Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2015 | 7(6): 7211–7222



# Sexual dimorphism in the Kudremukh Bush Frog (Anura: Rhacophoridae: *Raorchestes tuberohumerus*) of the Western Ghats, India, with a note on its distribution and conservation status

ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

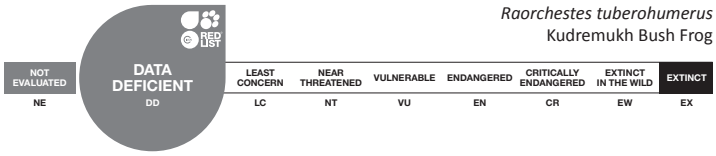
OPEN ACCESS

**Anand D. Padhye [1], Anushree Jadhav [2], Shauri Sulakhe [3] & Neelesh Dahanukar [4]**

[1,2] Department of Zoology, Abasaheb Garware College, Karve Road, Pune, Maharashtra 411004, India
[2] Present address: Department of Zoology, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra 411007, India
[3] Insearch Outdoors, C-26/9, Ketan Heights, Rahul Nagar Lane, Near Karve Statue, Kothrud, Pune. Maharashtra 411038, India
[4] Indian Institute of Science Education and Research (IISER), G1 Block, Dr. Homi Bhabha Road, Pashan, Pune, Maharashtra 411008, India
[4] Systematics, Ecology and Conservation Laboratory, Zoo Outreach Organization (ZOO), 96 Kumudham Nagar, Vilankurichi Road, Coimbatore, Tamil Nadu 641035, India
[1]anand.padhye@mesagc.org (corresponding author), [2]anushreejadhav@gmail.com, [3]shaurisulakhe@gmail.com, [4]n.dahanukar@iiserpune.ac.in

**Abstract:** *Raorchestes tuberohumerus* (Kuramoto & Joshy, 2003) was described based on three male specimens and was diagnosed mainly based on the presence of tubercle on the humerus. Here we describe the genetically confirmed female of the species and show that tubercle on the humeral bone is a sexually dimorphic character present only in males. Further, based on current collection and literature review we studied the distribution of the species using niche based modelling. Using the distributional range and our observations on the threats to the habitat we propose that *Raorchestes tuberohumerus*, currently assessed as Data Deficient, can fall under the 'Vulnerable' category of IUCN Red List of Threatened Species.

**Keywords:** Amphibians, IUCN Red List, molecular identification, osteology, threatened.




*Raorchestes tuberohumerus*
Kudremukh Bush Frog

**DOI:** http://dx.doi.org/10.11609/JoTT.o4192.7211-22 | **ZooBank:** urn:lsid:zoobank.org:pub:3C420A17-847A-4947-B82F-982698C4F51C

**Editor:** Anonymity requested. **Date of publication:** 26 May 2015 (online & print)

**Manuscript details:** Ms # o4192 | Received 28 November 2014 | Final received 20 May 2015 | Finally accepted 21 May 2015

**Citation:** Padhye, A.D., A. Jadhav, S. Sulakhe & N. Dahanukar (2015). Sexual dimorphism in the Kudremukh Bush Frog (Anura: Rhacophoridae: *Raorchestes tuberohumerus*) of the Western Ghats, India, with a note on its distribution and conservation status. *Journal of Threatened Taxa* 7(6): 7211–7222; http://dx.doi.org/10.11609/JoTT.o4192.7211-22



**Funding:** This work was partially supported by DST-INSPIRE Research Grant [IFA12-LSBM-21] to Neelesh Dahanukar.

**Competing Interest:** The authors declare no competing interests. Funding sources had no role in study design, data collection, results interpretation and manuscript writing.

For **Author Details** and **Author Contribution** see end of this article.

**Acknowledgements:** We thank Nikhil Modak for his help in field work. We are grateful to Dr. Asad Rahmani, Director; Dr. Deepak Apte, COO; Rahul Khot, incharge Natural History Collection department; Reshma Pitale, researcher and Vithoba Hegde, senior field assistant, for their help during study of the museum specimens from Bombay Natural History Society, Mumbai. We thank Dr. Sanjay Molur and Keerthi Krutha for their help in registration of specimens in the Wildlife Information Liaison Development Museum, Coimbatore. We are grateful to the Principal, Abasahab Garware College, Head Department of Zoology, Abasaheb Garware College and Indian Institute of Science Education and Research, Pune for providing infrastructure facilities. Anushree Jadhav is thankful to Dr. Narahari P. Gramapurohit for encouragement and support.

## INTRODUCTION

Kuramoto & Joshy (2003) described the shrub frog *Philautus tuberohumerus* (now *Raorchestes tuberohumerus* vide Biju et al. 2010), based on three male specimens collected from Kudremukh, Western Ghats of Karnataka, India. Kuramoto & Joshy (2003) primarily diagnosed the species based on what they considered as the unique feature - the presence of a tubercle on the humeral bone, the etymological feature. While describing the species *Raorchestes ghatei*, Padhye et al. (2013) suggested that tubercle on the humeral bone of *R. ghatei* is a sexually dimorphic character present only in males and not females. However, such information was not available for *R. tuberohumerus*, because neither the first description of the species by Kuramoto & Joshy (2003) nor the subsequent revision of the group by Biju & Bossuyt (2009) had any female specimens in their study.

In the present study we provide the morphometry of genetically identified female specimens of *R. tuberohumerus* for the first time. Further, based on osteological study of both male and female specimens we show that the tubercle on the humerus is a sexually dimorphic character present only in the males. In addition, based on the niche-based modelling we predict the probable distribution of the species. Owing to the fact that the habitat of the species is under threat we suggest that the current assessment of 'Data Deficient' (Das 2004) be changed to 'Vulnerable' category on the IUCN Red List of Threatened Species (see assessment box at the end of the article).

## MATERIALS AND METHODS

### Study area and specimen vouchers

Specimens (four males and two females) were collected from Nidigere, in Karnataka State. Collected specimens are deposited in the museum collection of the Wildlife Information Liaison Development (WILD), Coimbatore and Abasaheb Garware College, Zoology Research Laboratory (AGC-ZRL), Pune, India. Additional specimens were studied from the museum collection of Bombay Natural History Society (BNHS), Mumbai, India.

### Material examined

*Raorchestes tuberohumerus* (n=8): WILD-AMP-14-499 & 501 (males), WILD-AMP-14-500 & 502 (females), and AGC-ZRL-AMPHIBIA-201 & 202 (males), 29·vii.2014, (12.848$^0$N & 74.823$^0$E, 925m), on Madikeri-Sakleshpur Road, Nidigere Village, Karnataka, coll. Anand Padhye; Holotype, BNHS 4193, male, 15.vi.2000, from Kudremukh, Chikkamagalur, Karnataka, coll. by S.H. Joshy; Paratype, BNHS 4194, male, 15.vi.2000, from Kudremukh, coll. M. Kuramoto.

### Morphometry and analysis

Measurements were taken to the nearest 0.1mm using a digital calliper and using a binocular microscope. The following measurement were taken (after Biju & Bossuyt 2009): snout-vent length (SVL); head length (HL); head width (HW); rear of the mandible to the nostril (MN); rear of the mandible to the anterior orbital border of the eye (MFE); rear of the mandible to the posterior orbital border of the eye (MBE); snout length (SL); nostril to tip of the snout (SN); front of the eye to nostril (EN); eye length (EL); inter upper eyelid width (IUE); maximum upper eyelid width (UEW); internal front of eyes (IFE); internal back of eyes (IBE); forelimb length (FLL); hand length (HAL); third finger length (TFL); disc width on finger III (FDIII); width of finger III (FWIII); shank length (ShL); thigh length (TL); foot length (FOL); distance from the heel to the tip of the fourth toe (TFOL). Tympanum diameter was measured both vertically (TYDV) and horizontally (TYDH). Data of the four males from current study were included in the data set used by Padhye et al. (2013) and discriminant analysis was performed in PAST (Hammer et al. 2001; free software) to confirm the morphometric identity of the species.

### Osteology

Two specimens WILD-AMP-13-499 (male) and WILD-AMP-13-500 (female) were used for osteological study. Osteological clearing and staining procedure follow Potthoff (1984).

### Genetic analysis

Muscle tissue was harvested from fresh specimens of a male (WILD-AMP-14-499) and female (WILD-AMP-14-500) and preserved in absolute ethanol. DNA extraction, PCR amplification of 16S rRNA gene and sequencing protocols are as per Padhye et al. (2013). Sequences were analyzed by BLAST tool (Altschul et al. 1990). These sequences have been deposited in GenBank (accession numbers KP137387 & KP137388). GenBank accession numbers for specimens used for analysis are provided in Table 1. Gene sequences were aligned using MUSCLE (Edgar 2004). Molecular phylogenetic analysis was performed using the freeware MEGA 6 (Tamura et al. 2013). The best fit model for nucleotide substitution was selected from 24 models using MEGA 6 (Tamura et

**Table 1. Voucher and GenBank accession numbers for sequences used in molecular analysis.**

| Species | Voucher | Accession number |
|---|---|---|
| *Raorchestes tuberohumerus* Male | WILD-AMP-14-499 | KP137387 |
| *Raorchestes tuberohumerus* Female | WILD-AMP-14-500 | KP137388 |
| *Raorchestes tuberohumerus* | BNHS 4590 | EU450004 |
| *Raorchestes ghatei* | ZSI-WRC A/1484 | KF366384 |
| *Raorchestes ghatei* | WILD-AMP-13-100 | KF366385 |
| *Raorchestes bombayensis* | BNHS 4418 | EU450019 |
| *Raorchestes bombayensis* | WILD-13-AMP-230 | KF767502 |
| *Raorchestes akroparallagi* | 0317Phi018b* | EU450010 |
| *Raorchestes akroparallagi* | 0071Phi018* | EU450003 |
| *Raorchestes anili* | 1400PhiAni* | EU450024 |
| *Raorchestes anili* | 0307PhiAni* | EU450008 |
| *Raorchestes beddomii* | 0030PhiBed* | EU449998 |
| *Raorchestes beddomii* | 1153PhiBed* | EU450013 |
| *Raorchestes bobingeri* | BNHS 4273 | EU450014 |
| *Raorchestes charius* | - | AF249062 |
| *Raorchestes charius* | 0081PhiCha_type* | EU450007 |
| *Raorchestes chlorosomma* | BNHS 4426 | EU450017 |
| *Raorchestes chotta* | BNHS 4429 | EU450022 |
| *Raorchestes chromasynchysi* | BNHS 4433 | EU450018 |
| *Raorchestes coonoorensis* | BNHS 4446 | EU449999 |
| *Raorchestes dubois* | BNHS 5285 | EU449996 |
| *Raorchestes glandulosus* | 1369PhiGla* | EU450020 |
| *Raorchestes glandulosus* | 0077PhiGla* | EU450006 |
| *Raorchestes graminirupes* | BNHS 4266 | EU450015 |
| *Raorchestes griet* | BNHS 4455 | EU449997 |
| *Raorchestes griet* | - | AF536203 |
| *Raorchestes gryllus* | ROM 30288 | GQ285674 |
| *Raorchestes jayarami* | SDB 1379 | EU450023 |
| *Raorchestes kaikatti* | BNHS 4557 | EU450021 |
| *Raorchestes longchuanensis* | 7Rao | KC465839 |
| *Raorchestes longchuanensis* | 5Rao | GQ285675 |
| *Raorchestes luteolus* | BNHS 4478 | EU450005 |
| *Raorchestes marki* | BNHS 4537 | EU450028 |
| *Raorchestes menglaensis* | KIZ060821286 | EU924621 |
| *Raorchestes munnarensis* | BNHS 4481 | EU450016 |
| *Raorchestes nerostagona* | BNHS 4244 | EU450012 |
| *Raorchestes ponmudi* | 1451PhiPonb* | EU450026 |
| *Raorchestes ponmudi* | 1121PhiPon* | EU450011 |
| *Raorchestes resplendens* | SDB-2010 | GU808563 |
| *Raorchestes signatus* | - | GQ204684 |
| *Raorchestes signatus* | BNHS 4489 | EU450000 |
| *Raorchestes signatus* | - | AY141841 |
| *Raorchestes sushili* | BNHS 4544 | EU450027 |
| *Raorchestes tinniens* | BNHS 4548 | EU450001 |
| *Raorchestes travancoricus* | BNHS 4557 | EU450029 |
| *Pseudophilautus kani* | BNHS 4472 | EU449994 |
| *Pseudophilautus wynaadensis* | - | GQ204685 |
| *Pseudophilautus amboli* | BNHS 4399 | EU450025 |

* voucher numbers for these isolates cannot be determined based on information given in Biju & Bossuyt (2009).

al. 2013) based on the minimum Bayesian Information Criterion (BIC) value (Schwarz 1978; Nei & Kumar 2000). Best fit nucleotide substitution model was used to test the phylogenetic hypothesis using maximum likelyhood method implimented in MEGA 6 (Tamura et al. 2013). Genetic analysis was not carried out to thoroughly resolve the deep phylogeny of the genus but to assign individuals to genetically homogenous clusters for the purpose of species identification. Reliability of the phylogenetic tree was estimated using bootstrap values from 1000 replicates.

**Prediction of distribution and calculation of extent of occurrence**

Based on the present collection and point localities provided in Kuramoto & Joshy (2003), Das (2004), Biju & Bossuyt (2009), Gururaja (2012), Gururaja & Ramachandra (2012), we performed predictive niche-based distribution modelling to understand the probable distribution of *R. tuberohumerus* within the Western Ghats in the area between 8–22 $^0$N and 70–80 $^0$E. Predictive modelling was performed in DIVA-GIS (http://www.diva-gis.org/) using ~30 arc seconds data for altitude, precipitation and 19 bioclimatic parameters (Hijmans et al. 2005) available at the WorldClim website (http://www.worldclim.org/). Extent of occurrence (IUCN 2012) was estimated by overlaying the prediction map with hexagonal grid (Sahr et al. 2003) available online at the GLOBE website (http://globe.umbc.edu/documentation/foundational-data/globe-land-units-glus/). Each hexagonal cell has an area of approximate 100km$^2$. All the grids which had some prediction were counted for determining the possible distribution of the species.

## RESULTS AND DISCUSSION

### Conformation of species identity

Genetic analysis based on 16S rRNA gene showed that the female specimen, without the tubercle on the humeral bone, and male specimen, with tubercle on the humeral bone, were genetically 100% similar to each other and showed no genetic divergence from the known sequence of *R. tuberohumerus* (GenBank accession number EU450004) (Fig. 1).

Male specimens collected from Madikeri-Sakleshpur road (Image 1) were morphologically similar to the holotype (BNHS 4193) and paratype (BNHS 4194) (both males) of *Raorchestes tuberohumerus*. Morphometric data (Table 2) of the males collected in the present study showed that the specimens clustered with the *R. tuberohumerus* (Fig. 2). We considered only males for morphometric comparison as earlier taxonomic studies (Kuramoto & Joshy 2003; Biju & Bossuyt 2009; Padhye et al. 2013) have considered only males while describing species in the genus *Raorchestes*. Further the morphometric data of females of *R. bombayensis* are not available and of *R. tuberohumerus* we have data of only two specimens in the current study. For *R. tuberohumerus*, males and females were morphologically similar apart from the larger size and absence of tubercle on the humeral bone in females.

According to Kuramoto & Joshy (2003), head is broader than long while according to Biju & Bossuyt (2009), head is as wide as long. However we have observed that head is slightly longer than wide or as wide as long in both males and females (Table 2). The difference in description of these characters, as compared to that of Kuramoto & Joshy (2003), is probably due to population variations or difference in methodology of morphometry.

### Sexual dimorphism

Males posses a single, sub-gular vocal sac (Image 1b). We could not observe nuptial pads in males. Kuramoto & Joshy (2003) do not mention anything regarding nuptial pads in original species description; however,


**Image 1. *Raorchestes tuberohumerus* collected from Madikeri-Sakleshpur Road.**
**(a) Female with no tubercle on the humeral bone, (b) calling male showing single subgular vocal sac and (c) amplexus with male having tubercle on the humeral bone and female lacking it.**

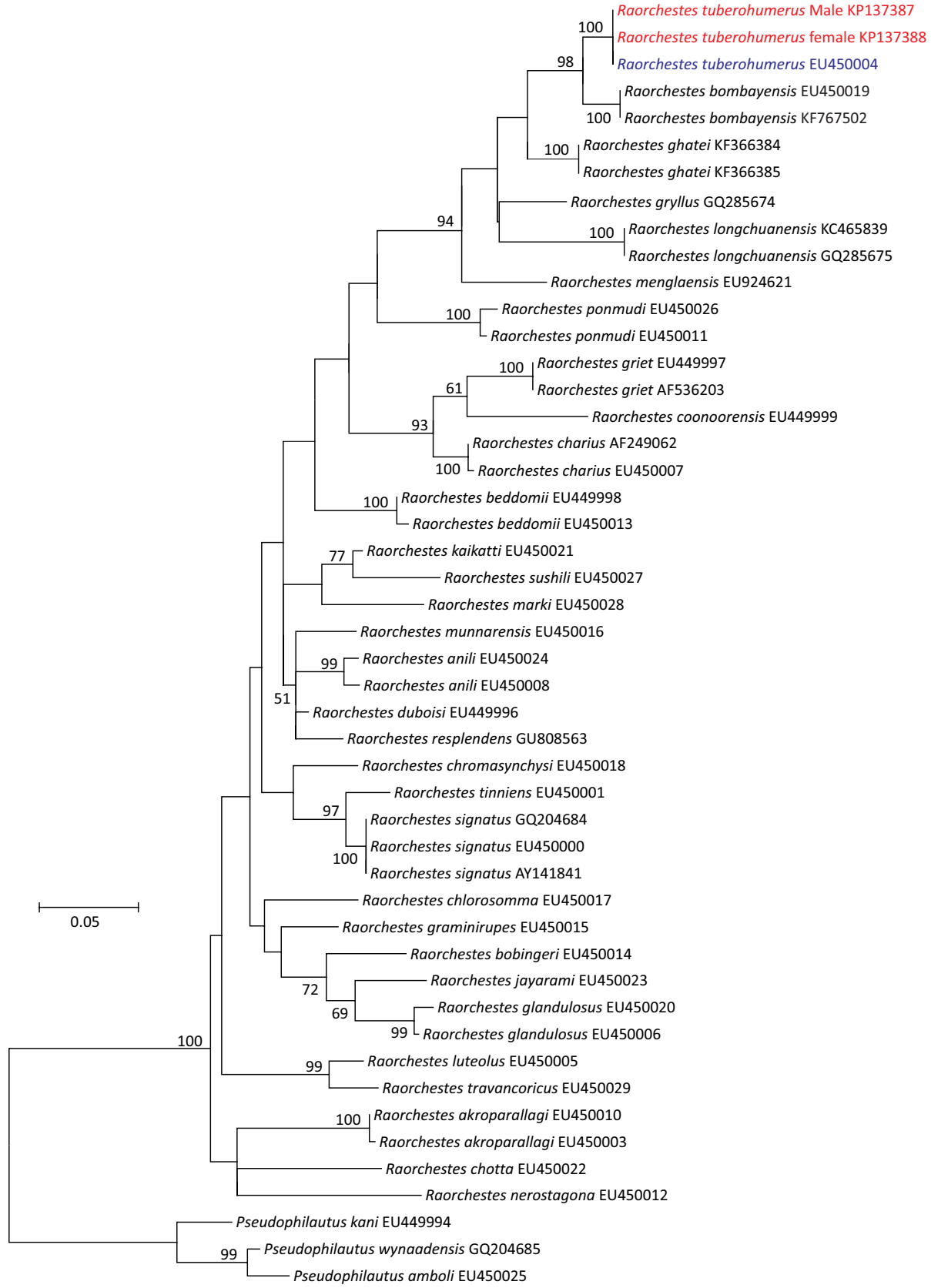



**Figure 1. Maximum likelihood tree based on 16S rRNA gene using general time reversal with gamma distribution and invariant sites (GTR+G+I, BIC = 7197.32, lnL = -2961.16, I = 0.44, G = 0.46) model of nucleotide substitution. Percent bootstrap values are for 1000 iterations.**

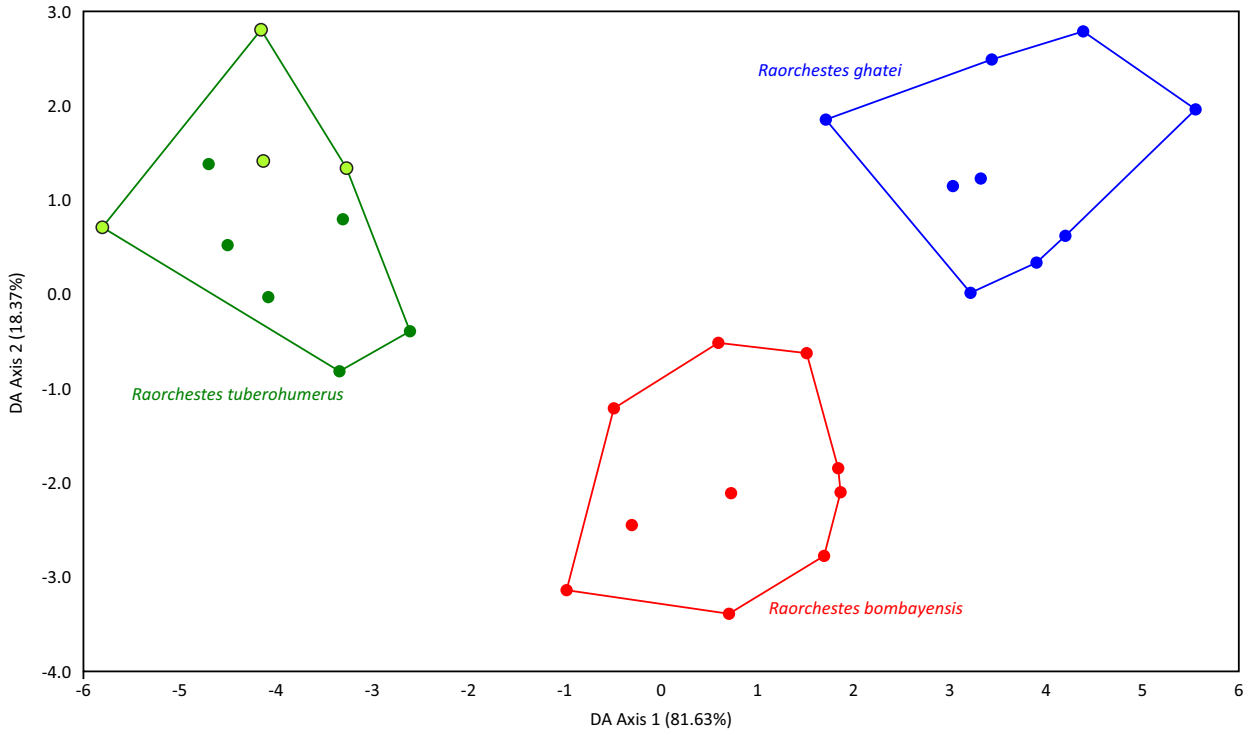



**Figure 2. Discriminant Analysis of morphological data of males of the three closely related species showing that the males in the current study are conspecific with *Raorchestes tuberohumerus*. Light green circles with black border are the male specimens within the *R. tuberohumerus* cluster.**

Biju & Bossuyt (2009) have mentioned the presence of slightly spinular nuptial pad in this species.

The most striking sexual dimorphism in this species is the presence of bony tubercle and two bony flaps in planes perpendicular to each other at the distal end of humeral bone in males and absent in females (Image 2). While describing *R. tuberohumerus*, Kuramoto & Joshy (2003) were not sure whether the presence of tubercle on humerus is male specific or not. In the revision of the taxa, Biju & Bossuyt (2009) also did not provide any information on the females of the species. Padhye et al. (2013), for the first time showed that similar to *R. tuberohumerus*, *R. ghatei* also possesses tubercle on the humerus; however, they noted the sexually dimorphic character present only in males. Padhye et al. (2013) predicted that even in *R. tuberohumerus*, the females may be devoid of the tubercle on the humeral bone. Current study confirms the humeral tubercle to be a sexually dimorphic character present only in males even in *R. tuberohumerus*.

A number of sexually dimorphic features in males are known in amphibians (Emerson & Voris 1992; Emerson 1996). However, modification of humeral bone as a sexually dimorphic character in bush frogs of the genus *Raorchestes* may have important ecological and evolutionary significance. Although, the exact role of the modified humeral bone is not clear, presence of tubercle as well as bony flaps in planes perpendicular

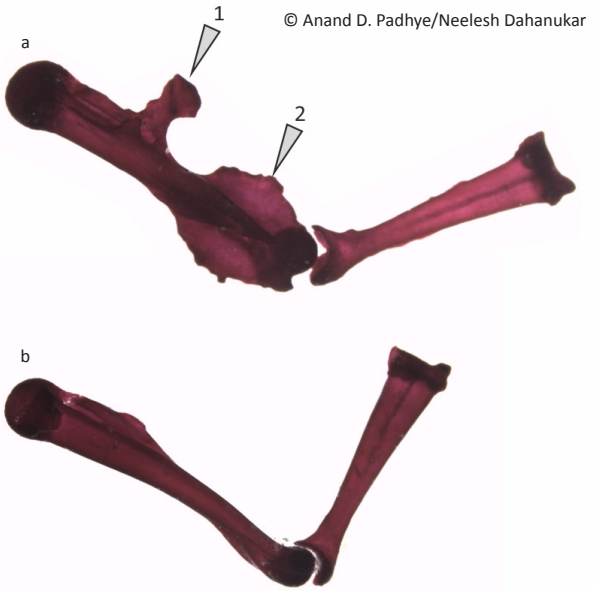



**Image 2. Humeral bone of male (a) and female (b) showing the absence of tubercle in females. Bony tubercle (1) and two bony flaps in planes perpendicular to each other at the distal end of humeral bone (2) are present only in males.**

**Table 2. Morphometry (in mm) of four males and two female specimens of *Raorchestes tuberohumerus* collected from Madikeri-Sakleshpura road.**

| Character | Female* | Female | Male* | Male | Male | Male |
|---|---|---|---|---|---|---|
| Voucher number | WILD-AMP-14-500 | WILD-AMP-14-502 | WILD-AMP-14-499 | WILD-AMP-14-501 | AGC- ZRL-AMPHIBIA-201 | AGC- ZRL-AMPHIBIA-202 |
| SVL | 20.5 | 21.6 | 18.9 | 18.2 | 16.6 | 16.8 |
| HL | 7.3 | 8.0 | 6.5 | 5.4 | 5.7 | 6.1 |
| HW | 6.9 | 7.4 | 6.4 | 5.4 | 5.5 | 5.3 |
| MN | 5.1 | 5.9 | 4.8 | 4.3 | 4.8 | 5.2 |
| MFE | 3.6 | 3.6 | 3.0 | 3.1 | 2.3 | 4.0 |
| MBE | 2.2 | 1.5 | 1.3 | 1.1 | 1.4 | 1.7 |
| SL | 3.3 | 3.0 | 1.4 | 2.2 | 2.3 | 2.2 |
| EL | 3.1 | 2.8 | 3.0 | 2.4 | 2.5 | 2.5 |
| SN | 1.2 | 1.1 | 0.7 | 1.0 | 0.6 | 0.8 |
| EN | 2.1 | 1.9 | 1.7 | 1.2 | 1.7 | 1.4 |
| IUE | 2.9 | 2.5 | 2.2 | 2.4 | 2.2 | 2.1 |
| UEW | 2.1 | 1.7 | 1.6 | 1.7 | 1.1 | 1.3 |
| IFE | 4.0 | 4.6 | 3.6 | 3.5 | 3.0 | 3.2 |
| IBE | 4.8 | 4.7 | 4.8 | 4.3 | 3.6 | 3.3 |
| FLL | 4.9 | 6.2 | 4 | 4.8 | 3.5 | 4.3 |
| HAL | 4.8 | 5.7 | 4.4 | 5.4 | 4.3 | 4.6 |
| TFL | 2.6 | 3.4 | 2.4 | 2.9 | 2.8 | 2.4 |
| FDIII | 1.0 | 1.0 | 0.7 | 0.9 | 0.7 | 0.8 |
| FWIII | 0.4 | 1.5 | 0.4 | 0.5 | 0.5 | 0.5 |
| ShL | 8.3 | 10.4 | 8.0 | 7.7 | 7.4 | 7.5 |
| TL | 9.5 | 11 | 8.7 | 9.1 | 7.6 | 7.9 |
| FOL | 7.3 | 8.2 | 7.1 | 7.4 | 6.2 | 6.1 |
| TFOL | 12.4 | 14.4 | 12.0 | 12.5 | 10.7 | 10.4 |
| TYDV | 1.2 | 1.2 | 0.9 | 1.0 | 0.7 | 1.0 |
| TYDH | 1.2 | 0.8 | 0.7 | 1.0 | 0.7 | 0.8 |

* used for genetic study and osteology

to each other at the distal end of humeral bone may provide additional anchorage to the forelimb muscles which in turn could help in firm grip. The firmness of this grip was also noted by Kuramoto & Joshy (2003) who mention '…. the arms were folded under the chest when the frog was anesthetized, so tightly folded that it was hard to extend them'. Padhye et al. (2013) suggested that the modified humerus might have dual function: (1) clasping the females during amplexus, and (2) clinging to the small shrubs in windy habitats.

Based on our observations on the sexual dimorphism in *Raorchestes tuberohumerus* it is obvious that tubercle on the humerus cannot be used as a diagnostic character as the female cannot be identified by it. Currently, we are not aware of whether the modification of humeral bone in males is always present, however, given that this is an osteological character, it may not be a seasonal trait. Ecology and evolution of such a sexually dimorphic character in two different species of bush frogs, *R. tuberohumerus* and *R. ghatei*, calls for further studies in these aspects.

**Species distribution, habitat, threats and conservation implications**

*Raorchestes tuberohumerus* is currently known to occur from 14 localities in the Western Ghats of Karnataka and northern Kerala (Image 3a) and include Anshi, Jog falls, Someshwar-Agumbe, Agumbe, Chikmagalur, Kudremukh, Malleshwaram, Kemphole, Sakleshwar, Madikeri-Sakleshpura Road, Kirundadu, Mercara, Muthunga and Wayanad (Kuramoto & Joshy 2003; Das 2004; Biju & Bossuyt 2009; Gururaja 2012;

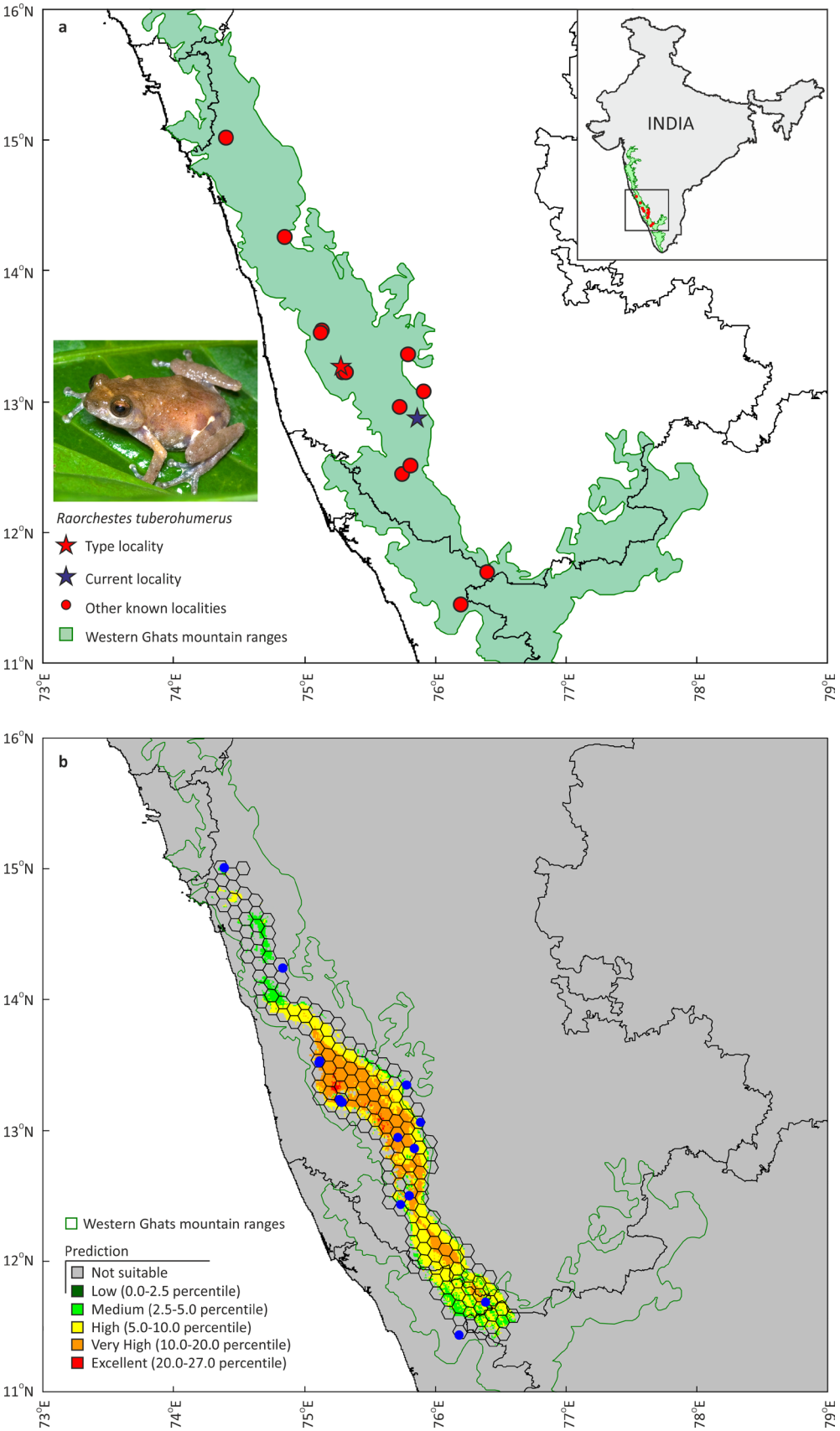



**Image 3. Distribution map of *Raorchestes tuberohumerus* (a) and prediction based on niche modelling using altitude, precipitation and 18 bio-climatic layers. Hexagonal grids shown in (b) were used to find the extent of occurrence of the species based on prediction model.**

Gururaja & Ramachandra 2012). Gururaja (2012) has shown more points in the map for the distribution of this species but the point localities are not mentioned. Records of this species from Maharashtra (Padhye & Ghate 2012) are not considered as Padhye et al. (2013) have mentioned that these records should be assigned to *R. ghatei*. Niche-based prediction model suggests that the species is restricted to the Western Ghats mountain ranges (Image 3b). Hexagonal grids that coincide with at least some prediction were considered for finding extent of occurrence (EOO) (for explanation of EOO see IUCN 2012) and this accounted for an area of approximately 18,565km$^2$. However, this was the maximum possible value as the prediction was not very high in all these hexagons. Considering only the very high prediction the EOO was estimated to approximate 10,966km$^2$. The true EOO is likely to be between these two values. Currently, the species is known from 14 localities, which can fall under 10 severely fragmented locations (for explanation of locations see IUCN 2012) based on the threats to the habitat—(1) Anshi, (2) Jog Falls, (3) Someshwar-Agumbe & Agumbe, (4) Chikmagalur, (5) Kudremukh & Malleshwaram, (6) Kemphole, (7) Sakleshwar & Madikeri-Sakleshpura road, (8) Kirundadu & Mercara (Madikeri), (9) Muthunga, and (10) Wayanad.

We observed that *Raorchestes tuberohumerus* is found in the undergrowth on shrubs not more than 2m in height. Similar observations were made by Biju & Bossuyt (2009). The reproductive biology of this species is in Bossuyt et al. (2001). The species is found calling from a height of 0.5–2 m on the shrubs and lays 26 or 27 eggs on the leaves (Bossuyt et al. 2001). As the undergrowth forms the major habitat for the breeding of these species threat to the undergrowth can have severe effects on the species population. Clearing of undergrowth for land levelling and plantation could be the most important threat.

Information on species specific threats are not available for *R. tuberohumerus*, however, habitat destruction, change in land use pattern, heavy traffic, recreational activities and agricultural, organic and inorganic pollution are general threats to the habitat of the species in most parts of its distribution (Kumara et al. 2000; Seshadri et al. 2009; Gururaja & Ramachandra 2012; Ramchurjee 2013). Within some of the sanctuary areas (like Wayanad and Muthanga), habitats of this species could be threatened by the road widening activities associated with the national highway passing through. Even though the highways are closed at night, the highway traffic might contribute to the increased pollution level. Further, presence of several private lands at these sites are subject to cultivation and tourism, which may lead to the change in land use pattern and habitat modification / destruction. Plantations (in places like Anshi, Wayanad, Kirundadu and Madikeri) also pose threats through agricultural pollution through runoffs. In areas like Jog falls, Chikmagalur, Sakleshpur and Madikeri recreational activities due to heavy tourism, increasing urbanization and runoffs from spice and coffee plantations could affect the habitat of this species.

Recently, based on niche-based predictive modelling, Molur et al. (2015) suggested that this area of Western Ghats has a high propensity of *Batrachochytrium dendrobatidis* (*Bd*) infection. Because other species of *Raorchestes* of the Western Ghats are already known to be vulnerable to the infection by *Bd* (Dahanukar et al. 2013; Molur et al. 2015), it is possible that *R. tuberohumerus* is also likely to have *Bd* infection. Although it is not known whether *Bd* has impacted amphibian populations in the Western Ghats, Dahanukar et al. (2013) argued that "…….it is possible that the fungal infection may manifest in the near future through increased stressors such as organic and inorganic pollution, which might increase the virulence of the fungal strain and/or decrease the immunity of amphibian host". If this is likely, then the presence of *Bd* could also be considered as a plausible threat to the species.

We do not have systematic sampling study to precisely pinpoint the threats to the populations of the species. Nevertheless, the fact that the species is restricted in distribution, has fragmented populations and there are several plausible threats to the habitat calls for raising conservation concern. *Raorchestes tuberohumerus* was assessed as Data Deficient (Das 2004) owing to lack of information. Based on the currently available data and above arguments we propose that the species is likely to be under the 'Vulnerable' category under the criteria B1ab(iii) for limited geographical range and threats to the habitat. A complete assessment is provided in Appendix A.

## REFERENCES

**Biju, S.D., Y. Shouche, A. Dubois, S.K. Dutta & F. Bossuyt (2010).** A ground-dwelling rhacophorid frog from the highest mountain peak of the Western Ghats of India. *Current Science* 98(8): 1119–1125.

**Biju, S.D. & F. Bossuyt (2009).** Systematics and phylogeny of *Philautus* Gistel, 1848 (Anura, Rhacophoridae) in the Western Ghats of India, with descriptions of 12 new species. *Zoological Journal of the Linnean Society* 155: 374–444; http://dx.doi.org/10.1111/j.1096-3642.2008.00466.x

## Appendix A. Red List Assessment: *Raorchestes tuberohumerus*

**Current Status:** Data Deficient

**Proposed Status:** Vulnerable B1ab(iii)

Kingdom: Animalia
Phylum: Chordata
Class: Amphibia
Order: Anura
Family: Rhacophoridae
Genus: *Raorchestes*
Species: *tuberohumerus*
Authority: (Kuramoto & Joshy, 2003)

Common name: Kudremukh Bush Frog

Synonyms: *Philautus tuberohumerus* Kuramoto & Joshy, 2003

Taxonomic notes: *Raorchestes tuberohumerus* was originally described as *Philautus tuberohumerus* from Kudremukh, Western Ghats of Karnataka, India, by Kuramoto & Joshy (2003). Species was assigned to the genus *Raorchestes* by Biju et al. (2010).

### Assessment Information

**Red List Category and Criteria (Version 3.1):** Vulnarable B1ab(iii)

**Justification:** *Raorchestes tuberohumerus* is assessed as Vulnerable owing to the fact that the extent of occurrence of the species is less than 19,000km$^2$ with a distribution restricted to about 10 severely fragmented locations and threats to the habitat and decrease in quality of habitat due to habitat destruction, change in land use, heavy traffic, recreational activities and agricultural, organic and inorganic pollution most parts of its distribution. Since the species breeds in the undergrowth, land leveling and plantation practices are potential threats throughout the species range.

### Geographic Range / Distribution Information

**Range description:** *Raorchestes tuberohumerus* is endemic to the Western Ghats of India. It in known from Anshi, Jog falls, Someshwar-Agumbe Road, Agumbe, Chikmagalur, Kudremukh, Malleshwaram, Kemphole, Sakleshwar, Madikeri - Sakleshpura Road, Kirundadu and Madikeri in Karnataka, and Muthunga and Wayannad in Kerala.

**Countries of occurrence:** Endemic to India

**Extent of Occurrence (EOO):** Based on the prediction map the maximum EOO is approximately 18,565km$^2$, while the very high prediction area is approximately 10,966km$^2$.

**Area of Occupancy (AOO):** The AOO is not estimated although the species is severely fragmented in distribution.

**Number of locations:** Currently, the species is known from 14 localities, which can fall under 10 severey fragmented locations based on the threats to the habitat such as habitat destruction through clearing of undergrowth for land levelling and plantation, change in land use, heavy traffic, recreational activities, and agricultural, organic and inorganic pollution.

**Range Map:** Image 3.

### Population Information

**Population:** Population status of the species is not properly known. However, calls are normally heard in isolated patches throughout the range of its distribution.

**Population trend:** The population is presumed to be declining due to general threats to the habitat throughout its distributional range.

### Habitat and Ecological Information

**Habitat and Ecology:** *Raorchestes tuberohumerus* is found in the undergrowth on shrubs. The males call from a height of 0.5m to 2m on the shrubs and females lay 26 or 27 eggs on the leaves.

**Systems:** Terrestrial

### Information on Threats

**Threats:** Since undergrowth forms the major habitat for the species, threats to the undergrowth can have severe effects of the species population. Clearing of undergrowth for land levelling and plantation could be the most important threat. Habitat destruction, change in land use, heavy traffic, recreational activities and agricultural, organic and inorganic pollution are general threats to the habitat of the species in most parts of its distribution. Plantations (in places like Anshi, Wayanad, Kirundadu and Madikeri) also pose threat through agricultural pollution through runoffs. In areas like Jog falls, Chikmagalur, Sakleshpur and Mercara recreational activities due to heavy tourism, increasing urbanization and runoffs from spice and coffee plantations could affect the habitat of this species. Presence of chytrid *Batrachochytrium dendrobatidis* (*Bd*) infection could also be a plausible threat to the species.

### Use and Trade Information

**Use:** The species is not in use.

**Livelihoods and sustenance:** There is no livelihood dependence on the species.

**Off take from the wild:** The species is not harvested.

**Commercial value:** The species has no local, domestic, national or international commercial value.

### Information on Conservation Actions

**Conservation actions:** No species specific conservation action plans are available for *Raorchestes tuberohumerus*. The species is found in some protected areas including Anshi, Agumbe, Kudremukh, Someshwar, Wayanad and Muthanga, however, populations in these areas could also be subjected to habitat modifications and recreational activities. Since the species lives in specific habitats on undergrowths, species and habitat specific conservation actions could be helpful. Research is also required to determine whether there is occurance of chytrid fungus on this species and its effects on the species in the wild.

**Research in place:** There is no systematic research in place other than opportunistic surveys.

**Research needed:** Systematic surveys, population monitoring, and effects of threats on populations and habitats, are some of the much needed research actions for the species.

**Monitoring in place:** Currently, there is no monitoring of the species, population or habitat in place.

**Monitoring needed:** Population and site monitoring are essential. Population monitoring for effects of chytrid is imperative.

**Education in place:** No formal or informal education about the species is in place.

**Education needed:** Outreach programmes to educate common people, environmental enthusiasts and forest officials are essential.

**Bossuyt, F., K. Roelants, L. Spithoven & M.H. Daro (2001).** *Philautus bombayensis* (Bombay Oriental Shrub Frog) reproduction. *Herpetological Review* 32(1): 34–35.

**Das, I. (2004).** *Raorchestes tuberohumerus*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 27 November 2014.

**Edgar, R.C. (2004).** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32: 1792–1797; http://dx.doi.org/10.1093/nar/gkh340

**Emerson, S.B. & H. Voris (1992).** Competing explanations for sexual dimorphism in a voiceless Bornean frog. *Functional Ecology* 6: 654–660; http://dx.doi.org/10.2307/2389960

**Emerson, S.B. (1996).** Phylogenies and physiological processes—the evolution of sexual dimorphism in southeast Asian frogs. *Systematic Biology* 45: 278–289; http://dx.doi.org/10.1093/sysbio/45.3.278

**Gururaja, K.V. (2012).** *Pictorial Guide to Frogs and Toads of the Western Ghats*. Gubbi Labs Publication, 154pp+xviii.

**Gururaja, K.V. & T.V. Ramachandra (2012).** Anuran Diversity and Distribution in Dandeli Anshi Tiger Reserve. Sahyadri Conservation Series: 8. ENVIS Technical Report 37. Sahyadri: Environmental Information System (ENVIS), Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India, 57pp.

**Hammer, Ø., D.A.T. Harper & P.D. Ryan (2001).** PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 1–9.

**Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones & A. Jarvis (2005).** Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978; http://dx.doi.org/10.1002/joc.1276

**IUCN (2012).** *IUCN Red List Categories and Criteria: Version 3.1*. Second edition. Gland, Switzerland and Cambridge, IUCN, UK, iv+32pp.

**Kumara, H.N., A.K. Sharma, A. Kumar & M. Singh (2000).** Roadkills of wild fauna in Indira Gandhi Wildlife Sanctuary, Western Ghats, India: implications for management. *Biological Conservation* 3: 41–47.

**Kuramoto, M. & S.H. Joshy (2003).** Two new species of the genus *Philautus* (Anura: Rhacophoridae) from the Western Ghats, southwestern India. *Current Herpetology* 22: 51–60.

**Molur, S., K. Krutha, M.S. Paingankar & N. Dahanukar (2015).** Asian strain of *Batrachochytrium dendrobatidis* is widespread in the Western Ghats, India. *Diseases of Aquatic Organisms* 112: 251–255; http://dx.doi.org/10.3354/dao02804

**Nei, M. & S. Kumar (2000).** *Molecular Evolution and Phylogenetics*.

Oxford University Press, New York, 333pp.

**Padhye, A.D. & H.V. Ghate (2012).** Amphibia. *Fauna of Maharashtra*, *Zoological Survey of India State Fauna Series* 20(1): 239–246.

**Padhye, A.D., A. Sayyed, A. Jadhav & N. Dahanukar (2013).** *Raorchestes ghatei*, a new species of shrub frog (Anura: Rhacophoridae) from the Western Ghats of Maharashtra, India. *Journal of Threatened Taxa* 5: 4913–4931; http://dx.doi.org/10.11609/JoTT.o3702.4913-31

**Ramchurjee, N.A. (2013).** Impacts of ecotourism in Rajiv Gandhi National Park (Nagarhole), Karnataka. *Environment, development and sustainability* 15(6): 1517–1525.

**Sahr, K., D. White & A.J. Kimerling (2003).** Geodesic discrete global grid systems. *Cartography and Geographic Information Science* 30 (2): 121–134.

**Schwarz, G. (1978).** Estimating the dimension of a model. *Annals of Statistics* 6: 461–464.

**Seshadri, K.S., A. Yadav & K.V. Gururaja (2009).** Road kills of amphibians in different land use areas from Sharavathi River basin, central Western Ghats, India. *Journal of Threatened Taxa* 1(11): 549–552; http://dx.doi.org/10.11609/JoTT.o2148.549-52

**Tamura, K., G. Stecher, D. Peterson, A. Filipski & S. Kumar (2013).** MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729; http://dx.doi.org/10.1093/molbev/mst197

**Author Contribution:** ADP and SS collected specimens; SS performed photography; AJ performed morphometry; ADP performed osteology; ND performed genetic and statistical analysis; ADP and ND wrote the manuscript.

**Author Details:** Anand D. Padhye is an Associate Professor in Zoology, Abasaheb Garware College, Pune. He works on systematics, ecology, diversity, distribution and evolution of amphibians. Anushree Jadhav has completed her Masters in Biodiversity at Abasaheb Garware College, Department of Biodiversity. She is currently pursuing her M.Phil from Department of Zoology, Savitribai Phule Pune University. Shauri Sulakhe is a Founder-Director of Insearch Outdoors, an environment tourism organization in Pune. He is also a freelance field worker interested in wildlife photography and natural history. Neelesh Dahanukar works in ecology and evolution with an emphasis on mathematical and statistical analysis. He is also interested in taxonomy, distribution patterns and molecular phylogeny.