

# Redescription and geographic distribution of *Raorchestes shillongensis* (Anura: Rhacophoridae) from Meghalaya, Northeast India

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## Abstract

**Redescription and geographic distribution of *Raorchestes shillongensis* (Anura: Rhacophoridae) from Meghalaya, Northeast India.** *Raorchestes shillongensis* is a threatened rhacophorid frog endemic to Northeast India. The species is poorly known and systematic information is lacking. We redescribe here the morphology of the species from topotypic material and compare with other Bush Frogs of the region. The locality records from the state of Meghalaya are new. We describe its advertisement call and discuss its phylogenetic position.

**Keywords:** advertisement call, conservation, morphology, phylogeny, Rhacophorinae.

## Resumo

**Redescrição e distribuição geográfica de *Raorchestes shillongensis* (Anura: Rhacophoridae) de Meghalaya, nordeste da Índia.** *Raorchestes shillongensis* é um anuro racoforídeo ameaçado e endêmico do nordeste da Índia. A espécie é pouco conhecida, não havendo informação sistemática. Redescrevemos aqui a morfologia da espécie a partir de material topotípico e a comparamos com outros racoforídeos da região. O registro da localidade no estado de Meghalaya é novo. Descrevemos ainda seu canto nupcial e discutimos sua posição filogenética.

**Palavras-chave:** canto nupcial, conservação, filogenia, morfologia, Rhacophorinae.

## Introduction

Sixty-two species of frogs of the genus *Raorchestes* are known from India, Nepal, Myanmar, Thailand, Laos, Southern China, Vietnam, Cambodia, and West Malaysia (Frost 2017). Four species are reported from Northeast India (Frost 2017). Pillai and Chanda (1973)

described *Philautus shillongensis* based on eight specimens collected from Malki Forest, Shillong (1,524 m a.s.l.), Meghalaya, India. Bossuyt and Dubois (2001) commented on the taxonomic identity of the species. Based on the assumptions of Biju *et al.* (2010), the species is placed in *Raorchestes* (Frost 2017) by implication. Mahony *et al.* (2013) considered the species endemic to the Shillong Plateau. Given the scanty information on distribution, ecology, reproductive behavior, and phylogeny of *Raorchestes shillongensis* (Pillai and Chanda, 1973), we redescribe the species and discuss its systematic relationships.

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## Materials and Methods

### Study Area

The study area is located in the state of Meghalaya in northeast India. The type locality of *Raorchestes shillongensis* in Malki forest (25°33'45" N, 91°53'19" E; WGS-84) lies in the Shillong City of East Khasi Hills District (Figure 1). The region receives about 9,000–11,000 mm of rain annually (Goswami *et al.* 2012). The Khasi Hills support a subtropical wet hill forest that is dominated by pine trees and considerable amount of grasslands with some scattered, broadleaf trees (Champion and Seth 1968, Mahony *et al.* 2013).

### Field Survey

We conducted our field study in May and August 2016. Nocturnal visual encounter and acoustic searches were used to locate calling aggregations (Heyer *et al.* 1994). We recorded data on habitat, microhabitat, perch height, color variation, and behavioral activities. Sex was determined by the presence/absence of a vocal sac and/or mature ova visible externally. Temperature and humidity were recorded with a hygrometer. We surveyed opportunistically to evaluate the distribution of the species, and determined geographical coordinates with a GPS. Adult frogs that were collected were fixed in formalin and preserved in 70% ethanol. Specimens are housed in the laboratory of Wildlife Institute of India (WII), Dehradun.

**Abbreviations.**—ZSI (Zoological Survey of India); V/ERS [Voucher/Eastern Regional Station, Shillong (ZSI)].

### DNA Extraction and Amplification

Total genomic DNA was extracted from liver tissues preserved in molecular grade ethanol using a DNeasy blood and tissue kit (Qiagen, Germany) following the manufacturer's protocol. Partial

gene fragments of 16S rDNA were amplified by polymerase chain reaction (PCR). Primers 5'-GCCTGTTTATCAAAAACAT-3' (16Sar-L) and 5'-CCGGTCTGAACTCAGATCACGT-3' (16Sbr-H) as forward and reverse for 16S (Palumbi *et al.* 1991), were used in the current study. Each PCR reaction was prepared in a 25 µl volume amplification mixture consisting of 2.5 µl MgCl<sub>2</sub>, 2.5 µl of Taq polymerase buffer, 2.5 µl of dNTPs mix, 0.25 µl of each primer (forward and reverse), 1 µl of purified DNA, 0.67 µl of Taq polymerase (Bangalore GeneiPvt. Ltd.) and Milli-Q water was used to make up the remaining 25 µl volume. We amplified the 16S rRNA gene sequences in steps described below.

An initial denaturing step at 94°C (4 min), 40 cycles of denaturing at 94°C (45 s), then annealing at 47.1°C (1 min) and extending at 72°C (1 min), and a final extension at 72°C (10 min). The PCR products thus generated were purified using spin columns. DNA sequences of only the forward strand were obtained using corresponding forward primers. Sequences are deposited in GenBank (accession numbers: SUB 3716157, SUB 3711598, SUB 3716694, SUB 3716575).

### Taxon Sampling and Phylogenetic Analyses

To infer phylogenetic position of the current species, homologous sequences for 16S r RNA gene for 15 species were downloaded from NCBI GenBank database of closely related clades and genera belonging to Asian Bush Frogs, from southern and southeastern Asia. The species for which sequences were downloaded represent Bush Frog clades from the work of Vijayakumar *et al.* (2016) and Rahim *et al.* (unpubl. data). *Kurixalus eiffingeri* (Boettger, 1895) (Rhacophoridae) was used as outgroup. We also used two sequences from the present study belonging to *R. shillongensis* collected from type locality (Malki Forest) and Risa Forest. And last, we added sequences from one specimen from Riwai and one specimen from Mawlynong, belonging to the genus

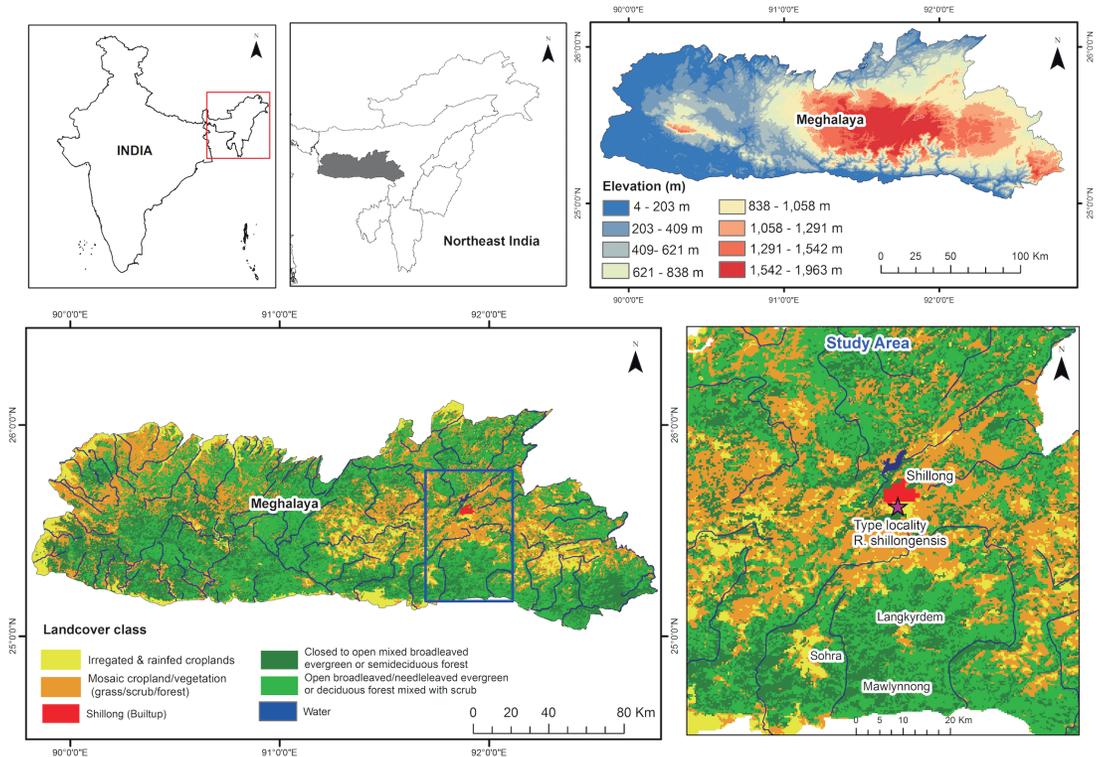


Figure 1. Map showing study area with type locality of *Raorchestes shillongensis*.

*Raorchestes* to include taxonomic representation of anurans from different locations within the distributional range of *R. shillongensis*. A total of 19 sequences was used in the current analysis.

Program Muscle (Edgar 2004) implemented in MEGA 7 was used to align our new sequences against these data manually. The alignments were checked visually and corrected manually if necessary. Alignment gaps were treated as missing data. The final alignment consisted of 486 base pairs. Accession numbers of new sequences generated in the study, as well as those that were downloaded from GenBank for phylogenetic analysis, are listed in Appendix I. The phylogenetic analysis was performed by Maximum Likelihood (ML). We performed

JModelTest to determine appropriate evolutionary models for the nucleotide and GTR+I was selected based on Akaike information Criterion (AIC). ML analyses were conducted using program RAxML 8.0.0 (Stamatakis 2014) on the dataset. GTR + I nucleotide substitution model was used for all subsets, and support for nodes of the resulting ML trees was assessed by analyses of 1000 bootstrap iterations.

### Morphometrics

Frogs were measured to the nearest 0.1 mm with digital callipers within 2 months of preservation. Abbreviations: SVL, snout–vent length (from tip of snout to vent); SL, snout

length (from anterior corner of eye to tip of snout); IOS, interorbital space (least distance between upper eyelids); ED, eye diameter (horizontal); UEW, upper eyelid width (maximum transverse distance of the upper eyelid measured from inner edge to outer edge); AJS, Angle of jaw to snout (distance between angle of jaws and tip of snout); HL, head length (distance between mandible and snout tip); HW, head width (at angle of jaw); FLL, forelimb length (from proximal end of junction of arm with the body to tip of the Finger III); F-I to F-IV, lengths of 1<sup>st</sup> to 4<sup>th</sup> fingers (from the base of the palm to the tip of the respective finger); HLL, hind-limb length (from midventral line of attachment of legs with body to tip of the 4<sup>th</sup> toe); TL, thigh length (distance from the middle of vent to knee); TBL, tibia length (distance between surface of knee and surface of heel, with both tibia and tarsus flexed); T-I to T-V, lengths of 1<sup>st</sup> to 5<sup>th</sup> toes (from the inner metatarsal tubercle region to tip of the respective toe); TTA, tibiotarsal articulation (not measured; character used to gauge position of the tibiotarsal articulation when hind limb is stretched parallel to the body).

#### Call Recording and Analysis

The call of a single uncollected male *Raorchestes shillongensis* was recorded with a digital recorder (Sony IC recorder 7.4.0) in Eastern Regional Station, Shillong (Zoological Survey of India) on 30 August 2016 at 19:41 h. The microphone was approximately 1–1.5 m away from the calling male. Ambient air temperature of the calling site was taken with a digital thermometer. We used Raven Pro Ver. 1.5 (Charif *et al.* 2010, Bioacoustics Research Program 2011) for call analysis. We measured a total of five temporal properties that included call-group duration, inter-call group interval, intra-call group interval, call duration, and call rate of a call bout comprising of five call groups. One spectral property (*viz.*, peak frequency) was measured for the entire series of calls.

Terminology and graphical representation of call properties analyzed follow those of Bee *et al.* (2013 a, b).

#### Maps and Distribution

Geographic range and point-location maps were generated in ArcGIS 10.3. Open source data from Global Administrative area ([www.gadm.org](http://www.gadm.org)) are used for an administrative boundary, and SRTM 90-m database (<http://srtm.csi.cgiar.org>) was used for elevation map. The area under minimum convex polygon (MCP) was computed by connecting the outermost occurrence points to estimate the extent of occurrence.

#### Results

*Raorchestes shillongensis* (Pillai and Chanda, 1973)

*Philautus shillongensis* Pillai and Chanda, 1973  
*Philautus (Philautus) shillongensis* Bossuyt and Dubois, 2001.

*Pseudophilautus shillongensis* Li, Che, Murphy, Zhao, Zhao, Rao, and Zhang, 2009.

*Raorchestes shillongensis* Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010.

*Holotype* ZSI A6971 (*ex* V/ERS 472).—Collector: R. Giri in 1971, from Malki Forest (GPS point not available), about 3 km E Risa Colony where Eastern Regional Station of ZSI is situated (Figure 2). Specimens were collected from a hill slope that was cut for the construction of road and about 100 m away from a moderate flowing stream.

Condition of the holotype: lower mandible broken below left eye, eye damaged; toe discs emaciated; and mouth open and tongue protruding. Color in preservative: body light brown; upper eyelids black; a dark line between upper eyelids; and bands on limbs dark brown.

The topotypic material collected from Risa Forest (WII 500–WII 522) and Malki (WII 523–WII 525) of the Shillong Plateau resembles the

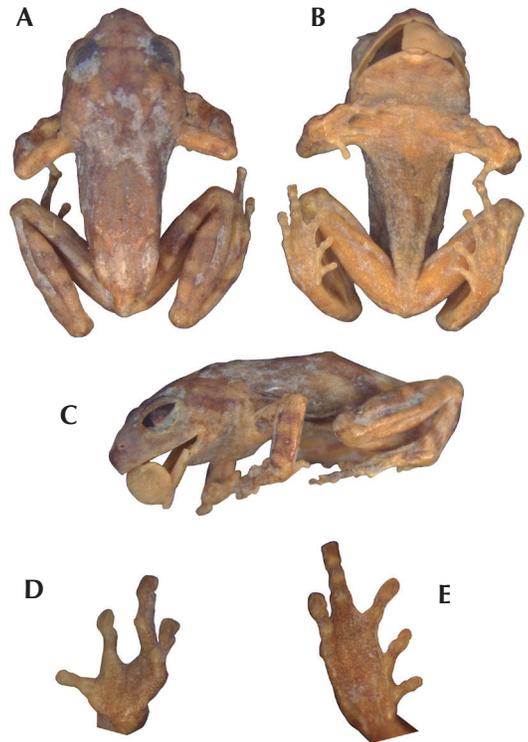
original description by Pillai and Chanda (1973), as follows: relatively small body (SVL of both males and females ranging from 14.21–21.26 mm); nostril closer to snout than eye; snout pointed and little longer than eye diameter; eye pupil horizontal; loreal concave; vomerine teeth absent; tongue without papilla and bifid; a dark line between upper eyelids; 3<sup>rd</sup> finger longest; 4<sup>th</sup> toe longest; finger and toe discs rounded. The original description reports a skin fold from eye to shoulder, perhaps about supratympanic fold. We observed the following differences from the original description: dorsolateral fold absent; tympanum indistinct; and both inner and outer metatarsal tubercles absent.

The frogs collected from Riwai, Mawlynnong and Nongkhylliem distinctly differ morphologically and genetically from *Raorchestes shillongensis* (Table 1).

*Redescription Based on Recently Collected Frogs*

*Voucher specimens*.—(WII 500–WII 525): collected from Risa Colony (25°33'41.8" N, 91°53'39.1" E; 25°33'24.4" N, 91°53'33.9" E; 25°32'39.7" N, 91°53'18.8" E) and Malki Forest (25°33'29.9" N, 91°53'3.8" E) during study period. Specimens are deposited in Wildlife Institute of India, Dehradun.

*Diagnosis*.—Redescription based on 25 males and 12 females. A small frog (male SVL 14.2–19.1 mm; female SVL 16.0–21.3 mm). Head length slightly greater than width; snout pointed, protruding slightly beyond mouth in ventral view; loreal region concave, SL slightly larger than ED; IOS larger than ES; IOS larger than UEW; pupil horizontal; nostril closer to tip of snout than to eye; tongue notched; tympanum indistinct; supratympanic fold distinct from posterior corner of upper eyelid to shoulder region; dorsal skin covered with tiny warts; warts may form middorsal ridge on head and forebody; venter and flanks smooth with black blotches; toes webbed, lateral dermal fringe absent along Toe V; discs rounded; tibia longer than thigh; reduced webbing; subarticular



**Figure 2.** Holotype (ZSI A6971) of *Raorchestes shillongensis* (17 mm SVL). (A) dorsal view, (B) ventral view, (C) lateral view, (D) ventral view of hand, and (E) ventral view of foot.

tubercle prominent, rounded; metatarsal tubercle absent; toe tips with distinct rounded discs; circummarginal grooves present on finger and toe discs.

*Description of an adult male*.—(Voucher number WII 503, Figure 3, all measurements in mm): small (SVL = 19.1); head longer than wide (HL = 7.0; HW = 6.7); snout pointed in both dorsal and ventral views; upper jaw protruding slightly in ventral view; snout slightly longer eye diameter (SL = 2.9; ED = 2.4); distance between angle of jaw to tip of the snout 5.2; canthus rostralis distinct; loreal region concave; interorbital space 1.9 × larger than upper eyelid width (IOS = 3.0; UEW = 1.4); nostrils oval, lacking flap, closer snout tip than to eye; tongue distinctly notched; lingual papillae

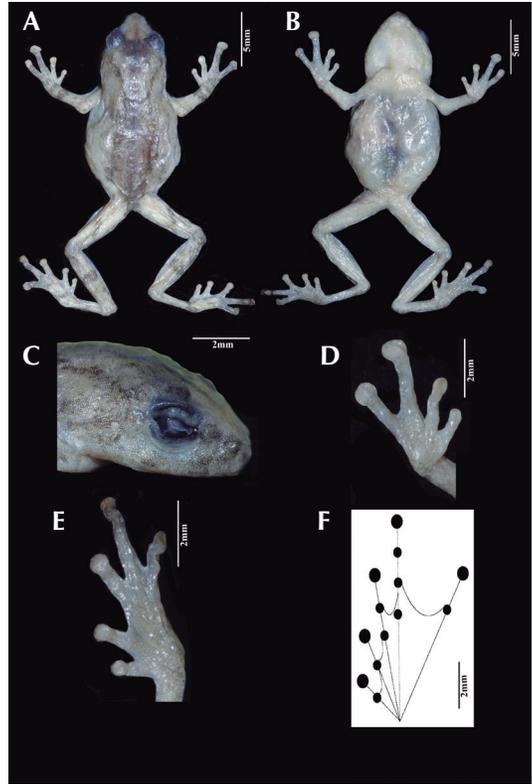
**Table 1.** Morphometric measurements (mm) of *Raorchestes shillongensis*.

Character	Female (N = 12)		Male (N = 25)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
SVL	15.99–21.26	18.47 $\pm$ 1.6	14.21–19.09	16.51 $\pm$ 1.29
HW	5.75–7.23	6.45 $\pm$ 0.53	4.33–6.68	5.81 $\pm$ 0.53
HL	5.79–7.24	6.65 $\pm$ 0.49	5.09–7.05	5.9 $\pm$ 0.49
ED	2.07–2.76	2.39 $\pm$ 0.2	1.56–2.68	2.08 $\pm$ 0.3
SL	2.1–3.23	2.58 $\pm$ 0.39	1.72–2.91	2.19 $\pm$ 0.32
AJS	4.47–6.02	5.41 $\pm$ 0.51	3.94–5.7	4.92 $\pm$ 0.4
IOS	2.01–3.27	2.54 $\pm$ 0.37	1.47–2.98	2.23 $\pm$ 0.33
UEW	1.08–1.61	1.32 $\pm$ 0.18	0.84–2.46	1.23 $\pm$ 0.31
AG	7.74–12.7	9.92 $\pm$ 1.25	6.44–10.62	8.39 $\pm$ 1.11
FLL	10.61–12.94	11.59 $\pm$ 0.75	8.16–12.91	11.34 $\pm$ 1.27
F I	2.11–3.57	2.81 $\pm$ 0.52	1.56–3.84	2.77 $\pm$ 0.62
F I DW	0.43–1	0.75 $\pm$ 0.16	0.31–1	0.63 $\pm$ 0.17
F II	3.29–5.49	4.4 $\pm$ 0.63	2.76–5.59	4.25 $\pm$ 0.68
F II DW	0.45–1.46	0.98 $\pm$ 0.3	0.4–1.43	0.81 $\pm$ 0.31
F III	4.97–8.65	6.76 $\pm$ 1.1	4.82–8.66	6.71 $\pm$ 0.93
F III DW	0.81–1.75	1.31 $\pm$ 0.29	0.38–1.75	1.13 $\pm$ 0.41
F IV	4.21–7.87	5.9 $\pm$ 1.08	3.76–7.34	5.68 $\pm$ 0.9
F IV DW	0.56–1.84	1.18 $\pm$ 0.37	0.4–1.76	1.01 $\pm$ 0.36
TBL	7.8–9.68	8.73 $\pm$ 0.58	6.37–8.66	7.76 $\pm$ 0.57
TL	5.99–8.37	7.18 $\pm$ 0.76	5.37–7.82	6.5 $\pm$ 0.53
HLL	23.92–30.03	26.92 $\pm$ 2.17	21.92–27.66	24.63 $\pm$ 1.98
T I	1.61–4.26	2.72 $\pm$ 0.79	1.64–2.98	2.47 $\pm$ 0.4
T I DW	0.49–1.02	0.71 $\pm$ 0.19	0.36–0.96	0.61 $\pm$ 0.16
T II	2.96–5.93	4.08 $\pm$ 0.89	2.61–4.47	3.73 $\pm$ 0.54
T II DW	0.54–1.19	0.84 $\pm$ 0.21	0.35–1.07	0.7 $\pm$ 0.2
T III	4.47–8.9	6.45 $\pm$ 1.17	4.52–7.12	5.87 $\pm$ 0.85
T III DW	0.43–1.37	0.89 $\pm$ 0.28	0.33–1.03	0.76 $\pm$ 0.22
T IV	5.9–10.92	8.76 $\pm$ 1.5	6.05–10.43	8.04 $\pm$ 1.28
T IV DW	0.51–1.76	0.97 $\pm$ 0.36	0.43–1.33	0.89 $\pm$ 0.26
T V	5.23–9.64	7.07 $\pm$ 1.22	4.74–8.37	6.57 $\pm$ 0.91
T V DW	0.39–1.53	0.99 $\pm$ 0.35	0.35–1.32	0.8 $\pm$ 0.3

absent; vomerine teeth absent; tympanum indistinct; supratympanic fold distinct; eyes small, pupil horizontal. Distance between axilla and groin 10.6. Forelimb length 11.9; fingers lacking lateral dermal fringe and web; subarticular tubercles prominent (fingers: I = 1, II = 1, III = 2, IV = 1); relative lengths of fingers: I < II < IV < III (FI = 3.6, FII = 5.6, FIII = 8.6, FIV = 6.9); discs rounded (finger: I DW = 0.7, II DW = 1.1, III DW = 2.0, IV DW = 1.3); circummarginal grooves present; nuptial pad absent. Tibia slightly longer than thigh (TBL = 8.5, TL = 6.8); supernumerary tubercles and subarticular tubercle present (toe: I = 1, II = 1, III = 2, IV = 3, V = 2); Toe V shorter than Toe III (TV = 6.4, TIII = 6.75); relative lengths of toes: I < II < III < V < IV (TI = 2.03, TII = 3.76, TIII = 6.75, TIV = 8.78, TV = 6.4); no lateral dermal fringe along Toe V; webbing reduced, reaching second subarticular tubercle medially, and below second subarticular tubercle along lateral edge of Toe IV; subarticular tubercles prominent, rounded; both inner and outer metatarsal tubercles absent; toe tips with distinct, rounded disks (toe: I DW = 0.76, II DW = 0.6, III DW = 0.91, IV DW = 1.2, V DW = 0.69); circummarginal grooves present.

*Color in life.*—Individuals vary in color (Figure 4). The most consistent and obvious character to identify *Raorchestes shillongensis* in the field is the presence of a )-( or )-(shaped dark brown mark on the dorsum. The pattern may be distinct or faint. Dark brown or black reticulations along the flank are more pronounced on the groin; dorsum is brown to dark brown.

The following patterns and colors may or may not be present in life: (1) band from axilla to dorsum; (2) dark brown below eye and tympanic fold; (3) fingers and toes discs reddish or whitish; (4) supratympanic fold whitish; (5) dark brown line from below the eye to upper lip; (6) brown line between the upper eyelids; (7) dorsum gray with yellowish hourglass-shaped marking; and (8) dorsum creamy white with dark brown hourglass-shaped marking.



**Figure 3.** *Raorchestes shillongensis* (voucher number WII 503). (A) dorsal view, (B) ventral view, (C) lateral view of head, (D) ventral view of hand, (E) ventral view of foot, and (F) web pattern in foot.

*Color in preservative.*—Dorsal color gray or light brown to dark brown, flanks pale. Prominent dorsal markings and bands on limbs dark brown or black. Anterior part of the head darker, or rarely paler, than rest of body. Triangular dark brown mark with posterior apex between upper eyelids, not visible in life. Eyelids and tympanic fold blackish or brown.

*Secondary sexual characters.*—Vocal sac transparent and extended. Males (14.2–19.1 mm;  $N = 25$ ) smaller than females (16.0–21.3 mm;  $N = 12$ ).



**Figure 4.** Different color morphs in *Raorchestes shillongensis*. Photographs were obtained from non-collected specimens at Risa Forest (A and C); Laitkor (B); Malki Forest (D).

#### *Molecular Phylogenetic Position*

The primers amplified approximately 550 bp long portions of the respective 16S gene; however, in the current analysis only 486 bp were used. ML analyses on the dataset yielded similar topologies having good bootstrap support at major nodes. In the phylogeny recovered from the ML analysis, *Raorchestes shillongensis* is a

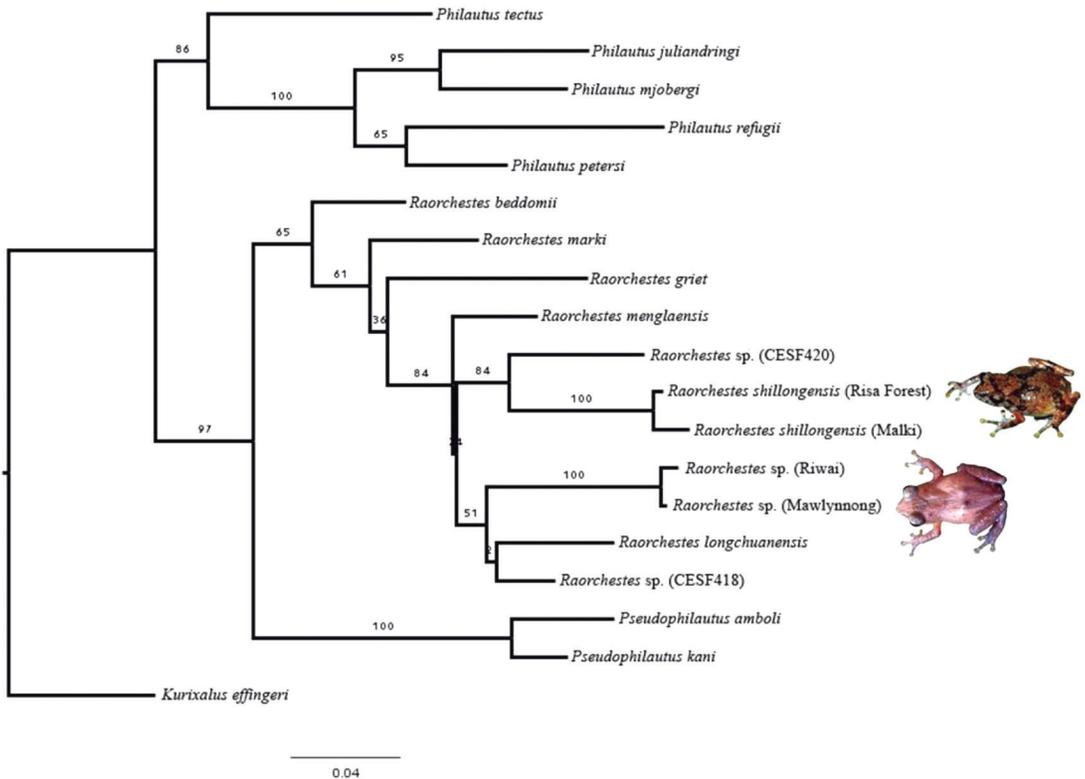
member of the south Asian clade (*Raorchestes*) rather than the Southeast Asian clade (*Philautus*); specimens collected from four sites (Malki, Risa Forest, Riwai, and Mawlynnong) represent two species. Risa Forest material (topotypic) resembles that of Malki Forest; thus, these populations are regarded as *R. shillongensis*, a species shown to be more closely related to *R. longchuanensis* (Yang and Li, 1978) in southern

China than to the peninsular Indian clade of *Raorchestes*. The results from ML analyses of concatenated gene sequences of 16S rRNA genes are shown in Figure 5.

*Differential Diagnosis*

*Raorchestes shillongensis* differs from other members of *Raorchestes* and *Philautus* in Northeast India; parenthetical characters refer to congener being compared with *R. shillongensis*. It can be distinguished from *Raorchestes* sp. 1 collected from Riwai and Mawlynnong by the following characters: smaller body size; tibia shorter than length of Toe IV; middorsal ridge absent; no ridge on thigh and tibia; dark reticulation in groin; TTA reaches tympanic

region [vs. large body (16.23–20.35 mm, *N* = 15), tibia longer than Toe IV, distinct or less distinct middorsal ridge from snout to vent, ridge present on thigh and tibia, reticulation absent, TTA reaches eye]. *Raorchestes shillongensis* differs from *Raorchestes* sp. 2 (from Nongkhyllem) by the following characters: smaller body size; head longer than wide; nostril closer to snout than eye; supratympanic fold distinct; TTA reaching tympanic region, )(–shaped mark present on back [vs. large body size (17.81–20.08 mm, *N* = 2), head wider than long, nostril equidistant from snout and eye, supratympanic fold indistinct, TTA reaching the eye, )(–shaped mark absent]. It can be distinguished from *R. manipurensis* (Mathew and Sen, 2009) by: head longer than wide, tongue



**Figure 5.** Maximum Likelihood tree for 16 Bush frog species and *Kurixalus effingeri* as outgroup. Numbers on nodes indicates bootstrap support for Maximum Likelihood.

bifid, tibia longer than thigh, nostril closer to snout than to eye (vs. head wider than long, tongue lobed, tibia shorter than thigh, nostril equidistant to eye and snout). The species differs from *R. annandalii* (Boulenger, 1906) by: nostril closer to snout than eye, TTA reaches tympanic region (vs. nostril equidistant from snout and eye, TTA reaches tip of snout) and from *R. sahai* (Sarkar and Ray, 2006) by: pointed snout, IOS larger than UEW, nostril closer to snout than to eye (vs. snout rounded, IOS smaller than UEW, nostril equidistant from snout and eye). *Raorchestes shillongensis* differs from *Phyllautus microdiscus* (Annandale, 1912) by: pointed snout, IOS broader than ED (vs. snout rounded, IOS is as wide as ED) and from *P. gario* (Boulenger, 1919) by: TTA reaching tympanic region (vs. TTA reaching anterior corner of the eye). It differs from *P. kempiae* (Boulenger, 1919) by: IOS broader than ED (vs. IOS smaller than ED) and from *P. namdaphaensis* Sarkar and Sanyal, 1985 by nostril closer to snout, TTA reaching tympanic region, middorsal line absent (vs. equidistant from eye and snout, TTA reaching between eye and nostril, mid dorsal line present). The species can be distinguished from *P. dubius* (Boulenger, 1882) by: absence of papilla in tongue, pointed snout (vs. papilla present, rounded snout) and from *P. kempii* (Annandale, 1912) by the pointed snout, distinct supratympanic fold, TTA reaching tympanic region (vs. rounded snout, supratympanic fold absent, TTA reaching tip of snout). Table 2 summarizes mensural and meristic data for adult *Raorchestes shillongensis* and those of other Bush Frogs (*Raorchestes* and *Phyllautus*) that occur in Northeast India.

#### Advertisement Call

Calling males emerge at dusk (18:30 h) and vocalize until 24:00 h. If it is raining, calling activity from bushy thickets often continues during the day. Advertisement calls occur in call groups (Figure 6) and consist of two to multiple

calls. Duration of the call analyzed is 22.62 s; the number of call group is five with call numbers varying from 3–5. The duration of call group is  $0.72 \pm 0.21$  s ( $N = 5$ ) and it varies with the number of calls. The interval between call groups is  $4.76 \pm 0.76$  s ( $N = 4$ ), whereas the interval within a call group is  $0.19 \pm 0.02$  s ( $N = 14$ ). Each call has a brief single pulse (non-pulsatile) of  $0.05 \pm 0.01$  s ( $N = 19$ ). Overall peak frequency of the calls is 3.62 kHz.

#### Natural History

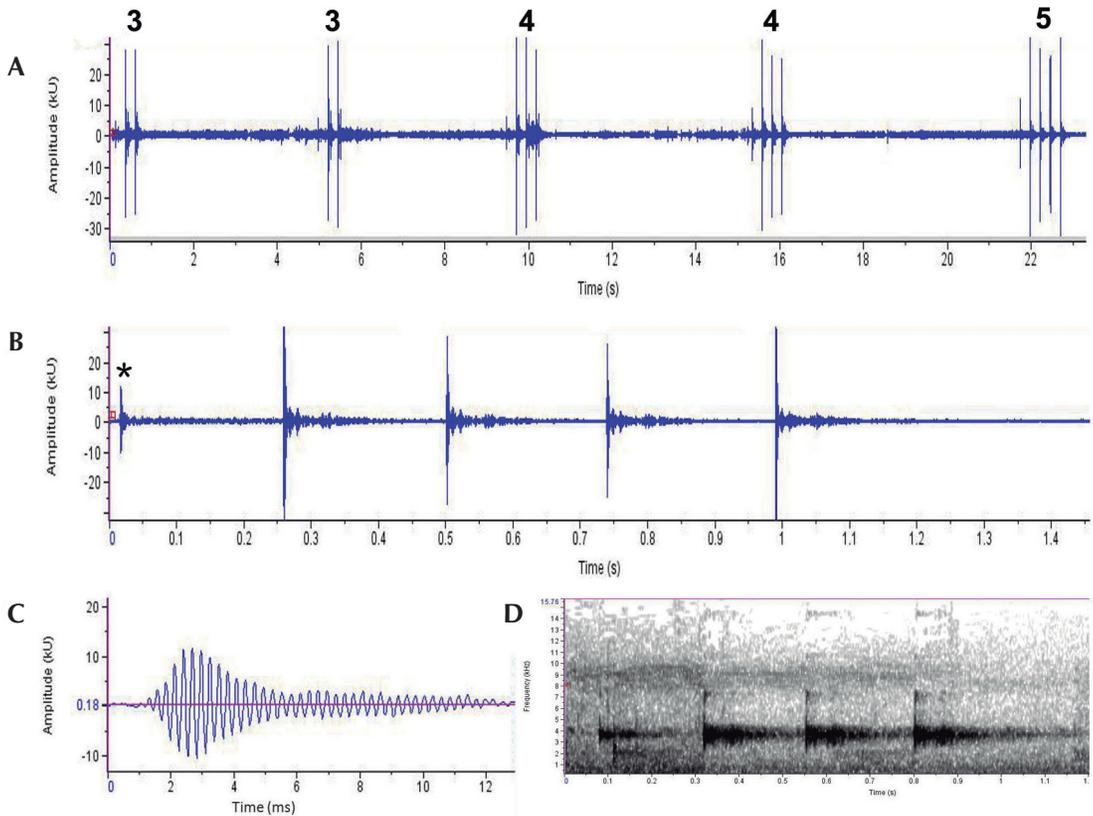
*Raorchestes shillongensis* became active with the onset of rain in the last week of April 2016. Frogs were found in human settlements, gardens, roadside shrubs (*Eupatorium* sp. and ferns), stream sides, and broadleaf forest areas. Individuals perch on leaves and branches of small trees and trunks of pine trees. Males perch  $69.2 \pm 38.73$  cm ( $N = 73$ ) above the ground, and females perch  $52.53 \pm 42.02$  cm ( $N = 15$ ) above the ground. The females lay 8–17 eggs on moist soil under leaf litter and the egg undergoes direct development for  $30.5 \pm 0.71$  days ( $N = 2$ ). The reproductive mode of *R. shillongensis* is Mode 17 (Duellman and Trueb 1986)—i.e. direct development on ground (Boruah et al. 2017).

#### Distribution

We added 82 new localities to the 531-km<sup>2</sup> range of *R. shillongensis* (Appendix II, Figure 7). The present distribution includes Mawlai Reserve Forest, Laitkor Reserve Forest, Mawpat Reserve Forest, and Upper Shillong Reserve Forest, along with some community conserved and semi-protected forests, such as Mawphlang Sacred Grove and North Eastern Hill University (NEHU) campus. Some tourist spots within the distribution range of *R. shillongensis* are Cherrapunjee, Elephant Falls, Shillong Peak, Happy Valley, and Dainthlen. The species is restricted to an altitudinal range from 1087–1924 m a.s.l.

**Table 2.** Mensural and meristic data for adults of *Raorchestes shillongensis*, compared with other Bush Frogs (*Raorchestes* and *Phyllautus*) that occur in Northeast India. References: A = male and female SVL (range is given in mm); B = ED [less than IOS (0), more than IOS (1)], equal to IOS (2); C = line between eyelids [absent (0), faint (1), conspicuous (2)]; D = nostril [equidistant from eye and snout (0), closer to snout (1)]; E = snout [pointed (0), rounded (1)]; F = Tympanum [indistinct (0), distinct (1)]; G = Tympanic fold [absent (0), distinct (1)]; H = tongue shape [lobed (0), notched (1)]; I = TTA reaching [tip of snout (0), nostril (1), middle of eye (2), anterior corner of eye (3), between nostril and eye (4), tympanic region (5)]; J = mid-dorsal line/ridge [absent (0), present on head and fore-body (1), present on snout to vent (2)]; K = line/ridge on thigh and tibia [absent (0), present (1)]; L = Inner metatarsal tubercle [absent (0), present (1)]; M = outer metatarsal tubercle [absent (0), present (1)].

Species	A	B	C	D	E	F	G	H	I	J	K	L	M	Source
<i>Raorchestes shillongensis</i> (M)	14.24–19.09 (25)	0	0,1	1	0	0	1	1	5	0,1	0	0	0	Present study
<i>R. shillongensis</i> (F)	15.99–21.26 (12)	0	0,1	1	0	0	1	1	5	0,1	0	0	0	Present study
<i>Raorchestes</i> sp.1 (M)	16.23–20.35 (15)	1	0,1	1	0	0	1	1	2	2	1	0	0	Present study
<i>Raorchestes</i> sp.2 (M)	17.81–20.08 (2)	1	1	1	1	0	1	1	2	0	0	0	0	Present study
<i>Raorchestes</i> sp.2 (F)	22.14 (1)	1	1	1	1	0	1	1	2	0	0	0	0	Present study
<i>R. sahai</i>	25–26	1	0	0	1	1	-	1	2	0	0	1	0	Sarkar and Ray (2006)
<i>R. manipurensis</i>	25	2	0	0	0	1	1	0	1	0	0	1	0	Mathew and Sen (2009)
<i>R. annandali</i>	16–20	0	1	0	0	1	1	-	0	0	0	0	0	Boulenger (1906), Chanda (2002)
<i>Phyllautus garo</i>	13–16	0	0	0	0	1	1	-	3	0	0	1	0	Boulenger (1919), Chanda (2002)
<i>P. kempiae</i>	17–18	1	2	0	1	0	1	-	0	0	0	0	0	Chanda (2002)
<i>P. namdaphaensis</i>	28	0	2	0	0	0	1	-	4	2	1	1	0	Sarkar and Sanjal (1985)
<i>P. dubius</i>	43	-	0	0	1	1	1	-	2	0	0	1	0	Günther (1876)
<i>P. kempii</i>	15	-	0	1	1	1	0	-	0	0	0	0	0	Annandale (1912)
<i>P. microdiscus</i>	29	2	0	1	1	1	1	-	2	0	0	1	0	Annandale (1912)



**Figure 6.** Advertisement call of *Raorchestes shillongensis* at ambient air temperature 24.1°C. (A) A call comprising five call groups (numbers indicate number of calls in the respective call group). (B) Showing of 5<sup>th</sup> (last) call group with five calls depicted in (A). (C) Showing 1<sup>st</sup> call (non-pulsatile) depicted in (B) (indicated by asterisk). (D) Spectrogram of 4<sup>th</sup> call group with four calls depicted in (A).

## Discussion

Northeastern Indian anurans are poorly known, as evidenced by the numerous discoveries of new amphibian species in the last 5 yr (Mahony *et al.* 2011, 2013, Sondhi and Ohler 2011, Kamei *et al.* 2012, 2013, Purkayastha and Matsui 2012, Das *et al.* 2013, Biju *et al.* 2016). Of the 62 species of *Raorchestes*, 45 have been described in the last 15 yr. This reflects increased research interest with this group of anurans in which much diversity probably remains to be revealed.

Photographs of living *R. shillongensis* are available in Ahmed *et al.* (2009) and Mathew and Sen (2010). Mathew and Sen (2010) mentioned the distribution of *R. shillongensis* in Mizoram, without providing a voucher specimen and collection locality; therefore, we could not include this record in the distribution map of the species. We have confirmed the distribution of the species from Mawlai Reserve Forest in the north to Cherrapunjee in the south of Meghalaya (Figure 7). The species is restricted to altitudes between 1000 and 1900 m a.s.l. Our frogs from the south (Riwai and Mawlynnong) and from

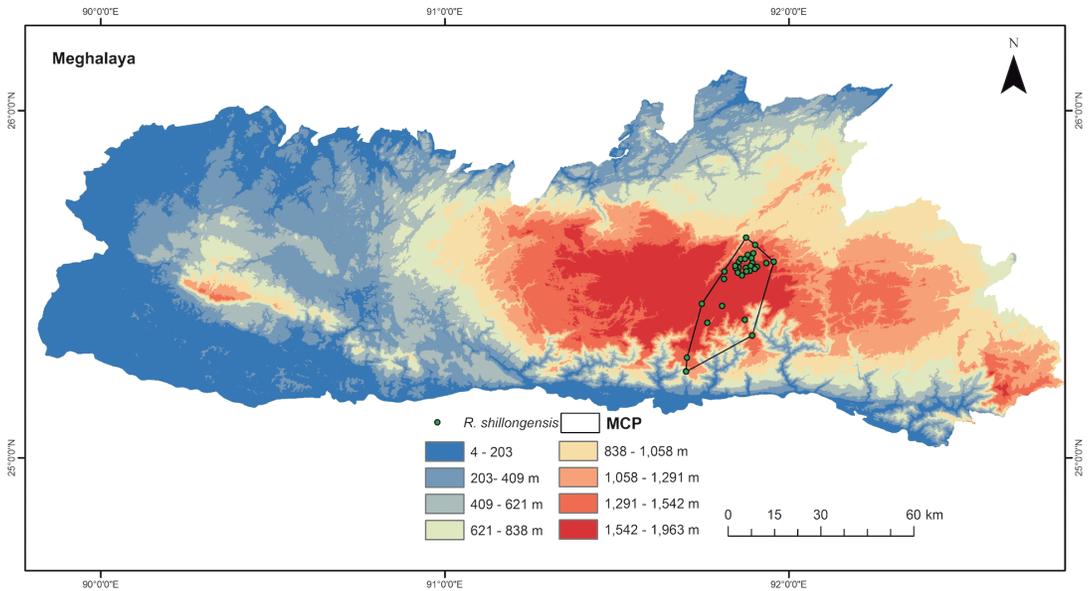


Figure 7. Current distribution map of *Raorchestes shillongensis*.

north (Ribhoi and Badapani) of Khasi Hills represent two separate lineages that are distinct from *R. shillongensis*. Both of these north and south locations are below 500 m a.s.l. Mathew and Sen (2009) reported *P. garo* from the ZSI, Risa Colony, Shillong, which is near the type locality of *R. shillongensis*. It may be misidentified because the population of *R. shillongensis* is variable in color. Nevertheless, the present molecular study reveals that the population from the Shillong is *R. shillongensis*. Our analyses support the findings of Vijaykumar *et al.* (2016) who showed that *R. shillongensis* is closely related to the Indochinese subclade of *Raorchestes*, rather than the clades of the Western Ghats (*Raorchestes*) and Southeast Asian (*Philautus*).

Though Northeast India is a major center of amphibian diversity, only the caecilian fauna of this region has been studied phylogenetically (Kamei *et al.* 2012, 2013, Biju *et al.* 2016). Moreover, rhacophorid frogs of Northeast India

have received little attention in contrast to the members of this family in the Western Ghats. In addition, we know little about the biogeography and taxonomy of the genus *Raorchestes* in the Indochina. Future research on this group from the region could uncover many undescribed species.

#### Conservation and Awareness

Khasi Hills has about 20 named species of anurans, some with defined and others with undetermined type localities. The Shillong Plateau has 11 taxa, the type localities of which lie specifically within its biogeographical limit (Mahony *et al.* 2013). Among them, *R. shillongensis* is categorized as “Critically Endangered” by IUCN (<http://www.iucnredlist.org>) because of its narrow distribution and the decline of its habitat. Future study may extend the range of its distribution. However, illegal tree felling, unregulated mining in protected and

unprotected areas in the state (Gilbert 2012) and in the Shillong Plateau, may have a considerable impact on the survival of amphibian fauna in the region (Mahony *et al.* 2013). Therefore, conservation of these remaining forests, which are the typical habitats of the threatened endemic species of the region, is urgent.

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**Appendix I.** Voucher numbers and GenBank accession numbers of the species used for phylogenetic analysis.

Sl. No.	Species name	GenBank # 16S accession numbers
1	<i>Raorchestes beddomii</i>	JX092653.1
2	<i>Raorchestes griet</i>	JX092654.1
3	<i>Raorchestes marki</i>	JX092719.1
4	<i>Raorchestes</i> sp. CESF418	JX092711.1
5	<i>Raorchestes</i> sp. CESF420	JX092712.1
6	<i>Raorchestes shillongensis</i> (Malki Forest)	SUB3716157
7	<i>Raorchestes shillongensis</i> (Risa Forest)	SUB3711598
8	<i>Raorchestes</i> spp. (Riwai)	SUB3716694
9	<i>Raorchestes</i> spp. (Mawlynnong)	SUB3716575
10	<i>Pseudophilautus kani</i>	JX092724.1
11	<i>Pseudophilautus amboli</i>	JX092698.1
12	<i>Raorchestes longchuanensis</i>	KC465839.1
13	<i>Philautus juliandringi</i>	KX440538.1
14	<i>Philautus mjobergi</i>	KX440533.1
15	<i>Philautus tectus</i>	KX440528.1
16	<i>Philautus petersi</i>	KX440527.1
17	<i>Philautus refugii</i>	KX440535.1
18	<i>Raorchestes menglaensis</i>	GQ285676.1
19	<i>Kurixalus eiffingeri</i>	AY880492.1

**Appendix II.** Gazetteer list of distribution of *Raorchestes shillongensis* in Meghalaya with locality records, GPS coordinates, elevation, and locality status (P = Protected forest, UP = Unprotected area, TP = Tourist place, CP = Community protected forest, SP = Semi-protected area).

Sl. No.	Latitude	Longitude	Elevation (m a.s.l.)	Locality	Status of the locality
1	25°32'20.2"	91°51'3.9"	1853	Shillong Peak	P
2	25°32'22.3"	91°51'2.5"	1800	Upper Shillong Forest	P
3	25°32'23.8"	91°50'59.1"	1791	Upper Shillong Forest	P
4	25°32'26.8"	91°50'59.7"	1803	Upper Shillong Forest	P
5	25°32'42.2"	91°51'14.4"	1682	Upper Shillong Forest	P
6	25°32'43"	91°51'13.4"	1711	Upper Shillong Forest	P
7	25°32'54"	91°51'14.6"	1694	Upper Shillong Forest	P
8	25°32'53.5"	91°51'3.9"	1676	Upper Shillong Forest	P
9	25°32'44.7"	91°50'46.1"	1781	Upper Shillong Forest	P
10	25°32'51.9"	91°51'1.1"	1747	Upper Shillong Forest	P
11	25°33'41.8"	91°53'39.1"	1577	Risa Colony	UP
12	25°33'34.2"	91°53'42.8"	1600	Risa Colony	UP

Appendix II. *Continued.*

Sl. No.	Latitude	Longitude	Elevation (m a.s.l.)	Locality	Status of the locality
13	25°33'16.5"	91°53'59.1"	1707	Motinagar Forest	P
14	25°33'24.7"	91°53'56.4"	1634	Motinagar Forest	P
15	25°33'33.6"	91°53'51.8"	1606	Motinagar Forest	P
16	25°33'23.7"	91°53'46.3"	1625	Motinagar Forest	P
17	25°33'25.8"	91°53'54.2"	1607	Motinagar Forest	P
18	25°33'19.8"	91°53'50.4"	1659	Motinagar Forest	P
19	25°33'16.3"	91°53'51.7"	1675	Motinagar Forest	P
20	25°33'14.6"	91°53'51.5"	1707	Motinagar Forest	P
21	25°33'10.8"	91°53'52.7"	1747	Motinagar Forest	P
22	25°33'6.5"	91°53'46.6"	1783	Laitkor Forest	P
23	25°33'39.3"	91°53'7.7"	1593	Malki Forest Office	P
24	25°33'29.9"	91°53'3.8"	1588	Malki Stream	P
25	25°33'25.8"	91°53'4.7"	1611	Malki Stream	P
26	25°33'22.4"	91°53'5.5"	1613	Malki Stream	P
27	25°33'31.7"	91°53'0"	1599	Malki Stream	P
28	25°33'31.2"	91°53'6.6"	1621	Laitkor Forest	P
29	25°33'33.7"	91°53'11"	1599	Laitkor Forest	P
30	25°33'18.4"	91°53'15.6"	1656	Laitkor Forest	P
31	25°33'9.5"	91°53'22.6"	1785	Laitkor Forest	P
32	25°32'59.7"	91°53'20.8"	1807	Laitkor Forest	P
33	25°32'50.4"	91°53'16.3"	1882	Laitkor Forest	P
34	25°32'39.7"	91°53'18.8"	1884	Near Laitkor Nursing Centre	P
35	25°33'24.4"	91°53'33.9"	1589	Risa Forest Stream	P
36	25°33'15.8"	91°53'38.9"	1639	Risa Forest Stream	P
37	25°33'11.3"	91°53'35"	1667	Risa Forest Stream	P
38	25°33'12"	91°53'33"	1700	Risa Forest	P
39	25°33'18.2"	91°53'36.2"	1655	Risa Forest	P
40	25°33'10.6"	91°53'27"	1770	Risa Forest	UP
41	25°33'42"	91°53'2.4"	1559	Malki	P
42	25°34'50.6"	91°53'17.5"	1418	Orchid Hotel (Polo)	UP
43	25°33'13"	91°53'32.3"	1719	Risa Forest	P
44	25°33'11"	91°53'26.2"	1762	Risa Forest	P
45	25°33'9.4"	91°53'22.6"	1784	Risa Forest	P
46	25°33'13.6"	91°53'23.4"	1753	Risa Forest	P
47	25°14'56"	91°42'3.1"	1171	Sohra	P
48	25°14'54.6"	91°42'6.2"	1101	Sohra	P
49	25°33'52.9"	91°51'15.4"	1607	Mawphlang Sacred Groove	CP

Appendix II. *Continued.*

Sl. No.	Latitude	Longitude	Elevation (m a.s.l.)	Locality	Status of the locality
50	25°33'10"	91°50'39.6"	1723	Mawphlang Sacred Groove	CP
51	25°26'36.2"	91°44'50.7"	1815	Mawphlang Sacred Groove	CP
52	25°26'14.5"	91°48'22"	1855	Cherrapunji Road	UP
53	25°23'19.7"	91°45'47.4"	1757	Cherrapunji Road	UP
54	25°16'31.67"	91°41'10.03"	-	Nohkalikai Falls	TP
55	25°23'51.4"	91°52'20.4"	1570	Mawllynnong Road	UP
56	25°21'8.4"	91°53'36.5"	1555	Pynursla	UP
57	25°30'54.2"	91°48'42.4"	1709	Upper Shillong	UP
58	25°32'11.9"	91°48'46"	1675	Sado, Upper Shillong	P
59	25°32'49.9"	91°52'29.5"	1912	Shillong Peak	P
60	25°33'55.4"	91°51'35.8"	1557	Upper Shillong	P
61	25°34'8.5"	91°51'50.8"	1497	Upper Shillong	P
62	25°34'21.3"	91°51'36.3"	1536	Nongsehrim	UP
63	25°34'21.6"	91°52'22.5"	1507	Relebang	UP
64	25°34'21.6"	91°52'41.4"	1500	Police Bazar	UP
65	25°34'29.5"	91°52'46"	1464	Police Bazar	UP
66	25°35'4.1"	91°52'50.9"	1431	Lawmali	UP
67	25°34'31.3"	91°53'30"	1480	Municipal	UP
68	25°34'48.4"	91°53'25.3"	1431	Polo	UP
69	25°34'43.8"	91°53'35.2"	1440	Polo	UP
70	25°35'21.3"	91°53'47.8"	1425	Golf Link	UP
71	25°36'46.6"	91°54'7.2"	1400	Northeast Hill University	SP
72	25°17'17.1"	91°42'13.6"	1418	Dainthlen	UP
73	25°38'4"	91°52'32.6"	1087	Mawlai Forest	P
74	25°33'53.6"	91°57'23.1"	1475	Sweet Fall	TP
75	25°33'37.3"	91°56'3.2"	1660	Everliving Museum	UP
76	25°32'58.3"	91°54'27"	1754	Laitkor	UP
77	25°32'17.4"	91°53'12.5"	1872	Laitkor	UP
78	25°32'38.2"	91°54'5.2"	1829	Laitkor	UP
79	25°32'11.8"	91°52'37.3"	1879	Laitkor	UP
80	25°31'31.2"	91°51'49.7"	1847	Laitkor	UP
81	25°32'0.5"	91°51'6.3"	1924	Shillong Peak	TP
82	25°32'13.23"	91°49'20.85"	-	Elephant Falls	TP