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"Endangered or an artifact of unsound taxonomy? Case of the critically endangered bush frog Philautus sanctisilvaticus Das and Chanda, 1997"

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Abstract

We investigated the phylogenetic relationship of the Critically Endangered bush frog *Philautus sanctisilvaticus* Das and Chanda, 1997 and other species distributed across the fragmented forests of Deccan Peninsula and the northern Eastern Ghats. A short fragment of the mitochondrial 16S rRNA gene was employed to assess phylogenetic relationships across *Philautus sanctisilvaticus* Das and Chanda, 1997, *Raorchestes terebrans* (Das and Chanda, 1998) and *Philautus similipalensis* Dutta, 2003. All sequenced specimens, including material from near the type localities of *P. sanctisilvaticus* (Amarkantak) and *P. similipalensis* (Simlipal) were genetically extremely similar, with pairwise uncorrected distances <1% in the 16S gene, and were phylogenetically placed within the genus *Raorchestes*. The results based on morphology are ambiguous and do not go hand in hand with molecular data, which however do not provide support for a three species hypothesis either. our findings advocate the need for making nomenclatural amendments. *Philautus sanctisilvaticus* Das and Chanda, 1997, is the first available nomen for this taxon, and we propose to include this species in *Raorchestes* as *Raorchestes sanctisilvaticus* (Das and Chanda, 1997), and to consider the nomina *Philautus terebrans* Das and Chanda, 1998 **syn. nov.** and *Philautus similipalensis* Dutta, 2003 **syn. nov.** as junior subjective synonyms for this nomen following the Principle of Priority in article 23.1 of the ICZN. The findings are notable from the point of conservation of the species and present a novel case with remarkable genetic homogeneity across the fragmented forests of Deccan Peninsula and Eastern Ghats.

Key words: IUCN, Critically Endangered, conservation, Amphibia, Anura, taxonomy, 16S, phylogeny, *Philautus terebrans* **syn. nov.**, *Philautus similipalensis* **syn. nov.**

Introduction

Bush frogs distributed across South and Southeast Asia were until recently grouped under the genus *Philautus* Gistel (Biju & Bossuyt 2009). However, the genus was shown to be polyphyletic and was subsequently split into three genera *Raorchestes* Biju, Shouche, Dubois, Dutta, and Bossuyt, *Pseudophilautus* Laurent and *Philautus* (Biju *et al.* 2010; Yu *et al.* 2009). Species currently known from the Western Ghats of India were assigned to *Raorchestes*, *Pseudophilautus*, and those from Northeast India and the Eastern Ghats to the genus *Philautus* (vijayakumar *et al.* 2014, 2016). The amendment was based on molecular data for species from Western Ghats and Southeast Asia, but there is no data available on species distributed in the Eastern Ghats. The Eastern Ghats are discontinuous chain of hills extending from Odisha running through parts of Jharkhand, Chhattisgarh, Odisha, Andhra Pradesh, and Telangana up to central Tamil Nadu. Forest covering this region also extends to the Deccan Plateau, however the connectivity is poor between patches. This landscape, although considered less biodiverse that that of the Western Ghats, is home to some unique species of reptiles and amphibians, namely, *Calodactylodes aureus* Beddome, *Sepsophis punctatus*

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Beddome, *Cyrtodactylus jeyporensis* (Beddome), *Barkudia melanosticta* (Schneider), *B. insularis* Annandale, *Geneophis orientalis* Agarwal, Wilkinson, Mohapatra, Dutta, Giri & Gower etc. Only a few opportunistic surveys have been conducted across the eastern Ghats and species that have been described from this region, remain poorly studied.

There are three species of bush frogs described from the Eastern Ghats and the adjoining Deccan Peninsula, namely: *Philautus sanctisilvaticus* Das and Chanda, 1997, *Philautus similipalensis* Dutta, 2003, and *Philautus terebrans* Das and Chanda, 1998. Of these the Amphibian Species of the World database (Frost, 2019) considers the former two in the genus *Philautus*, and the latter as belonging to the genus *Raorchestes*. *Philautus sanctisilvaticus* is evaluated as Critically Endangered concerning its conservation status (Das *et al.* 2004) and hence for better conservation planning it is imperative to ascertain its taxonomic status, distribution range and biology to contribute towards its conservation. The two species, *R. terebrans* and *P. similipalensis* on the other hand are treated as Data Deficient. All these three species are distributed across the fragmented landscape of the Eastern Ghats and Deccan Plateau. A study of these frogs would help understand genetic structure across such fragmented landscapes.

Philautus sanctisilvaticus (Fig 1a–c) was described from Amarkantak, Madhya Pradesh based on three specimens (one male and two females). The species was distinguished from congeners in bearing a head wider than long; tympanum small, concealed; webbing on toe IV up to basal subarticular tubercle on the inner side and to the distal subarticular tubercle on the outer side. This species is morphologically similar to *Raorchestes bombayensis* (Annandale) from which it differs in lacking a median papilla on tongue and absence of dermal fringes on outer toe (Das & Chanda 1997). *Raorchestes terebrans* (Fig 1d–f) was described a year later from Visakhapatnam district of Andhra Pradesh based on eight specimens (3 males and 5 females, holotype from Peddavalasa and paratypes from Golconda hills); however the authors did not compare it with *Philautus sanctisilvaticus*. Dutta (2003) described *Philautus similipalensis* (Fig 1g–i) based on six female specimens from Similipal Biosphere Reserve, Odisha. In his paper, Dutta (2003) compared his new species with the former two species, which were described based on both sexes. *Philautus similipalensis* purportedly differs from *P. sanctisilvaticus* and *R. terebrans* in the webbing on toe IV being broad, reaching up to the basal subarticular tubercle on the inner side.

We undertook fieldwork across known localities of these frog species and other localities across their distribution range to obtain additional specimens, call recordings and tissue samples for molecular analysis, with the aim to ascertain their generic allocation and elucidate their phylogenetic relationship. Results from this work are herein presented with notes on the biology of the three species.

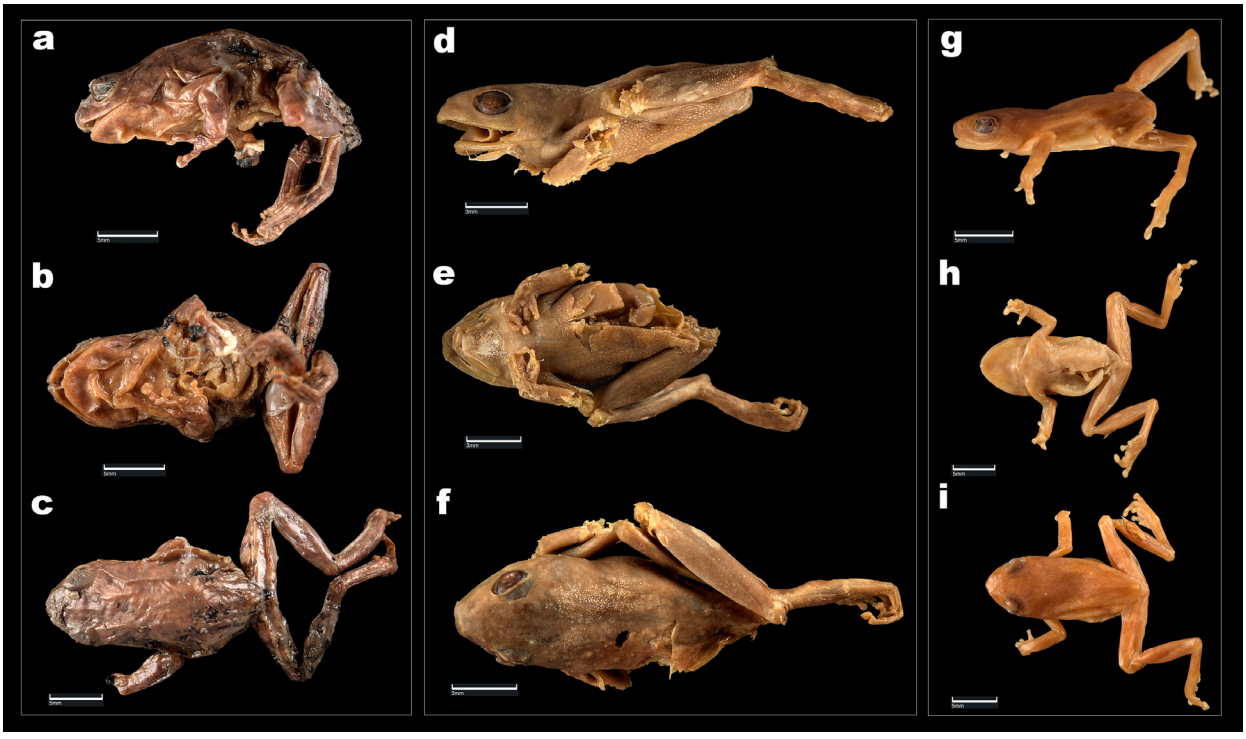

FIGURE 1. Images of examined type specimens—*Philautus sanctisilvaticus* holotype ZSIA 1778 SVL 20.8mm (a–c), *Raorchestes terebrans* paratype ZSIA 2868 SVL 16.2mm (d–f) and *Philautus similipalensis* holotype ZSIA 9061 SVL 21mm (g–i). Images courtesy Zoological Survey of India, Kolkata. Scale bar a–c & g–i 5mm, d–f 3mm.

Material and methods

Survey: Two of the type specimens of *P. sanctisilvaticus* were collected in the month of May, those of *P. terebrans* in the months of October and July; January and February for *P. similipalensis*. Types of these species that were collected in non-monsoon seasons under boulders or heap of leaf litter and most of these were not mature indicating that these were not breeding. Dutta (2015) also recorded a bush frog species (=*P. sanctisilvaticus*) in the months of March and August which he found to be morphologically similar to the types of the three species discussed above. Deuti *et al*. (2014) collected 12 specimens from Araku valley, Andhra Pradesh in the month of September 2009, which they attributed to *P. sanctisilvaticus*. We conducted surveys at all these sites in the months of June and July, given that all known species of bush frogs from India breed during the monsoons.

Morphology and call: Specimens in field were caught by hand, photographed and euthanized by exposure to halothane. Collected specimen were fixed in 4% formalin and stored in 70% ethanol, and are deposited in the collection of the National Centre for Biological Sciences, Bangalore (NCBS) and Bombay Natural History Society (Mumbai). Liver/muscle tissue were dissected before fixation and stored in >95% molecular grade ethanol. Measurements were taken with a Mitutoyo™ digital caliper to the nearest 0.1 mm. Call was used as a proxy for identification of the species in field and the same specimen observed calling was collected. Calls of a few specimens were recorded at Amarkantak with a handheld ZOOM™ H2n Handy Recorder and were used as reference at all survey sites to verify they were similar to the calls heard at the respective sites. The call can be accessed at the following link: https://figshare.com/articles/Call_of_raorchestes_sanctisilvaticus/9783752. Calls were used to rule out the chance of missing sympatric species if any, during fieldwork. Multiple calling individuals were observed in the field and recorded to avoid undersampling of existing species. We also reviewed existing literature for morphological data, and conducted surveys at different times of the monsoon season to strengthen our results. Calls were analyzed with Raven™ Pro v.1.5. (https://ravensoundsoftware.com/). Details of each specimen used for morphology, call and molecular data are summarized in Appendix II. Calls for *R. bombayensis* were recorded to compare it with *P. sanctisilvaticus* from Amboli, southern Maharashtra (https://figshare.com/articles/Call_of_Raorchestes_bombayensis/9821126). Temperature data was not recorded while recording calls.

Multivariate Principal Component Analysis (PCA) was performed on standard morphometric values to assess distinctness of the species presented in Das and Chanda (1997 & 1998) and Dutta (2003). PCA was performed on data presented in the original descriptions. The following measurements were taken following Dutta (2003): SVL (snout-vent length: from tip of snout to vent); HL (head length: distance from angle of jaw to tip of snout); HW (head width: distance between angle of jaws); HD (head depth: greatest transverse depth of head, taken beyond orbital region); A-G (axilla to groin length: distance from posterior base of forelimb to anterior base of hind limb); ED (eye diameter: greatest diameter of orbit); UEW (upper eyelid width: greatest width of upper eyelids); IND (internarial distance: distance between nostrils); IOW (inter orbital width: least distance between upper eyelids); END (eye to nostril distance: distance from anterior margin of eye to nostril); ESD (eye to tip of snout distance: distance from anterior margin of eye to tip of snout); and TBL (tibia length: distance between surface of keen to surface of heel, with both tibia and tarsus flexed). Webbing formula was recorded as per guidelines presented by Savage & Heyer (1997). Data for Principal Component Analysis (PCA) was taken from the original descriptions and four freshly collected specimens. All morphometric values were corrected for varying SVL by dividing the morphometric values by SVL and later log transformed to normalize the data, and the analysis was performed in Past v.3.20 (Hammer *et al.* 2001). Institutional acronyms are as follows: BNHS—Bombay National History Society (Mumbai), ZSIK—Zoological Survey of India (Kolkata), NCBS—National Centre for Biological Sciences (Bangalore), CES-Centre for Ecological Sciences (Bangalore).

Molecular methods and analysis: Genomic DNA was extracted from liver/muscle tissue of six specimens using HiMedia™ Mammalian genomic DNA extraction kit following the protocol directed by the manufacturers. We amplified a partial segment of mitochondrial 16S rRNA gene using published primers. 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. The 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 products were cleaned using QIAquick PCR Purification Kit and sequenced with an ABI 3730 DNA Analyzer. Additional sequences of related bush frog species required for phylogenetic analysis for the 16S rRNA gene were taken from

Vijayakumar *et al.* (2016). Downloaded sequences were aligned in MegaX (Kumar *et al.* 2018) using ClustalW (Thompson & Gibson 2002) with default settings. The Maximum Likelihood (ML) method was implemented to assess phylogenetic relationships with RAxML (Silvestro & Michalak 2012). Data were subjected to phylogenetic reconstruction with Generalized time-reversible (GTR) model as the sequence substitution model for both ML and Bayesian Inference. Maximum likelihood analysis was run for 1000 non-parametric bootstrap replicates with rapid ML search option. Bayesian Inference (BI) was implemented in MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003) and was run for 10 million generations and sampled every 1000 generations. The run was terminated after the analysis reached a standard split frequency of 0.01. Twenty five percent of trees generated were discarded as burn-in. Sequences generated in the present study have been uploaded on GenBank with the following accession numbers MK188863–MK188866, MH915506–MH915508. Uncorrected p-distance (sequence divergence) was calculated in MegaX with pairwise deletion option for treatment of gaps and missing data (Table 1).

TABLE 1. Pairwise uncorrected p-distance (genetic divergence) for the "Bombayensis" clade of *Raorchestes* for the mitochondrial 16S rRNA gene with pairwise deletion of gaps and missing data.

		1	**2**	**3**	**4**	**5**	**6**	**7**
1	Simlipal							
2	Papikonda	0.005						
3	Mahendragiri 2	0.004	0.003					
4	Mahendragiri 1	0.006	0.008	0.006				
5	Kanger	0.006	0.005	0.002	0.008			
6	Amarkantak 1	0.006	0.005	0.002	0.008	0.000		
7	Amarkantak 2	0.004	0.003	0.000	0.006	0.002	0.002	
8	*Raorchestes* aff. *terebrans*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	*Raorchestes bombayensis*	0.010	0.014	0.010	0.010	0.012	0.012	0.010
10	*Raorchestes bombayensis*	0.010	0.014	0.010	0.010	0.012	0.012	0.010
11	*Raorchestes bombayensis*	0.010	0.014	0.010	0.010	0.012	0.012	0.010
12	*Raorchestes bombayensis*	0.013	0.019	0.013	0.015	0.015	0.015	0.013
13	*Raorchestes tuberohumerus*	0.020	0.028	0.020	0.022	0.022	0.022	0.020
14	*Raorchestes ghatei*	0.052	0.069	0.052	0.054	0.054	0.054	0.052

TABLE 1. (Continued)

		8	**9**	**10**	**11**	**12**	**13**	**14**
1	Simlipal							
2	Papikonda							
3	Mahendragiri 2							
4	Mahendragiri 1							
5	Kanger							
6	Amarkantak 1							
7	Amarkantak 2							
8	*Raorchestes* aff. *terebrans*							
9	*Raorchestes bombayensis*	0.010						
10	*Raorchestes bombayensis*	0.010	0.000					
11	*Raorchestes bombayensis*	0.010	0.000	0.000				
12	*Raorchestes bombayensis*	0.010	0.000	0.000	0.000			
13	*Raorchestes tuberohumerus*	0.017	0.019	0.019	0.019	0.018		
14	*Raorchestes ghatei*	0.052	0.048	0.048	0.048	0.046	0.038	

Results

Type material newly examined for this study: Philautus similipalensis holotype female ZSIK A9061 from Gurguria

ca. 770m altitude, Simlipal Biosphere Reserve, Orissa state (now Odisha), south-eastern India on 13 January 1988; *Philautus sanctisilvaticus* holotype male ZSIK A1778 collected from Kapildhara Falls, Amarkantak ca. 190km SE Jabalpur city, Shahdol, Jabalpur district, Madhya Pradesh by P. Singh on 23 May 1962; *Philautus terebrans* paratype female ZSIK A2868 collected from Golconda Hills, Vishakhapatnam district, Andhra Pradesh collected by R H. Beddome on 9 July 1877. Measurements of additional type specimens were taken from the literature; see Appendix II).

Freshly collected material: New specimens and/or tissue samples were collected from the following localities (1) Three males BNHS 6065–6067 from Amarkantak town, Madhya Pradesh on 10 June 2018, corresponding to a site very close to the type locality of *P. sanctisilvaticus*; (2) one male NCBS-BH648 from Kanger Valley National Park, Bastar District, Chhattisgarh collected on 14 June 2018; (3) one male ZSI from Papikonda Wildlife Sanctuary, Andhra Pradesh collected on 7 March 2018.; (4) only tissue samples of two specimens from Mahendragiri hills, Odisha, and (5) SKD F-240 from Simlipal (close to the type locality of *P. similipalensis*). We could not get tissue from the exact type locality of *Philautus terebrans*, but the localities Peddavalasa (type locality), Araku (cf. Deuti et al. 2014) and Papikonda (sampled herein) are in the same hill range which during the colonial period was likely called Golconda hills.

Morphology: The putative three species are similar in general appearance and morphology. The coloration varies between individual from being paly brown to dark with distinct polygonal marks on the dorsum and banded legs. Close scrutiny of the type specimens and description of the three species (Das *et al.* 2004; Das & Chanda 1998; Dutta 2003) show that the only characters that separate these individuals morphologically is the extent of webbing on toe IV. The type series of *R. similipalensis* (excluding the holotype) comprises of only sub-adult females and the holotype of *P. terebrans* too is a female. Webbing formula for the species is I2-2II2-2III2-3IV2-2V (Fig. 2d). After examination of all relevant material, the webbing is identical in same sexes across type and non-type material implying that the species inhabiting the Deccan peninsular and norther Eastern Ghats do not show any distinct morphological feature to diagnose the three species. Morphometry of specimens used for PCA is presented in Table 2.

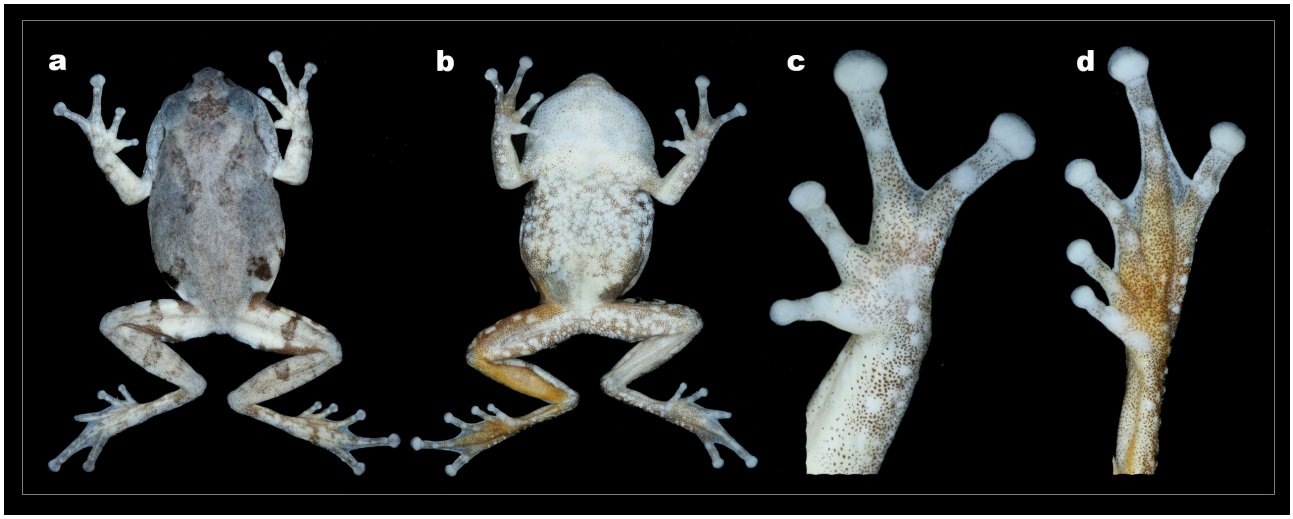

FIGURE 2. Images of *Raorchestes sanctisilvaticus* NCBS-BH648 male from Kanger Valley National Park, Chhattisgarh

Advertisement call of BNHS 6065 (Fig. 4)*:* Phonetically, the call resembled a metallic "*tik tik tik*" with varying call duration and frequency and is quite similar to that of *R. bombayensis*. Each call comprises 4–6 pulses. The lowest frequency of the sampled call of an individual from Amarkantak was 2165.9 Hz, highest 3429.4 Hz and the delta frequency was 1263.5 Hz. Call duration was between 0.022–0.036s; inter-call duration was 0.08–0.1s and one call consisted of 40–42 pulses.

Molecular analysis: Seven sequences were obtained from specimens collected from Amarkantak, Kanger, Simlipal, Mahendragiri and Papikonda, which include specimens from type localities of two of the relevant *nomina* (Fig. 3). A sequence of *R.* aff. *terebrans* (Vijayakumar *et al.* 2014) was downloaded from GenBank and was found to be identical to the sequences generated in the present work. The sequences were generated for mitochondrial 16S rRNA gene that comprised 523bp of which 457 sites were conserved, 66 variable and 53 parsimoniously informative sites. All the sequences were found to be embedded within the genus *Raorchestes* and were members of the

'*bombayensis* clade' (Vijayakumar *et al.* 2014). They formed a well-supported clade sister to *R. bombayensis* with high support values (ML bootstrap >90, BI posterior probability > 0.97) (Fig. 5). Samples from sites across northern Eastern Ghats and Deccan Peninsula show 0–0.008 divergence, suggesting that these may represent a single species and not three. All eight sequences of the species show no to a few mutations across 523 sites representing a remarkable case despite the wide geographic span of the samples, with maximum pairwise sequence divergences of 0.8%. From *R. bombayensis*, the samples collected for the study differ in showing 1–1.5% p-distance (uncorrected), 1.7–2.2% from *R. tuberohumerus* (Kuramoto & Joshy) and 5.2–6.9% from *R. ghatei* Padhye, Sayeed, Jadhav, and Dahanukar (Table 1).

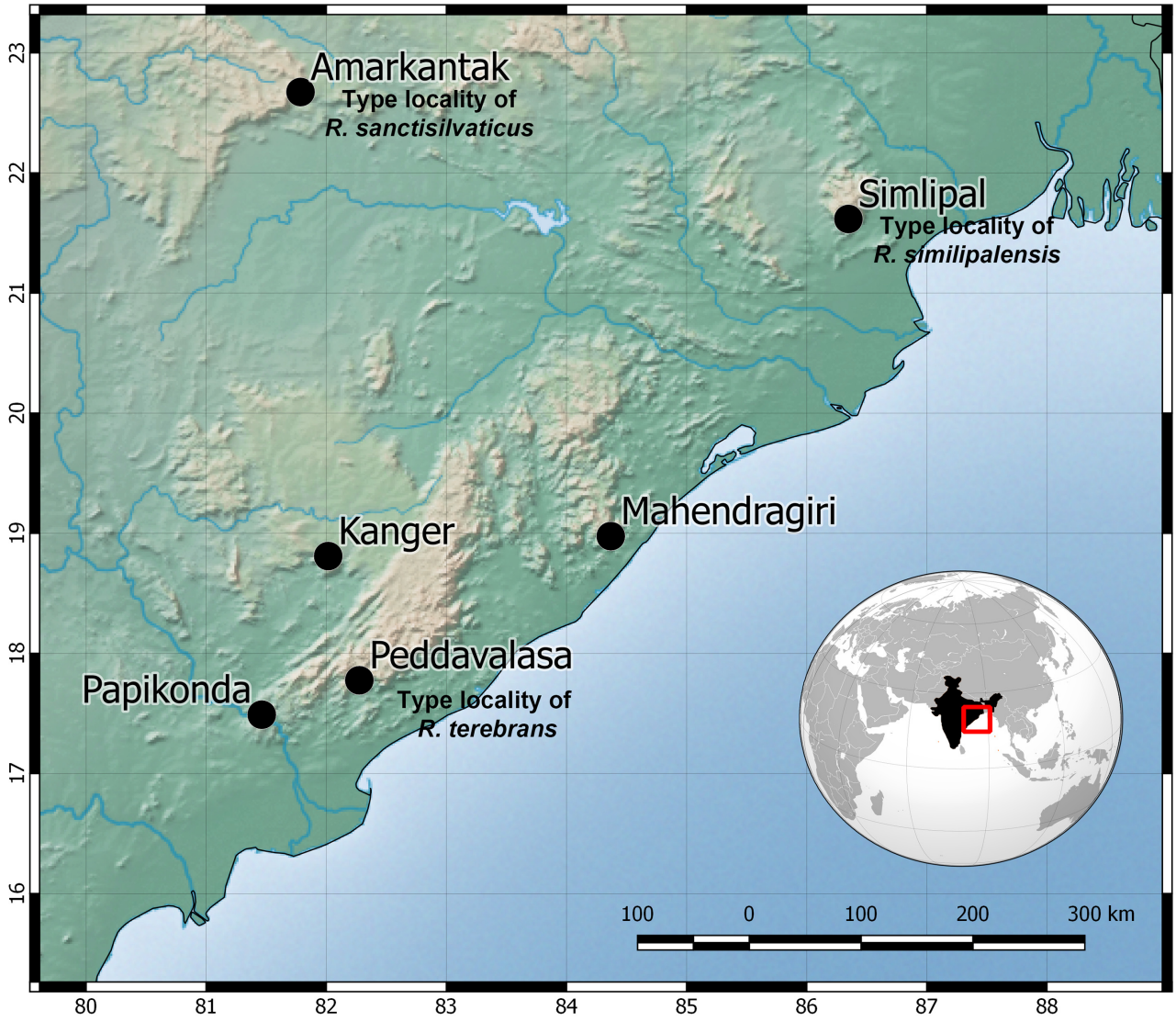

FIGURE 3. Map of eastern India showing collection localities of three species of the genus *Raorchestes.*

Multivariate analysis (Fig. 6)*:* PCA recovered two clusters, (Cluster A) one that correspond to specimens representing *R. sanctisilvaticus* and *R. terebrans* and the second cluster (Cluster B) of *R. similipalensis* and specimens of R. *sanctisilvaticus* collected in the present work. The variance explained by PC1 + PC2 is 76.77+8.57 (Fig. 5, Table 3 & 4). Cluster A corresponds to data for type specimens measured by different researchers, which are not in a prime state and are not well-preserved which in turn might lead to inaccurate measurements. Furthermore, only the holotype of *R. terebrans* is an adult as all the paratypes measure 12.8–16.2 mm SVL, suggesting that they are subadults. Cluster B on the other hand was based on fresh collection and measurements are more accurate and form a single cluster evident in PC1. The clusters observed in PC1 could also be a result of differences in the way data was recorded by observers.

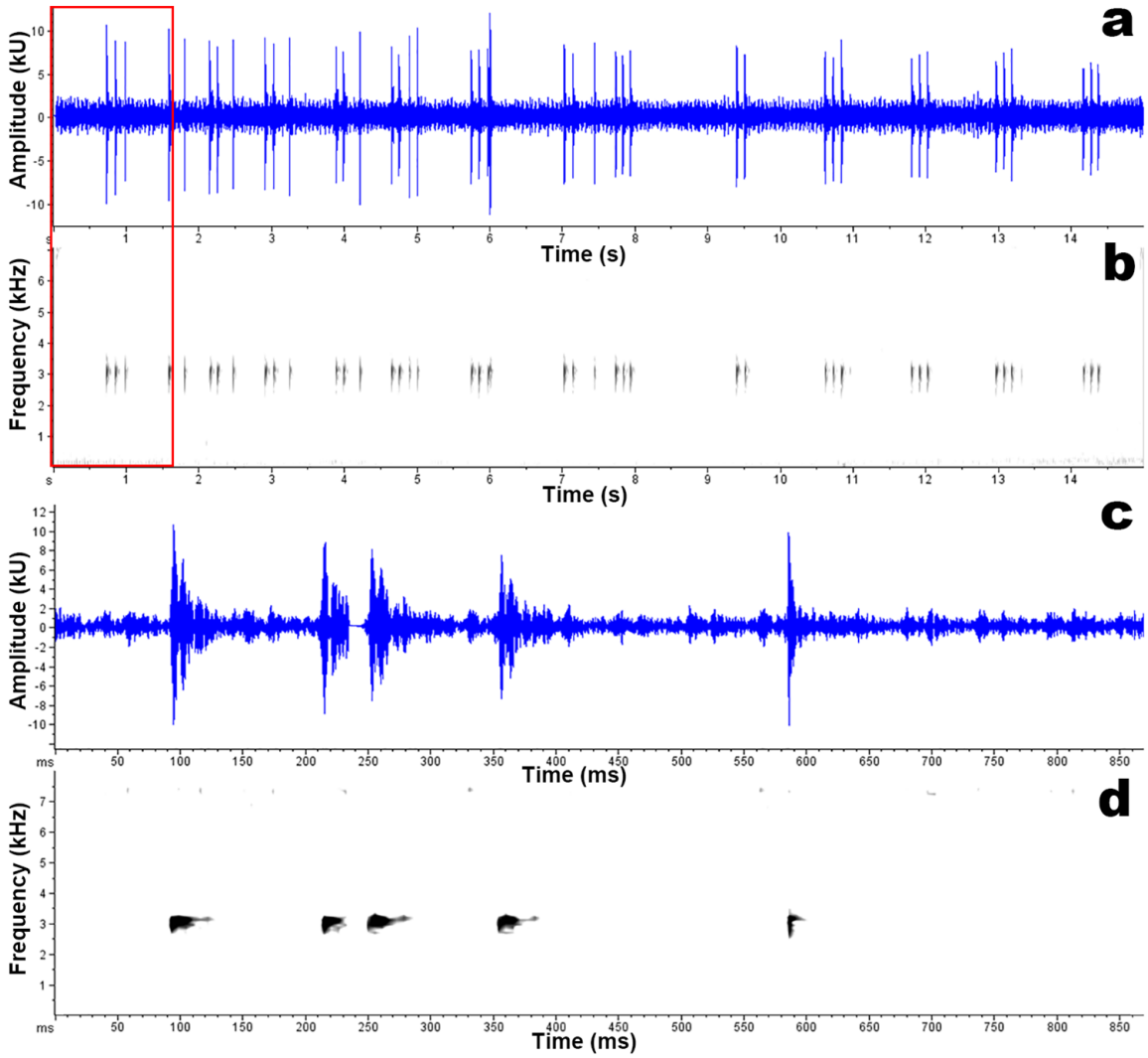

FIGURE 4. Advertisement call of *Raorchestes sanctisilvaticus* recorded at Amarkantak, Entire call Amplitude (a) & Spectrogram (b), Single call Amplitude (c) & Spectrogram (d). No data on temperature available.

TABLE 2. Morphometry of type specimens of the three species of *Raorchestes* taken from their original descriptions and of freshly collected specimens. All measurements in mm (rounded to the nearest 0.1 mm).

	R. sanctisilvaticus ZSI A1778 ♂ Holotype	***R. sanctisilvaticus*** ZSI A1777 ♀ Paratype	***R. sanctisilvaticus*** ZSI A1779 ♀ Paratype	***R. similipalensis*** ZSI A9061 ♀ Holotype	***R. similipalensis*** ZSI A9062 ♀ Paratype
SVL	20.8	19.3	23.8	21	16
TL	7.3	8.7	10.9	6.0	5.4
HL	6.5	6.4	7.3	7.0	6.1
HW	7.2	7.2	9.3	4.0	3.2
HD	4.1	4.2	4.7	9.1	7.2
ED	3.2	3.2	3.5	3.0	2.5
UE	1.8	1.7	1.8	1.7	1.3
IO	4.1	4.1	4.3	2.6	2.3
IN	2.9	2.7	2.8	3.6	3.2
E-S	2.7	2.6	2.7	2.0	1.4
E-N	1.5	1.9	1.4	3.1	2.6
TBL	10.1	10	10.9	10.8	8.3
FIIID	1.3	1.0	1.1	-	-

TABLE 2. (Continued)

	R. similipalensis ZSI A9063 ♀ Paratype	*R. similipalensis* ZSI A9064 ♀ Paratype	*R. similipalensis* ZSI A9065 ♀ Paratype	*R. similipalensis* USNM 217503 ♀ Paratype	*R. terebrans* USNM 239428 ♂ Holotype	*R. terebrans* ZSI A2868 ♀ Paratype
SVL	16.8	15.8	17.5	14.5	21.6	16.2
TL	5.5	5.3	5.7	5.0	10	6.8
HL	6.3	6.0	6.5	5.7	5.7	4.6
HW	3.4	3.1	3.6	3.0	8.8	5.0
HD	7.5	7.0	8.0	6.5	3.5	3.1
ED	2.6	2.4	2.7	2.3	3.1	2.1
UE	1.4	1.3	1.5	1.2	2.2	1.3
IO	2.3	2.2	2.4	2.1	4.2	3.0
IN	3.3	3.2	3.3	3.2	2.4	1.8
E-S	1.4	1.3	1.5	1.2	3.6	2.2
E-N	2.7	2.5	2.7	2.4	2.0	1.4
TBL	8.4	8.1	9.0	7.7	9.5	9.1
FIIID	-	-	-	-	1.3	0.6

TABLE 2. (Continued)

	R. terebrans ZSI A2869 ♂ Paratype	*R. terebrans* ZSI A2870 ♀ Paratype	*R. terebrans* ZSI A2871 ♂ Paratype	*R. terebrans* ZSI A2872 ♀ Paratype	*R. terebrans* ZSI A2873 ♀ Paratype
SVL	14.8	14.2	15.2	12.8	15
TL	6.2	4.9	7.5	5.9	7.4
HL	4.3	4.3	4.9	4.2	4.1
HW	4.5	5.1	5.0	4.6	4.2
HD	3.2	3.1	3.3	3.1	3.3
ED	2.1	2.1	2.3	2.1	1.9
UE	1.1	1.2	0.9	0.7	1.1
IO	2.8	3.1	3.2	2.8	2.5
IN	1.2	1.9	1.7	1.4	1.3
E-S	2.0	2.3	2.0	1.8	2.2
E-N	1.3	1.5	1.7	1.3	1.2
TBL	6.7	8.2	7.2	7.0	7.0
FIIID	0.5	0.6	0.6	0.4	0.5

TABLE 2. (Continued)

	R. terebrans ZSI A2874 ♀ Paratype	*R. sanctisilvaticus* BNHS 6065	*R. sanctisilvaticus* BNHS 6066	*R. sanctisilvaticus* BNHS 6067	*R. sanctisilvaticus* NCBS BH648
SVL	14.8	21.3	20.8	21.9	21.9
TL	7.2	6.2	4.8	5.9	6.2
HL	4.7	7.5	6.4	7.3	7.5
HW	4.8	4.0	3.9	4.3	4.0
HD	3.2	11.9	9.2	12.0	9.0
ED	1.9	2.8	2.5	2.5	2.5
UE	1.2	2.2	1.6	1.9	2.4
IO	3.2	2.8	2.4	2.6	2.5
IN	1.9	4.6	4	4.4	4.1
E-S	2.3	1.6	1.4	1.4	1.5
E-N	1.7	2.8	2.1	2.4	2.9
TBL	7.9	13.0	13.0	13.6	15.6
FIIID	0.5	-	-	-	-

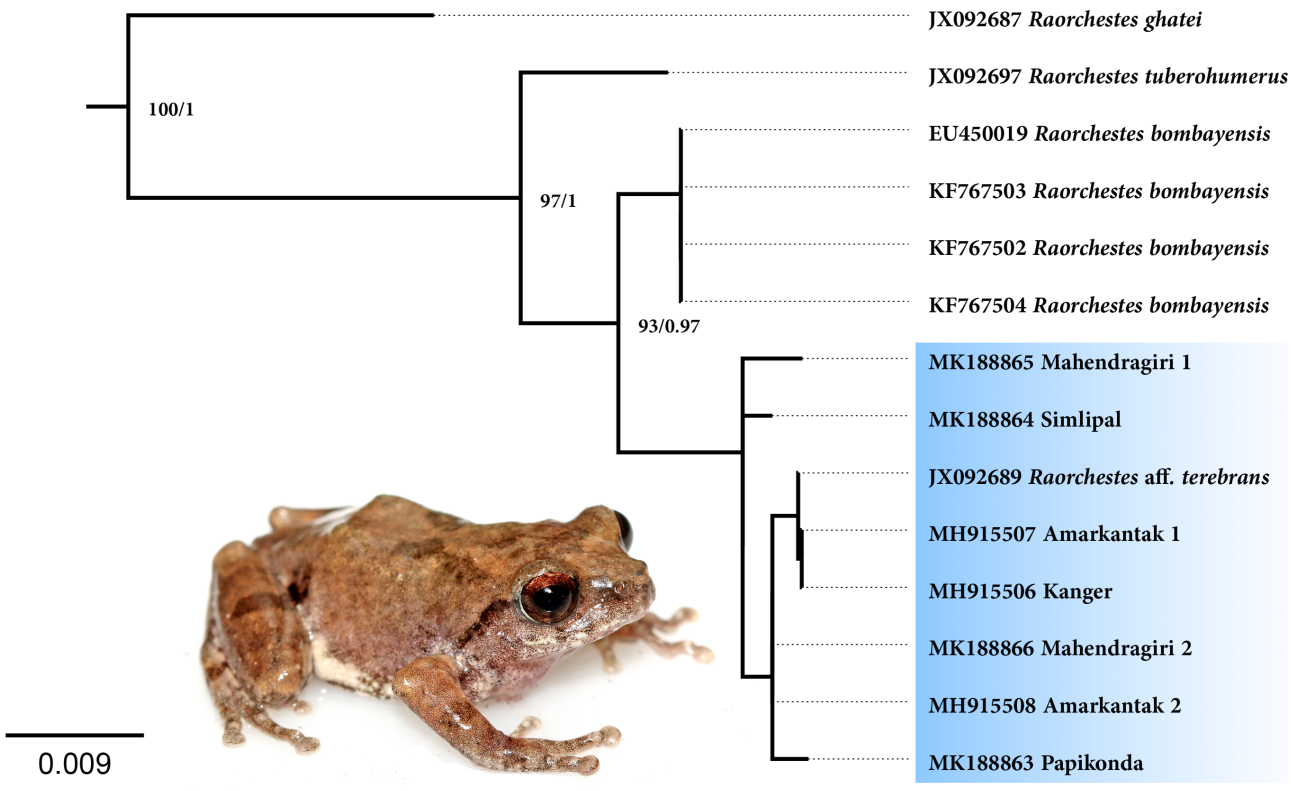

FIGURE 5. Maximum likelihood phylogeny of members of the "Bombayensis" clade of *Raorchestes* base on 16S rRNA gene reconstructed through 1000 non-parametric bootstrap replicates. Numbers at nodes represent bootstrap support and Bayesian posterior probability. Inset image of *Raorchestes sanctisilvaticus* from Kanger Valley National Park, Chhattisgarh.

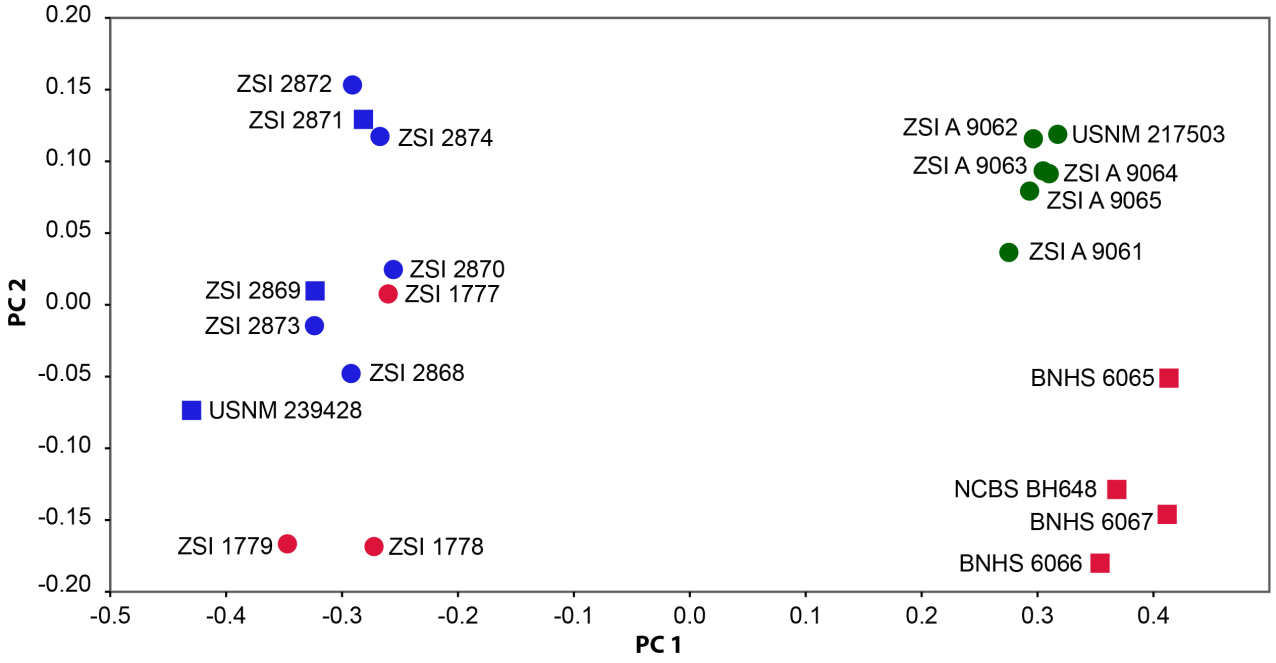

FIGURE 6. Multivariate Principal Component Analysis plot. Shapes of the data points indicate sex circles (females), squares (males) and colors indicate different species, *Philautus sanctisilvaticus*—red, *Raorchestes terebrans*—blue and *Philautus similipalensis*—green. Numbers with each point indicate specimen numbers.

Discussion

Phylogenetic relationships within members of the clade containing *Raorchestes* and *Pseudophilautus* are well resolved, especially for the ones distributed in the Western Ghats and Sri Lanka (Biju *et al.* 2010; Vijayakumar *et al.* 2016). However, our knowledge of species inhabiting Eastern Ghats, Deccan Peninsula and Northeast India is very limited. The present study merely fills a small gap in our knowledge of bush frog species distributed in the former range, as much of it remains unexplored. Sampling conducted in the present work, examination of museum material and literature review are in agreement with a hypothesis of a single species distributed at all the localities sampled, and inclusion of this species in the genus *Raorchestes*.

TABLE 3. Summary of Principal Component Analysis based on morphometric data for species of bush frogs of the genus *Raorchestes* from Eastern Ghats.

PC	Eigenvalue	% variance
1	0.093432	47.83
2	0.0862908	44.18
3	0.00432014	2.21
4	0.00411256	2.11
5	0.00284423	1.46
6	0.00227454	1.16
7	0.00121897	0.62
8	0.00035572	0.18
9	0.00021324	0.11
10	0.00019938	0.10
11	6.63E-05	0.03
12	6.68E-06	0.00
13	3.05E-07	0.00

TABLE 4. Character loadings for the Principal Component Analysis

	PC 1	**PC 2**
SVL	0.082809	0.23169
HL	-0.12488	0.27771
HW	0.21923	0.15908
HD	-0.23384	0.45253
A-G	0.55527	-0.05066
ED	0.13053	0.23667
UEW	0.17198	0.33791
IND	-0.15652	0.3031
IOW	0.48105	0.16663
END	-0.24524	0.34121
ESD	0.40972	-0.03274
TBL	0.094642	0.18067
FIIID	0.16428	0.44336

The results presented in the present study, despite ambiguities, advocate the need for making nomenclatural amendments. *Philautus sanctisilvaticus* Das and Chanda, 1997, is the first available nomen for this taxon, and we propose to treat *Philautus terebrans* Das and Chanda, 1998 **syn. nov.** and *Philautus similipalensis* Dutta, 2003 **syn. nov.** as junior subjective synonyms for this nomen following the Principle of Priority in article 23.1 of the ICZN (International Commission on Zoological Nomenclature 1999). Furthermore, according to this taxonomic hypothesis, the species is abundant and widespread across the northern Eastern Ghats and Deccan Peninsula and hence it is recommended that the species be reclassified as Data Deficient concerning its conservation status till the further assessment and not Critically Endangered as per IUCN guidelines.

We also stress that *Raorchestes sanctisilvaticus* shows a low genetic divergence from *R. bombayensis;* however, their calls appear to slightly differ and we therefore propose that both should for now be retained as distinct species (Appendix I).

Funding agencies are generally biased in their approach and award funds to projects working on IUCN classified "threatened species" with the aim to prioritize conservation of these species. However, unsound taxonomy would work against the goal of conservation as funds might be allocated to species, which might not be under immediate threat. In the present case, the species appears to be widespread, abundant and also distributed across large network of protected areas and does not face immediate concern of extinction. On the other hand, cryptic species that are merged under a single taxon (Mirza *et al.* 2018) suffer as they are usually not evaluated by IUCN or are classified as Least Concern or Data Deficient. It is hoped that taxonomic investigations are encouraged to help understand biodiversity, which in many cases is either underrated or overrated due to unsound taxonomy.

Conclusion

The bush frogs studied herein, distributed in the northern part of the Eastern Ghats and the Deccan Peninsula, probably represent a single species, to be allocated to *Raorchestes sanctisilvaticus*. The species, according to the taxonomic hypothesis herein, is locally abundant and can be met within forests across eastern Madhya Pradesh, Chhattisgarh, Odisha, eastern Telangana and northern Andhra Pradesh along an altitudinal gradient of 500–1700 m asl. The current distribution hints on its possible presence in Jharkhand and maybe West Bengal.

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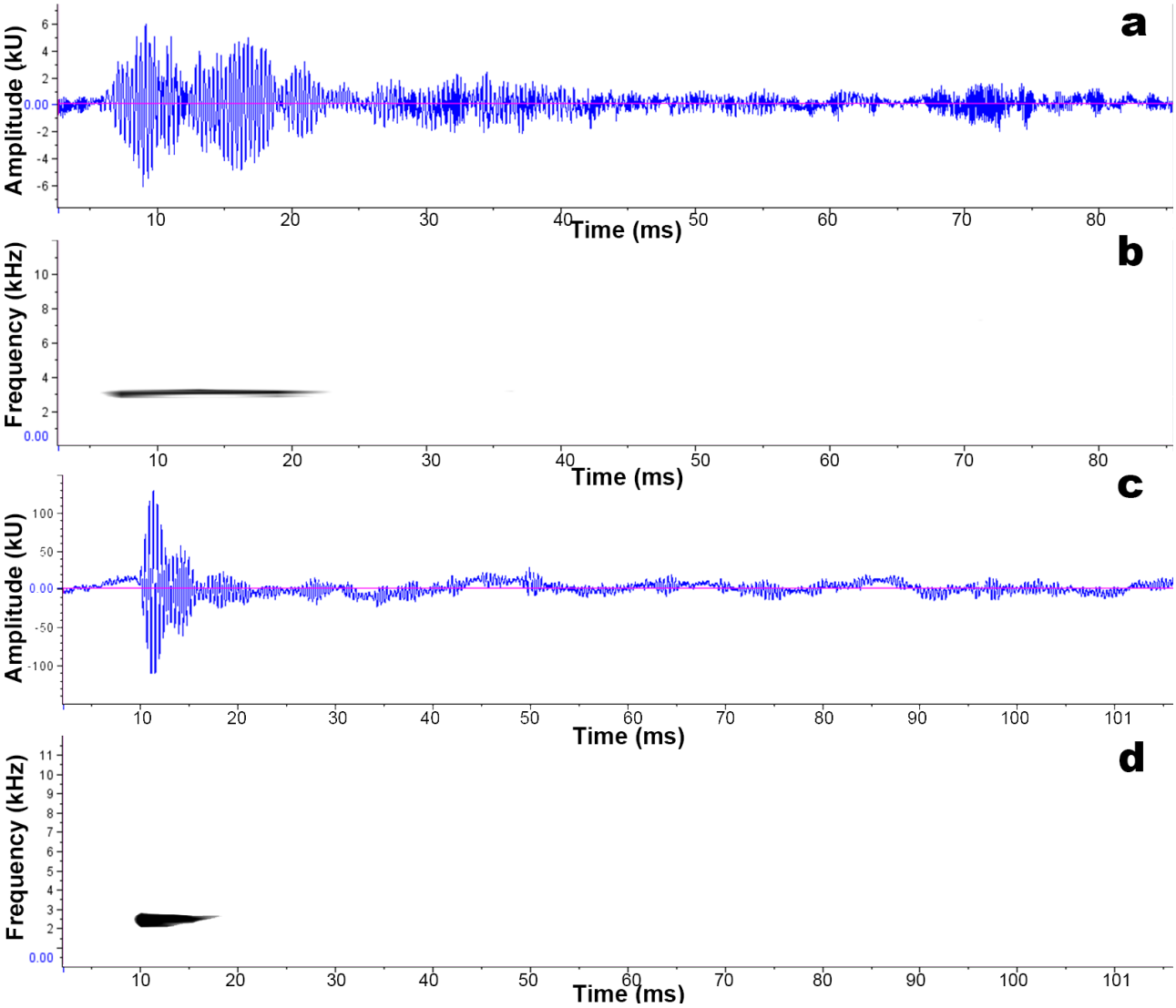

LEGEND TO APPENDIX I. Advertisement call of a single call for *R. sanctisilvaticus* (a & b) *R. bombayensis* (c & d); Amplitude (a & c) & Spectrogram (b & d). Note the duration of the note per call for *R. sanctisilvaticus* (15ms) vs. less duration call in *R. bombayensis* (5ms). No data on temperature available.

LEGEND TO APPENDIX II. Summary of specimens used in the study. Superscript denotes the researcher who measured the specimens, 'a' ZAM, 'b' SP and the rest taken from the original description ('c' Das and Chanda 1997;'d' Das and Chanda 1998; 'e' Dutta 2003).

Specimen voucher	Locality	Accession number for 16S	Morphometry	Call
SKD F-242	Mahendragiri, Odisha	MK188865	NA	NA
SKD F-244	Mahendragiri, Odisha	MK188866	NA	NA
SKD F-240	Simlipal, Odisha	MK188864	NA	NA
NCBS BH648	Kanger Vally National Park, Chhattisgarh	MH915506	+[a]	NA
BNHS 6065	Amarkantak, Madhya Pradesh	MH915507	+[b]	+
BNHS 6066	Amarkantak, Madhya Pradesh	MH915508	+[b]	NA
BNHS 6067	Amarkantak, Madhya Pradesh	NA	+[b]	NA
-	Papikonda Wildlife Sanctuary, Andhra Pradesh	MK188863	NA	NA
ZSI A1777	Amarkantak, Madhya Pradesh	NA	+[c]	NA

.....continued on the next page

APPENDIX II. (Continued)

Specimen voucher	Locality	Accession number for 16S	Morphometry	Call
ZSI A1778	Amarkantak, Madhya Pradesh	NA	+[c]	NA
ZSI A1779	Amarkantak, Madhya Pradesh	NA	+[c]	NA
USNM 239428	Peddavalasa, Andhra Pradesh	NA	+[d]	NA
ZSI 2868	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI 2869	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI 2870	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI 2871	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI 2872	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI 2873	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI 2874	Golconda hills, Andhra Pradesh	NA	+[d]	NA
ZSI A9061	Simlipal Biosphere Reserve, Odisha	NA	+[e]	NA
ZSI A9062	Simlipal Biosphere Reserve, Odisha	NA	+[e]	NA
ZSI A9063	Simlipal Biosphere Reserve, Odisha	NA	+[e]	NA
ZSI A9064	Simlipal Biosphere Reserve, Odisha	NA	+[e]	NA
ZSI A9065	Simlipal Biosphere Reserve, Odisha	NA	+[e]	NA
USNM 217503	Simlipal Biosphere Reserve, Odisha	NA	+[e]	NA