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# Integrative taxonomy of the backswimmer genus *Enithares* (Hemiptera: Notonectidae) from India, with two new records

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## ABSTRACT

*The present study made an integrative taxonomic assessment of the backswimmer genus Enithares Spinola, 1837 (Hemiptera: Notonectidae) from the Eastern Ghats of India, combining morphological, morphometric, and molecular evidence. Two species, Enithares intricata Breddin 1905 and Enithares metallica Brooks, 1948, are reported for the first time from the Indian subcontinent. Morphological differentiation was established using diagnostic characters, viz., body size, pronotal structure, eye morphology, metaxyphus, mid femur, male genitalia, etc. Phylogenetic reconstruction using the Maximum Likelihood method revealed pairwise genetic divergences among the species ranging from 4.7% to 16.2%. Morphometric variation among the species was further evaluated using Principal Component Analysis (PCA), which revealed clear clustering of the three taxa (E. fusca, E. metallica, and E. intricata) based on measured morphological traits. In addition, violin plot analyses morphometric parameters such as body shape and head length, highlighting differences in their morphological ranges. The integration of morphological, molecular, and morphometric evidence confirms the taxonomic identity of the species and highlights the importance of integrative approaches in resolving species boundaries within aquatic Heteroptera. Furthermore, the number of Enithares species known from India increases to ten.*

**Keywords:** *Enithares metallica, Enithares intricata, Taxonomy, DNA barcoding, new records, Eastern Ghats.*

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## Introduction

The Notonectidae family (Hemiptera: Heteroptera), or backswimmers, is a unique lineage of freshwater insects found extensively throughout tropical and temperate areas. These insects are fully adapted for life within the water and are frequently found in still or slowly moving freshwater bodies such as ponds, pools, and streams [1,2]. Notonectids can be easily identified by their convex dorsum, paddle-shaped hind legs adapted for paddling, and their mode of behavior of swimming on their backs [3]. Being voracious predators, they perform an important ecological function in controlling aquatic invertebrate populations and act as indicators of freshwater habitat quality. Taxonomically, Notonectidae includes several genera placed in various subfamilies, and the genus *Enithares* Spinola, 1837, is one of the most heterogeneous and morphologically striking in the Oriental and Australasian fauna [4,5,6]. Species of *Enithares* are of medium to large size, with an elongate-oval body, convex and keeled dorsum, and characteristic male genitalia diagnostic at the species level [7,8]. The adult is a vigorous flyer and swimmer and is usually found in shaded forest pools, rice paddies, and sluggish streams, frequently sympatric with congeners or other Notonectidae.

There are more than sixty species of *Enithares* reported worldwide, of which the Oriental region has the most diversity [6,9].

Although the recent taxonomic revisions and faunal surveys have produced new distributional records and expanded the group's recognized diversity [10,11,12,13]. Despite their morphological diversity and ecological value, Indian species of *Enithares* are still not well understood, and many regions lack comprehensive inventories. Therefore, further integrative research combining morphological and molecular approaches is essential. This study was made an attempt on the genus *Enithares* from the Eastern Ghats of India.

## Materials and Methods

The *Enithares* specimens were collected from the Eastern Ghats of Telangana and Andhra Pradesh, India (Fig. 1). Collected specimens were preserved in 90% ethanol and observed under Leica EZ4 and photographed through Leica M205A stereo-zoom microscopes. Identification was done following standard literature [7,8,14]. The identified specimens were registered and deposited in the National Zoological Collections of Zoological Survey of India, Freshwater Biology Regional Centre, Hyderabad. Patterns of morphometric variation among the examined species were assessed using Principal Component Analysis (PCA) implemented in R version 4.3.1. The morphometric dataset was standardized before analysis to minimize the influence of differences in measurement scales among variables.

PCA was applied to reduce the dimensionality of the dataset and to visualize patterns of morphological differentiation among the studied taxa [15]. Additionally, violin plots were generated to illustrate the distribution of selected morphometric variables among species [16]. These plots combine kernel density estimation with summary statistics to represent the range, variability, and density of measurements within each taxon. The violin plots were produced in RStudio using the ggplot2 package, providing a clear visualization of interspecific variation in the measured morphological characters.

The genomic DNA was extracted from the base of the mid and forelegs of the preserved specimens by using Dneasy® Blood & Tissue kit (Qiagen, Germany). Amplification was done through primer LCO-1490 5'-GGTCAACAAATCATAAAGATATTGG-3', HCO-2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' for the mitochondrial Cytochrome Oxidase Subunit I (COI) gene (~620bp) [17]. Polymerase chain reaction (PCR) was prepared for 25µl, which contains 12.5µl of 2x PCR Taq mixture, 0.5µl (10 pmol/µl) of each primer, 10.5µl of molecular grade water, and 1µl of template DNA used for both genes. The thermal cycle profile was set as 95ºC for 1 min, 94ºC for 30sec, 48ºC for 30sec, 35 cycles, 72ºC for 1 min. 0.5% Agarose gel was used to check the DNA [18]. The PCR products were purified using the NucleoSpin® Gel and PCR Clean-up Kit, following the manufacturer's protocol. Forward and reverse sequences were generated using the Sanger sequencing method by Eurofins Genomics (Ebersberg, Germany). The resulting chromatogram files from three species were processed in MEGA 11 [19], a software package used for molecular evolutionary genetics analysis. The molecular sequence data generated in the present study have been deposited in the GenBank database. Details of these newly generated sequences, along with sequences retrieved from NCBI GenBank, are provided in Table 1. Following sequence generation, each sequence was quality-checked and trimmed to remove ambiguous base calls using MEGA 11. The processed sequences were then subjected to BLAST (Basic Local Alignment Search Tool) searches on the NCBI website (https://www.ncbi.nlm.nih.gov) to identify homologous sequences and confirm taxonomic identity. BLAST results were carefully reviewed, and sequences showing high similarity (≥98%) and appropriate taxonomic resolution were selected from the NCBI GenBank database. A total of 15 such sequences were retrieved and included in the final dataset for phylogenetic analysis (Tab. 1). All sequences, both newly generated and GenBank-derived, were aligned using the ClustalW algorithm implemented in MEGA 11. The alignment was manually checked and adjusted as necessary to ensure homology across taxa. Genetic divergences were then calculated using the Kimura-2-Parameter (K2P) model in IQTREE to assess inter- and intra-specific variability. To infer evolutionary relationships, a phylogenetic tree was constructed using the Maximum Likelihood (ML) method under the Kimura-2-parameter model [20], as implemented in IQTREE. The tree with the highest log likelihood value (-2566.14) is presented in the results. Statistical support for internal nodes was assessed using a bootstrap analysis with 1000 replicates. *Metrocoris communis* (Distant, 1910) was included as an outgroup to root the tree and clarify phylogenetic relationships among the ingroup taxa.

## Results

**Key to the species of *Enithares***

1. Dorsal surface of hemelytra with distinct metallic bluish or greenish sheen; body relatively large; body broad and robust....................................................*Enithares metallica* (Fig. 3)

Dorsal surface without metallic sheen; body smaller or moderately sized ........................................................ 2

2. Hemelytra uniformly dark brown to blackish, lacking pale or yellow maculation; vertex comparatively broader; dorsal pattern almost uniform ....................*Enithares fusca* (Fig. 4)

Hemelytra with distinct pale yellow or ochraceous markings on basal or lateral regions .........................................................................3

3. Hind legs bearing conspicuous fringe-like hairs (ciliation); body comparatively larger; hemelytral markings usually pale ochraceous; vertex moderately narrow ..........................................4

4. Hind legs without conspicuous ciliation; body comparatively smaller; hemelytra with characteristic pale-yellow basal patches; vertex relatively narrow.........*Enithares intricata* (Fig. 2)

**Taxonomic Hierarchy**

**Order** Hemiptera Linnaeus, 1758
**Suborder** Heteroptera Latreille, 1810
**Infraorder** Nepomorpha Popov, 1971
**Superfamily** Notonectoidae Latreille, 1802
**Family** Notonectidae Latreille, 1802
**Subfamily** Notonectinae Latreille, 1802
**Genus** *Enithares* Spinola, 1837

***1. Enithares intricata* Breddin 1905 (Fig. 2A-E)**

**Material examined:** 5 exs, 23.viii.2025, Mallela Theertham waterfalls, Amrabad Tiger Reserve, Telangana; 1 ex, 04.xi.2025, Papavinashanam; 9 exs, 20.ii.2026, Mori kinda bavi, KG penta beat; 7 exs, 19.ii.2026, Palakondalu waterfalls, Seshachalem Biosphere Reserve, Andhra Pradesh, Coll. S. Banerjee & Deepa J.

**Diagnosis:** Small-sized species with an elongate-oval body and strongly convex dorsum. Head broad with large, prominent compound eyes and a relatively narrow vertex. Pronotum trapezoidal with slightly rounded lateral margins. Scutellum triangular and moderately elevated. Hemelytra smooth and glossy, tapering posteriorly to form a pointed apex when closed. Distinct pale-yellow patches are present near the basal region of the hemelytra and along the lateral margins, contrasting with the darker central disc. Rostrum short and adapted for predatory feeding. Legs slender; hind legs elongated with dense natatorial hairs facilitating efficient swimming.

**Measurements (mm):** Body size ranges (♂) from 9.09-9.23, body width 3.23-3.53, head length 2.01-2.38, head width 2.67-2.78, pronotum length 0.93-1.21, eye length 1.87-2.03, eye width 1.27-1.36, interocular space 0.83-0.97, scutellum length 1.66-1.81.

**Coloration:** Dorsal surface predominantly dark brown to blackish with contrasting yellow to pale ochre markings. Head bearing a conspicuous yellow band across the anterior region between the compound eyes. Pronotum dark with faint pale margins. Hemelytra glossy blackish with distinct yellow patches at the basal and lateral regions, occasionally forming irregular triangular markings. Ventral surface yellowish to light brown. Legs pale yellow to yellowish-brown, with hind femora slightly darker. Compound eyes dark reddish-brown to black.

**Habitat:** A typical aquatic predator inhabiting freshwater lentic environments such as ponds, marshes, slow-moving streams, irrigation tanks, and paddy fields. The species prefers vegetated shallow margins where it preys upon small aquatic insects, larvae, and other invertebrates.

**Distribution: India:** Seshachalam Biosphere Reserve (Andhra Pradesh) and Amrabad Tiger Reserve (Telangana).

**Elsewhere:** Java and Sumatra [7].
**Note:** The species can be readily distinguished from other Oriental congeners by its characteristic dorsal coloration pattern with pale yellow patches on the hemelytra and the relatively narrow posterior body apex. Its occurrence in the Eastern Ghats indicates that the species may be more widely distributed in suitable freshwater habitats across the Indian peninsula than previously documented.

***2. Enithares metallica*** **Brooks, 1948 (Fig. 3A-E)**
**Material examined:** 5 exs, 04.xi.2025, Papavinashanam; 1 ex, 04.xi.2025, KP Dam, Seshachalam Biosphere Reserve, Tirupati, Andhra Pradesh, Coll. S. Banerjee & Deepa J.

**Diagnosis:** Medium sized species, elongate body and distinctly convex dorsal surface. The head is relatively broad with large hemispherical compound eyes and a narrow vertex. Pronotum trapezoidal with gently rounded lateral margins and slightly broader posterior margin. Scutellum triangular and moderately elevated. Hemelytra smooth, shining, and distinctly tapering posteriorly, forming a rounded apex when closed. The dorsal surface is notable for its metallic sheen, which is a key feature distinguishing this species from many congeners. The clavus and corium are well developed with a clear claval commissure. Hind legs elongated and equipped with dense natatorial setae that facilitate efficient swimming.

**Measurements (mm):** Body size ranges (♂) from 12.01-12.22, body width 5.11-5.18, head length 2.39-2.48, head width 3.74-3.83, pronotum length 1.38-1.40, eye length 1.84-1.87, eye width 1.60-1.69, interocular space 0.85-0.86, scutellum length 2.91-2.93.

**Coloration:** Dorsal surface generally dark brown to blackish with a distinct metallic bluish or greenish sheen, especially visible on the hemelytra. Head usually dark with pale yellow or ochraceous markings on the anterior region near the eyes. Pronotum dark brown to black with faint pale margins. Hemelytra glossy with metallic reflections and sometimes with faint pale patches near the basal region. Ventral surface yellowish to light brown. Legs pale yellow to yellowish-brown; hind femora may appear slightly darker. Compound eyes dark reddish-brown to black.

**Habitat:** Like most members of Notonectidae, *E. metallica* inhabits freshwater lentic habitats including ponds, marshes, lakes, irrigation tanks, and slow-flowing streams. The species typically swims upside down just below the water surface. Individuals are often found near submerged or marginal aquatic vegetation, which provides shelter and hunting sites.

**Distribution: India:** Andhra Pradesh (Seshachalam Biosphere Reserve).

**Elsewhere:** Myanmar, Thailand, Malaysia, Indonesia [6].

**Note:** The specimens examined correspond well with the original description of *E. metallica* and subsequent taxonomic treatments of the genus (Brooks, 1951; Lansbury, 1965).

The species is readily recognized by its elongate-oval body, strongly convex dorsum, and the distinctive metallic sheen on the hemelytra, which differentiates it from many other Oriental species of *Enithares*. The combination of smooth hemelytral surface, narrow posterior body outline, and characteristic dorsal coloration further supports its identification. The discovery of this species in the Eastern Ghats suggests a broader biogeographic distribution within the Oriental region and highlights the importance of systematic surveys in Indian freshwater ecosystems.

***3. Enithares fusca*** **Brooks, 1948 (Fig. 4A-E)**
**Material examined:** 3 exs, 24.xii.2021, Mallela Theertham waterfalls (Amrabad Tiger Reserve), Nagarkurnool, Telangana; 3 exs, 19.vi.2024, Jamiguda, Narsipatnam; 11 exs, 20.vi.2024, Galikonda viewpoint, Visakhapatnam, Andhra Pradesh, Coll. S. Banerjee & Deepa J.

**Diagnosis:** Body elongate-oval, moderately depressed; head narrow with moderately large, prominent compound eyes; vertex moderately broad. Antennae slender, segments distinctly setose. Pronotum trapezoidal with gently rounded lateral margins; anterior and posterior margins nearly straight. Hemelytra elongate with slightly convex corium, surface finely punctate; membrane well developed. Corium generally unicolorous, dark brown to blackish, lacking conspicuous pale maculation seen in some congeners. Legs relatively slender, with hind femora and tibiae bearing fine setae; hind legs adapted for swimming with dense natatorial hairs. Rostrum long, reaching posterior margin of the prosternum.

**Measurements (mm):** Body size ranges (♂) from 12.30-12.41, body width 5.52-5.60, head length 2.69-2.73, head width 3.75-3.83, pronotum length 1.62-1.65, eye length 2.80-2.85, eye width 1.65-1.71, inter ocular space 1.03-1.06, scutellum length 1.90-1.94.

**Coloration:** Dorsum uniformly dark brown to blackish; head dark brown with genae slightly paler. Antennae brown with gradual darkening toward apical segments. Pronotum dark brown, margins slightly lighter. Hemelytra dark brown to black throughout, without contrasting light patches; veins sometimes slightly paler. Ventral surface dark brown, sternites and mesosternum slightly lighter. Legs dark brown with coxae and bases of femora lighter brown. Eyes dark reddish-brown to black.

**Habitat:** This species inhabits freshwater lentic ecosystems, including ponds, marshes, irrigation tanks, lakes, and slow-moving streams. Individuals are commonly found in shallow vegetated margins of water bodies where aquatic plants provide shelter and prey availability. Adults are capable of flight and are frequently attracted to artificial lights during the night, which facilitates dispersal between water bodies.

**Distribution: India:** Andhra Pradesh (Present record), Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, Telangana [21].

**Note:** Although *E. fusca* has been previously reported from several regions of central and peninsular India, the present study provides the first molecular confirmation of this species from the country using mitochondrial (COI) DNA barcoding.

Morphologically, the specimens examined match the diagnostic features described in earlier taxonomic works [7,8], including the elongate body form, pale basal hemelytral markings and the characteristic fringe-like setae along the hind legs.

**Molecular Identification**

Molecular identification of the examined specimens was performed using mitochondrial cytochrome c oxidase subunit I (COI) sequences, and genetic divergences were estimated using the Kimura-2-Parameter (K2P) model. The analysis revealed clear genetic separation among the examined *Enithares* species (Fig. 2). The sequences of *Enithares intricata* (PZ325614 and PZ325615) showed very low intraspecific divergence (0.1%), confirming their conspecific identity and indicating minimal genetic variation within the species. In contrast, *E. intricata* exhibited substantial interspecific divergence from other congeners, ranging from 13.6-14.1% with *E. fusca*, 16.2-16.8% with *E. metallica*, 14.5-15.1% with *E. sinica*, 14.9-15.1% with *E. woodwardi,* and 15.7% with *E. hungerfordi*. Similarly, sequences of *Enithares metallica* (PZ325616 and PZ325617) formed a well-defined genetic group with a very low intraspecific divergence of 1.1%. However, *E. metallica* showed considerable genetic divergence from other *Enithares* species, including 16.5-16.8% from *E. intricata*, 7.1-8.2% from *E. fusca*, 11.8-12.4% from *E. sinica*, 10.6-11.3% from *E. woodwardi,* and 9.9-11% *E. hungerfordi*. The sequences of *Enithares fusca* (MN939472 and Pz325613) also displayed minimal intraspecific divergence (1.6%), supporting their taxonomic conspecific nature. Interspecific divergence between *E. fusca* and other congeners ranged from 13.7-14.1% with *E. intricata*, 7.0–8.2% with *E. metallica*, 10.2–11.2% with *E. sinica*, 14.9-15.7% with *E. hungerfordi*, and 6.4–7.1% with *E. woodwardi*.

Overall, the observed interspecific genetic divergence among *Enithares* species ranged from approximately 6.4% to 16.8%, clearly exceeding the low intraspecific divergence values (0.8–1.6%) observed within species. These divergence levels are consistent with typical COI barcode thresholds for species-level differentiation in aquatic Heteroptera and strongly support the distinct taxonomic status of *E. intricata*, *E. metallica*, and *E. fusca*. Furthermore, the much higher divergence observed between *Enithares* species and the outgroup taxa (*Anisops sardeus* and *Metrocoris communis*; 14.6–24.0%) confirms the phylogenetic separation of these genera and validates the reliability of COI barcoding for species delimitation within the family Notonectidae.

**Principal Component Analysis (PCA)**

Morphometric variation among the species of *Enithares* was analyzed based on selected morphological traits using Principal Component Analysis (PCA). The PCA ordination revealed clear separation among the three species studied (*E. fucsa*, *E. metallica*, and *E. intricata*), indicating distinct morphometric patterns among the taxa. The first two principal components accounted for the majority of the total variance in the dataset and effectively discriminated against the species in the ordination space (Fig. 6). Specimens of each species formed relatively discrete clusters, demonstrating that the measured morphometric variables contribute significantly to species differentiation within the genus. In the PCA scatter plot, specimens of *E. metallica* and *E. fusca* showed partial proximity along the first principal component, suggesting certain morphological similarities between these species, whereas *E. intricata* formed a more distinct cluster separated along the second principal component.

This pattern indicates that while some morphometric traits overlap among closely related species, the overall morphometric structure remains sufficiently distinct to separate the taxa.

The distribution patterns of key morphometric characters were further visualized using violin plots. The violin plot of body size (BS) demonstrated clear differences in the range and distribution of measurements among the three species (Fig. 7). *Enithares metallica* generally exhibited relatively larger body size compared to the other species, whereas *E. intricata* and *E. fusca* showed comparatively narrower ranges. Similarly, the violin plot of head length (HL) revealed distinct variation among species (Fig. 8). The distribution patterns indicate species-specific ranges in head length measurements, with limited overlap among the taxa. These results highlight the usefulness of quantitative morphometric characters in supporting species delimitation within the genus.

**Discussion**

The present study provides new taxonomic and molecular insights into the genus *Enithares* Spinola, 1837 from the Eastern Ghats of peninsular India. *Enithares metallica* Brooks, 1948 and *E. intricata* Breddin, 1905, were recorded for the first time from the Indian subcontinent. Previous records indicate that both species are primarily distributed across Southeast and East Asia, including Myanmar, Thailand**,** Malaysia, Indonesia [2,6,7]. Their occurrence in the Eastern Ghats considerably extends the known distribution range westwards within the Oriental biogeographic region and highlights the region's importance for aquatic insect diversity. Diagnostic characters such as body shape, pronotal structure, dorsal coloration, hemelytral pattern, metaxyphus, mid femur, and configuration of male genitalia are widely regarded as reliable taxonomic features in *Enithares* [4,7,8]. In the present study, *E. metallica* was readily identified by the characteristic metallic sheen of the dorsal surface and its overall body proportions, whereas *E. intricata* was distinguished by its distinctive hemelytral markings and specific morphological traits including the structure of the paramere, metaxyphus, mid femur, and male genitalia. These characters correspond closely with earlier descriptions and taxonomic revisions of the genus [7,8], confirming the continued reliability of traditional morphological approaches for species identification.

DNA barcoding has become an important tool in insect taxonomy and biodiversity studies, particularly for groups in which morphological characters may be subtle or overlapping [22]. The sequences of *Enithares intricata* (PZ325614 and Pz325615) formed a distinct and well-supported clade, indicating strong genetic similarity and confirming their conspecific status. The sequences of *E. intricata* clustered together as a strongly supported monophyletic clade and occupied an independent branch within the broader *Enithares* clade. This phylogenetic placement indicates a comparatively higher genetic divergence of *E. intricata* from other studied *Enithares* species. The distinct branching pattern suggests that *E. intricata* may represent an early diverging lineage among the sampled *Enithares* taxa in the present analysis. Such separation is consistent with the morphological differentiation observed in this species, particularly in characters such as the hemelytral pattern, structure of the paramere, metaxyphus, and mid femur, which clearly distinguish *E. intricata* from its congeners [7,8]. The congruence between molecular phylogenetic results and morphological diagnostic characters therefore reinforces the taxonomic validity of *E. intricata* and demonstrates the effectiveness of COI-based phylogenetic analysis for resolving species-level relationships within the genus *Enithares* [22,23]. Similarly, the sequences of *E. metallica* (PZ325616 and Pz325617) clustered together in a separate monophyletic clade, clearly separated from the other *Enithares* species included in the analysis. A distinct clade was also recovered for *E.* (PZ325613), where the newly generated sequence grouped closely with the previously published GenBank reference sequence (MN939472), further validating its taxonomic identification. The outgroup taxa *Anisops sardeus* formed a clearly separated clade, confirming the phylogenetic distinction between the genera *Enithares* and *Anisops* within the family Notonectidae. The more distantly related outgroup taxa *Metrocoris communis* was placed as the basal lineage in the tree, providing an appropriate external reference for rooting the phylogeny. Overall, the phylogenetic topology recovered in the present study is largely congruent with morphological species boundaries and supports the effectiveness of COI-based DNA barcoding in resolving taxonomic relationships within *Enithares*.

In addition to qualitative morphological characters, morphometric analyses provided further support for species differentiation. Principal Component Analysis (PCA) revealed distinct clustering among the three examined species (*E. fusca*, *E. metallica*, and *E. intricata*) based on multiple morphometric variables (Fig. 6). Multivariate morphometric approaches have been widely applied in insect systematics to evaluate morphological variation and delimit closely related species. Wing morphometric analyses, in particular, have proven useful for clarifying species boundaries within groups such as Aphididae, demonstrating the effectiveness of statistical methods in resolving patterns of morphological differentiation and supporting taxonomic classification [24]. In the present study, a similar analytical approach was applied to aquatic Hemiptera for the first time, demonstrating its usefulness in supporting species delimitation within *Enithares*. The PCA ordination showed clear separation among species, indicating that quantitative morphological traits contribute significantly to interspecific discrimination. Although *E. metallica* and *E. fusca* showed partial proximity along one of the principal component axes, suggesting certain morphological similarities, *E. intricata* formed a relatively distinct cluster, reflecting its unique morphometric characteristics. These results indicate that multivariate statistical analyses can effectively complement traditional morphological methods in taxonomic studies. The violin plot analyses further illustrated the distribution patterns of selected morphometric variables, including body size and head length. These plots revealed differences in measurement ranges and density distributions among the studied taxa, providing a clear visualization of interspecific variation.

The comparatively larger body size observed in *E. fusca* and the relatively narrower measurement ranges in the other species indicate measurable morphological differentiation (Fig. 7). Similarly, head length measurements indicated that *Enithares fusca* possesses a comparatively larger head than *E. metallica* and *E. intricata*. This morphometric distinction provides an additional diagnostic feature separating *E. fusca* from the other examined species and supports the differentiation observed in the multivariate and graphical analyses (Fig. 8). Such graphical approaches allow more detailed visualization of morphometric variability and are increasingly used in modern taxonomic studies to support species delimitation [24].

The integration of morphological, morphometric, and molecular evidence therefore provides a robust framework for species identification and delimitation within the genus *Enithares*. Integrative taxonomic approaches are increasingly recommended because they reduce the likelihood of misidentification and facilitate the detection of cryptic diversity [23]. In aquatic insects, where morphological variation may be subtle or influenced by environmental factors, combining traditional taxonomy with molecular and quantitative analyses can substantially improve taxonomic resolution [23,25]. The present findings also emphasize the biogeographic and ecological significance of the Eastern Ghats as an important center of freshwater biodiversity. Although several studies have documented aquatic Heteroptera from India [10,11,13,21,26], many areas of the Eastern Ghats remain poorly explored. With the inclusion of *E. metallica* and *E. intricata*, the number of species of *Enithares* recorded from India increases to ten. This updated inventory highlights the need for further integrative taxonomic studies combining morphological, morphometric, and molecular data to better understand the diversity, evolutionary relationships, and biogeographic patterns of this ecologically important group of aquatic bugs.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Ethical approval

No ethical issues were raised during our research.


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***Table 1: Sequence divergence of COI gene (K2P) for Enithares species and out groups***

| Sl. No. | Genebank No. Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *PZ325614.1_Enithares_intricata* | | | | | | | | | | | | | | |
| 2 | *PZ325615.1_Enithares_intricata* | 0.008 | | | | | | | | | | | | | |
| 3 | *PZ325616.1_Enithares_metallica* | 0.168 | 0.162 | | | | | | | | | | | | |
| 4 | *PZ325617.1_Enithares_metallica* | 0.165 | 0.155 | 0.011 | | | | | | | | | | | |
| 5 | *MN939472.1_Enithares_fusca* | 0.140 | 0.136 | 0.082 | 0.075 | | | | | | | | | | |
| 6 | *PZ325613.1_Enithares_fusca* | 0.141 | 0.137 | 0.078 | 0.071 | 0.016 | | | | | | | | | |
| 7 | *LC921600.1_Enithares_sinica* | 0.151 | 0.145 | 0.124 | 0.118 | 0.112 | 0.102 | | | | | | | | |
| 8 | *KP697588.1_Enithares_woodwardi* | 0.149 | 0.143 | 0.111 | 0.106 | 0.070 | 0.064 | 0.105 | | | | | | | |
| 9 | *KP697510.1_Enithares_woodwardi* | 0.151 | 0.145 | 0.113 | 0.107 | 0.071 | 0.066 | 0.107 | 0.002 | | | | | | |
| 10 | *KP697452.1_Enithares_woodwardi* | 0.151 | 0.145 | 0.113 | 0.107 | 0.071 | 0.066 | 0.107 | 0.002 | 0.000 | | | | | |
| 11 | *KF638569.1_Enithares_hungerfordi* | 0.157 | 0.149 | 0.110 | 0.099 | 0.072 | 0.066 | 0.114 | 0.088 | 0.090 | 0.090 | | | | |
| 12 | *ON406849.1_Anisops_sardeus* | 0.153 | 0.149 | 0.167 | 0.163 | 0.146 | 0.146 | 0.166 | 0.161 | 0.163 | 0.163 | 0.136 | | | |
| 13 | *ON406699.1_Anisops_sardeus* | 0.157 | 0.153 | 0.171 | 0.167 | 0.153 | 0.152 | 0.168 | 0.163 | 0.165 | 0.165 | 0.145 | 0.011 | | |
| 14 | *MW536012.1_Anisops_sardeus* | 0.151 | 0.147 | 0.165 | 0.161 | 0.146 | 0.146 | 0.162 | 0.157 | 0.159 | 0.159 | 0.136 | 0.006 | 0.008 | |
| 15 | *PV041414.1_Metrocoris_communis* | 0.240 | 0.229 | 0.224 | 0.222 | 0.214 | 0.210 | 0.222 | 0.203 | 0.203 | 0.203 | 0.240 | 0.214 | 0.210 | 0.208 |

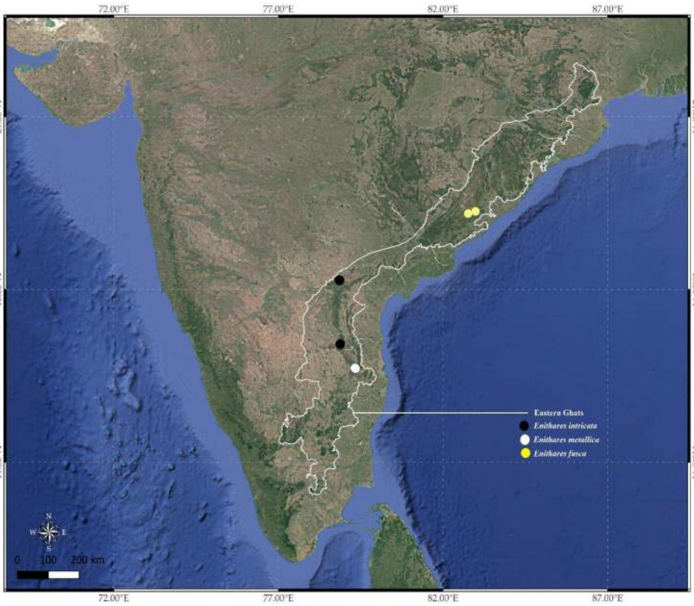


***Figure 1:*** *Collection localities of the three Enithares species from Eastern Ghats*

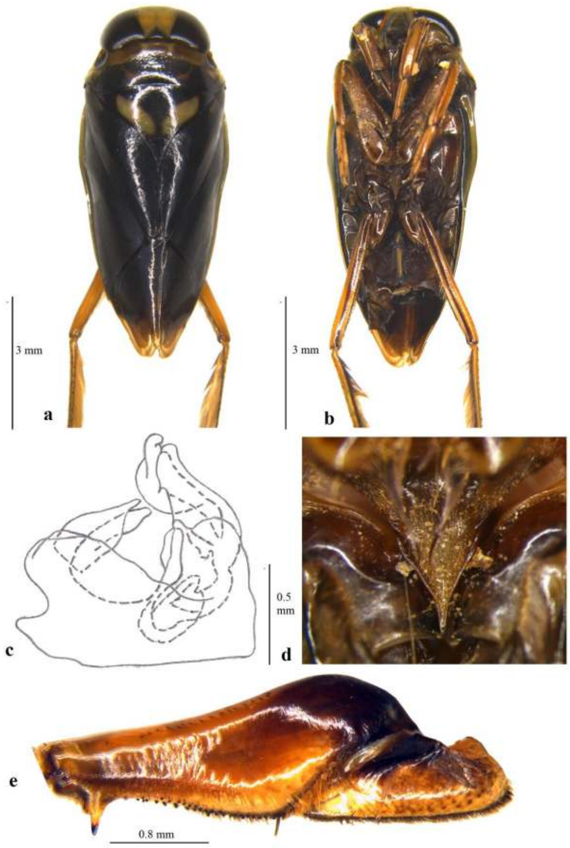


***Figure 3:*** *Enithares metallica Brooks, 1948 (Figure 3a-e); a – male habitus (dorsal view), b – male habitus (ventral view), c – male genital capsule (line drawing), d – metaxyphus, e – mid-femur*

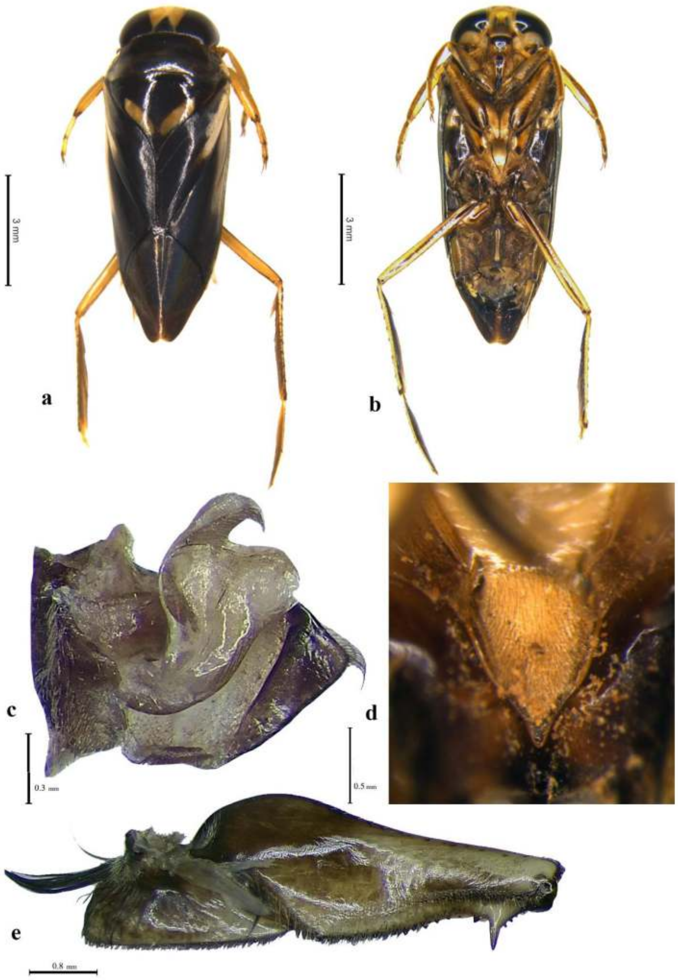


***Figure 2:*** *Enithares intricata Breddin, 1905 (Figure 2a-e); a – male habitus (dorsal view), b – male habitus (ventral view), c – male genital capsule, d – metaxyphus, e – mid-femur*

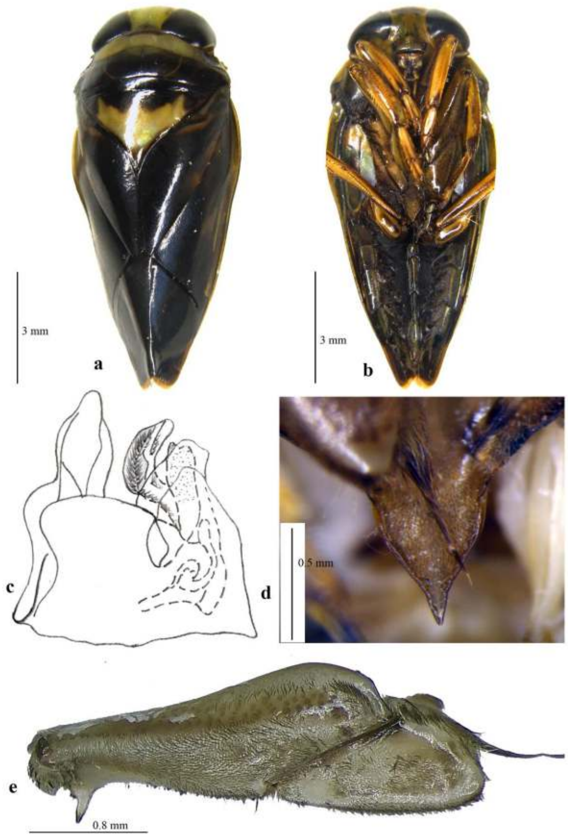


***Figure 4:*** *Enithares fusca Brooks, 1948 (Figure 4a-e); a – male habitus (dorsal view), b – male habitus (ventral view), c– male genital capsule (line drawing), d – metaxyphus, e – mid-femur*

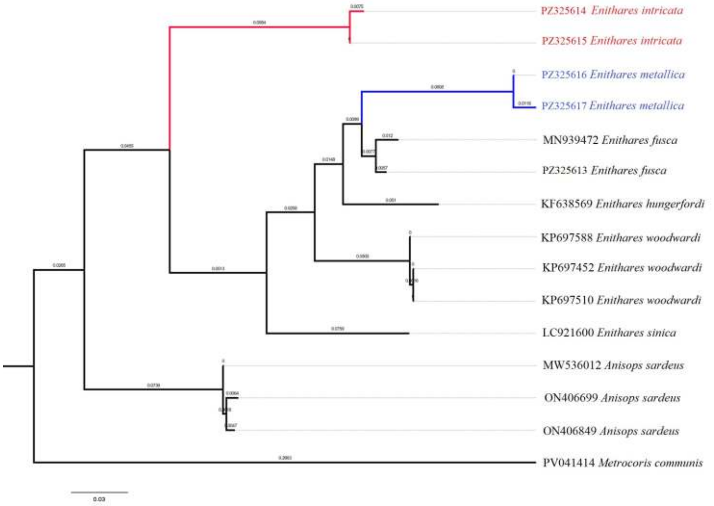


*Figure 5: Maximum likelihood phylogenetic tree for Enithares species through mtCOI gene, generated in the present study*

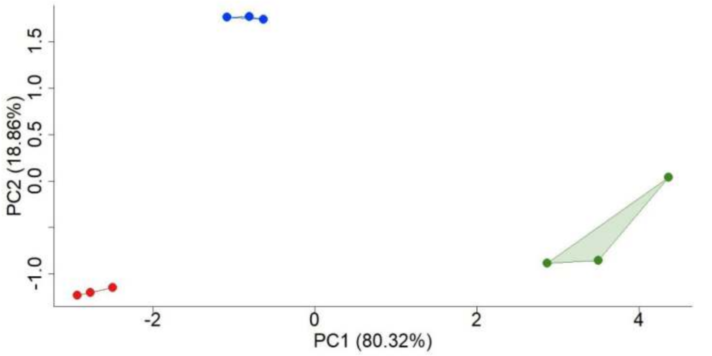


*Figure 6: Principal Component Analysis (PCA) based on morphometric measurements of three Enithares species illustrating patterns of morphological differentiation. Each point represents an individual specimen, and colors indicate species identity: green – Enithares fusca, red – Enithares metallica, and blue – Enithares intricata. The ordination plot shows distinct clustering of specimens corresponding to species, indicating that the selected morphometric variables effectively discriminate among the taxa.*

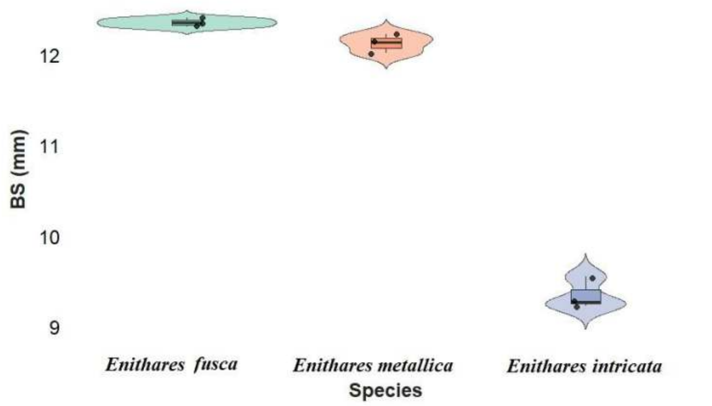


*Figure 7: Violin plots showing the distribution of body size (BS, mm) among the three Enithares species examined in the present study. The width of each violin represents the density of measurements across the observed range, illustrating interspecific variation and differences in size distribution among E. ciliata, E. metallica, and E. sinica.*

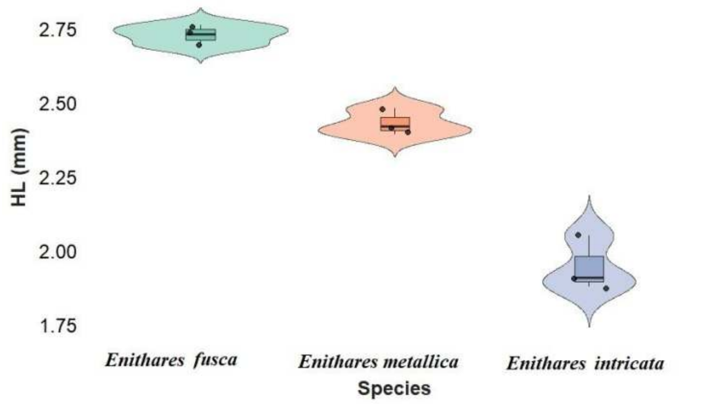


*Figure 8: Violin plots showing the distribution of head length (HL, mm) among three Enithares species. The plots depict the range and density of morphometric measurements for each species, highlighting interspecific variation in head morphology and supporting morphometric differentiation among the examined taxa.*

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