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A NEW TOAD SPECIES (AMPHIBIA: BUFONIDAE: *Duttaphrynus*) FROM THE KHASI HILLS, MEGHALAYA, NORTHEASTERN INDIA

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Abstract

We describe a new bufonid toad from Mawphlang in the Khasi Hills, Meghalaya, northeastern India, based on an integrative taxonomic assessment combining external morphology and mitochondrial 16S rRNA sequence data. Phylogenetic analyses recover the Khasi Hills specimens as a distinct and well-supported monophyletic lineage within *Duttaphrynus*, forming a clade sister to an undescribed lineage from Chin State, Myanmar, and clearly separated from the most closely related congeners, *D. stuarti* and *D. chandai*. Uncorrected mitochondrial 16S sequence divergence between the new species and these taxa ranges from 4.4–6.7%, consistent with interspecific differentiation in Asian bufonids. Morphometric analyses based on allometrically size-corrected measurements further support its distinctiveness. The new species is characterized by a small adult body size (male SVL 40.0–41.8 mm), absence of cranial ridges, a distinct but small tympanum, moderate toe webbing, specific digital length proportions, and a dorsum bearing numerous pointed keratinized warts. The concordant molecular, multivariate morphometric, and qualitative morphological evidence demonstrates that the Khasi Hills population represents a previously unrecognized evolutionary lineage. The species is currently known only from its type locality in montane habitats of the East Khasi Hills, highlighting the continued importance of the Meghalaya Plateau as a center of amphibian diversity and endemism within the Indo-Burma biodiversity hotspot.

Keywords: 16S rRNA, India, integrative taxonomy, Meghalaya, PCA, systematics, taxonomy

Introduction

The Old-World toad family Bufonidae Gray, 1825 constitutes one of the most species-rich and geographically widespread radiations of anurans, currently comprising more than 650 recognized species distributed across all continents except Antarctica (Frost 2024). Bufonids occupy a remarkable breadth of ecological niches, ranging from tropical rainforests and montane systems to arid environments and heavily modified anthropogenic landscapes. This ecological versatility, together with a long evolutionary history and repeated continental dispersal events, has resulted in extensive diversification within the family (Bossuyt & Milinkovitch 2001, Van Bocxlaer *et al*. 2009, Pyron & Wiens 2011). Recent phylogenetic studies integrating multilocus and genomic datasets have further revealed that Asian bufonids represent several independent evolutionary radiations shaped by complex geological and climatic processes across the Indo-Malayan region (Van Bocxlaer *et al*. 2009, Portik & Papenfuss 2015).

Within Bufonidae, the genus *Duttaphrynus* Frost *et al*., 2006 represents a major lineage of Asian toads distributed across the Indian subcontinent, mainland Southeast Asia, southern China, and parts of the Sundaic region (Van Bocxlaer *et al*. 2009). Species of *Duttaphrynus* are typically characterized by a moderately robust body, well-developed parotoid glands, variable cranial ridges, and differences in the degree of digital webbing (Dubois & Ohler 1999, Frost *et al*. 2006). Although the genus contains several widely distributed species, particularly the Asian common toad *Duttaphrynus melanostictus* (Schneider, 1799), recent molecular investigations have demonstrated that several nominal taxa encompass deeply divergent evolutionary lineages, suggesting that species diversity within the genus remains underestimated (Van Bocxlaer *et al*. 2009, Pyron & Wiens 2011, Portik & Papenfuss 2015).

Northeastern India represents one of the most biogeographically complex regions of South Asia and forms part of the Indo-Burma biodiversity hotspot, one of the most species-rich yet threatened regions on Earth (Myers *et al*. 2000). The region lies at the intersection of the Indian, Indo-Chinese, and Himalayan biogeographic realms and is characterized by a heterogeneous landscape of plateau systems, deeply dissected river valleys, and montane evergreen forests (Mani 1974, Pawar *et al*. 2007). These landscapes have promoted high levels of endemism and phylogenetic diversification among amphibians and reptiles (Pawar *et al*. 2007, Biju *et al*. 2014). During the past two decades, integrative taxonomic research combining molecular phylogenetics with detailed morphological analyses has revealed a considerable number of previously unrecognized amphibian lineages from northeastern India, indicating that the region harbours substantial undocumented biodiversity (Kamei *et al*. 2012, Biju *et al*. 2014, Garg & Biju 2019, Mahony *et al*. 2018).

Despite this increasing recognition of amphibian diversity in the region, the taxonomy of *Duttaphrynus* in northeastern India remains comparatively understudied. Only a few species have been documented from the region, including the widespread *D. melanostictus*, the montane species *D. stuarti* (Smith, 1929), and the more recently described *D. chandai* Das, Dutta, Sengupta & Gayen, 2013 (Das *et al*. 2013). However, morphological similarity among species, coupled with limited comparative material, has historically complicated species delimitation within the genus. Phylogenetic studies have demonstrated that morphological conservatism can obscure substantial genetic divergence in bufonids, emphasizing the need for integrative approaches that combine molecular and morphological data when assessing species boundaries (Van Bocxlaer *et al*. 2009, Pyron & Wiens 2011, Portik & Papenfuss 2015).

During recent field surveys conducted in the Khasi Hills of Meghalaya, northeastern India, we collected specimens of a small forest-associated *Duttaphrynus* that differed from other regional congeners in several aspects of external morphology and colouration. Preliminary molecular analysis of mitochondrial 16S rRNA sequences indicated that these specimens represent a distinct evolutionary lineage within the genus. Phylogenetic comparisons including closely related taxa revealed clear genetic divergence from both *D. stuarti* and *D. chandai*, the most morphologically similar congeners known from the region. The combined molecular, morphometric, and qualitative morphological evidence supports the recognition of the Khasi Hills population as a previously undescribed species, which we formally describe herein as a new species.

Materials and methods

Field sampling and specimen preservation. Field surveys were conducted in montane forest habitats of the Khasi Hills in Mawphlang (25.45329°N, 91.75443°E; 1854 m a.s.l.; Fig. 1), Meghalaya, northeastern India. Specimens were encountered during nocturnal visual encounter surveys along forest trails and within leaf-litter microhabitats. Individuals were photographed in life to document colouration and pattern prior to preservation. Voucher specimens (ADBU-HW0180 and ADBU-HW0182; ADBU = Assam Don Bosco University, India) were euthanized using a tricaine methanesulfonate (MS-222) solution following accepted ethical standards for amphibian research (Simmons 2015). Tissue samples (liver or thigh muscle) were removed immediately after euthanasia and preserved in 95–100% ethanol for molecular analysis. Specimens were subsequently fixed in 10% buffered formalin and later transferred to 70% ethanol for long-term storage. Voucher specimens were deposited in the institutional herpetological collection of Assam Don Bosco University (see Systematics section). Sex was determined by examination of secondary sexual characters and, when necessary, by dissection of gonads.

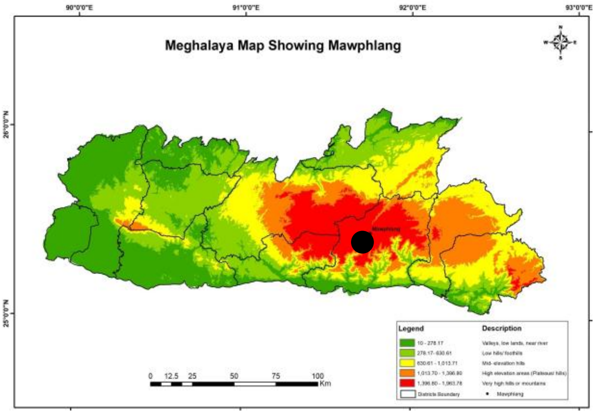

Figure 1. Map showing the type locality of the new species in Mawphlang, East Khasi Hills District, Meghalaya, northeastern India.

Comparative material and taxon sampling. Comparative morphological data were obtained from examined museum specimens and from published descriptions of congeners occurring in South and Southeast Asia. Particular attention was given to species that are morphologically or geographically close to the newly collected population, including *Duttaphrynus stuarti* and *D. chandai*. Additional comparative information was compiled from the taxonomic literature and from original species descriptions where necessary. Comparative specimens examined are listed in the Appendix. Taxonomic nomenclature and species assignments follow the amphibian taxonomy of Frost (2024).

Morphological measurements. Morphological measurements were taken with digital callipers to the nearest 0.01 mm under a stereomicroscope when necessary. Terminology and measurement definitions largely follow previous studies on Asian bufonids and anuran morphometrics (Duellman & Trueb 1994, Das *et al*. 2013). The mensural characters were recorded following Watters *et al*. (2016): snout−vent length (SVL); head length (HL); head width (HW); snout length (SL); interorbital distance (IOD); internarial space (INS); eye−nostril distance (EN); snout−nostril length (NS); eye diameter (ED); upper eyelid width (UEW); mandible to eye distance (MBE); mandible−nostril distance (MN); tympanum diameter (TD); parotid gland width (PGW); parotid gland length (PGL); upper arm length (UAL); forearm length (FLL); lower arm length (LAL); hand length (HAL); finger I length (Fin1L); finger II length (Fin2L); finger III length (Fin3L); finger IV length (Fin4L); snout−urostyle length (SUL); body width (BW); hindlimb−length (HLL); thigh length (THL); tibia length (TL); tarsus length (TSL); foot length (FL); toe I length (Toe1L); toe II length (Toe2L); toe III length (Toe3L); toe IV length (Toe4L); toe V length (Toe5L). Additional qualitative morphological characters, including cranial ridges, webbing formulae, relative finger and toe lengths, skin texture, and colouration, were recorded following standard bufonid descriptive terminology (Duellman & Trueb 1994, Frost *et al*. 2006).

Sexual dimorphism analysis. To assess whether morphometric data from males and females could be combined in multivariate analyses, sexual dimorphism was tested in the comparative species *Duttaphrynus chandai* using Welch two-sample t-tests for each morphometric variable. Statistical analyses were conducted in R version 4.x (R Core Team 2024). Because no variables showed statistically significant differences between sexes ($p > 0.05$ for all characters), male and female measurements were pooled for subsequent analyses.

Allometric size correction and principal component analysis. Morphometric traits typically scale allometrically with body size; therefore, raw measurements were adjusted to remove the effects of overall body size prior to multivariate analyses. Size correction was performed using the allometric equation originally proposed by Thorpe (1975) and implemented following the framework of Chan & Grismer (2022). The size-adjusted variable was calculated as: $X_{adj} = \log(X) - b[\log(SVL) - \log(SVL_{mean})]$, where Xadj is the size-corrected value, X is the original measurement, b is the regression slope of log-transformed trait values against log-transformed SVL, and SVL mean represents the mean snout–vent length for each species. For interspecific datasets such as the present study, SVL mean was calculated separately for each species in order to avoid conflating among-species size variation with within-species growth patterns (Chan & Grismer 2022).

Principal component analysis (PCA) was used to explore patterns of morphometric variation among species and to assess whether the newly sampled population forms a morphologically distinct cluster. PCA was performed using the correlation matrix of size-corrected variables to account for differences in measurement scale. Snout–vent length was excluded from the analysis after size correction because it served as the reference variable for allometric adjustment. Eigenvalues and factor loadings were examined to determine which characters contributed most strongly to the major axes of variation.

Molecular data and phylogenetic analysis. Genomic DNA was extracted from two ethanol-preserved tissue samples (ADBU-HW0180 and ADBU-HW0182) using standard phenol–chloroform extraction or commercial DNA extraction kits. A fragment of the mitochondrial 16S rRNA gene was amplified using the primer pair AH-16S_S (5'-CGC CTG TTT ACC AAA AAC ATC GCC T-3') and AH-16S_R (5'-TGC GCT GTT ATC CCY RGG GTA ACT-3') following Grosjean *et al*. (2015). The 16S marker is widely used in amphibian systematics and provides reliable phylogenetic resolution at the species level (Vences *et al*. 2005). PCR products were purified and sequenced in both directions using automated sequencing platforms.

Sequences were edited and assembled in sequence-editing software and aligned using the MUSCLE algorithm (Edgar 2004) implemented in MEGA 7 (Tamura & Nei 1993, Kumar *et al*. 2016). Phylogenetic relationships were inferred using maximum likelihood (ML) methods. A maximum likelihood phylogeny was reconstructed in IQ-TREE (Nguyen *et al*. 2015) using an unpartitioned dataset. The best-fitting nucleotide substitution model (TIM2+F+R2) was selected using ModelFinder based on Bayesian Information Criterion (BIC) scores (Kalyaanamoorthy *et al*. 2017). Node support was evaluated using ultrafast bootstrap approximation with 1,000 replicates (Minh *et al*. 2013). Uncorrected pairwise genetic distances (p-distances) were calculated to quantify sequence divergence among species of *Duttaphrynus*.

Comparative sequences representing additional species of *Duttaphrynus* were obtained from GenBank. Outgroup taxa representing related bufonid genera were included to root the phylogeny. Sequence accession numbers and voucher information for all taxa included in the analysis are provided in the Sup. Table 1.

Results

Molecular phylogenetic relationships. The aligned mitochondrial 16S rRNA dataset comprised sequences of *Duttaphrynus* and related bufonid taxa retrieved from GenBank together with two newly generated sequences from the Khasi Hills specimens (ADBU-HW0180 and ADBU-HW0182).

Maximum likelihood analysis recovered the Khasi Hills samples as a well-supported monophyletic lineage within the genus *Duttaphrynus* (Fig. 2). The two newly sequenced specimens formed a distinct clade with strong bootstrap support, indicating that they represent a genetically cohesive population. This lineage was recovered as sister to an undescribed *Duttaphrynus* lineage represented by sequence KF665335 from Chin State, Myanmar. Together, these taxa formed a clade that was clearly separated from other congeners included in the analysis.

Among the named species sampled in the phylogeny, the Khasi Hills lineage was most closely related to *D. stuarti* and *D. chandai*. However, the new lineage was clearly distinct from both species and formed an independent evolutionary branch within the genus.

Genetic divergence. Uncorrected pairwise genetic distances (p-distances) based on the 16S rRNA fragment revealed substantial sequence divergence between the Khasi Hills lineage and its closest relatives. The genetic divergence between the new lineage and *Duttaphrynus stuarti* ranged from 4.4–5.5%, whereas divergence from *D. chandai* ranged from 4.4–6.7%. These levels of mitochondrial sequence divergence are comparable to, or greater than, those typically observed among recognized species of Asian bufonids, further supporting the distinctiveness of the Khasi Hills population.

Sexual dimorphism. Statistical tests conducted on the comparative dataset of *Duttaphrynus chandai* revealed no significant sexual dimorphism in the measured morphometric variables (Welch two-sample t-tests; $p > 0.05$ for all characters). Because no statistically significant differences were detected between males and females, measurements from both sexes were pooled for subsequent multivariate analyses.

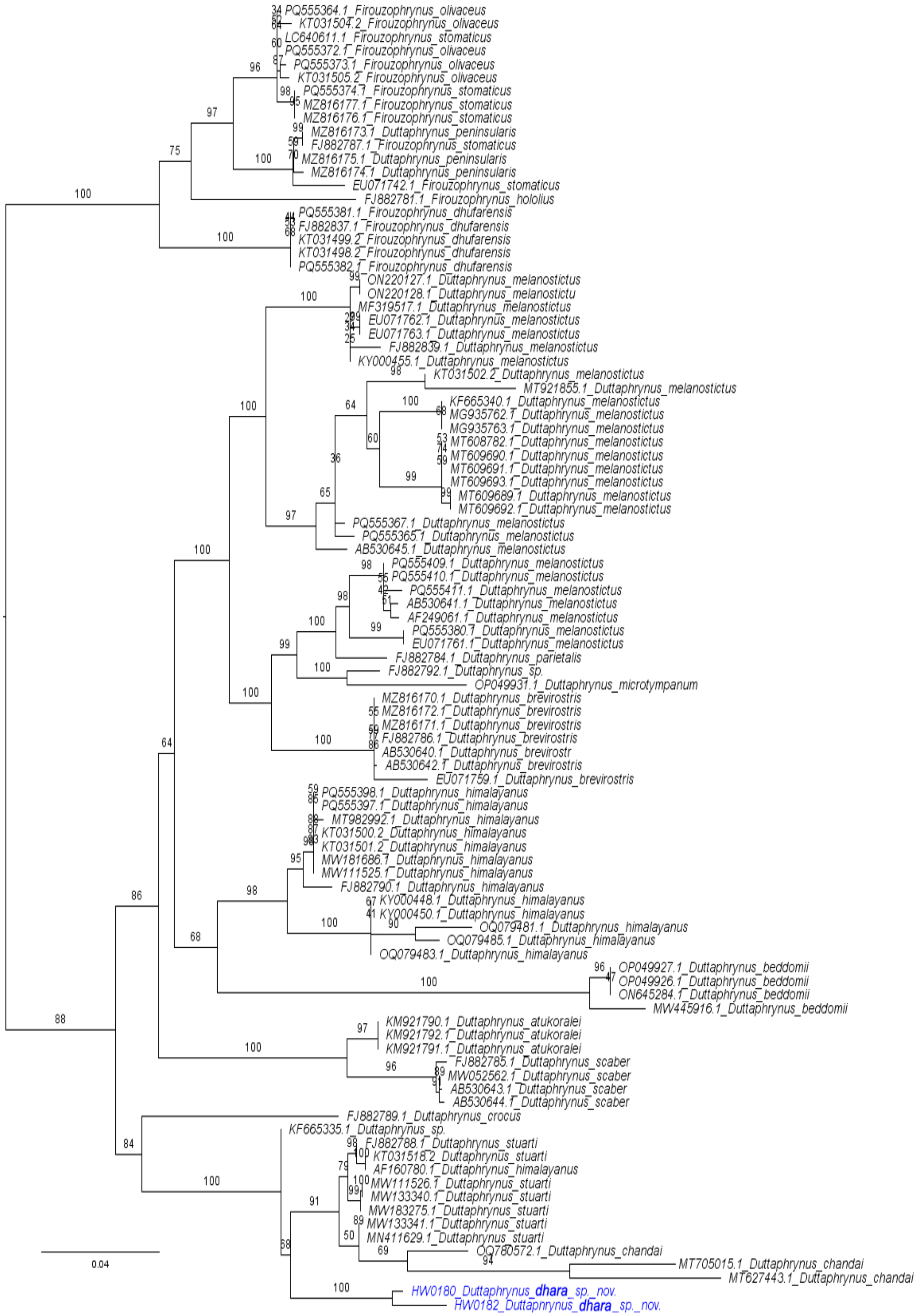

Figure 2. Maximum likelihood phylogeny inferred using IQ-TREE under the TIM2+F+R2 model from mitochondrial 16S rRNA sequences showing the placement of the new species within the genus *Duttaphrynus*. Node support values represent ultrafast bootstrap percentages based on 1,000 replicates. Branches with support values <70% are not shown.

Multivariate morphometric analyses. Principal component analysis of size-corrected morphometric variables revealed that the first two principal components accounted for the majority of variation in the dataset. PC1 explained 85.6% of the total variance, and PC2 explained 9.8%, together accounting for 95.4% of the total morphological variation. The first principal component was strongly influenced by overall body proportions, with high positive loadings for head length (HL), mandible–nostril distance (MN), snout length (SL), and tibia length (TL). The second principal component was primarily associated with variation in snout–eye proportions, with strong positive loading for eye–nostril distance (EN) and negative loading for internarial spacing (INS). In morphospace defined by the first two axes, specimens of the new species form a distinct cluster separated from both *D. chandai* and *D. stuarti*, indicating consistent morphological differentiation after accounting for body-size effects (Fig. 3).

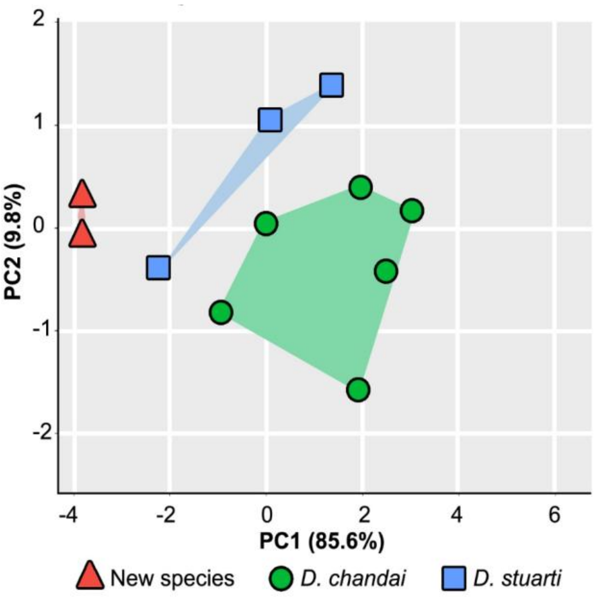

Figure 3. Principal component analysis (PCA) of size-corrected morphometric variables comparing the new species, *D. chandai*, and *D. stuarti*.

Taxonomy

Duttaphrynus ***dhara*** sp. nov.
[urn:lsid:zoobank.org:act:174AA912-9BCF-4E4E-9FBA-5E2DE7094C49]
(Figs. 4, 5; Sup. Tables 4, 5)

Holotype. ADBU-HW0180, adult male, collected from Mawphlang (25.45329°N, 91.75443°E; alt. 1854 m asl.), East Khasi Hills District, Meghalaya, India, on 25 July 2024 at ~17:50 hr by H. Warjri, W. Wahlang & R. Warjri.

***Paratype* (*n*=1).** ADBU-HW0182, adult male, collection data similar to the holotype.

Diagnosis. *Duttaphrynus dhara* sp. nov. is assigned to the genus *Duttaphrynus* by the presence of well-developed parotoid glands, a tuberculate dorsum bearing keratinized warts, distinct canthus rostralis, and the general bufonid body form. The new species is distinguished from congeners by its phylogenetic position and by the following combination of morphological characters: medium body size (male SVL 40.05–41.76 mm); head wider than long; tympanum distinct but small; canthus rostralis and canthal ridge present; preorbital, supraorbital, orbitotympanic, parietal, and postorbital ridges absent; fingers free of webbing; toe webbing moderate with formula I 1–2 II 2–2.5 III 2–3.5 IV 3.5–1.5 V; relative finger lengths I < II < IV < III; relative toe lengths I < II < III < V < IV; outer palmar tubercle distinct and rounded, inner palmar tubercle present but smaller; inner metatarsal tubercle oval and prominent, outer metatarsal tubercle rounded; subarticular tubercles distinct with supernumerary tubercles of varying sizes present on the digits; tibiotarsal articulation reaching the posterior tip of the parotoid gland when the hindlimb is adpressed along the body; dorsum bearing numerous pointed keratinized warts of varying sizes with irregular black patches; and nuptial pad present on Finger I, whitish in colour.

Description of holotype. Medium-sized specimen (SVL 41.76 mm) with a robust body. Head large (HL/SVL 0.27), wider than long (HW/HL 1.21). Cranial ridges indistinct. Canthus rostralis distinct; loreal region slightly concave and nearly vertical. Canthal ridge present; preorbital, supraorbital, orbitotympanic, parietal, and postorbital ridges absent. Interorbital space flat. Nostrils rounded, positioned closer to the snout tip than to the eye. Snout longer than eye diameter (SL/ED 1.34) and approximately half of head length (SL/HL 0.47). Interorbital distance larger than internarial space (IOD/INS 1.74). Eye large (ED/HL 0.35); eye diameter greater than upper eyelid width (ED/UEW 1.09) and greater than eye–nostril distance (ED/EN 1.15). Pupil round. Tympanum round, distinct, and very small relative to eye diameter. Pineal ocellus absent. Tongue elongated and slightly rounded posteriorly. Choanae small, round; vomerine teeth absent. Supratympanic fold absent. Parotoid glands present, elongated (PGL/PGW 1.73), prominent and perforated; almost twice as long (PGL 7.8 mm) as wide (PGW 4.5 mm), and longer than the distance separating them (PD 5.61 mm).

Dorsal skin bearing numerous glandular warts of varying sizes. Snout and interorbital region slightly smoother than the remainder of the dorsum. Spinules present on the temporal region and around the tympanum. Lower flank warts capped with keratinized (horny) tips and spinules. Latero-dorsal folds absent. Dorsal surfaces of limbs with numerous pointed spinules rather than granular tubercles. Ventral surfaces of head, body, and limbs granular. Macroglands represented by the parotoid glands.

Forelimbs strong; forearm longer than upper arm (FLL/UAL 1.33). Fingers relatively long and slender; relative finger lengths I < II < IV < III. Finger tips rounded, not expanded into discs, lacking grooves; dermal fringes absent. Webbing between fingers absent. Outer palmar tubercle distinct and rounded; inner palmar tubercle present but smaller than outer palmar tubercle. Nuptial pad present on Finger I. Subarticular tubercles distinct; numerous small pointed tubercles present on palm and fingers. Hindlimbs relatively long; tibia length slightly exceeding foot length (TL/FL 1.03). Relative toe lengths I < II < III < V < IV. Toe tips rounded, lacking discs. Toe webbing moderate with formula I 1–2 II 2–2.5 III 2–3.5 IV 3.5–1.5 V. Inner metatarsal tubercle oval and prominent (2.15 mm); outer metatarsal tubercle rounded (1.96 mm). Subarticular tubercles well developed; supernumerary tubercles present in various sizes. When the hindlimb is adpressed along the body, the tibiotarsal articulation reaches the posterior tip of the parotoid gland.

Colouration of holotype. After preservation (Fig. 4), the dorsal colouration fades to pale brown to greyish-brown, while the darker dorsal patches remain visible but less contrasting. The pale mid-dorsal line becomes faint but remains distinguishable. The distal portions of the fingers and toes remain darker than the surrounding surfaces. Ventral surfaces become off-white to pale cream with the dark blotches remaining clearly visible on the throat, chest, abdomen, and ventral surfaces of the limbs.

In life (Fig. 5), dorsal surfaces brown with irregular black patches scattered across the head, body, and limbs. A narrow pale mid-dorsal line is present, extending from the snout along the dorsum toward the vent. The distal portions of the fingers and toes are dark brown to black. Ventral surfaces creamy white, with numerous black, rounded-to-elongated blotches distributed across the throat, chest, abdomen, and ventral surfaces of the limbs.

Comparison. Herein, *Duttaphrynus dhara* sp. nov. is compared primarily with its most morphologically and geographically similar congeners, *D. chandai* and *D. stuarti*. Comparisons with additional species of *Duttaphrynus* and related bufonid taxa occurring in the Indo-Burma region are summarized in Table 3. *Duttaphrynus dhara* sp. nov. differs from *D. chandai* Das, Chetia, Dutta & Sengupta, 2013 by the following combination of characters: (1) smaller body size, SVL 40.05–41.76 mm vs. 61.2–88.1 mm in *D. chandai*; (2) tympanum distinct vs. indistinct or partly visible in *D. chandai*; (3) supraorbital ridge absent vs. present in *D. chandai*; (4) relative finger lengths I < II < IV < III vs. II < I < IV < III in *D. chandai*; (5) relative toe lengths I < II < III < V < IV vs. I < II < V < III < IV in *D. chandai*; (6) webbing formula I 1–2 II 2–2.5 III 2–3.5 IV 3.5–1.5 V vs. I 1.5–1.5 II 1.5–2.5 III 1.5–3.5 IV 3.5–1.5 V in *D. chandai*; and (7) tarsometatarsal articulation reaching the posterior end of the parotoid gland vs. reaching the middle of the parotoid gland in *D. chandai*.

Duttaphrynus dhara sp. nov. differs from *D. stuarti* (Smith, 1929) by the following characters: (1) smaller body size, SVL 40.05–41.76 mm vs. 54.06–71.3 mm in *D. stuarti*; (2) relative finger lengths I < II < IV < III vs. II < I < IV < III in *D. stuarti*; (3) webbing formula I 1–2 II 2–2.5 III 2–3.5 IV 3.5–1.5 V vs. I 1–2.5 II 2–2.5 III 2–3 IV 2.5–1.5 V in *D. stuarti*; and (4) tarsometatarsal articulation reaching the posterior end of the parotoid gland vs. reaching the tip of the snout in *D. stuarti*.

Diagnostic differences between *Duttaphrynus dhara* sp. nov. and other congeners from the Indo-Burma region, including *D. himalayanus*, *D. crocus*, *D. scaber*, *D. melanostictus*, *D. mamitensis*, *D. manipurensis*, *D. nagalandensis*, *D. wokhaensis*, *D. kiphirensis*, and *D. mizoramensis*, are summarized in Sup. Table 5.

Etymology. The specific epithet *dhara* is used as a singular noun in apposition and refers to the traditional Khasi female attire known as the “Dhara” (locally corresponding to the Jainsem). This garment, commonly woven from Muga silk, is worn by knotting the fabric over both shoulders and draping it to the ankles, and is typically characterized by horizontal and vertical border patterns with fringes along the lower margin. The name is proposed in recognition of the cultural heritage of the Khasi community of

Plate 16

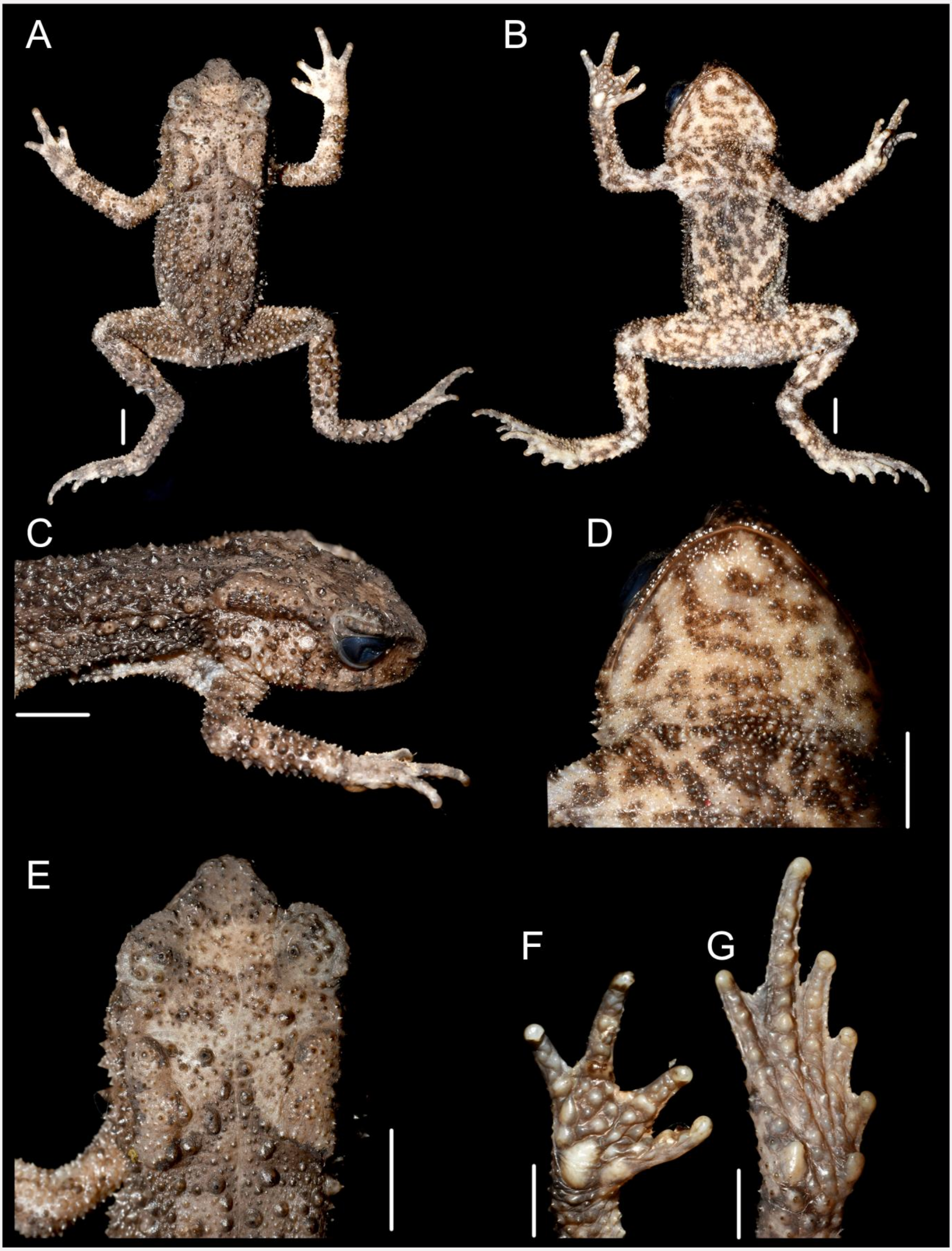

Figure 4. Holotype of *Duttaphrynus dhara* sp. nov. in preservative. **(A)** dorsal and **(B)** ventral views of the full body; **(C)** lateral view of the anterior body; **(D)** ventral and **(E)** dorsal views of the head; ventral views of **(F)** manus and **(G)** pes (scale = 5 mm)

Plate 17

Figure 5. *Duttaphrynus dhara* sp. nov. adult male in life: **(A)** holotype (ADBU-HW0180), and **(B)** Paratype (ADBU-HW 0182) collected from Mawphlang, East Khasi Hills District, Meghalaya, India

Meghalaya, whose traditions and way of life remain closely connected to the forests and landscapes of the region. By invoking *dhara*, a symbol of identity and tradition, the epithet honours the cultural legacy, stewardship of nature, and enduring relationship between people and biodiversity in the Khasi Hills, where this species occurs.

Natural history. All individuals of *Duttaphrynus dhara* sp. nov. were encountered in fallow agricultural fields situated adjacent to the montane forest in Mawphlang, East Khasi Hills. These fields are periodically cultivated by local indigenous communities for vegetable farming but were uncultivated at the time of collection. The specimens were observed during the late afternoon to early evening (approximately 17:50 h), indicating crepuscular activity. Individuals were found on the ground among low vegetation and leaf litter, suggesting a primarily terrestrial lifestyle.

The surrounding habitat consisted of forest edges interspersed with semi-natural open patches, characterized by relatively low levels of permanent human disturbance. The only other bufonid species observed in the surrounding landscape was *D. melanostictus*, a widespread species typically associated with human settlements. In contrast to the new species, *D. melanostictus* was abundant in villages and disturbed habitats but was rarely encountered near the collection site of *D. dhara* sp. nov. This pattern suggests ecological differentiation between the two species, with *D. dhara* sp. nov. appearing to prefer forest-adjacent or semi-natural habitats, whereas *D. melanostictus* is strongly synanthropic. The diet of the new species was not directly observed but is presumed to consist primarily of small terrestrial invertebrates, as in other members of the genus.

Distribution. *Duttaphrynus dhara* sp. nov. is currently known only from its type locality in Mawphlang, East Khasi Hills District, Meghalaya, northeastern India, at an elevation of approximately 1854 m a.s.l. The species appears to inhabit montane forest margins and adjacent semi-natural open habitats. Additional surveys in similar habitats across the Khasi Hills and neighbouring plateau systems are required to determine the full extent of its distribution.

Discussion

The evidence presented here unequivocally supports the recognition of *Duttaphrynus dhara* sp. nov. as a distinct species within the genus *Duttaphrynus*. The Khasi Hills population forms a well-supported monophyletic lineage in the mitochondrial 16S phylogeny and exhibits levels of genetic divergence from its closest congeners (*D. stuarti* and *D. chandai*) that fall within the range typically observed among recognized species of Asian bufonids. Morphometric analyses based on allometrically size-corrected characters further demonstrate clear separation of the new species from these taxa in multivariate morphospace, indicating consistent differences in body proportions independent of overall size. These quantitative results are congruent with a suite of diagnostic morphological traits, including small adult body size, absence of cranial ridges, distinctive digital proportions, moderate toe webbing, and a dorsum bearing numerous pointed keratinized warts. The concordance of molecular phylogenetic, multivariate morphometric, and qualitative morphological evidence provides a robust basis for delimiting the Khasi Hills population as a previously unrecognized evolutionary lineage within *Duttaphrynus*.

The genus *Duttaphrynus* includes several widely distributed species whose conservative external morphology has historically obscured species boundaries across large geographic ranges (Frost *et al*. 2006, Pyron & Wiens 2011). Increasingly, molecular phylogenetic studies have revealed that multiple nominal species within Asian bufonids represent complexes of deeply divergent lineages (Van Bocxlaer *et al*. 2009, Portik & Papenfuss 2015). Such cryptic diversity is particularly common in tropical regions where historical climatic oscillations, complex topography, and geographic barriers promote lineage diversification. The discovery of *D. dhara* sp. nov. adds to the growing body of evidence that the diversity of *Duttaphrynus* in South and Southeast Asia remains underestimated and that additional lineages likely await discovery, especially in poorly surveyed montane areas.

Northeastern India occupies a pivotal position in the biogeography of Asian amphibians, forming part of the Indo-Burma biodiversity hotspot, one of the most species-rich yet threatened regions on the planet (Myers *et al*. 2000). The region lies at the confluence of the Indian, Indo-Chinese, and Himalayan biogeographic realms and is characterized by a complex geological history and pronounced environmental heterogeneity (Mani 1974, Pawar *et al*. 2007). These factors have contributed to the exceptional amphibian diversity documented in the region, with recent integrative taxonomic studies revealing numerous previously unrecognized lineages (Kamei *et al*. 2012, Biju *et al*. 2014, Garg & Biju 2019). The Khasi Hills plateau of Meghalaya, where *D. dhara* sp. nov. occurs, represents an especially important center of montane biodiversity. Its high-elevation plateaus and dissected valleys create isolated ecological systems that may promote population divergence and local endemism.

The discovery of *D. dhara* sp. nov. further underscores the importance of montane habitats in driving amphibian diversification in northeastern India. Montane environments often function as "sky islands," where isolated populations may experience reduced gene flow and local adaptation, eventually leading to speciation. Similar patterns have been documented in several amphibian groups across the Indo-Burma region, where montane lineages frequently exhibit restricted distributions and high levels of endemism (Pawar *et al*. 2007, Biju *et al*. 2014). However, such elevational restricted systems are also vulnerable to climate change, as rising temperatures may force species to shift to higher elevations where suitable habitat may be limited or absent. The occurrence of *D. dhara* sp. nov. at elevations around 1850 m suggests that montane forest edges and associated semi-natural habitats in the Khasi Hills may harbour additional undescribed amphibian taxa.

Ecological observations from the type locality suggest that *D. dhara* sp. nov. occupies habitats at the interface between montane forest and semi-natural open areas with relatively low levels of permanent anthropogenic disturbance. This ecological preference contrasts with that of the widespread Asian common toad, *D. melanostictus*, which is strongly synanthropic and typically abundant in urban and village environments (Van Bocxlaer *et al*. 2009). The apparent scarcity of *D. melanostictus* near the type locality of *D. dhara* sp. nov. suggests ecological segregation between the two species. Such habitat partitioning between forest-associated and synanthropic bufonids has been observed in other parts of South and Southeast Asia and may reduce interspecific competition while allowing closely related species to coexist within the same broader landscape.

The morphometric analyses further reinforce the distinctiveness of *D. dhara* sp. nov. Principal component analysis of size-corrected morphological variables revealed clear separation between the new species and its closest congeners. The use of allometric size correction prior to multivariate analysis ensures that the observed differences reflect variation in body shape rather than simple size differences, thereby providing a more robust framework for morphological comparison (Chan & Grismer 2022). Integrating such quantitative morphometric approaches with molecular phylogenetic evidence represents an increasingly important methodological framework in modern taxonomy, allowing species boundaries to be evaluated using multiple independent lines of evidence.

Despite recent progress in documenting amphibian diversity in northeastern India, large portions of the region remain insufficiently surveyed, particularly in montane and forest-edge habitats. Continued integrative surveys combining field exploration, molecular phylogenetics, and quantitative morphological analyses will likely reveal additional undescribed taxa and refine our understanding of amphibian biogeography in the Indo-Burma region. Such work is especially urgent given the rapid environmental changes occurring throughout the region, including land-use transformation and climate-driven habitat shifts.

At present, *Duttaphrynus dhara* sp. nov. is known only from its type locality in Mawphlang, East Khasi Hills District, Meghalaya, at approximately 1854 m a.s.l. Although the species appears to occupy semi-natural habitats adjacent to montane forests, its full geographic distribution, ecological requirements, and population status remain unknown. Additional field surveys across the Khasi Hills and adjacent plateau systems are necessary to determine whether the species is narrowly endemic or more broadly distributed within northeastern India. Given its apparently restricted range and specialized habitat association, further ecological and conservation assessments will be essential to evaluate its chances of long-term persistence.

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Author contributions

All the authors contributed equally.

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Supplemental data

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Appendix. Comparative material examined

Duttaphrynus chandai: India – Nagaland: ZSIC A 11453 (Holotype), 11454–6; Mizoram: MZMU 884–887.

Duttaphrynus stuarti: Myanmar – Kachin State: BMNH 1947.2.1.73 (holotype), CAS 213826–8, 221485.