



Research Article

Morphological and molecular characterization of predatory aquatic and semi-aquatic bugs of India

SRIMOYEE BASU^{1*}, K. CHANDRA¹ and T. VENKATESAN²

¹Zoological Survey of India, M Block, New Alipore, Kolkata – 700053, West Bengal, India

²ICAR-National Bureau of Agricultural Insect Resources, H.A. Farm Post, Bellary road, Hebbal, Bangalore – 560024, Karnataka, India

*Corresponding author E-mail: srimoyeebasu3422@gmail.com

ABSTRACT: In the present study, a total of eight species of aquatic and semi-aquatic Hemiptera collected from India were identified based on morphology and mitochondrial cytochrome oxidase gene I (COI). Details of morphology based identification and the utility of COI gene to identify the aquatic Hemiptera is discussed in this paper.

KEY WORDS: Aquatic and semi-aquatic bugs, cytochrome oxidase I, Hemiptera, taxonomy

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INTRODUCTION

The aquatic and semi-aquatic hemipterans, commonly known as ‘water bugs’, are essential components of freshwater ecosystem. They play an important role in the food web of aquatic ecosystems as predators or scavengers. Knowledge of them is essential to study the fish biology and proper management of hatcheries and also to control the mosquito population as they were proved as efficient bio-control agents of mosquito larvae (Saha *et al.*, 2010). Besides, poor dispersal capabilities of these insects, they also serve as zoogeographical indicators (Jordon, 1951; Hungerford and Matsuda, 1958; Thirumalai, 1999) and they are important indicators of long-term environmental changes. Most of them are predatory except the members of the family Corixidae. Because of their immense importance, the study on the diversity and distribution of this group is required in understanding the functional aspects of community structure in the freshwater ecosystem.

Taxonomic identification through morphological characters of these bugs is well attempted in India. But, molecular identification using the universal primer mitochondrial gene, i.e., Cytochrome c oxidase subunit I (COI) is poorly attempted for these bugs. The mitochondrial DNA is more abundant than the nuclear genome as it evolves more rapidly (Wang *et al.*, 2009). Hence, the mitochondrial DNA is more applicable (Masters *et al.*, 2007) to characterize the species level. In the present study, keeping the above in view, an integrated approach using morpho- and

molecular-taxonomy were used to characterize eight species of aquatic and semi-aquatic Heteroptera of India based on mitochondrial COI gene.

MATERIAL AND METHODS

Collection and preservation of samples

Insect samples were collected from various wetlands such as rivers, streams, lakes, pools by a long handled rectangular net and were preserved in absolute alcohol in 5 ml glass vials. The male genitalia were dissected by using the stereoscopic binocular microscope Leica M205A and identified up to the species level and the voucher specimens were deposited in the NBAIR repository. The photographs of those eight species are taken using the same and provided in Plate 1.

Isolation of genomic DNA

Genomic DNA was isolated using Qiagen DNeasy blood and tissue kit following the established protocols. Bug samples preserved in absolute alcohol, were first washed well with distilled water followed by dissection of the leg. The dissected leg was dried and collected in 1.5 ml eppendorf tube to which 180 µl of ATL buffer was added and homogenized using micro pestle. 20µl of proteinase K was added to it and mixed methodically by vortexing and then incubated in water bath at 56°C overnight. 100µl of AL buffer was added and kept further for incubation at 56°C for 10 minutes, after incubation 100µl of absolute ethanol was added to the eppendorf and vortexed.

The solution was then transferred into the mini spin columns with silica member that binds the genomic DNA. The columns were then centrifuged at 8000 rpm for 5 minutes and the flow-through collected in a tube was discarded. The column was then further given two washes with two different buffers present in kit i.e., AW1 and AW2 and centrifuged at 8000 rpm for 5 minutes simultaneously. These columns were then transferred to a new fresh 1.5 ml eppendorf tubes in order to elute out the DNA bound to the Silica member in the column. The elution was carried out by pipetting 100 µl of sterile water to the columns, centrifuged at 8000 rpm for 5 minutes. The DNA was then checked at 1% agarose gel and stored in 4°C until PCR was done.

PCR amplification of CO1 gene

The DNA obtained was then amplified for a portion of mitochondrial CO1 gene fragment, using the universal primers CO-1 F 5'GGTCAACAAATCATAAAGATATTGG 3' and CO-1 R 5'TAACTTCAGGCTGACCAAAAAATCA3', (M/SBioserve biotechnologies (India) Pvt. Ltd). Each PCR reaction mixture of 25 µl consisted 2.5 µl of 10x PCR buffer with 15mM MgCl₂, 2.0 µl of dNTP's mix, 1 µl of each forward and reverse primer, 1 µl of *Taq* Polymerase (1U/µl), 2.5 µl of template DNA and 15 µl of sterile water. The PCR was carried out in thermal-cycler (BioRad, USA) and the conditions set were initial denaturation at 95°C for 4 minutes, denaturation at 95°C for 30 seconds, annealing and extension at 50°C and 72°C for 1 minute, respectively and final extension for 7 minutes at 72°C. The PCR was carried out for a total of 34 cycles. Amplified DNA was then checked on 1% agarose gel using a DNA ladder of 250 bp and the gel was visualized in gel dock (Fig. 1).

Sequencing

The sequencing of amplified CO1 product was carried out at M/S. Eurofins Pvt. Ltd., Bangalore. The sequence data was retrieved in the form of chromatograms which was then submitted to genbank for obtaining the accession numbers.

Sequence analysis and data interpretation

The ambiguous bases were removed by chromatogram editing. These edited sequences were then aligned using Basic Local Alignment Search Tool (BLAST), with the sequences of same or related species retrieved from the nucleotide database (PUBMED) of National Centre for Biological Information (NCBI). The CO1 nucleotide sequences of the hemipteran species were aligned and compared with the species obtained from PUBMED, using CLUSTAL W alignment (Thompson *et al.*, 1994).

RESULTS AND DISCUSSION

Infraorder Nepomorpha

Family Nepidae

1. *Laccotrephes griseus* (Guerin-Meneville, 1844) (Fig. 1)

Material examined: 2 M, 1F, India, Sikkim, Teesta River, Rangpo, East Sikkim District, 28.iii.2016, Coll: S. Basu.

Diagnostic characters: Male body length generally ranges between 16.7–19.2 mm. Female may attain 18.3–20 mm. Respiratory siphon always shorter than body, length 12.9–14.5 mm. Small relative to other species. Antennae hidden, three segmented, with stout hairs. Hemelytra parallel, thick, membrane thin with numerous reticulate veins. Abdomen beneath wings bluish in color. Male parameres symmetrical, slightly hooked and articulated in anterior region. Female genitalia triangular, two pairs of plate like structures forming slender ovipositor.

Molecular identification: The size of the COI PCR product of *L. griseus* was 604 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

Infraorder Gerromorpha

Family Gerridae

2. *Amemboa kumari* (Distant, 1910) (Fig.2.)

Material examined: 2M, India, Odisha, Gadgati Nala, Junaghati, Banuguelia forest watch tower, 17.xii.2015, Coll: K. Valarmathi and party.

Diagnostic characters: Body length of male 3.12–3.3 mm and body length of female 3.46–3.52 mm. Body dark black with distinct yellowish orange markings. Antennal tubercle small, but visible from above. Metanotum and abdomen dorsally dark, marked with yellow markings. Mesopleura with longitudinal dark stripe, Venter pale yellow, marked with one median and two sublateral dark markings. Male fore femur relatively slender, with two separate hair tufts beyond middle.

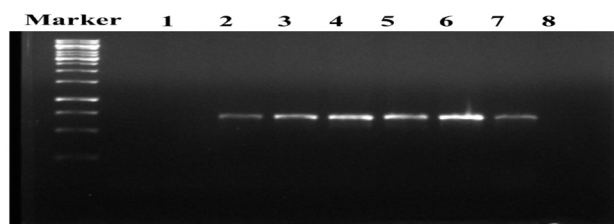


Fig. 1. PCR amplified products of CO1 gene of Gerromorphan population, Lane M: 250 bp DNA ladder.

Molecular identification: The size of the COI PCR product of *A. kumari* was 458 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

3. *Chimarrhometra orientalis* (Distant, 1879) (Fig. 3)

Material examined: 3M, 1F, India, Sikkim, Banjhakri waterfalls, Gangtok, East Sikkim District, 27.iii.2016, Coll: S. Basu.

Diagnostic characters: Males may attain a length of about 8.0 mm. Length of female insects vary from 8–9.2 mm. Body yellowish brown to reddish brown dorsally. Head with a broad median band and narrow lateral stripes. Pronotum truncate anteriorly with mid-longitudinal pale line and mesonotum with paired oblique depression near the anterior margin. Male genital segment more or less depressed posteriorly, sternite VII a little longer than preceding two sterna together. Parameres falciform, large, with apices crossing beneath pygophore.

Molecular identification: The size of the COI PCR product of *C. orientalis* was 607 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

4. *Ptilomera (Ptilomera) himalayensis* Hungerford and Matsuda, 1958 (Fig. 4)

Material examined: 2M, 1F, India, Sikkim, Mangan, North Sikkim District, 23.3.2016, Coll: S. Basu.

Diagnostic characters: Body length of macropterous male 14.2–150 mm. Body length of macropterous female 12.2–14.5. Head in front of eyes little swollen, with a few trichobothrial setae; vertex with a pair of backward angling dark spots on either sides of midline. First antennal segment longer than the length of rests together. Metanotum dark brown with a prominent dark suture medially. Metacoxal spine absent. Fore leg light to dark brown. Fore trochanter with four prominent setae. Fore tibiae with two small teeth followed by a small curvature, marked with another small tooth towards its anterior margin. Pygophore small, simple and broad, the lateral lobes of pygophore slender and hidden inside except their tips, without dorsolateral projections.

Molecular identification: The size of the COI PCR product of *P. himalayensis* was 608 bps and the sequences obtained were compared with the homologous sequences

available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

5. *Ptilomera (Ptilomera) agriodes* Schmidt, 1926 (Fig. 5)

Material examined: 1M, 1F, India, Odisha, Gadgati Nala, Junaghati, Banuguelia forest watch tower, 17.xii.2015, Coll: K. Valarmathi and party.

Diagnostic characters: Adults attain 16–17 mm in body length. Colour is typical for this species i.e.; usually dark brown approaching black. Thoracic region with a black longitudinal band laterally. Fore femur of male stout and with two black shiny protuberances near its middle; Silvery pilosity in the venter is prominent. Median lobe of suranal plate is broadly rounded; Dorso-lateral projection of pygophore surpasses the lateral wings of suranal plate by half of their length and with this character it can be easily separated from the other species. Parameres are long with pointed tip turning forward. Connexival spines of female arise beneath the edge of connexivum with its tip reaching the caudal end of dorso-lateral lobe. The lateral projections of seventh abdominal segment extend beyond the connexival spine.

Molecular identification: The size of the COI PCR product of *P. agriodes* was 690 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

6. *Metrocoris dinendrai* Basu, Polhemus and Subramanian, 2016 (Fig. 6)

Material examined: 1M, 2F, India, Sikkim, Waterfalls, Lungchowk, North Sikkim district, 24.iii.2016, Coll: S. Basu.

Diagnostic characters: Male attains a length 5.4 mm, maximum width across mesoacetabula 2.5mm. Body length of female ranges from 4.4–4.5, maximum width across mesoacetabula 2.2–2.3. Interocular dark mark rectangular and bifid posteriorly, anterior margin not connected with dark mark of postclypeus. Pronotum slightly bulbous in male, wider than long, slightly wider than head. Meso- and metanota 1.12 times wider than combined length. Fore femur slender and slightly curved at middle, ratio of length/width approximately 6.5, ventral surface with small constriction near middle and short dense hair fringe ventrally near apex, but without any tooth, inner margin hairy. Inner margin of fore tibia not modified, clothed with hairs. Male pygophore

elongate, heavily setiferous, apex truncate. Proctiger moderately elongated, lateral margins slightly convex, apex broadly rounded, posterior margin with dense hair fringe. Parameres symmetrical strongly curved near midpoint, apical section expanded to small head with outer margin concave, apex blunt, inner and outer margins with long distinct setae, several whitish dots distributed throughout.

Molecular identification: The size of the COI PCR product of *M. dinendrai* was 584 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

7. *Metrocoris deceptor* Basu, Polhemus and Subramanian, 2016 (Fig.7)

Material examined: 13M, 11F, India, Sikkim, Rothak Khola, Legship road, West Sikkim district, 9.v.2016, Coll: S. Basu.

Diagnostic characters: Body length of apterous male 6.1- 6.9mm, maximum body width 2.6–3.3 mm. Body length of apterous females 5.3–5.7 mm, maximum body width 3.2–3.4 mm. Interocular area with broad arrow shaped marking medially. Fore femur strongly incrassate, ratio length/ width: 3.22(2.68/0.83), constricted in apical third, with bipartite apical tooth, without any ventral indentation. Length of abdomen 2.87 and width 1.62, abdominal tergites black with dense golden pubescences. In macropterous forms, wing surpassing apex of abdomen. Male paramere long, hook shaped, pointed apically. Female genital segment VII with large medial lobe, with longitudinal ridge laterally from anterior end of hind margin and with small wing shaped lobes, medial lobe sub-trapezoidal, with distinctly notched posterior margin.

Molecular identification: The size of the COI PCR product of *M. deceptor* was 608 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is given in Table 1.

Family Hydrometridae

8. *Hydrometra greeni* Kirkaldy, 1898 (Fig.8)

Material examined: 2M, 1F, India, Sikkim, Teesta River, Rangpo, East Sikkim District, 28. iii. 2016, Coll: S. Basu.

Table 1. Aquatic hemipteran species with their accession numbers

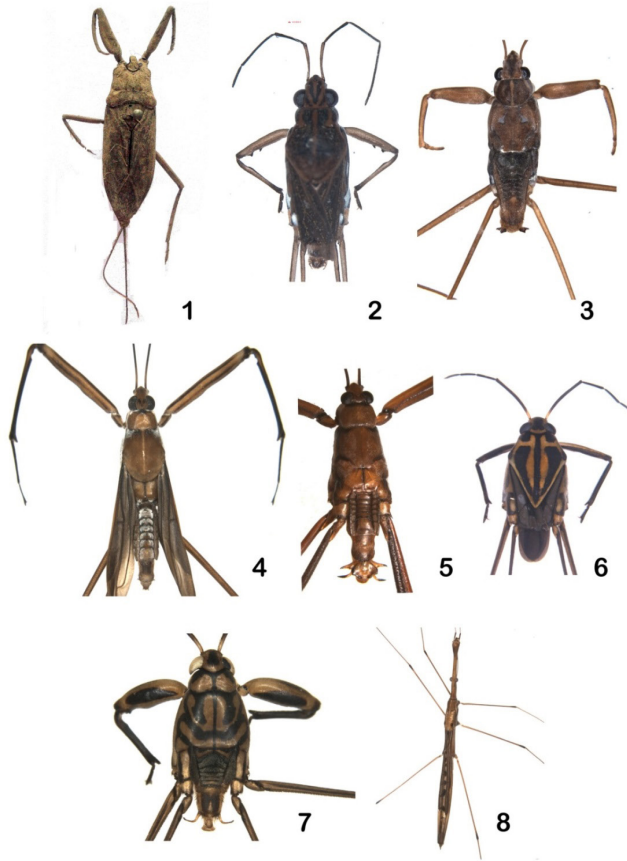
Sl. No.	Species	SampleID	GenBank Acc. No.
1.	<i>Laccotrephes griseus</i> (Guerin-Meneville, 1844)	AIN19H002	KX365491
2.	<i>Amemboa kumari</i> (Distant, 1910)	AIN19H003	KX775121
3.	<i>Chimarrhometra orientalis</i> (Distant, 1879)	AIN19H004	KY018602
4.	<i>Ptilomera (Ptilomera) himalayensis</i> Hungerford and Matsuda, 1958	AIN19H005	KY018603
5.	<i>Ptilomera (Ptilomera) agriodes</i> Schmidt, 1926	AIN19H009	KY556677
6.	<i>Metrocoris dinendrai</i> Basu, Polhemus and Subramanian, 2016	AIN19H007	KY212124
7.	<i>Metrocoris deceptor</i> Basu, Polhemus and Subramanian, 2016	AIN19H008	KY283957
8.	<i>Hydrometra greeni</i> Kirkaldy, 1898	AIN19H006	KY041641

Diagnostic characters: Body length of male ranges from 10.2 to 11.4 mm. Female may attain a length of about 11.2–11.8 mm. The general colour of the body brownish yellow. Clypeus bluntly conical. First antennal segment short, third antennal segment longer than second. Pronotum with an encircling row of pits parallel to the anterior margin, posterior lobe of pronotum with a median longitudinal row of pits. The hemelytra large and long, extending to the posterior margin of fourth abdominal segments in brachypterous forms. Seventh sternite of male is transversely depressed and hairy as in the posterior half of sixth abdominal segment. Posterior dorsal margin of seventh segment is fringed with short stiff hairs. The terminal dorsal process of male sharp. In female, the last dorsal abdominal segment broader behind than in front and with a sharp ovipositor.

Molecular identification: The size of the COI PCR product of *H. greeni* was 578 bps and the sequences obtained were compared with the homologous sequences available at GenBank (<https://www.ncbi.nlm.nih.gov/>). The accession number is provided in Table 1.

Aquatic and semi-aquatic Heteroptera, especially of the family Notonectidae, Belostomatidae, Nepidae, Naucoridae, Gerridae and Veliidae etc., which inhabit paddy

Plate 1



1. Dorsal view of *Laccotrephes griseus*; 2. view of male of *Amemboa kumari* 3. Dorsal view of male of *Chimarrhometra orientalis*; 4. Dorsal view of macropterous male of *Ptilomera himalayensis*; 5. Dorsal view of male of *P. agriodes*; 6. Dorsal view of male of *Metrocoris dinendrai*; 7. Dorsal view of male of *M. deceptor*; 8. Dorsal view of male of *Hydrometra greeni*

fields and marshes, are found to be as ecologically important mosquito predators (Mogi, 2007; Ohba *et al.*, 2011). The species *Micronecta quadristrigata* Breddin, 1905 was found to be a successful predator of *Aedes aegypti* and *A. albopictus* (Nam *et al.*, 2000). Because of their immense importance, their study should be taken in consideration and for management of freshwater ecosystems as well as control of mosquitoes and pests in paddy fields. In the present study, an integrated approach of using morphology and molecular based (using COI gene) identification of eight species of aquatic and semi-aquatic bugs for the first time in India. Furthermore, mitochondrial COI sequence for these specimens would be a reference source in the GenBank database. The study also indicates that these bugs can be effectively identified using COI gene. There are still many known and unknown species of Indian aquatic Hemiptera, several of which are not molecularly characterized. Hence, this study will be

the first record and the reference for further analysis in future.

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