A new species of cryptic Bush frog (Anura, Rhacophoridae, Raorchestes) from northeastern Bangladesh

Hassan Al-Razi¹, Marjan Maria¹, Sabir Bin Muzaffar²

¹ Faculty of Life and Earth Science, Department of Zoology, Jagannath University, Dhaka, Bangladesh
² Department of Biology, United Arab Emirates University, Al Ain, United Arab Emirates

Corresponding author: Hassan Al-Razi (chayan1999@yahoo.com)

Academic editor: A. Herrel | Received 23 November 2019 | Accepted 26 February 2020 | Published 16 April 2020

http://zoobank.org/C8772A41-F588-406F-B1CD-FA139ED8CE9A

Citation: Al-Razi H, Maria M, Muzaffar SB (2020) A new species of cryptic Bush frog (Anura, Rhacophoridae, Raorchestes) from northeastern Bangladesh. ZooKeys 927: 127–151. https://doi.org/10.3897/zookeys.927.48733

Abstract

Raorchestes is a speciose genus of bush frogs with high diversity occurring in the Western Ghats of India. Relatively fewer species have been recorded across India, through Bangladesh, southern China, into Vietnam and Peninsular Malaysia. Many bush frogs are morphologically cryptic and therefore remain undescribed. Here, a new species, Raorchestes rezakhani sp. nov., is described from northeastern Bangladesh based on morphological characters, genetics, and bioacoustics. The 16S rRNA gene distinguished this species from 48 known species of this genus. Bayesian Inference and Maximum Likelihood analyses indicated that the new species was most similar to R. tuberohumerus, a species found in the Western Ghats, and to R. gryllus, a species found in Vietnam. Bioacoustics indicated that their calls were similar in pattern to most Raorchestes species, although number of pulses, duration of pulses, pulse intervals and amplitude differentiated it from a few other species. It is suggested that northeastern India, Bangladesh, northern Myanmar, and southern China represent important, relatively unexplored areas that could yield additional species of Raorchestes. Since many remaining habitat patches in Bangladesh are under severe threat from deforestation, efforts should be made to protect these last patches from further degradation.

Keywords

Amphibian, bush frog, DNA, herpetofauna, Raorchestes rezakhani sp. nov.
Introduction

Raorchestes Biju et al., 2010, is a genus of bush frogs belonging to the family Rhacophoridae, that extends in distribution from southwestern India through northeastern India, Bangladesh, Myanmar, southern China, and into Laos, Vietnam, and Peninsular Malaysia (Biju and Bossuyt 2009; IUCN 2016; Vijayakumar et al. 2016; Frost 2019). The genus is particularly speciose in the Western Ghats of India, where more than 50 of the 63 recorded species occur (Biju et al. 2010; Vijayakumar et al. 2014; Priti et al. 2016; Vijayakumar et al. 2016; Boruah et al. 2018). In addition, a few species have been recorded from the Eastern Ghats, Eastern Himalayas, and northeastern India, southern China and adjoining regions (Vijayakumar et al. 2016; Boruah et al. 2018; Wu et al. 2019, Frost 2020). Many species in the genus are cryptic (morphologically difficult to distinguish from congenerics) and, as a result, remain undescribed (Priti et al. 2016; Vijayakumar et al. 2016; Boruah et al. 2018; Wu et al. 2019). The genus is characterized within the Rhacophoridae by small size (15–45 mm snout-vent length), absence of vomerine teeth, transparent gular pouch, and direct development (Biju et al. 2010). Their advertisement calls consists of repetitive ‘treenk.. treenk.. treenk’ with variation in number of pulses, duration of calls, interval between calls and amplitude, which may be used to distinguish between species (Priti et al. 2016). Thus, an integrative approach using morphological traits, bioacoustics and molecular variation has been used to distinguish cryptic species (Priti et al. 2016; Boruah et al. 2018).

Bangladesh falls within the Indo-Malayan realm, with forests classified as tropical moist, tropical evergreen and several other less-extensive forest types (Champion and Seth 1968; Slik et al. 2018). Broad similarities exist between forest patches in northeastern and southeastern Bangladesh and the surrounding Indian States of Meghalaya, Tripura, Mizoram and Nagaland, and adjoining northern Myanmar and southern China (Slik et al. 2018). Three species of Raorchestes, namely the Darjeeling bush frog Raorchestes annandalii (Boulenger, 1906), the Karin bubble-nest frog R. parvulus (Boulenger, 1893) and, most recently, the Longchuan bush frog R. longchuanensis (Yan and Li 1978) have been recorded from Bangladesh (Ghose and Bhuiyan 2012; IUCN 2015; Khan 2015; Al Razi et al. 2020). Raorchestes parvulus has a distribution from northeastern India, Bangladesh through Southeast Asia extending up to Vietnam and Peninsular Malaysia (Khan 2015; IUCN 2015; IUCN-SSC 2016). In fact, R. parvulus has been confused with R. longchuanensis reported originally from southern China (Frost 2020) and recently from northeastern Bangladesh (Al-Razi et al. 2020). Furthermore, Thai populations of R. parvulus are possibly separate species (Frost 2020). Thus, R. parvulus has been regarded as members of a species complex (Khan 2015; IUCN 2015; IUCN-SSC 2016). It has been speculated that R. longchuanensis has a wider distribution in northeastern India and northern Myanmar (Al-Razi et al. 2020). Raorchestes annandalii, on the other hand, has a more restricted distribution in southeastern Bangladesh, northeastern India, and Nepal (Bardoloi et al. 2004; IUCN 2015). The region of northeastern India that surrounds Bangladesh
hosts at least four species of *Raorchestes* and other related genera. This suggests that the region represent a zone of diversification of bush frogs. It is possible that *R. annandalii* represents a northern complex of related species and *R. parvulus* are part of a more southern species complex (Frost 2020). Here we describe a new species of bush frog from northeastern Bangladesh based on bioacoustics, morphology, and molecular characterization.

**Materials and methods**

**Study area**

We conducted this study in Adampur Reserve Forest (24°13.410′N, 91°54.836′E) and Lawachara National Park (24.330755N, 91.789396E), two small forest patches of northeastern Bangladesh (Fig. 1). Both forests are semi-evergreen, and local climate and hydrologic patterns are similar, but their sizes and disturbance patterns differ (Quazi and Ticktin 2016). The topography of the study area is hilly, with elevations ranging from 50–100 m a.s.l. (Islam et al. 2007). Annual temperature ranges from 9 °C (January) to 32 °C (August–October), and nearly 80% of the annual average rainfall (3,334 mm) occurs between the months of May and October (Quazi and Ticktin 2016). Numerous streams and swampy areas crisscross the region. The landscape is categorized into hill forests, scrublands, and mixed bamboo forests (IUCN 2015). Northeast Bangladesh shares an international border with India, and two of the Indian states, Tripura, and Assam, are adjacent to northeast Bangladesh (Fig. 1).

**Specimen collection**

We collected four adult calling males from April to October 2019. We euthanized and fixed the specimens in 95% ethanol for 5 hrs and stored them in 70% ethanol. We tentatively designated the specimens to the genus *Raorchestes* based on small size (18.85–20.90 mm snout-vent length), absence of vomerine teeth, transparent gular pouch, and advertisement calls consisting of repetitive ‘treenk.. treenk.. treenk’ which is characteristic of *Raorchestes* (following Biju et al. 2010). Thigh-muscle samples for genetic analysis were collected before fixing the specimen. We recorded the color of living specimens and recorded natural history observations at the type locality during specimen collections. As the frog was very small, cryptic, and very difficult to find, we were able to collect only four specimens. We deposited the specimens in the Shahid Rafique Special Specimen Collection (SRSSC), Department of Zoology, Jagannath University, Dhaka. Since the SRSSC is a newly established part of the Zoological Museum, the catalogue numbers for specimens retain the original codes adopted by the Zoological Museum, namely JnUZool.
Figure 1. Map showing the type location of *Raorchestes rezakhani* sp. nov. in northeastern Bangladesh as well as adjoining areas.

**Morphometrics**

We measured the following from the left side of the specimens with digital calipers (to the nearest 0.10 mm):

- **ED** eye diameter (horizontal diameter of the eye);
- **EN** eye-nostril distance (distance between anterior canthus of eye and the posterior edge of nostril);
- **FD I to IV** width of 1\(^{st}\) to 4\(^{th}\) finger disks (measured at the widest point on the finger disk);
- **FL I to IV** lengths of 1\(^{st}\) to 4\(^{th}\) fingers (from the tip of the respective finger to where it connects with the palm);
- **FOL** foot length (from the distal end of tarsus tip of Toe IV);
- **HAL** hand length (from distal end of radioulna to tip of distal finger III);
- **HL** head length (distance between tip of the snout to the rear of the mandible);
- **HW** head width (at angle of jaw);
- **IND** internarial distance (least distance between inner edge of the nostrils);
IOD  interorbital distance (least distance between proximal edges of upper eyelids);
NS   nostril-Snout distance (distance from the anterior edge of nostril to the tip of the snout);
ShL  shank length (distance between knee and heel);
SL   snout length (from anterior canthus of eye to tip of snout);
SVL  snout-vent length (from tip of snout to vent);
TD   tympanum diameter (maximum diameter of the tympanum);
TD I to V width of 1st to 5th toe disks (the greatest horizontal distance between the edges of toe disks);
TL   thigh length (distance from the middle of vent to knee);
TL I to V lengths of 1st to 5th toes (from base of proximal subarticular tubercle to tip of the respective toe);
UEW  upper eyelid width (maximum transverse distance of the upper eyelid).

We compared morphological characters based on morphometric measurements provided in the following published papers (Al-Razi et al. 2020; Kuramoto and Joshy 2003; Padhye et al. 2015; Orlov et al. 2012):

In addition, we compared eleven linear, morphometric variables of four species using Principle Components Analysis (PCA) (McGarigal et al. 2000; Sokal and Rohlf 2012), using PAST (version 3.8). All linear morphometric variables were transformed by subtracting each variable from the mean of that variable (McGarigal et al. 2000; Sokal and Rohlf 2012). We derived eleven principle components, since there were eleven variables, each representing a linear combination of all eleven variables. We generated Eigenvalues and their relative weightings to determine the relative contribution of the variables towards each principle component (McGarigal et al. 2000). Loadings of each of the eleven variables in relation to each of the eleven principle components were used to determine relative effect of individual morphological characters on each principle component. We visualized the differences in the species compared using a scatter plot of principle components that explained the greatest variance in the data (McGarigal et al. 2000).

DNA Extraction and amplification

We extracted DNA from the muscle samples using a standard protocol described in Vences et al. (2012) for DNA extraction. We amplified mitochondrial 16S ribosomal RNA gene. The PCR amplification and sequencing of the 16S rRNA gene were done following Palumbi et al. (1991) and Bossuyt et al. (2004) respectively. We used primers 5’-GCCTGTTTATCAAAAACAT-3’ (16Sar-L) and 5’-CCGGTCTGAACTCAGATCACGT-3’ (16Sbr-H) as forward and reverse primers for 16S (Palumbi et al. 1991) for this study. We performed PCR amplifications in a 20 μl reaction volume; Master Mix 10 μl, T DNA (Concentration 25–65 ng/μl) 1 μl, Primer F (Concentration 10–20 pMol) 1 μl, Primer R (Concentration 10–20 pMol) 1 μl and nuclease-free
water 7 μl with the following cycling conditions: an initial denaturing step at 95 °C for 3 min; 40 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s and extending at 72 °C for 45 s, and a final extension step of 72 °C for 5 min. We sent the amplified product to First Base Laboratories, Malaysia for sequencing. The sequences were checked manually using the program Chromas lite 2.01 (http://www.technelysium.com.au/chromas_lite.html). The sequences were submitted to GenBank (Accession no: MN072374, MN072375, MN615901, MN615902).

**Phylogenetic analyses**

We compared the new sequences to the GenBank sequences using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to confirm their genetic identity and determine similar species that allow the evaluation of the phylogenetic position of the new taxon. Homologous sequences of other *Raorchestes* species were obtained from GenBank (Table 1). *Kurixalus eiffingeri* Boettger, 1895 was selected as outgroup based on Yu et al. (2013). Sequences were aligned using the MUSCLE tool in MEGA 7 (Kumar et al. 2016), alignments were checked visually, and both ends of the sequence were trimmed to avoid low quality base pairs. Alignment gaps were treated as missing data. The best substitution model (GTR+I+G) was selected using the Akaike Information Criterion (AIC) and Bayesian information criteria (BIC) in jModelTest v2.1.2. Maximum likelihood phylogenetic analyses were performed using the RAxML v4.0 Geneious plugin (Stamatakis 2006) with 1,000 bootstrap replicates. Bayesian phylogenetic inference analysis were performed in MrBayes 3.2.4 (Ronquist et al. 2012). We performed an MCMC Bayesian analysis that consisted of two simultaneous runs of 1 million generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs). The trees were visualized and edited in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree). Additionally, pairwise genetic distances (uncorrected $p$) of 21 species under the genus *Raorchestes* including the new species were calculated for 16S using MEGA 7.0 (Kumar et al. 2016).

**Call recording and analysis**

The call of a single male individual (JnUZool- A0519) was recorded with a Sony ICD-PX240 digital sound recorder with sampling rate of 48 kHz and 32-bit resolution on 10 May 2019. The device was approximately 1–1.5 m away from the calling male. Air temperature and humidity were taken by a digital hygrometer. For the call analysis we used Raven Pro Ver. 1.5 (Charif et al. 2010; Bioacoustics Research Program 2011). We measured call-group duration, inter-call group interval, duration of intervals between pulses, call duration, pulse rate and dominant frequency comprising of 25 call groups.
Table 1. Species of *Raorchestes* and the outgroup and their associated GenBank accession numbers that were used in the phylogenetic analysis.

| Species                  | Location                  | Voucher                | GenBank 16S rRNA accession numbers | Source                  |
|--------------------------|---------------------------|------------------------|------------------------------------|-------------------------|
| 1. R. rezakhani sp. nov. | Maulovibazar, Bangladesh | JnUZool-A0319          | MN072374                           | This study              |
| 2. R. rezakhani sp. nov. | Maulovibazar, Bangladesh | JnUZool-A0419          | MN072375                           | This study              |
| 3. R. rezakhani sp. nov. | Maulovibazar, Bangladesh | JnUZool-A0619          | MN615901                           | This study              |
| 4. R. rezakhani sp. nov. | Maulovibazar, Bangladesh | JnUZool-A0519          | MN615902                           | This study              |
| 5. *longchuanensis*      | Habigonj, Bangladesh      | JnUZool-A0317          | MN193414                           | Al-Razi et al. 2020     |
| 6. *longchuanensis*      | Habigonj, Bangladesh      | JnUZool-A0117          | MN193412                           | Al-Razi et al. 2020     |
| 7. *ghatei*              | Satara, Maharashtra, India| WILD-AMP-13-100        | KF366385                           | Padhye et al. 2013      |
| 8. *ghatei*              | Satara, Maharashtra, India| ZSI-WRC A/1484         | KF366384                           | Padhye et al. 2013      |
| 9. *ghatei*              | Satara, Maharashtra, India| WILD-AMP-13-104        | KF366387                           | Padhye et al. 2013      |
| 10. *Raorchestes* sp. R3 | Riwi, Meghalaya, India   | –                      | MG980284                           | Boruah et al. 2018      |
| 11. *Raorchestes* sp. R4 | Mawlynong, Meghalaya, India| –                      | MG980285                           | Boruah et al. 2018      |
| 12. *shillongensis*      | Malki forest, Meghalaya, India| –                      | MG980282                           | Boruah et al. 2018      |
| 13. *shillongensis*      | Malki forest, Meghalaya, India| –                      | MG980283                           | Boruah et al. 2018      |
| 14. *gyllus*             | Pac Ban, Vietnam          | ROM30288               | GQ285674                           | Li et al. 2009          |
| 15. *menglaensis*        | Yunnan, China             | KIZ060821286          | EU924621                           | Yu et al. 2009          |
| 16. *bombayensis*        | Utara Kannada, Karnataka, India| 1362PhiBom             | EU450019                           | Biju and Bossuyt 2009   |
| 17. *bombayensis*        | Utara Kannada, Karnataka, India| WILD-13-AMP-230        | KF767502                           | Padhye et al. 2013      |
| 18. *tuberolimnaria*     | Western Ghats, India      | CESF424 16S            | KM596574                           | Vijayakumar et al. 2009 |
| 19. *tuberolimnaria*     | Western Ghats, India      | 0073PhiTub             | EU450004                           | Biju and Bossuyt 2009   |
| 20. *sanciisivaticus*    | Eastern Ghats, India      | SKD244                 | MH915511                           | Mirza et al. 2019       |
| 21. *sanciisivaticus*    | Eastern Ghats, India      | SKD240                 | MH915509                           | Mirza et al. 2019       |
| 22. *ponnudi*            | Western Ghats, India      | 1451PhiPonb            | EU450026                           | Biju and Bossuyt 2009   |
| 23. *ponnudi*            | Western Ghats, India      | 1121PhiPon             | EU450011                           | Biju and Bossuyt 2009   |
| 24. *ponnudi*            | Western Ghats, India      | 0030PhiBed             | EU449998                           | Biju and Bossuyt 2009   |
| 25. *indigo*             | Western Ghats, India      | CESF138                | KM596557                           | Vijayakumar et al. 2009 |
| 26. *partulus*           | southern Yunnan, China   | KIZ 20160374           | KM564634                           | Yu et al. 2019          |
| 27. *partulus*           | southern Yunnan, China   | KIZ 20160366           | KM564630                           | Yu et al. 2019          |
| 28. *theuerkaufi*        | Western Ghats, India      | CESF1342               | JX092693                           | Vijayakumar et al. 2009 |
| 29. *signatus*           | Western Ghats, India      | CESF1666               | KM596562                           | Vijayakumar et al. 2009 |
| 30. *signatus*           | Western Ghats, India      | CESF1662               | KM596561                           | Vijayakumar et al. 2009 |
| 31. *sinniens*           | Munnar, Kerala, India     | SDBDU2010.274          | KU169991                           | Biju et al. 2016        |
| 32. *sinniens*           | Western Ghats, India      | 0058PhiTin             | EU450001                           | Biju and Bossuyt 2009   |
| 33. *marki*              | Western Ghats, India      | CESF467                | JX092719                           | Vijayakumar et al. 2009 |
| 34. *chromasynchysi*     | Western Ghats, India      | CESF1127               | JX092667                           | Vijayakumar et al. 2009 |
| 35. *chromasynchysi*     | Western Ghats, India      | CESF1203               | KM596543                           | Vijayakumar et al. 2009 |
| 36. *chattus*            | Karnataka, India          | SDBDU2011.814          | KU169985                           | Biju et al. 2016        |
| 37. *charius*            | Sri Lanka                 | –                      | AY141840                           | Meegaskumbura et al. 2002 |
| 38. *primarrunuf*        | Western Ghats, India      | CESF442                | KM596575                           | Vijayakumar et al. 2009 |
| 39. *chatelazoides*      | Western Ghats, India      | BRA-2014               | KJ619643                           | Unpublished             |
| 40. *sp.*                | Western Ghats, India      | CESF403                | JX092710                           | Vijayakumar et al. 2009 |
| 41. *sp.*                | Western Ghats, India      | CESF427                | JX092714                           | Vijayakumar et al. 2009 |
| 42. *lechiya*            | Western Ghats, India      | SPV-2014b              | KM596563                           | Vijayakumar et al. 2009 |
| 43. *lechiya*            | Western Ghats, India      | CB-2015a               | KT359622                           | Zachariah et al. 2016   |
| 44. *lechiya*            | Western Ghats, India      | CB-2015a               | KT359623                           | Zachariah et al. 2016   |
| 45. *sp.*                | Western Ghats, India      | SPV-2014b              | KM596563                           | Vijayakumar et al. 2009 |
| 46. *karixalus effingeri*| Okinawa Islands, Japan    | A120                   | DQ468673                           | Wu et al. 2016          |

*A new species of cryptic Bush frog...* 133
**Results**

**Molecular data**

The ML and BI analyses resulted in essentially identical topologies and were integrated in the consensus tree (Fig. 2), in which the maximum nodes were sufficiently supported with the Bayesian posterior probabilities (BPP) > 0.90 and the bootstrap supports (BS) for maximum likelihood analysis > 70 and a few poorly supported basal nodes. Both Bayesian and Maximum Likelihood analyses strongly supported that the new species is in the genus *Raorchestes*. The uncorrected p-distances for the 16S rRNA gene

![Figure 2](image-url). Bayesian Inference and Maximum Likelihood phylogenies, showing the placement of *Raorchestes rezakhani* sp. nov. in relation to other congeneric species. The Bayesian Posterior Probabilities (BPP) > 0.75 and the bootstrap supports for Maximum Likelihood analysis (ML) > 60 were retained.
A new species of cryptic Bush frog...

that are interpreted as interspecific distances were lowest between *R. bombayensis* Annandale 1919 and *R. sanctisilvaticus* Das and Chanda 1997 ($p = 1.4\%$, Table 2). The highest interspecific distances were between *R. tinniens* (Jerdon 1854) and *R. ghatei* Padhye et al. 2013 ($p = 10.6\%$, Table 2). The newly discovered species was most similar to *R. tuberohumerus* (Kuramoto and Joshy 2003) ($p = 4.6\%$) followed by *R. gryllus* (Smith 1924) ($p = 4.7\%$) at the gene fragment examined. In addition, *R. rezakhani* sp. nov. differed considerably from *R. longchuanensis* ($p = 5.5\%$), *R. shillongensis* ($p = 5.5\%$) and *R. parvulus* ($p = 6.6\%$, Table 2). The average divergence ($p$-distance) between the new species and other congeneric species ranged from 4.6\% to 9.6\% (Table 2). This level of divergence in the 16S rRNA gene is typically seen in many other frog species pairs, thereby justifying the status of *R. rezakhani* sp. nov. as a new species (Fouquet et al. 2007).

**Raorchestes rezakhani** sp. nov.

http://zoobank.org/CDDA555B-9B29-4D94-B5E6-BA560ECD8DB3

Figures 4, 5

Suggested English name: Reza Khan’s bush frog

**Type. Holotype** (Figs 4A, B, 5). JnUZool-A0419, an adult male from Lawachara National Park, Kamalgonj, Moulavibazar, Bangladesh (24°20.746ʼN, 91°47.945ʼE, ca. 59 m a.s.l., Fig. 1), collected on 26 April 2019 by Hassan Al-Razi and Marjan Maria.

**Paratypes** (Fig. 4C, D). Three specimens: adult male (JnUZool-A0319) same locality as the holotype; two adult males (JnUZool-A0519, JnUZool-A0619) from the Adampur, Rajkandhi Reserved Forest, Kamalgonj, Moulavibazar (24°14.878ʼN, 91°54.002ʼE, ca. 64 m a.s.l., Fig. 1), on 10 May 2019 by Hassan Al-Razi.

**Generic placement.** We assign this species to *Raorchestes* based on molecular characterization of the 16S rRNA gene.

**Etymology.** We take great pleasure in naming the new species as a patronym for one of the pioneers in the field of wildlife research in Bangladesh, Dr. Mohammad Ali Reza Khan.

**Diagnosis.** A species of *Raorchestes* having the following unique combination of characters: (1) relatively small size (adult males = 18.85–20.90 mm SVL); (2) head wider than long (HW/HL 1.55; range 1.53–1.56, $N = 4$); (3) dark brown, granular dorsum bearing small, horny spicules; (4) vomerine teeth absent; (5) single transparent vocal sac while calling; (6) snout projecting, sub-elliptical in ventral aspect, and subequal to or smaller than horizontal diameter of eye; (7) tympanum indistinct; (8) supratympanic fold weakly distinct; (9) finger and toe discs well developed and rounded; (FD IV 0.50–0.60, TD IV 0.56–0.65 mm); (10) both inner and outer metacarpal and metatarsal tubercles absent; (11) nostril is closer to tip of snout than to eye (NS 0.63–0.90, EN 1.10–1.25 mm); (12) Tongue without papilla (13) venter pale white, with minute dark gray flecks present in the vocal sac region. Details of these measurements are provided in Table 3.
Table 2. Intraspecific and interspecific genetic divergence. Uncorrected p-distances between 16S rRNA sequences of closely related 20 species of *Raorchestes*.

| Species name            | 1  | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  |
|-------------------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1. *R. rezakhani* sp. nov. |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2. *R. longchuanensis*    | 0.055 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3. *R. shillongensis*     | 0.055 | 0.072 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4. *R. ghatei*            | 0.065 | 0.085 | 0.061 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5. *R. gryllus*           | 0.047 | 0.067 | 0.054 | 0.058 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6. *R. menglaensis*       | 0.059 | 0.066 | 0.054 | 0.065 | 0.065 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 7. *R. bombayensis*       | 0.061 | 0.074 | 0.044 | 0.071 | 0.056 | 0.063 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 8. *R. tuberohumerus*     | 0.046 | 0.058 | 0.037 | 0.063 | 0.053 | 0.051 | 0.019 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 9. *R. sanctisilvaticus*  | 0.061 | 0.067 | 0.047 | 0.070 | 0.058 | 0.062 | 0.014 | 0.014 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 10. *R. ponmudi*          | 0.074 | 0.077 | 0.067 | 0.096 | 0.087 | 0.086 | 0.084 | 0.076 | 0.084 |     |     |     |     |     |     |     |     |     |     |     |     |
| 11. *R. beddomii*         | 0.072 | 0.082 | 0.07 | 0.095 | 0.084 | 0.087 | 0.081 | 0.07 | 0.082 | 0.061 |     |     |     |     |     |     |     |     |     |     |     |
| 12. *R. indigo*           | 0.054 | 0.063 | 0.039 | 0.069 | 0.059 | 0.058 | 0.055 | 0.053 | 0.054 | 0.064 |     |     |     |     |     |     |     |     |     |     |     |
| 13. *R. parvulus*         | 0.066 | 0.065 | 0.06 | 0.072 | 0.058 | 0.047 | 0.044 | 0.045 | 0.042 | 0.083 | 0.088 | 0.061 |     |     |     |     |     |     |     |     |     |
| 14. *R. theuerkaufi*      | 0.078 | 0.088 | 0.078 | 0.093 | 0.088 | 0.088 | 0.077 | 0.064 | 0.075 | 0.067 | 0.021 | 0.062 | 0.082 |     |     |     |     |     |     |     |     |     |
| 15. *R. signatus*         | 0.082 | 0.097 | 0.073 | 0.088 | 0.087 | 0.076 | 0.078 | 0.074 | 0.078 | 0.072 | 0.060 | 0.043 | 0.081 | 0.063 |     |     |     |     |     |     |     |
| 16. *R. tinniens*         | 0.084 | 0.105 | 0.075 | 0.106 | 0.099 | 0.088 | 0.078 | 0.072 | 0.08 | 0.082 | 0.071 | 0.048 | 0.097 | 0.077 | 0.028 |     |     |     |     |     |     |
| 17. *R. marki*            | 0.069 | 0.069 | 0.067 | 0.077 | 0.071 | 0.066 | 0.067 | 0.061 | 0.062 | 0.064 | 0.066 | 0.047 | 0.059 | 0.064 | 0.051 | 0.056 |     |     |     |     |
| 18. *R. chromasynchysi*   | 0.082 | 0.084 | 0.064 | 0.078 | 0.085 | 0.068 | 0.08 | 0.074 | 0.083 | 0.073 | 0.060 | 0.055 | 0.085 | 0.064 | 0.044 | 0.050 | 0.051 |     |     |     |
| 19. *R. charius*          | 0.096 | 0.105 | 0.071 | 0.096 | 0.091 | 0.08 | 0.084 | 0.070 | 0.087 | 0.085 | 0.063 | 0.064 | 0.091 | 0.073 | 0.078 | 0.080 | 0.058 | 0.075 |     |
| 20. *R. primarrumfi*      | 0.073 | 0.085 | 0.059 | 0.084 | 0.073 | 0.076 | 0.063 | 0.070 | 0.066 | 0.058 | 0.049 | 0.046 | 0.077 | 0.054 | 0.021 | 0.012 | 0.054 | 0.038 | 0.058 |     |
| 21. *R. chalazodes*       | 0.081 | 0.091 | 0.085 | 0.085 | 0.085 | 0.079 | 0.079 | 0.076 | 0.079 | 0.087 | 0.058 | 0.065 | 0.085 | 0.058 | 0.044 | 0.057 | 0.057 | 0.052 | 0.077 | 0.049 |
**Table 3.** Morphological measurements (in mm) of the four specimens of *Raorchestes rezakhani* sp. nov.

| Characters                  | Abbreviation | Holotype       | Paratype       | Mean ± SD        |
|-----------------------------|--------------|----------------|----------------|------------------|
| 1 Snout–vent length         | SVL          | 20.30          | 20.90          | 20.06 ± 0.87     |
| 2 Head length               | HL           | 4.50           | 4.60           | 4.54 ± 0.05      |
| 3 Head width                | HW           | 7.00           | 7.05           | 7.03 ± 0.06      |
| 4 Eye diameter              | ED           | 2.45           | 2.70           | 2.58 ± 0.12      |
| 5 Tympanum diameter         | TD           | 1.10           | 1.22           | 1.17 ± 0.05      |
| 6 Eye–nostril distance      | EN           | 1.25           | 1.20           | 1.19 ± 0.06      |
| 7 Snout length              | SL           | 2.24           | 2.25           | 2.24 ± 0.01      |
| 8 Nostril-Snout distance    | NS           | 0.80           | 0.90           | 0.80 ± 0.12      |
| 9 Interorbital distance     | IOD          | 2.20           | 2.40           | 2.26 ± 0.09      |
| 10 Internarial distance     | IND          | 1.70           | 1.65           | 1.66 ± 0.05      |
| 11 Upper eyelid width       | UEW          | 1.45           | 1.50           | 1.48 ± 0.06      |
| 12 Thigh length             | TL           | 10.03          | 9.20           | 9.83 ± 0.42      |
| 13 Shank length             | ShL          | 10.10          | 10.10          | 10.58 ± 0.88     |
| 14 Foot length              | FOL          | 7.95           | 6.60           | 7.59 ± 0.66      |
| 15 Hand length              | HAL          | 4.90           | 4.35           | 4.78 ± 0.28      |
| 16 Fore limb length         | FLL          | 4.70           | 4.70           | 4.85 ± 0.17      |
| 17 Finger I disk width      | FD I         | 0.25           | 0.20           | 0.21 ± 0.02      |
| 18 Finger II disk width     | FD II        | 0.45           | 0.40           | 0.41 ± 0.03      |
| 19 Finger III disk width    | FD III       | 0.75           | 0.70           | 0.73 ± 0.03      |
| 20 Finger IV disk width     | FD IV        | 0.50           | 0.50           | 0.53 ± 0.05      |
| 21 Finger I length          | FL I         | 1.20           | 1.05           | 1.14 ± 0.07      |
| 22 Finger II length         | FL II        | 1.75           | 1.80           | 1.76 ± 0.05      |
| 23 Finger III length        | FL III       | 3.40           | 3.05           | 3.39 ± 0.24      |
| 24 Finger IV length         | FL IV        | 2.15           | 1.95           | 2.14 ± 0.13      |
| 25 Toe I length             | TL I         | 1.15           | 1.00           | 1.08 ± 0.09      |
| 26 Toe II length            | TL II        | 2.10           | 1.90           | 1.99 ± 0.10      |
| 27 Toe III length           | TL III       | 3.20           | 2.90           | 3.05 ± 0.13      |
| 28 Toe IV length            | TL IV        | 4.25           | 4.00           | 4.16 ± 0.14      |
| 29 Toe V length             | TL V         | 3.05           | 2.95           | 3.05 ± 0.08      |
| 30 Toe I disk width         | TD I         | 0.30           | 0.20           | 0.26 ± 0.05      |
| 31 Toe II disk width        | TD II        | 0.35           | 0.25           | 0.31 ± 0.05      |
| 32 Toe III disk width       | TD III       | 0.50           | 0.40           | 0.46 ± 0.05      |
| 33 Toe IV disk width        | TD IV        | 0.65           | 0.56           | 0.60 ± 0.04      |
| 34 Toe V disk width         | TD V         | 0.60           | 0.45           | 0.51 ± 0.06      |

**Description of holotype.** A small frog (SVL = 20.30, Fig. 5, Table 3, all measurements in mm); head wider than long (HW = 7.0; HL = 4.5); snout sub-elliptical in ventral aspect, shorter than eye diameter (ED = 2.45; SL = 2.24). Canthus rostralis sharply rounded; loreal region slightly concave. Interorbital region flat and larger (IOD = 2.20) than the upper eyelid (UEW = 1.45 mm) or internarial distance (IND = 1.70). Nostrils oval (dorsally compressed), without flap, directed laterally, closer to tip of snout than to eye (NS = 0.80; EN = 1.20). Tympanum indistinct, oval (TD = 1.10), close to eye, supratympanic fold weakly distinct, extends from eye to the end of the tympanum. Vocal sac single, sub-gular, translucent. Tongue bifid, lingual papilla absent. Eyes relatively large (ED = 2.45), protruding; pupil horizontal.
Forelimb length shorter than hand length (FLL = 4.70; HAL = 4.90). Relative lengths of fingers I < II < IV < III (FL I = 1.20; FL II = 1.75; FL III = 3.40; FL IV = 2.15). Fingertips with well-developed discs (FD I = 0.25, FD II = 0.45, FD III = 1.1, FD IV = 1.2) bearing circum-marginal grooves. Dermal fringe absent on fingers. Webbing between fingers absent. Subarticular tubercles weak, number of subarticular tubercles in fingers: I = 1, II = 1, III = 1, IV = 1, rounded. Supernumerary tubercles indistinct. Nuptial pad absent.

Hind limbs long, shank shorter than thigh (ShL = 10.03; TL = 10.10), longer than foot (FOL = 7.95). Relative toe length I < II < V < III < IV (ToL I = 1.15; ToL II = 2.10, ToL III = 3.20; ToL IV = 4.25; ToL V = 3.05). Toes with well-developed discs (TD I = 0.30, TD II = 0.35, TD III = 0.50, TD IV = 0.65, TD V = 0.60). Webbing moderate, webbing formula (fingers: I2-2•II1¾-2•III1½-3IV2¾-2•V) (Fig. 5D, E). Inner and outer metatarsal tubercles absent, subarticular tubercle present (toe: I = 1, II = 1, III = 2, IV = 3, V = 2). Supernumerary tubercles absent.

In preservative, dorsum dark gray; loreal and tympanic regions lighter; forelimbs and hind limbs with black bands. Venter uniform cream white, vocal sac with dark gray flecks. Webbing cream; ventral side of feet and hands light gray with small black spots.

In life, dorsum grayish brown with dark brown specks; “)-(" or ")(“ shaped blackish mark present on the mid dorsum; blackish line between upper eyelids; snout much darker, loreal and tympanic region blackish; iris dark golden brown. Dorsal side of hind limbs with several black bands; forelimbs with single band these bands are also present in the other members of this genus. Fingers and toes discs reddish or whitish. Abdomen brownish, with few black spots. Vocal sac translucent whitish, with a few black flecks. A few dark spots present near fore limbs. Foot webbing grayish.

**Variation.** Because all specimens were males, sexual dimorphism could not be determined. Details of morphometric variation observed in four individuals are provided in Table 3. All of the specimens are almost similar except the size and the coloration. One of the four specimens (JnUZool-A0619) is smaller than others. For two specimens (JnUZool-A0619, JnUZool- A0519) the ventral dark gray flecks are more than others. The )(" shape is present on the dorsum of three specimens where for one specimen (JnUZool- A0519) it is shaped ")-(". Some individuals have a greater proportion of dark gray spots on the ventral surface. Detailed comparisons between *R. rezakhani* sp. nov. and other species of *Raorchestes* are provided below.

**Bioacoustics analyses.** An advertisement call of the paratype (JnUZool-A0519) from the Lawachara National Park were recorded at an ambient air temperature of 27.8 °C, 97% relative humidity. Advertisement calls occurred without call groups (Fig. 3). The duration of the analyzed call was 16 s. The number of notes within this call was 25, and number of pulses within a note varied from 5–11 (8.84 ± 1.70 SD). Note duration was 0.183 – 0.379 s. The interval between notes was 0.222 – 0.592 s (0.323 ± 0.098 SD, N = 24). These intervals increase gradually within a call (mean interval for first five notes = 0.2422, mid five notes = 0.2784, last five notes = 0.4754). Pulse duration was 0.003–0.029 s (0.013 ± 0.007 SD, N = 205 pulses), duration of intervals between pulses was 0.005–0.127 s (0.027 ± 0.017 SD, N = 179 intervals).
A new species of cryptic Bush frog...

Figure 3. Advertisement call of *Raorchestes rezakhani* sp. nov. showing 25 notes that vary in amplitude. **A** waveform of 25 notes **B** shows variation in frequency **C** shows waveform of first six notes of the call; and **D** shows a spectrogram of the six notes **E** shows a pulse of fourth note and **F** shows the spectrogram of pulse of fourth note.

Pulse rate was 10–19/s (14.27 ± 2.49 SD, *N* = 15 seconds interval). The advertisement call had a dominant frequency at 4.32–4.77 kHz (4.55 ± 0.12 SD, *N* = 25). To the human ear, the calls sounded similar to cricket calls.

**Distribution and natural history.** *Raorchestes rezakhani* sp. nov. was recorded from the semi-evergreen forests of northeastern Bangladesh. They were active with the onset of the rainy season in the month of April. We did not hear calls of this species after August. Frogs were found inside the primary and secondary forest mainly on the edge of streams and near man-made trails. They often use the hilly slopes during calling. Individuals perch on leaves and branches of small trees and on bamboo trunks (with diameters of 1.5–4 cm). Vocalizing individuals were perched 1–1.5 m above the forest floor. We usually heard the calls immediately after the sunset (ca. 1815 h in April) although calling activity started a little earlier when it was raining.

**Comparisons.** Based on morphology, we compared *Raorchestes rezakhani* sp. nov. with some other member of this genus. This new species is differs from *R. amboli* (Biju & Bossuyt, 2009), *R. anili* (Biju & Bossuyt, 2006), *R. charius* (Rao, 1937),
R. chlorosomma (Biju & Bossuyt, 2009), R. flaviventris (Boulenger, 1882), R. glandulosus (Jerdon, 1853), R. jayarami (Biju & Bossuyt, 2009), R. kaikatti (Biju & Bossuyt, 2009), R. luteolus (Kuramoto & Joshy, 2003), R. munnarenis (Biju & Bossuyt, 2009), R. nero stagna (Biju & Bossuyt, 2005), R. ochlandrae (Gururaj et al., 2007), R. ponmudi (Biju & Bossuyt, 2005), R. signatus (Boulenger, 1882), R. susbili (Biju & Bossuyt, 2009), R. wynaadensis (Jerdon, 1853), R. kakachi Seshadri et al., 2012, R. crustai Zachariah et al., 2011, R. johnceei Zachariah et al., 2011, R. theuerkaufi Zachariah et al., 2011, R. thodai Zachariah et al., 2011, R. gryllus (Smith 1924) by its smaller size. SVL of male individuals of these species ranged from 24.9–36.8 mm whereas Raorchestes rezakhani sp. nov. is 20.06 mm. Raorchestes rezakhani sp. nov. is quite similar to R. longchuanensis Yang et al. 1979 but differs for the following characters:

Figure 4. Color variation in R. rezakhani sp. nov. A holotype, showing single transparent vocal sac during advertisement call (B holotype with brown dorsum and “)- (“ mark; C dorsolateral view of paratype (JnUZool- A0519) D ventral view of paratype (JnUZool- A0519), showing small dark brown spots.
A new species of cryptic Bush frog...

Raorchestes rezakhani sp. nov. differs from R. tuberohumerus in: snout sub-elliptical (vs. slightly pointed); relative lengths of fingers I < II < IV < III (vs. I < IV < II < III); thigh shorter than the tibia/shank, TL/ShL = 93% (vs. thigh longer than the tibia/shank, ShL/TL = 96%); inner metatarsal tubercles absent (vs. present); supernumerary tubercles feebly distinct (vs. distinct) [Kuramoto and Joshy 2003; Padhye et al. 2015]. Raorchestes rezakhani sp. nov. differs from R. gryllus in: snout sub-elliptical (vs. pointed); tympanum indistinct in males (vs. large and rounded); relative toe lengths I < II < V < III < IV (vs. I < II < III < V < IV); subarticular tubercles

Figure 5. Holotype of R. rezakhani sp. nov. A dorsal view B ventral view C ventral view of right hand D ventral view of right foot E web pattern in foot.
in finger weakly distinct I = 1, II = 1, III = 1, IV = 1 (vs. distinct I = 1, II = 1, III = 2, IV = 1) [Smith 1924; Orlov et al. 2012]. Raorchestes rezakhani sp. nov. is also similar to R. shillongensis (Pillai & Chanda, 1973) but differs in: SVL of male 20.06 ± 0.87 (vs. 16.51 ± 1.29); head wider than long, HL/HW = 61% (vs. length slightly greater than the width, HW/HL = 98%); snout length shorter than the eye diameter (vs. slightly longer than eye diameter); subarticular tubercles in finger weakly distinct, I = 1, II = 1, III = 1, IV = 1 (vs. distinct, I = 1, II = 1, III = 2, IV = 1) [Pillai and Chanda 1973; Boruah et al. 2018]. Raorchestes rezakhani sp. nov. is very similar to R. parvulus but differs in: forearm and hand length (9.05–9.95 mm) generally shorter than half body size (vs. longer than the half body size); relative toe length I < II < V < III < IV (vs. I < II < III < V < IV); toe subarticular tubercle: I = 1, II = 1, III = 2, IV = 2 (vs. I = 1, II = 1, III = 2, IV = 2, V = 1); inner metatarsal tubercles absent (vs. present) [Boulenger 1893; Yu et al. 2019]. Raorchestes rezakhani sp. nov. differs from R. sahai (Sarkar & Ray, 2006) in: smaller SVL (18.85–20.90 vs. 25–26 mm); nostril closer to tip of snout than to eye, NS/EN = 67% (vs. equidistance from the tip of the snout and the eye NS/EN = 100%); snout length shorter than the eye diameter, SL/ED = 87% (vs. slightly longer than eye diameter, ED/SL = 81%); interorbital distance larger than the upper eyelid UEW/IOD= 65% (vs. equal to the upper eyelid, UEW/IOD = 100%) [Sarkar and Ray 2006]. Raorchestes rezakhani sp. nov. differs from R. annandalii in: snout sub-elliptical (vs. pointed); nostril closer to tip of snout than to eye, NS/EN = 67% (vs. equidistant from the tip of the snout and the eye, NS/EN = 100%); inner metatarsal tubercles absent (vs. feebly distinct); ShL longer than TL, TL/ShL = 93% (vs. ShL shorter than TL) [Boulenger 1906; Chanda 1994]. Raorchestes rezakhani sp. nov. differs from R. menglaensis (Kou 1990) in: male with external single subgular vocal sac (vs. internal single subgular vocal sac); outer metatarsal tubercle absent (vs. present); [Padhye et al. 2013; Kou 1990]. This new species differs from R. garo (Boulenger 1919) in: SVL 18.85–20.90 (vs. 13–16 mm); eye diameter larger than the interorbital distance, IOD/ED = 88% (vs. less than interorbital distance, ED/IOD = 92%); dark line present between eyelids (vs. absent); nostril closer to tip of snout than to eye, NS/EN = 67% (vs. equidistance from the tip of the snout and the eye or slightly closer to the tip of snout); tympanum indistinct (vs. distinct); inner metatarsal tubercles absent (vs. present) [Boulenger 1919; Chanda 2002]. Raorchestes rezakhani sp. nov. differs from R. kempeia (Boulenger 1919) in: SVL 18.85–20.90 (vs. 13–17.5 mm); nostril closer to tip of snout than to eye (vs. equidistant from the tip of the snout and the eye); tympanic fold indistinct (vs. distinct) [Boulenger 1919, Chanda 1994, 2002].

Principle Components Analysis showed that the specimens of R. rezakhani sp. nov. did not overlap with R. longchuanensis, R. tuberohumerus, or R. gryllus (Fig. 6). Eigenvalues indicated that PC1 accounted for more than 91% of the variation in the data while PC2 contributed another 5% (Table 4). Thus, the inclusion of further principle components would not add substantially to the characterization of these species based on these variables. Loading of individual morphological variables indicated that SVL, HL, HW, THL and TL strongly influenced PC1, ED, SL, UEW and THL strongly influenced PC2, while HL and TL strongly influenced PC3, that helped to segregate the R. rezakhani sp. nov. from the remaining three species (Table 5).
A new species of cryptic Bush frog...

**Figure 6.** A Scatterplot of principle component axes 1 and 2 and B principle component axes 2 and 3. *R. tuberohumerus* (light blue), *R. gryllus* (green), *R. longchuanensis* (black), and *R. rezakhani* sp. nov. (purple).

**Table 4.** Eigen analysis showing relative contributions of each Principle Component towards the characterization of each species.

| Principle component | Eigen value | % variance |
|---------------------|-------------|------------|
| 1                   | 26.07       | 91.15      |
| 2                   | 1.57        | 5.50       |
| 3                   | 0.47        | 1.66       |
| 4                   | 0.28        | 0.98       |
| 5                   | 0.07        | 0.25       |
| 6                   | 0.06        | 0.21       |
| 7                   | 0.04        | 0.15       |
| 8                   | 0.01        | 0.04       |
| 9                   | 0.0064      | 0.02       |
| 10                  | 0.0043      | 0.01       |
| 11                  | 0.0019      | 0.006      |

**Table 5.** Loading plot showing individual loadings of each measured variable in *Raorchestes tuberohumerus*, *R. gryllus*, *R. longchuanensis*, and *R. rezakhani* sp. nov. against four principle components.

| Variable | PC 1  | PC 2  | PC 3  | PC 4  |
|----------|-------|-------|-------|-------|
| SVL      | 0.60  | -0.24 | -0.25 | -0.69 |
| HL       | 0.40  | 0.40  | 0.47  | -0.07 |
| HW       | 0.31  | 0.04  | 0.12  | 0.10  |
| ED       | 0.23  | 0.39  | -0.36 | 0.21  |
| EN       | 0.09  | -0.03 | 0.23  | 0.03  |
| SL       | 0.18  | 0.27  | 0.11  | 0.19  |
|IOD      | 0.15  | 0.06  | 0.24  | 0.01  |
|IND      | 0.04  | -0.05 | 0.32  | 0.00  |
|UEW      | 0.18  | 0.50  | -0.26 | 0.14  |
|THL      | 0.32  | -0.43 | 0.33  | 0.41  |
|TL       | 0.35  | -0.34 | -0.41 | 0.49  |
Figure 7. Plot showing individual loadings of each morphometric variable in relation to PC1, which accounted for over 91% of the variation in the data.

Discussion

Our discovery of a new species of *Raorchestes* is not unexpected (Reza 2014; Khan 2015; IUCN 2015). Our recent report of *R. longchuanensis* from northeastern Bangladesh (Al-Razi et al. 2020) supports the suggestion of the authors of the species, who stated that it was very likely to occur outside of Longchuan (the type locality) as well as nearby provinces in southern China (Yang et al. 2004). We suggested that the broad similarities between southern China, northern Myanmar, several northeastern states of India, and northeastern Bangladesh with their relative proximity to each other would suggest that many species may occur across this region (Al-Razi et al. 2020). Slik et al. (2018) recently classified the world’s forest types using phylogenetic similarities into five floristic regions. Two of these five regions, namely the Indo-Pacific and the Subtropical floristic regions, are of interest. The Indo-Pacific region spans across the Indian subcontinent and through Myanmar into the rest of Southeast Asia. In addition, the Subtropical floristic region spans from northeastern India, northern Myanmar, through southern China (where it has significant overlaps with the Indo-Pacific floristic region) further into eastern China (Slik et al. 2018). The Indian subcontinental fauna differs considerably compared to the Southeast Asian fauna, making the entire region of great interest to diversification of biota.
The Western Ghats region of India is a global biodiversity hotspot (Gadgil 1996). The region has undergone biodiversity loss along with changes in land use that has contributed towards the creation of geographic barriers within the last few decades (Gadgil 1996). The diversification of frogs in the Western Ghats has generally been attributed to long-term ecological change over extended geological time scales (Priti et al. 2016). Due to its status as a biodiversity hotspot, considerable research attention has been placed on this region, resulting in more discoveries in the anuran fauna. On the other hand, the taxonomic challenges as well as the lack of funding for dedicated studies examining species diversity in Bangladesh could have precluded the detection of cryptic species until recently (Reza 2014; Khan 2015; IUCN 2015; Frost 2020). This is also true for the northeastern regions of India, where relatively few studies have been done on cryptic anurans (Ao et al. 2003; Vijayakumar et al. 2016; Boruah et al. 2018). Myanmar has only recently been opened up to biological exploration and we anticipate that more species will be found from this region. Renewed interest, especially with respect to anuran biodiversity and the relative availability and cost-effectiveness of molecular tools, have made it easier to target cryptic species for identification. We anticipate that further extensive surveys followed by molecular characterization, and bioacoustics data could aid in discovering additional species and delineating their occurrence in the region (Vijaykumar et al. 2014; Priti et al. 2016).

Northeastern India, particularly Meghalaya and parts of Assam, are separated by the river Brahmaputra, that effectively creates differences in forest type (Champion and Seth 1968). Areas south of the river have more subtropical influence, compared to areas north of the river, which are climatically affected by the Himalayas and its foothills, due to variation in local climatic patterns (Champion and Seth 1968). Thus, there are forested areas in Meghalaya, Assam, Tripura and Mizoram states of India and Bangladesh with variation in niche types affected by local climates that could have encouraged diversification of *Raorchestes* or other forest-dwelling genera (Ahmed et al. 2009; Vijayakumar et al. 2016).

The similarities between *R. rezakhani* sp. nov., *R. tuberohumerus*, and *R. gryllus* could offer some insight into the diversification of the *Raorchestes* in the region. *Raorchestes tuberohumerus* is distributed in the Western Ghats while *R. gryllus* is limited in distribution to central Vietnam and Laos (Frost 2020). *Raorchestes shillongensis*, restricted to a small part of Megalaya state of India, is mostly closely related to either *R. tuberohumerus* ($p = 3.7\%$) and *R. indigo* ($p = 3.9\%$), a species also found in the Western Ghats (Frost 2020). Thus, we suggest that the *Raorchestes* species in northeastern India and surrounding regions may have separated from Western Ghats species giving rise to *R. shillongensis* and *R. rezakhani* sp. nov. relatively recently (Vijayakumar et al. 2016). Ancestors of *Raorchestes parvulus* may have diverged from Western Ghats stock even earlier (difference compared to *R. bombayensis* was 4.4%) and from Eastern Ghats and Deccan plateau species (difference compared to *R. sanctisilvaticus* was 4.2%). Our analysis indicates that *R. longchuanensis* is quite distinct from *R. parvulus* ($p = 6.5\%$). *Raorchestes rezakhani* sp. nov. is also significantly different both morphologically and genetically from *R. parvulus*, providing support of the idea that *R. parvulus* is part of a Southeast Asian species
complex. The status of *Raorchestes annandalii* is not clear since there are no sequences of 16S rRNA genes for this species in GenBank. They are morphologically distinct from *R. rezakhani* sp. nov. and we speculate that they could be part of a species complex associated with northeast India and northern Myanmar, and may include species such as *R. shillongensis*, *R. longchuanensis*, and *R. rezakhani* sp. nov. as closely related congeners. Further genetic analyses could clarify their status in relation to the evolution and biogeography of *Raorchestes* in the region. We speculate that *R. rezakhani* sp. nov. may be found in other adjoining areas including the northeastern states of India and northern Myanmar due to close affiliations of the habitat types in this floristic region.

It is also important to note that Bangladesh retains some forest patches that are of high value to biodiversity. The two areas in the northeast, Lawachara National Park and Adampur reserve forest contain high bird and mammal diversity. Six of the ten species of primates in Bangladesh occur there in numbers higher than elsewhere in the country (Al-Razi et al. 2019; Al-Razi and Maria 2019). Lawachara is legally protected whereas Adampur is under the management of the Forest Department of the Ministry of Environment and Forests of Bangladesh, but not under formal protected areas status. Illegal logging, fuel wood collection, and hunting occurs in these areas (Muzaffar et al. 2011; Islam et al. 2013). Although a signatory to the Convention on Biological Diversity, all forested and other wilderness areas suffer from poor implementation of the principles of ecosystem management (Muzaffar et al. 2011). Lawachara has a total area of about 12 km\(^2\) and Adampur has an area of about 71.9 km\(^2\), making both of them relatively small patches. Despite all odds, our finding of new species and previous studies on primates suggest that viable populations of varied species persist in these areas (Muzaffar et al. 2007, 2011; Al-Razi et al. 2020). Thus, efforts must be made to protect these remaining forest patches, which may still retain undiscovered new species, as documented in this study.

**Ethics statement**

Fieldwork and sampling were carried out in Adampur Reserve Forest and Lawachara National Park, with permission from Forest Department Bangladesh (Permit no. 22.01.0000.101.23.2019.2940). Individuals were euthanized and muscle tissue was collected in strict accordance with protocols approved by the Forest Department solely for scientific research. The sampling is unlikely to affect population size of the species since the bare minimum of specimens were collected.

**Acknowledgments**

We thank the Forest Department for providing the necessary permission and for approving our protocol. We thank Ranjit Vijayan, Department of Biology, United Arab Emirates University, for providing helpful comments on an earlier version of the manuscript. The authors are also thankful to Amaël Borzée, College of Biology and the Environment, Nanjing Forestry University for his assistance with the phylogenetic analy-
A new species of cryptic Bush frog... 147

ses. The authors are grateful to Professor Dr. M. Saiful Islam, Chairman, Department of Zoology, Jagannath University, for his support to keep the specimens in Department of Zoology, Jagannath University. We are obliged to INVENT TECHNOLOGY for permission to use their laboratory. We are especially appreciative of Habibun Nobi Al Hasib (Joy), Faiyaz Hassan, and Jafar Bhai for their support during field work. Lastly, we are thankful to Tanvir Ahmed who provided materials for the map.

References

Ahmed MF, Das A, Dutta SK (2009) Amphibians and Reptiles of Northeast India, A Photographic Guide. Aranyak, Guwahati, India, 170 pp.

Al-Razi H, Maria M, Hasan S, Muzaffar SB (2020) First record of *Raorchestes longchuanensis* Yang & Li, 1978 (Anura: Rhacophoridae) from northeastern Bangladesh suggests wide habitat tolerance. Amphibian & Reptile Conservation 14(1): 119–131.

Al-Razi H, Maria M, Hasan S, Muzaffar SB (2019) Mortality of primates due to roads and power lines in two forest patches in Bangladesh. Zoologia 36: 1–6 https://doi.org/10.3897/zoologia.36.e33540

Al-Razi H, Maria M (2019) Population status of Particolored Flying Squirrel (*Hylopetes Alboginger*) in four forest patches of Northeast Bangladesh. NeBIO 10(2): 77–79.

Ao JM, Bordoloi S, Ohler A (2003) Amphibian fauna of Nagaland with nineteen new records from the state including five new records for India. Zoos’ Print Journal 18(6): 1117–1125.

Bordoloi S, Ohler A, Shrestha TK (2004) *Raorchestes annandali*. The IUCN Red List of Threatened Species 2004: e.T58816A11844262. https://doi.org/10.2305/IUCN.UK.2004.RLTS.T58816A11844262.en [downloaded 12 February 2020]

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

Biju SD, Bossuyt F (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 1848: 374–444. https://doi.org/10.1111/j.1096-3642.2008.00466.x

Biju SD, Senevirathne G, Garg S, Mahony S, Kamei RG, Thomas A, Shouche Y, Raxworthy CJ, Meegaskumbura M, Van Bocxlaer I (2016) *Frankixalus*, a new rhacophorid genus of tree hole breeding frogs with oophagous tadpoles. PLoS ONE 11 (1): e0145727. https://doi.org/10.1371/journal.pone.0145727

Bioacoustics Research Program (2011) Raven Pro: Interactive Sound Analysis Software Version 1.5. URL: http://www.birds.cornell.edu/raven [accessed 18 July 2019]

Boruah B, Raj P, Dutta SK, Das A (2018) Redescription and geographic distribution of *Raorchestes shillongensis* (Anura: Rhacophoridae) from Meghalaya, Northeast India. Phylomedusa 17: 3–20. https://doi.org/10.11606/issn.2316-9079.v17i1p3-20

Bossuyt F, Meegaskumbura M, Beenerts N, Gower DJ, Pethiyagoda R, Roelants K et al. (2004) Local endemism within the Western Ghats-Sri Lanka biodiversity hotspot. Science 306: 479–481. https://doi.org/10.1126/science.1100167
Boulenger GA (1906) Description of two new Indian frogs. Journal of the Asiatic Society of Bengal. 2(2): 385–386.

Boulenger GA (1919) Descriptions of three new batrachians from the Garo Hills, Assam. Records of the Indian Museum 16: 207–208. https://doi.org/10.5962/bhl.part.25921

Boulenger GA (1893) Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea dealing with the collection made in Pegu and the Karin Hills in 1887–88. Annali del Museo Civico di Storia Naturale di Genova 2(13): 304–347.

Champion HG, Seth SK (1968) A Revised Survey of the Forest Types of India. Government of India Press, New Delhi, India, 27 + 404 pp.

Chanda SK (2002) Handbook, Indian amphibians. Zoological Survey of India, Calcutta, 335 pp.

Chanda SK (1994) Anura (Amphibia) of northeastern India. Memoirs of the Zoological Survey of India 19: 1–143.

Charif RA, Waack AM, Strickman LM (2010) Raven Pro 1.4 User's Manual. Ithaca. Cornell Laboratory of Ornithology, 379 pp.

Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. PLoS ONE 2(10): e1109. https://doi.org/10.1371/journal.pone.0001109

Frost DR (2020) Amphibian species of the World 6.0, an online reference. New York, USA: American Museum of Natural History. http://research.amnh.org/herpetology/amphibia/index.html [last accessed 10 November 2019].

Gadgil M (1996) Western Ghats: a lifeescape. Journal of Indian Institute of Sciences 76: 495–504.

Islam MA, Uddin M, Aziz MA, Muzaffar SB, Chakma S, Chowdhury SU, Chowdhury GW, Rashid MA, Mohsanin S, Jahan I, Saif S, Hossain MB, Chakma D, Kamruzzaman M, Akter R (2013) Status of Bear in Bangladesh: Going, Going, Gone? Ursus 24 (1): 83–90. https://doi.org/10.2192/URSUS-D-12-00010.1

IUCN Bangladesh (2015) Red List of Bangladesh Volume 4: Reptiles and Amphibians. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, 16 + 320 pp.

IUCN SSC Amphibian Specialist Group (2016) Raorchestes parvulus. The IUCN Red List of Threatened Species 2016: e.T58886A55068080. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T58886A55068080.en [downloaded on 17 November 2019]

Khan MH (2018) Photographic Guide to the Wildlife of Bangladesh. Arannayk Foundation, Dhaka, 488 pp.

Khan MAR (2015) Wildlife of Bangladesh: Checklist-cum-guide. Chayabithi, Dhaka, Bangladesh, 568 pp.

Kou Z-T (1990) A new species of genus Philautus (Amphibia: Rhacophoridae) from Yunnan, China. In: Zhao E-M (Ed.) From Water onto Land, China Forestry Press, Beijing, 210–212.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054
A new species of cryptic Bush frog...

Kuramoto M, Joshy SH (2003) Two new species of the genus Philautus (Anura: Rhacophoridae) from the Western Ghats, southwestern India. Current Herpetology 22: 51–60. https://doi.org/10.5358/hsj.22.51

Li JT, Che J, Murphy RW, Zhao H, Zhao EM, Rao DQ, Zhang YP (2009) New insights to the molecular phylogenetics and generic assessment in the Rhacophoridae (Amphibia: Anura) based on five nuclear and three mitochondrial genes, with comments on the evolution of reproduction. Molecular Phylogenetics and Evolution 53: 509–522. https://doi.org/10.1016/j.ympev.2009.06.023

McGarigal K, Cushman SA, Stafford S (2000) Multivariate Statistics for Wildlife and Ecology Research, Springer New York, 233 pp. https://doi.org/10.1007/978-1-4612-1288-1_6

Meegaskumbura M, Bossuyt F, Pethiyagoda R, Manamendra-Arachchi K, Bahir M, Milinkovitch MC, Schneider CJ (2002) Sri Lanka: an amphibian hot spot. Science 298: 379–379. https://doi.org/10.1126/science.298.5592.379

Muzaffar SB, Islam MA, Kabir DS, Khan MH, Ahmed FU, Chowdhury GW, Aziz MA, Chakma S, Jahan I (2011) The endangered forests of Bangladesh: why the process of implementation of the Convention on Biological Diversity is not working. Biodiversity and Conservation 20(7): 1587–1601. https://doi.org/10.1007/s10531-011-0048-6

Orlov NL, Poyarkov AN, Vassilieva AB, Ananjeva NB, Nguyen TT, Sang NV, Geissler P (2012) Taxonomic notes on rhacophorid frogs (Rhacophoridae: Rhacophoridae: Anura) of southern part of Annamite Mountains (Truong Son, Vietnam), with description of three new species, Russian Journal of Herpetology 19(1): 23–64.

Padhye AD, Sayyed A, Jadhav A, Dahanukar N (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. https://doi.org/10.11609/JoTT.o3702.4913-31

Padhye AD, Jadhav A, Sulakhe S, Dahanukar N (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. https://doi.org/10.11609/JoTT.o4192.7211-22

Palumbi SR, Martin AP, Romano SL, McMillan WO, Stice L, Grabowski G (1991) The Simple Fool’s Guide to PCR. Special Publications, Department of Zoology, University of Hawaii, Honolulu, 45 pp.

Pillai RS, Chanda SK (1973) Philautus shillongensis, a new frog (Ranidae) from Meghalaya, India. Proceedings of the Indian Academy of Sciences B 79: 30–36.

Priti H, Roshmi RS, Ramya B, Sudhira HS, Ravikanth G, Aravinda NA, Gururaja KV (2016) Integrative taxonomic approach for describing a new cryptic species of bush frog (Raorchestes: Anura: Rhacophoridae) from the Western Ghats, India. PLoS ONE 11(3): e0149382. https://doi.org/10.1371/journal.pone.0149382

Quazi SA, Ticktin T (2016) Understanding Drivers of Forest Diversity and Structure in Managed Landscapes: Secondary Forests, Plantations, and Agroforests in Bangladesh. Forest Ecology and Management 366: 118–34. https://doi.org/10.1016/j.foreco.2016.01.024

Reza AHMA (2014) Status, distribution and conservation of the Amphibians of Bangladesh. In: Heatwole H, Das I (Eds) Conservation Biology of Amphibians of Asia: Status of Con-
servation and Decline of Amphibian: Eastern Hemisphere. Natural History Publications (Borneo), Kota Kinabalu, 382 pp.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sarkar AK, Ray S (2006) Amphibia. Zoological Survey of India, Fauna of Arunachal Pradesh, State Fauna Series 13(1): 285–316.

Slik JWF, Franklin J, Arroyo-Rodriguez V et al. (2018). Phylogenetic classification of the world's tropical forests. PNAS 115(13): 1–6. https://doi.org/10.1073/pnas.1803346115

Smith MA (1924), New tree-frogs from Indo-China and the Malaya Peninsula, Proceedings of the Zoological Society of London 1924: 225–234. https://doi.org/10.1111/j.1096-3642.1924.tb01499.x

Sokal RR, Rohlf FJ (2012) Biometry: the principles and practice of statistics in biological research. 4th edition. W. H. Freeman and Co. New York, 937 pp.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Vences M, Nagy ZT, Sonet G, Verheyen E (2012) DNA barcoding Amphibians and reptiles. In: Kress WJ, Erickson DL (Eds) DNA Barcodes: Methods and Protocols, Methods in Molecular Biology, Springer Science + Business Media, LLC, 79–108. https://doi.org/10.1007/978-1-61779-591-6_5

Vijayakumar SP, Dinesh KP, Prabhu MV, Shanker K (2014) Lineage delimitation and description of nine new species of bush frogs (Anura: Raorchestes, Rhacophoridae) from the Western Ghats Escarpment. Zootaxa 3893: 451–488. https://doi.org/10.11646/zootaxa.3893.4.1 [PMID: 25544534link?]

Vijayakumar SP, Menezes RC, Jayarajan A, Shanker K (2016) Glaciations, gradients, and geography: multiple drivers of diversification of bush frogs in the Western Ghats Escarpment. Proceedings of the Royal Society B. Biological Sciences 283: 20161011. https://doi.org/10.1098/rspb.2016.1011

Wu Y-H, Suwannapoom C, Xu K, Chen J-M, Jin J-Q, Chen H-M, Murphy RW, Che J (2019) A new species of the genus Raorchestes (Anura: Rhacophoridae) from Yunnan Province, China. Zoological Research 40: 558–563. https://doi.org/10.24272/j.issn.2095-8137.2019.066

Yang D, Lu S, Chou W (2004) Raorchestes longchuanensis. The IUCN Red List of Threatened Species 2004: e.T58864A11841186. https://doi.org/10.2305/IUCN.UK.2004.RLTS.T58864A11841186.en [downloaded on 20 November 2019]

Yang D-T, Su CY, Li SM (1979) New species and new subspecies of amphibians and reptiles from Gaoligong Shan, Yunnan. Acta Zootaxonomica Sinica 02: 185–188.

Yu G, Liu S, Hou M, Li S, Yang J (2019) Extension in distribution of Raorchestes parvulus (Boulenger, 1893) (Anura: Rhacophoridae) to China. Zootaxa 4577(2): 381–391. https://doi.org/10.11646/zootaxa.4577.2.10
Yu G, Zhang M, Yang J (2013) Molecular evidence for taxonomy of *Rhacophorus appendiculatus* and *Kurixalus* species from northern Vietnam, with comments on systematics of *Kurixalus* and *Gracixalus* (Anura: Rhacophoridae). Biochemical Systematics and Ecology 47: 31–37. https://doi.org/10.1016/j.bse.2012.09.023

Yu G, Rao D, Zhang M, Yang J (2009) Re-examination of the phylogeny of Rhacophoridae (Anura) based on mitochondrial and nuclear DNA. Molecular Phylogenetics and Evolution 50: 571–579. https://doi.org/10.1016/j.ympev.2008.11.023

Zachariah A, Cyriac VP, Chandramohan B, Ansil BR, Mathew JK, Raju DV, Abraham RK (2016) Two new species of *Raorchestes* (Anura: Rhacophoridae) from the Silent Valley National Park in the Nilgiri Hills of the Western Ghats, India. Salamandra 52(2): 63–76.