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Morphological and molecular characterization of *Limnometra fluviorum* (Fabricius) (Hemiptera: Heteroptera: Gerridae)

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ABSTRACT: The species *Limnometra fluviorum* (Fabricius) 1798 is redescribed with morphological and molecular taxonomic approach. The molecular work involving CO1amplification is the first time approach for this species from India. The diagnostic characters with measurements of different body parts are provided. © 2017 Association for Advancement of Entomology

KEY WORDS: Gerridae, *Limnometra fluviorum*, Taxonomy, DNA Barcoding

INTRODUCTION

Genus *Limnometra* Mayr, 1865 (Hemiptera: Gerridae) consisting of a group of large, black to brown colored, long legged water striders which are widely distributed and occur in a wide variety of freshwater habitats across Oriental, Malaysian, Southern and West Pacific and Northern Australia (Polhemus and Polhemus, 1997). The gerrids are predatory in nature and play a major role in the food chain of freshwater ecosystem (Thirumalai, 2002). The genus *Limnometra* Mayr was attracted the attention of taxonomists. The major revisionary work on this genus was carried out by Hungerford and Matsuda (1958) revised the genus, Nieser and Chen (1992) worked on Indo-Australian fauna, Andersen and Weir (1997) documented on Australian fauna, Polhmeus and Polhemus (1997) studied on fauna of New guinea. Zettel (2001) described one new species from India and Thirumalai (1986, 2002) provided the distribution of the genus *Limnometra* across the Indian States, which shows only three species from India. All these scientists described species of *Limnometra* through morpho-taxonomy especially based on male genitalia and mesofemoral armature. The detailed morphological characteristics with the measurements of different body parts of this species were not presented so far after the taxonomic work of Hungerford and Matsuda (1958). Furthermore, if only females and nymphs are found, they are not identifiable using morphological keys. In order to solve this difficulty, DNA barcoding technique through amplification of mitochondrial gene Cytochrome c oxidase I (COI) will be very useful to identify species irrespective of its stage and rapid, simple and widely applicable now a days (Raupach *et al.*, 2014).

Cytochrome c oxidase I (COI) of the mitochondrial DNA has been widely used as popular marker for identification and understanding the evolutionary

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relationships among the insects. Mitochondrial gene COI can be considered as the core of a universal bio-identification system for animals. COI has been already proved to be a standardized DNA Barcode for identification of Heteroptera (Damgaard *et al*., 2000; Park *et al.*, 2011; Raupach *et al.*, 2014) species regardless of their developmental stage. Keeping the above in view, an integrated approach using morpho-taxonomy and DNA barcoding was used to characterize *Limnometra fluviorum* (Fabricius) from India.

MATERIALS AND METHODS

Collection and Preservation of samples

During the recent entomological survey to the State of Odisha, India, samples were collected using net from the streams flowing across Satkosia Tiger Reserve of Angul District of Odisha. The collected samples were preserved in absolute alcohol. The samples were identified using binocular microscope Leica M205A and photographed using the same. The measurements of different body parts were taken in millimeters (mm). The male genitalia was dissected and cleared in 10% KOH. The genitalia was mounted on glass slide using Canada Balsam. The samples were stored at -20^{o} C for molecular studies.

Molecular analysis: DNA isolation, PCR amplification and sequencing

Identified gerrid samples stored in absolute alcohol were first washed well with distilled water followed by dissection of the fore leg. Fore leg samples were homogenized using a micro pestle in 50 µl of lysis buffer and treated with proteinase K at 37°C. The genomic DNA was isolated using a DNA extraction kit (QIAGEN DNeasy blood and tissue kit Cat. 69504, Germany). The barcode region of COI gene was amplified using Advantage 2 Polymerase PCR kit (Takara Clontech Japan). Standard primers for amplifying the COI region such as LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (reverse) were used in the PCR. The PCR reaction was performed using a thermal cycler (c-1000 Thermal Cycler, Biorad Laboratories, California) with the following cycle conditions: 95°C for 5 minutes, followed by 34 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for one minute and a final extension at 72°C for 5 minutes. Amplified products were separated on 1.2% agarose. Gels were stained using ethidium bromide (1%). 1 kbp DNA standards were run along with the samples for reference. The PCR products were gel eluted with QIAquick Gel Extraction Kit (Cat. No. 28704, Germany) and direct sequenced using a BigDye terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster city, CA) and Applied Biosystems prism 310 Genetic Analyzer. The sequence information of individual specimens are publically available at NCBI GenBank nucleotide sequence database (Table 1) and Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert, 2007).

RESULTS AND DISCUSSION

Morphological identification of *Limnometra fluviorum* (Fabricius) (Fig.1-9)

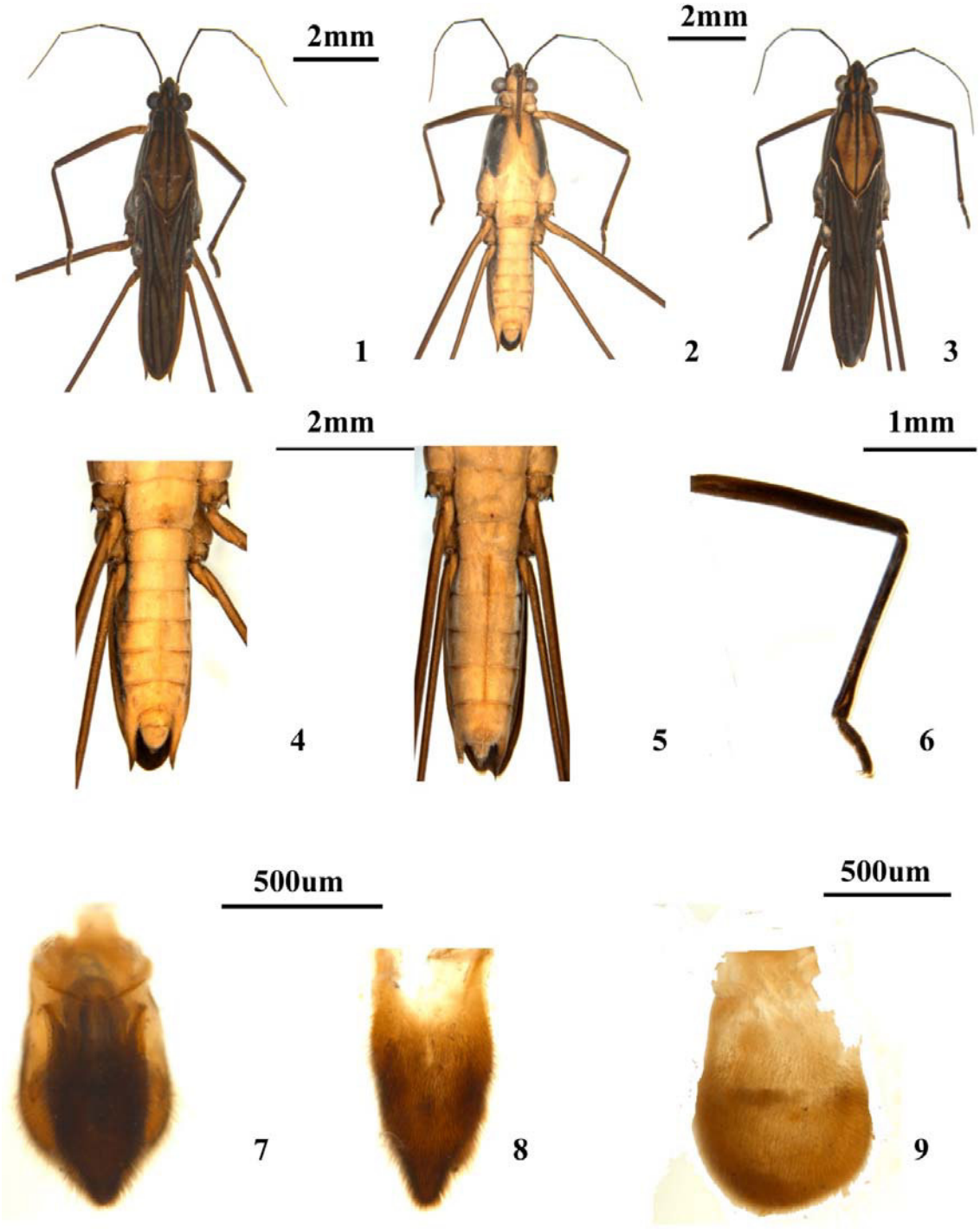
Material examined: 2M, 3F, from Katarang Nala, Satkosia Tiger Reserve, Angul District, Odisha, 21.706084N, 86.447282E, alt: 759 ft; date: 22.12.2015; coll: Dr. K. Valaramathi, Accession no. KX300087.

Description:

Size: Male attains a body length of 10.12 mm to 10.60mm. Female generally attains a length of 10.71mm to 11.54mm.

Table 1. Details of sample used in the study

Species	Sample ID	GenBank Accession	BIN number
L. fluviorum	AIN19H001	KX300087	BOLD:ACM1305



1. Dorsal view of macropterous male
2. Ventral view of male
3. Dorsal view of macropterous female
4. Male abdominal sternite
5. Female abdominal sternite
6. Male foreleg
7. Dissected genital segment
8. Proctiger of male
9. Pygophore of male

Colour: Usually dark brown to black with black markings. Antennae, rostrum and legs dark brown. Eyes black. Head black with typical orange markings. Pronotum brown with black longitudinal marking, extending up to metanotum. Connexival spines dark brown. Wings dark brown with prominent black markings. Ventrally yellowish. Fore wings dark brownish with black venation.

Structural characteristics: Head length 0.69 and width 0.78. Length of antennomere 1st to 4th: 2.01, 1.41, 1.45, 1.91. Eye length 0.59 and width 0.43. Interocular width 0.75. Humeral length 3.24 and width 1.87. Rostrum 2.53 in length. Male fore femur slender and unarmed, length 3.02 and width 0.33. Meso coxa with distinct spine like projection on its rear margin. Length of abdomen in male 4.22 and width 1.45, whereas in female, length of abdomen 6.15 and width 1.64. Female sternite with a distinct median ridge; however in male the median ridge is not so prominent. Connexival spines long and curved in both sexes. Length of wings 7.64.

Male genital segment: Male genital segment little elongated and 0.89 in length and 0.53 in width. Male proctiger (Fig.8) long and 'v' shaped, narrow, hairy. Pygophore (Fig. 9) truncated towards apex and broad, with hairs. Paramere vestigial. Endosomal sclerite well sclerotised.

Diagnostic characters: This species can be easily identified by presence of its spine like projection on dorso-lateral rear margin of mesocoxa of mid leg. Mesopleura black, especially with large black marking.

Distribution: India (Karnataka, Kerala, Maharashtra, Pondicherry, Tamil Nadu, West Bengal), Philippines, Sri Lanka.

Molecular identification

The size of the COI PCR product of *L. fluviorum* was 650 bps and the sequences obtained were compared with the homologous sequences (KC880894, KC880945, KC880945) available at GenBank (https://www.ncbi.nlm.nih.gov/) and species sequences reported in BOLD databases (http://www.boldsystems.org/). The accession number and Bin number are provided in Table 1. Multiple sequence alignment of the COI showed a total of 780 bp. We found larger gaps in alignment due to the length differences in sequences. To deal with this issue, we screened alignment by using trimAL software to remove the unreliable region and obtained column which are having reliable phylogenetic signals. After refinement, we got a total of 540 bps for COI. The Barcode index numbers (BINs) analysis of COI sequences was performed using BOLD Systems v3 and all (100%) fulfilled BOLDs quality criteria (Ratnasingham and Hebert, 2007).

The study described morphological and DNA barcode of mitochondrial CO1 of *L. fluviorum* for the first time in India. Furthermore, mitochondrial CO1 sequence for this specimen would be a reference source in the GenBank and BOLD database. The study also indicates that the barcoding technique can be used effectively for identification of semi-aquatic bugs. There are still many known and unknown species of Indian semi-aquatic hemiptera, several of which are not molecularly characterized, Hence this study will be the first record and the reference for further analysis.

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