); lower arm slightly longer than upper arm (UAL:LAL 0.93); palm a little longer (UAL:PAL 0.77). Fingers partially webbed, webbing formula  $I_{0-1} II_{0-2} III_{1-1} IV$ ; relative length of fingers  $III > IV > II > I$ . Outer metacarpal tubercle large & evident. Thigh relatively short (FEL:SVL 0.39); tibia a little longer than the thigh (FEL:TBL 0.94); foot nearly as long as the thigh (FEL:FOL 0.98). Toes partially webbed, webbing formula:  $I_{0-0} II_{0-0} III_{0-0.5} V_{0.5-0} V$ , a relatively large inner and a slightly smaller ovoid metatarsal tubercles at the base of the foot. Toe tips with discs as broad as the toes; tarsal ridge not discernible. Finger and toe-tips bearing slightly expanded terminal discs lacking circum-marginal grooves. Dorsal colouration uniform black, with irregular feeble yellow markings; venter grey with fine white spots.

#### Description of calls

The call of *B. meghalayanus* recorded during this study comprised of a series of high pitched syllables of 'ti-tuk' that lasted for a duration of 20 s and was composed of eight separated notes, each of which comprise two pulses. The mean duration of each note was 0.28 ms, with a mean interval of 2.45 s in between. Maximum amplitude of the call was -7 dB with a dominant frequency of 1.0 kHz (Image 8).

#### Distribution: (Table 4)

During this study, *B. meghalayanus* was recorded from a few locations in the Khasi hills within an altitudinal range of 1060–1240 m that are mapped in Figure 3. Additional localities were provided by Deuti et al. (2012).

#### Abundance

The rate of encounter of *B. meghalayanus* was 0.875 / hour, or about an hour of effort to locate one individual in this region. The encounter rate across the survey duration of 38 h ranged from 0.67–5.33 (Table 4).

#### DISCUSSION

*B. kemp*i was described by Boulenger in 1919 based on the two specimens collected by S.W. Kemp, and presented to him by Nelson Annandale from 'above Tura, 2,500 ft' in the Garo Hills. Since its description, no further records or observations of this species have been made until now. Studies conducted in this region have uncovered several new and noteworthy species but *B. kemp*i remained elusive to scientists until now (Datta-Roy et al. 2013; Deuti et al. 2012; Biju et al. 2016; Giri et al. 2019). Although Das et al. (2009) mentioned a specimen (MFA 10134) of *Bufo*ides collected from Tura, Garo Hills, no taxonomic assessment of this specimen has been made until now, which still remains unidentified. Therefore, with the results of the present study, we announce the authentic rediscovery of *B. kemp*i after a period of more than a century (1919–2022) from near the type locality, Garo Hills. First ever field observations on its ecology, behaviour, breeding biology, and natural history have been presented here. Observation on their breeding in phytotelmata and multiple males participating in amplexus with a single female have been made for the first time. Das & Dutta (2007) noted the absence of any larval descriptions for *B. kemp*i, which has now been provided for the first time, across three developmental stages. Based on our field observations, the distribution of *B. kemp*i mapped here shows that it is restricted to the lower reaches of a small hillock in the western part of Meghalaya, south of the Garo Hills, across an elevation range of 100–250 m. above which *B. kemp*i was not detected despite intensive surveys. In comparison, *B. meghalayanus* was found to be a strictly montane species occurring only on the hilltops between elevations of 1,000–1,240 m. Further surveys north of the Garo ridge could possibly uncover additional locations characterized by lowland evergreen forests with perennial streams and rock boulders, that could potentially be occupied by *B. kemp*i and we recommend additional surveys in such localities in the future.

Unlike *B. kemp*i, for *B. meghalayanus*, the original descriptions of the species as well as the genus were comprehensive in terms of both morphology & natural history (Yazdani & Chanda 1971; Pillai & Yazdani 1973).





Image 7. Adult male *Bufoides meghalayanus* in life (dorsal and ventral views). © S.R. Chandramouli.

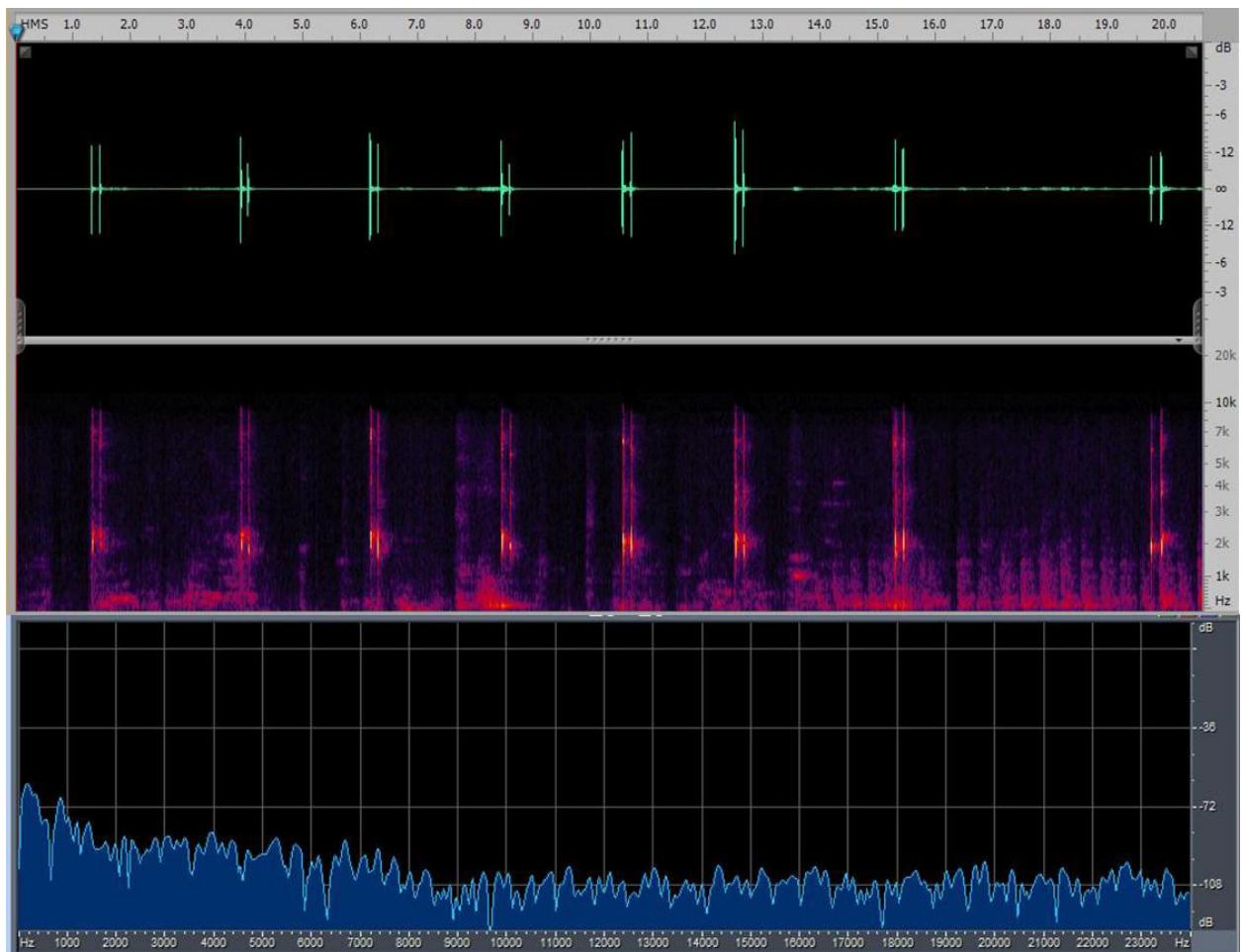


Image 8. Oscillogram, spectrogram and power spectrum of the call of *B. meghalayanus*.



Table 4. Abundance estimates of *B. kempfi* and *B. meghalayanus*.

<i>Bufoides kempfi</i>												
Site	Lat. (°N)	Long. (°E)	Elevation (m asl.)	time	Duration (h)	No. of ind.	ER	Microhabitat	Forest type	Habitat	Canopy cover	Ambient temperature (°C)
Eman Asakgre	25.40	90.54	225	day	3	1	0.33	Under boulder	Evergreen	Dry stream	90	26
Eman Asakgre	25.36	90.53	122	day	6	1	0.17	Under boulder	Evergreen	Stream	90	27
Eman Asakgre	25.37	90.54	202	day	6	5	0.83	Tree hole	Evergreen	Forest	90	26
Eman Asakgre	25.36	90.53	122	night	6	2	0.33	Tree hole	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	122	night	5	1	0.20	on leaf	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	220	night	6	2	0.33	Tree hole	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	220	night	6	2	0.33	on leaf	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	220	night	6	4	0.67	on leaf/under boulder	Evergreen	Forest	90	23
Eman Asakgre	25.34	90.51	250	night	6	1	0.17	Under boulder	Evergreen	Forest	90	23
Eman Asakgre	25.37	90.54	202	night	6	3	0.50	on leaf	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	122	night	1	2	2.00	Tree hole/on leaf	Evergreen	Forest	90	23
					<b>57</b>	<b>24</b>	<b>0.53</b>					
<i>Bufoides meghalayanus</i>												
Stream behind Mawsmi cave	25.25	91.72	1200	Day/ Night	1	0	0		Montane	Stream	70	21
Stream behind Mawsmi cave	25.25	91.72	1200	Day/ Night	1	0	0		Montane	Stream	70	21
Stream 1 behind Mablang village	25.24	91.74	1200	Day/ Night	1	0	0		Montane	Stream	80	21
Stream 1 behind Mablang village	25.23	91.74	1200	Day/ Night	2	1	0.67	Rock Crevice	Montane	Stream	80	21
Stream behind Mawsmi cave	25.25	91.72	1200	Night	1	0	0		Montane	Stream	70	20
Stream 1 behind Mablang village	25.23	91.74	1200	Day/ Night	4	0	0		Montane	Stream	80	20
Stream behind Mawsmi cave	25.25	91.72	1200	Day/ Night	5	0	0		Montane	Stream	70	20
Stream 2 behind Mablang village	25.23	91.74	1200	Day/ Night	4	0	0		Montane	Stream	80	20
Stream 2 behind Mablang village	25.23	91.74	1200	Day	4	0	0		Montane	Stream	80	20
Stream 1 on-route to Thangkarank park	25.239	91.73	1200	Day	6	4	1.33	Rock Crevice	Montane	Dry stream	90	18
Stream 2 on-route to Thangkarank park	25.23	91.74	1200	Day/ Night	6	32	10.67	Rock Crevice / Pandanus tree	Montane	Dry stream	95	21
Stream 1 on-route to Thangkarank park	25.23	91.73	1200	Day	6	19	3.17	Rock Crevice	Montane	Dry stream	90	22
					<b>38</b>	<b>56</b>	<b>0.875</b>					

Subsequent studies have supplemented information on its morphology (Das et al. 2009), osteology (Chandramouli & Amarasinghe 2016); ecology and distribution (Deuti et al. 2012). Das et al. (2009) after examining the specimen reported by Pawar & Birand (2001) from Mizoram, opined that it is not conspecific with *B. meghalayanus*. Hence, we do not include that record within the range of *B. meghalayanus*. Therefore, the Mizoram population of *Bufoides* reported by Das et al. (2009) still needs a proper taxonomic assessment.

#### Availability of Data

Specimens collected and studied are deposited in the collections of SACON. DNA sequence generated in this study has been deposited in the genbank under the NCBI voucher number OP920605.

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## Communications

### A preliminary survey of moss flora of Chail Wildlife Sanctuary, Himachal Pradesh, India

– Meenal Sharma, Anju Rao & S.S. Kumar, Pp. 22207–22214

### New distribution record and DNA barcoding of *Sapria himalayana* Griff. (Rafflesiaceae), a rare and endangered holoparasitic plant from Mizoram, India

– Laldinfeli Ralte, Hmingremhlua Sailo, Sagolshem Priyokumar Singh, Laldinliana Khiangte & Y. Tunjinba Singh, Pp. 22215–22220

### Species distribution modeling of a cucurbit *Herpetospermum darjeelingense* in Darjeeling Himalaya, India

– Debasruti Boral & Saurav Moktan, Pp. 22221–22231

### An updated catalogue of true flies (Insecta: Diptera) from northern Pakistan

– Noor Fatima & Ding Yang, Pp. 22232–22259

### Desert Carabidae (Insecta: Coleoptera) of India

– S.V. Akhil, Sabu K. Thomas & Sanjeev Kumar, Pp. 22260–22269

### Photographic evidence of fish assemblage in artificial reef site of Palk Bay - an implication for marine resource management

– Koushik Sadhukhan, T. Shanmugaraj, Ramesh Chatragadda & M.V. Ramana Murthy, Pp. 22270–22276

### Systematics of the enigmatic and narrowly endemic toad genus *Bufoides* Pillai & Yazdani, 1973: rediscovery of *Bufoides kempfi* (Boulenger, 1919) and expanded description of *Bufoides meghalayanus* (Yazdani & Chanda, 1971) (Amphibia: Anura: Bufonidae) with notes on natural history and distribution

– R.S. Naveen, S.R. Chandramouli, Gautam Kadam, S. Babu, P.V. Karunakaran, H.N. Kumara & N. Parthasarathy, Pp. 22277–22292

### Avifaunal diversity in Indian Institute of Technology Guwahati Campus, Assam, India

– Umang H. Rathod & Rupam Bhaduri, Pp. 22293–22308

## Reviews

### Threatened flora of Uttarakhand: an update

– D.S. Rawat, Satish Chandra & Preeti Chaturvedi, Pp. 22309–22328

### A systematic review on the feeding ecology of Sloth Bear *Melursus ursinus* Shaw, 1791 in its distribution range in the Indian subcontinent

– Vasantkumar Rabari & Nishith Dharaiya, Pp. 22329–22336

## Short Communications

### Mercury in tuna from the western equatorial Atlantic Ocean and health risk assessment

– Ana Paula Madeira Di Benedetto, Inácio Abreu Pestana, Igor David da Costa, Marcelo Gomes de Almeida, Bráulio Chereze Vaz de Oliveira & Carlos Eduardo de Rezende, Pp. 22337–22340

### First photographic record of Spotted Deer *Axis axis* (Erxleben, 1777) (Artiodactyla: Cervidae) in Great Indian Bustard Sanctuary, Maharashtra, India

– Shaheer Khan, S. Ramesh Kumar & Bilal Habib, Pp. 22341–22345

### Comparative study of morphology and keratin levels in hair from deer and goat

– Sangeeta Patle, Divya Bagchi & K.P. Singh, Pp. 22346–22350

## Response & Reply

### Is trade the reason for the unusual colour morph of Cobra from Goa? Response to Sawant et al.

– Raju Vyas & Harshil Patel, Pp. 22351–22353

### Corrections to 'An unusual morph of *Naja naja* (Linnaeus, 1758) from Goa, India (Serpentes: Squamata)'

– Nitin Sawant, Amrut Singh, Shubham Rane, Sagar Naik & Mayur Gawas, P. 22354

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