

**Occurrence of *Minervarya asmata* (Howlader, 2011): A new state record of
Meghalaya, Northeast India**

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ABSTRACT

The Bangladeshi Cricket Frog (*Minervarya asmata*), previously recorded in Bangladesh, Manipur, and Mizoram, is newly reported from Shillong and Cherrapunjee, Meghalaya, India. During a herpetological survey in March 2024, three male specimens were collected from paddy fields and ephemeral pools. Morphological traits matched existing descriptions, and phylogenetic analysis of 16S rRNA sequences confirmed species identity with 100% bootstrap support. This finding extends the known range northward by 260 km. With *M. asmata*, Meghalaya's amphibian diversity reaches 62 species. The study emphasizes the need for further surveys to document amphibian distribution and assess conservation priorities in the region.

Keywords: Amphibian diversity, Meghalaya, *Minervarya asmata*, Phylogenetics

INTRODUCTION

Meghalaya, located within the Eastern Himalayas and the Indo-Myanmar Biodiversity Hotspots, boasts an exceptional diversity of flora and fauna. This unique geographical position facilitates ecological connectivity between species from Southeast Asia and Peninsular India, thereby contributing to the region's high biodiversity. The state is recognized for hosting one of the most diverse amphibian populations in India, thriving within its varied landscapes, including hills, valleys, rivers, waterfalls, and grasslands. These diverse

~~habitats support a high degree of endemism among different amphibian groups, such as anurans (frogs and toads), salamanders (tailed amphibians), and caecilians (limbless amphibians).~~

~~Earlier, Mathew & Sen (2010) documented a total of 49 amphibian species in Meghalaya [3]. However, with continuous research and extensive surveys, the number of recorded amphibians in the state has significantly increased. Recent documentation has raised this count to 61 species, found across various forest habitats, including sacred groves and protected areas. Surveys conducted since 2015 (Tron et al., 2021) [8] have further underscored the rich amphibian diversity in Meghalaya, emphasizing its ecological importance and the need for conservation efforts.~~

Meghalaya remains a focal point for amphibian discoveries, with new species frequently being recorded. Among the notable discoveries ~~is-include~~ *Raorchestes shillongensis* from Shillong [1]. Several species of the genus *Leptolalax* have also been identified, including *Leptolalax khasiorum* from Mawphlang [2] and *Leptolalax nokrekensis* from the Nokrek Biosphere Reserve [3]. A particularly significant find is *Chikila gaiduwani*, a new genus of limbless amphibian from Tura, Garo Hills [4]. Additionally, recent studies have revealed new species of megophryid frogs, such as *Xenophrys megacephala* from Ri Bhoi [5], *Xenophrys oropedion* from Malki Forest [6], and *Xenophrys falvipunctata* from Mawphlang Sacred Grove, along with *Xenophrys oreocrypta* from Tura, Garo Hills [7].

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Moreover, emphasize that more discoveries are likely to be made in the future such as the case for the *Minervarya* species.

~~count to 61 species, found across various forest habitats, including sacred groves and protected areas. Surveys conducted since 2015 (Tron et al., 2021) [8] have further underscored the rich amphibian diversity in Meghalaya, emphasizing its ecological importance and the need for conservation efforts.~~

The genus *Minervarya*, belonging to the Dicroglossidae family, was first described by Dubois, Ohler, & Biju in 2001 [9]. This genus comprises 31 species distributed across Eastern India, the Andaman Islands, Nepal, Bangladesh, and Thailand [10]. Commonly referred to as cricket frogs, these species have been classified into different groups, namely *M. nilagirica*, *M. rufescens*, *M. sahyadris*, and *M. syhadrensis*, as proposed by Yadav et al. [11].

The Bangladeshi Cricket Frog, *Minervarya asmata*, was first described from Hathazari in the Chittagong District of Bangladesh. Subsequent records also confirmed its presence in Dhaka District and Nazipur, Bangladesh, as documented by Ahmad & Alam in 2015 [12]. More recently, sightings of *M. asmata* have been reported from Manipur and Mizoram in Northeast India by Decemson et al. in 2021 [13]. Despite these findings, the conservation status of *Minervarya asmata* remains unevaluated by the IUCN Red List [14].

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This study provides new evidence of *M. asmata*'s presence in Shillong and Cherrapunjee, Meghalaya, Northeast India, extending the known range of this species. These findings contribute to the growing body of knowledge regarding amphibian diversity in the state and reinforce the importance of continued research and conservation efforts to safeguard Meghalaya's amphibian populations.

MATERIALS AND METHODS

During a herpetological survey in Meghalaya, two adult male frog specimens, each with a snout-vent length (SVL) of 30.2 ± 0.5 mm ($N = 2$), were collected on March 16, 2024, at

11:00 AM. The collection site was a paddy field near North Eastern Hill University, Mawlai Umshing Mawkynroh, Shillong (25.3627°N, 91.5400°E) at an elevation of 1,520 meters above sea level (m asl) (Fig. 1A & 1B). An additional male specimen, measuring 29.76 mm in SVL, was collected two days later, on March 18, 2024, at 4:00 PM from an ephemeral pool in Cherrapunjee (25.1633°N, 91.4309°E; elevation: 1,484 m asl) (Fig. 1A & 1C).

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The collected specimens were euthanized using MS222, with liver tissue preserved in molecular-grade ethanol at -20°C for subsequent genetic analysis. The specimens were then fixed in 10% formalin, transferred to 70% ethanol for long-term preservation, and deposited in the Museum of the Department of Zoology, St. Edmund's College, Shillong. The catalog numbers assigned were SECZOO-AN-22 and SECZOO-AN-23 for the specimens from Mawlai Umshing Mawkynroh and SECZOO-AN-24 for the specimen from Cherrapunjee.

Phylogenetic analysis was performed following the protocols outlined by Gopalan et al. (2014) [15]. DNA was extracted from the preserved liver tissue, and the mitochondrial 16S rRNA gene was amplified through polymerase chain reaction (PCR). Partial sequences of the 16S rRNA gene were retrieved from the National Center for Biotechnology Information (NCBI) database and submitted to GenBank under accession numbers ATE1 and ATE2 for the specimens from Mawlai Umshing Mawkynroh, and ATE3 for the specimen from Cherrapunjee.

For comparative analysis, 13 previously published 16S rRNA sequences from the NCBI database were utilized. *Megophrys montana* (MZB-Amp: 30897) [16] and *Boulenophrys daweimontis* (KIZ048920) [17] were included as out-group taxa. Maximum Likelihood (ML) phylogenetic analysis was conducted on a sequence dataset comprising 568 base pairs (bp) of 16S rRNA using MEGA 11.0 software [18].

RESULTS AND DISCUSSION

The obtained results from both morphological and genetic analyses strongly support the identification of the collected specimens as *Minervarya asmata*. Morphological examinations of the male individuals from Mawlai Umshing Mawkynroh (Fig. 2) revealed defining characteristics consistent with the descriptions provided by Howlader (2011) [19] and later reaffirmed by Decemson et al. (2021) [13]. These characteristics include a distinct lateral line along both sides of the belly, smooth skin with small warts and folds, the absence of a rectal gland at the mouth corner, slightly barred lips, unwebbed fingers, partially webbed toes, and a butterfly-shaped vocal marking in males. These key morphological traits strongly align with previously described specimens, thereby confirming the identification of *M. asmata* in Meghalaya.

This morphological identification was further reinforced by molecular evidence. Phylogenetic analysis of the mitochondrial 16S rRNA sequences (ATE1, ATE2, ATE3) showed a strong genetic affinity with *M. asmata* voucher sequences (FaCSE 17) [20]. The specimens formed a well-supported monophyletic group with 100% bootstrap support (Fig. 3), clearly distinguishing them from other closely related species. The high confidence in this clustering mirrors the findings of Decemson et al. (2021) [13], further validating the taxonomic placement of the specimens. This molecular evidence highlights the reliability of 16S rRNA sequencing as a tool for amphibian taxonomy, especially in regions with high biodiversity like Meghalaya.

These results align with the broader taxonomic revisions concerning cricket frogs, particularly the ongoing debate surrounding the classification of the family Dicroglossidae. The discovery of *M. asmati* within Meghalaya strengthens the argument for recognizing *Minervarya* as a distinct genus from *Fejervarya*, a division that has been widely supported by strong phylogenetic evidence [21]. The separation of South Asian species into *Minervarya* and Southeast Asian species into *Fejervarya* highlights their evolutionary divergence and reinforces the monophyly of each genus [21]. This finding supports previous studies that advocate for a clearer taxonomic distinction between these genera, thereby contributing to a more refined understanding of amphibian systematics.

The identification of *M. asmati* in Shillong and Cherrapunjee also carries significant biogeographical implications. It extends the species' known range by approximately 260 km and 170 km northward, respectively, from Chittagong, Bangladesh. This range expansion is notable as it bridges the geographical gap between the previously recorded populations in Bangladesh and the newly documented occurrences in Northeast India. This extension of its known distribution contributes to the growing documentation of amphibian diversity in Meghalaya, raising the total recorded species count from 61 (Tron et al., 2021) [8] to 62 with the addition of *M. asmati*. The discovery of this species in Meghalaya underscores the state's importance as a biodiversity hotspot for amphibians.

Despite these significant findings, the ecological and behavioral aspects of *M. asmati* remain largely unexplored. The observed presence of this species in lateritic plateaus and paddy fields suggests an affinity for open, water-associated environments. However, there is a need for further research to investigate its habitat preferences, reproductive behavior, and population dynamics. Understanding these ecological parameters will be crucial for assessing the conservation needs of *M. asmati* within Meghalaya's diverse landscape. Given the

ongoing habitat alterations due to agricultural expansion and urbanization, future conservation efforts should focus on habitat protection and monitoring population trends to ensure the long-term survival of *M. asmati* in Meghalaya.

CONCLUSION

This study marks a significant milestone in the documentation of amphibian diversity in Meghalaya by reporting the first recorded occurrence of *Minervarya asmati* in the region. The findings highlight the continued expansion of known amphibian species distributions in Northeast India and reinforce the critical role of systematic field surveys, morphological assessments, and molecular phylogenetics in understanding biodiversity.

The identification of *M. asmati* in Shillong and Cherrapunjee represents an approximately 260 km and 170 km northward extension of its previously known range from Chittagong, Bangladesh. Such discoveries emphasize Meghalaya's strategic biogeographical position within the Eastern Himalayas and Indo-Myanmar Biodiversity Hotspot, acting as a corridor for species dispersal between South and Southeast Asia. Phylogenetic analysis demonstrated a strong genetic affinity between the collected specimens and previously documented *M. asmati* sequences, forming a well-supported monophyletic group. These findings contribute to the ongoing taxonomic revisions of *Minervarya* and its differentiation from closely related genera such as *Fejervarya*.

The confirmation of *M. asmati* in Meghalaya also has broader implications for amphibian conservation. As habitat fragmentation, agricultural expansion, and climate change continue to threaten amphibian populations worldwide, the discovery of new or previously unrecorded species underscores the importance of habitat protection. The presence of *M. asmati* in

lateritic plateaus and paddy fields suggests an ecological preference for open, water-associated environments. However, additional research is needed to better understand its habitat specificity, reproductive biology, and seasonal activity patterns. Conservation initiatives must be strengthened to ensure the preservation of these vital ecosystems, enabling future generations to study and appreciate the remarkable amphibian diversity of the region.

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UNDER PEER REVIEW

Figures

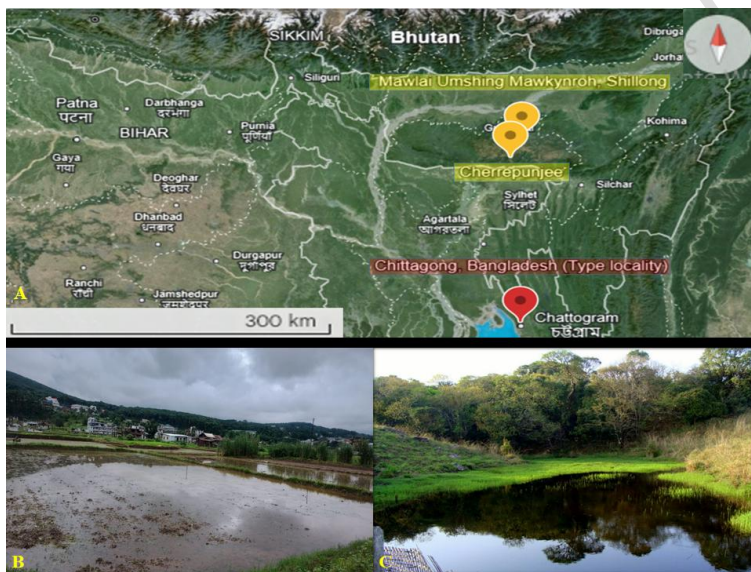


Figure 1 (A-C): A. A map illustrating the collection sites of *Minervarya asmati* from Mawlai Umshing Mawkynroh in Shillong (25.3627°N, 91.5400°E; Elevation: 1520 m asl) and Cherrapunjee (25.1633°N, 91.4309°E; Elevation: 1484 m asl) in Meghalaya, along with the type locality in Chittagong, Bangladesh. B. Collection site at Mawlai Umshing Mawkynroh. C. Study site at Cherrapunjee.



Figure 2: A male *M. asmati* observed at the ephemeral pool in Cherrapunjee, Meghalaya (SVL 29.76 mm).

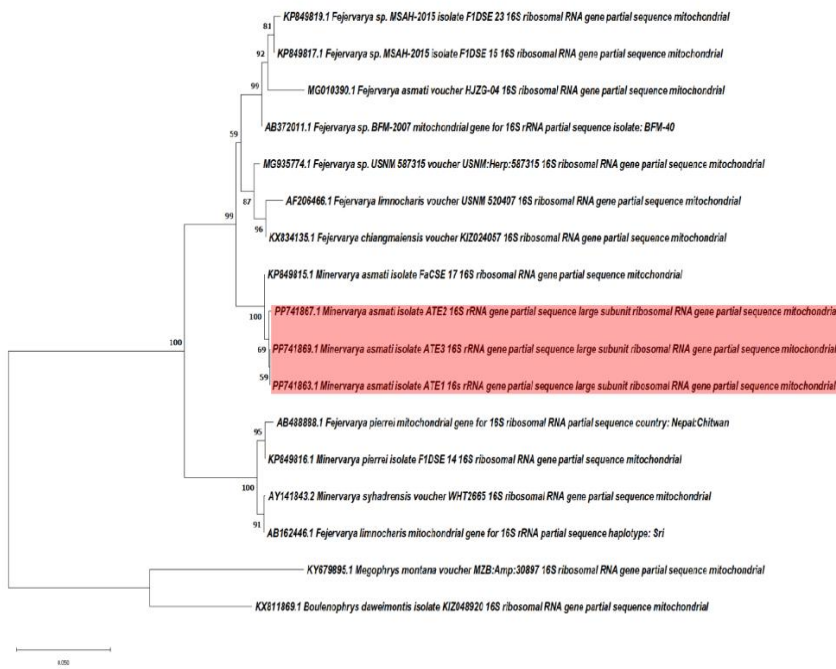


Figure 3: A Maximum Likelihood (ML) phylogenetic tree constructed using 568 bp sequences of the mitochondrial DNA (16S rRNA) gene.