

Morphological and molecular characterization of two species of *Laccotrephes* (Hemiptera, Nepidae) from Eastern Ghats, India

Somesh Banerjee^{1,2*}, Devadoss Kumar² and Deepa Jaiswal¹

¹Freshwater Biology Regional Centre, Zoological Survey of India, Hyderabad 500048, Telangana, India.

²Department of Zoology, Virudhunagar Hindu Nadars Senthikumara Nadar College, Virudhunagar 626001, Madurai Kamaraj University, Tamil Nadu, India.

Email: banerjeesomesh49@gmail.com*, kumar@vhnsnc.edu.in, deepajzsi@gmail.com

ABSTRACT: This study presents an integrative approach combining morphological and molecular techniques to identify and characterize two species of the genus *Laccotrephes* Stål — *L. griseus* (Guérin-Méneville) and *L. ruber* (Linnaeus) from the Eastern Ghats, India. Morphological differentiation was based on key characters such as body length, respiratory siphon length, prosternal structure, and male parameres. For molecular analysis, partial mitochondrial COI gene sequences (~620 bp) were amplified and analyzed using the Maximum Likelihood method in IQTREE. The resulting phylogenetic tree supported species-level divergence, with pairwise genetic distances ranging from 15.5 to 17.7 per cent between *L. griseus* and *L. ruber*, confirming their distinct taxonomic status. Intraspecific variation was minimal (<1%), while interspecific distances among other congeners showed clear genetic gaps. The integration of morpho-taxonomy and DNA barcoding underscores the value of a combined approach for accurate species delimitation and reveals potential cryptic diversity within the genus. This work enhances the understanding of aquatic Hemiptera biodiversity and provides a foundation for further taxonomic revisions in *Laccotrephes*. © 2026 Association for Advancement of Entomology

KEY WORDS: Water scorpion, taxonomy, DNA barcodes, mtCOI gene, Eastern Ghats

INTRODUCTION

Among true bugs (Heteroptera), the infraorders Nepomorpha and Gerromorpha have a close association with aquatic habitats, with the former completely submerged and the latter prospering on the water surface or along shorelines. *Laccotrephes* Stål, 1866 is the major genus in the subfamily Nepinae of the family Nepidae in the insect suborder Heteroptera. It is a large genus (roughly 60 species, Keffer 2004) that is primarily

restricted to the Old-World tropics (Neiser *et al.*, 2009). Poisson (1965) published a catalogue of African Nepidae, which included African *Laccotrephes* parameres. Polhemus and Keffer (1999) published an overview of the *L. grossus* subgroup, however, there has been no recent survey of Asian species (Neiser *et al.*, 2009).

The water scorpion (Nepidae), notably the species *Laccotrephes*, is a crucial ecological predator of crustaceans, fish, amphibians, and mosquito larvae.

* Author for correspondence

The long-term predation experiment (up to 30 days) revealed that *L. griseus* is an excellent predator of mosquito larvae such as *C. quinquefasciatus* (Ghosh and Chandra, 2011). The majority of *Laccotrephes* species, like other members of the Nepinae, reside near the shallow border of stagnant waters, even virtually static bays of streams, where they maintain contact with atmospheric air by respiratory siphons. In India, seven *Laccotrephes* species have been recorded (Thirumalai, 2007), with only three species, *L. elongatus*, *L. griseus*, and *L. ruber*, being spread throughout the country, while the remaining species are uncommon and have no documentation after Thirumalai (2007). Traditional taxonomy, while valuable, can be subjective and limiting, especially when specimens are damaged or missing. DNA barcoding based on mitochondrial COI gene sequences has emerged as a dependable and effective approach for species identification, allowing for correct classification with minimal tissue samples (Hebert *et al.*, 2003; Ward *et al.*, 2005). This molecular technique, paired with morphological taxonomy, provides a solid framework for distinguishing *Laccotrephes* species with precision. The present study integrates morpho-taxonomy and DNA barcoding to establish a definitive identification of *Laccotrephes* species in India, addressing taxonomic ambiguities and contributing to a deeper understanding of aquatic Hemiptera diversity.

MATERIALS AND METHODS

Laccotrephes specimens have been collected from several locations in Eastern Ghats, India (Fig. 1, Table 1). The collected specimens were kept in ethanol (90%) and examined with Leica EZ4W and Olympus SZX10 stereo-zoom microscopes, as well as photographed with a Nikon D500 Nikon AF-S DX Micro Nikkor 40mm f/2.8G Lens. Morphological identification is done using relevant literature (Thirumalai, 2007; Chandra and Jehamalar, 2020). The identified specimens were placed in the National Zoological Collection of the Zoological Survey of India, Freshwater Biological Regional Centre, Hyderabad. DNA was extracted from tissues from the base of the chosen specimens' mid and fore legs using the Dneasy®

Blood & Tissue kit (Qiagen, Germany).

Amplification done through primer LCO-1490 5'-GGTCAACAAATCATAA AGATATTGG-3', HCO-2198 5'-TAAACTTCAGGGTGACCAAA AAATCA-3' for the mitochondrial Cytochrome Oxidase Subunit I (COI) gene (~620bp) (Folmer *et al.*, 1994). Polymerase chain reaction prepared for 25µl which contains 12.5µl of 2x PCR Taq mixture, 0.5µl (10pmol Con) 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 95°C for 1 min, 94°C for 30sec, 48°C for 30 sec 35 cycles, 72°C for 1 min. Agarose gel (0.5%) was used to check the DNA which also contains Ethidium bromide (10mg ml⁻¹). The PCR products were purified using NucleoSpin® Gel and PCR clean up kit protocol were followed. Forward and reverse sequences were generated using the Sanger method by Eurofins Genomics (Ebersberg, Germany). Generated chromatogram files of three species have been handled in MEGA 11 (Kumar *et al.*, 2018) phylogenetic analyzing software. The generated sequences were searched in NCBI (<https://www.ncbi.nlm.nih.gov>) to prepare final dataset. A total of 4 sequences were acquired from the NCBI database to compare and prepare final database for the study. MEGA 11 software was used to align the generated sequences and genetic divergence were calculated by using Kimura-2-Parameter (K2P) model in MEGA 11 (Kumar *et al.*, 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1982) in both MEGA 11 and IQTREE for better results. The tree with the highest log likelihood (-3369.75) is shown. Branch support was estimated using the Bootstrap value analysis with 1000 replicates. *Metrocoris communis* (Distant, 1910) was used as an out-group for better understanding (Table 2).

RESULTS AND DISCUSSION

Class Insecta Linnaeus, 1758; **Subclass** Pterygota Brauer, 1885

Order Hemiptera Linnaeus, 1758; **Suborder** Heteroptera Latreille, 1810

Table 1. Survey locations throughout the Eastern Ghats

No.	Survey locations	Latitude	Longitude	MSL
1	Koyalagudem	17.4985N	81.6043E	410
2	Rollapadu Wildlife Sanctuary	15.4514N	78.2122E	321
3	Ananthavoram	16.5887N	80.4362E	45
4	Kalab Dam	18.6116N	82.6242E	868
5	Mandrikalapadu	17.7226N	80.6224E	137
6	Bairampally	16.1427N	78.6813E	755
7	Bolghatpally	16.4022N	78.8861E	587
8	Octopus viewpoint	16.2227N	78.8313E	817
9	Mallela Theertham waterfalls	16.2657N	78.8541E	729
10	Mudasarlova reservoir	17.7641N	83.2962E	65
11	Kasipatnam	18.6521N	83.3034E	123
12	Kondakarla ava lake	17.6035N	82.9981E	33
13	KP Dam	13.7245N	79.3250E	927
14	Cheyeru river	14.1280N	79.0166E	228
15	Adidavaram cheruvu	17.7898N	83.2541E	108
16	AP Secretariat	16.5186N	80.5201E	45
17	Meghadri Gadda reservoir	17.7747N	83.1511E	33
18	Relli Lake	17.8093N	83.3298E	86
19	Muran Dam	19.1058N	82.7618E	643
20	Thatipudi reservoir	18.1722N	83.1913E	120
21	Stream near Maredumilli	17.5518N	81.7273E	342
22	Musurumilli reservoir	17.3881N	81.7691E	128
23	Tadikonda	16.4149N	80.4529E	41
24	Bhadrachalam	17.6743N	80.8812E	58
25	Narsipatnam	17.6652N	82.6047E	88
26	Ethakayala Mandapam	13.6807N	79.3506E	881

Infraorder Nepomorpha Popov, 1971;
Superfamily Nepoidea Latreille, 1802

Family Nepidae Latreille, 1802; **Subfamily** Nepinae Latreille, 1802

Genus *Laccotrephes* Stål, 1865

Laccotrephes is a genus of “water scorpion”. They are carnivorous, hunting at the water surface or

submerged in aquatic plants. They are dark brown to rufous brown in color, with an elongated and flattened body, hooked raptorial forelegs, and a long, thin respiratory siphon extending from the abdomen tip (Polhemus and Keffer, 1999). The respiratory siphon consists of two filaments that extend from the eighth abdominal tergum.

Table 2. Specimen sequences generated in current study, and additional sequences obtained from GenBank

Accession no.	Species	Collection locality as per NCBI GenBank	Source
KY320485	<i>Laccotrephes japonensis</i>	Japan: Kumamoto	GenBank
KF966560	<i>L. japonensis</i>	South Korea	GenBank
KY320469	<i>L. aff. pfeifferiae</i>	China: Tianjin	GenBank
KP274069	<i>L. maculatus</i>	India: Imphal, Manipur	GenBank
OR056392	<i>L. maculatus</i>	Kenya: Tharaka Nithi	GenBank
OR056393	<i>L. maculatus</i>	Kenya: Tharaka Nithi	GenBank
OR056394	<i>L. maculatus</i>	Kenya: Tharaka Nithi	GenBank
PP409484	<i>L. maculatus</i>	Kenya: Nyeri	GenBank
PV041420	<i>L. ruber</i>	India: Umri village, Satara, Maharashtra	Current study
PV603800	<i>L. griseus</i>	India: Kinnerasani Wildlife Sanctuary, Bhadradi Kothagudem, Telangana	Current study
PP032035	<i>L. griseus</i>	India: Chennai, Tamil Nadu	GenBank
PQ436053	<i>Diplonychus annulatus</i>	India: Kinnerasani Wildlife Sanctuary, Bhadradi Kothagudem, Telangana	Current study
PV041409	<i>D. molestus</i>	India: Pakhal Wildlife Sanctuary, Warangal, Telangana	Current study
KY451618	<i>D. rusticus</i>	India: Dindi reservoir, Koratikal, Nizamabad, Telangana	GenBank
PP885728	<i>D. rusticus</i>	India: Pakhal Wildlife Sanctuary, Warangal, Telangana	Current study
PV041413	<i>D. rusticus</i>	India: Pakhal Wildlife Sanctuary, Warangal, Telangana	Current study
MN053270	<i>D. esakii</i>	South Korea: Upo wetlands, Yueo-myeon, Changnyeong-gun, Yibang-myeon, Gyeongsangnam-do	GenBank
MN053261	<i>D. esakii</i>	South Korea: Jeju Islands	GenBank
MN053268	<i>D. esakii</i>	South Korea: Upo wetlands, Yueo-myeon, Changnyeong-gun, Yibang-myeon, Gyeongsangnam-do	GenBank
PV041414	<i>Metrocoris communis</i>	India: Goutami Dam, Nashik, Maharashtra	Current study

Key to the species of *Laccotrephes* from Eastern Ghats of India

1. Small sized with body size 13.7-15.21mm in males and 15-16.43mm in females, respiratory siphon shorter than body in both sexes, fore femora with blunt process, apex of paramere hooked and sub-apex with blunt protrusion..... (Fig. 3A-F) ***L. griseus***
2. Elongated species with body size 27.41-31.23mm in males and 28.79-33.33mm in females, respiratory siphon longer than body length, respiratory siphon longer than body length, paramere with hooked apex and a

small process at tip..... (Fig. 3G-L) ***L. ruber***

A. *Laccotrephes griseus* (Guérin-Méneville, 1835)

1844. *Nepa griseus* Guérin-Méneville, *Iconogr. Regne. Anim. Ins.*, 352.

1910. *Laccotrephes griseus* (Guérin-Méneville, 1844): Distant, *Fauna of Brit. India*, 5: 314.

Material examined: 1♂1♀, FBRC/ZSI/INS/2479, 15.vii.2021, Koyalagudem; 1♂, FBRC/ZSI/INS/2510, 16.viii.2024, Rollpadu Wild life somctuary; 2♂, FBRC/ZSI/INS/2770, 7.iv.2024, Ananthavorum; 2♂1♀, FBRC/ZSI/INS/2840,

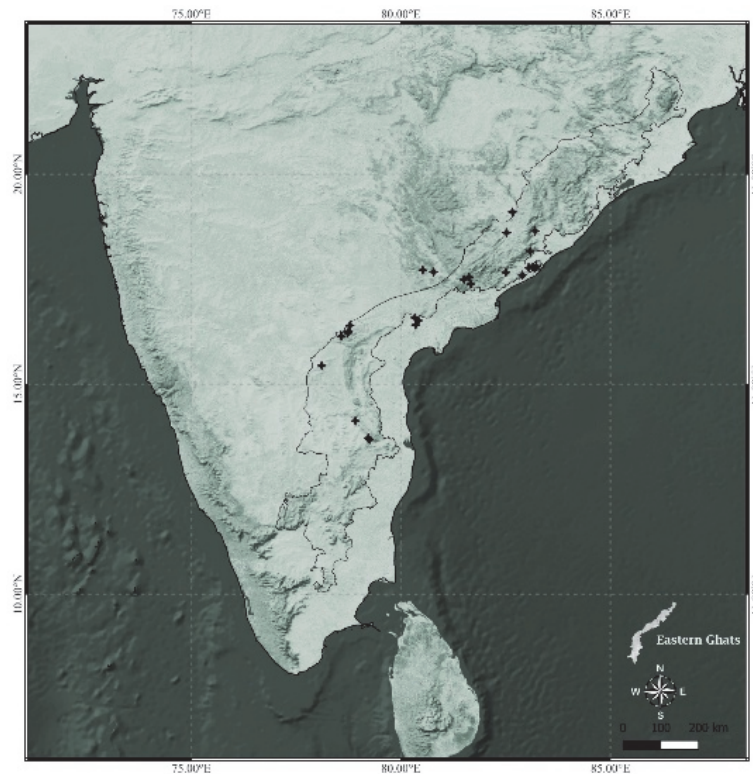


Fig. 1 Survey locations throughout the Eastern Ghats (parts of Odisha, Andhra Pradesh and Telangana)

18.i.2022, Kalab dam; 1♂, FBRC/ZSI/INS/2844, 20.i.2022, Saradapur; 2♂1♀, FBRC/ZSI/INS/2855, 22.xii.2021, pond near Bairamrampally;

2♂, FBRC/ZSI/INS/2883, 20.xii.2021, Stream near Bolghatpalli; 1♀, FBRC/ZSI/INS/2890, 3.x.2021 Octopus view point; 1♂, FBRC/ZSI/INS/4447, 8.iii.2023, Mudasarlova resarvoir; 1♂, FBRC/ZSI/INS/3612, 29.vii.2022, Kasipatnam, 1♀, FBRC/ZSI/INS/3760, 17.x.2024, Konndakara pava Lake Coll. Deepa J Somesh B.

Diagnostic characters: Small species with body length 13.7-15.21mm in males and 15-16.43mm in females. Abdomen above with bluish tinge. Anterior area of prosternum provided with a strong acute spinelike structure. Respiratory siphon shorter than body length, 10.81-12.07mm in males and 12.46-14.12mm in females. Pygophore subquadrate in ventral view, posterior margin shallowly concave, latero-posterior angles rounded, anal segment short, slightly exposed dorsally. Parameres are

symmetrical and slightly hook shaped at apex, while sub-apex has blunt protrusion, inner margin smooth, lacking teeth. In females' sub-genital plate broad triangular, ovipositor slender, elongate with narrow base. Gonapophyses with two pairs of plate-like structures.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Delhi, Gujarat, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Manipur, Meghalaya, Nagaland, Odisha, Pondicherry, Punjab, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh and West Bengal (Jaiswal and Somesh, 2023).

Elsewhere: Malaysia, Myanmar, Seychelles, Sri Lanka and Thailand (Jaiswal and Somesh, 2023).

Note: This species can be found in moderate numbers from all types of wetlands, small streams. It inhabits the bottom littoral zone weedy zone of stagnant or slow flowing waters. It is very common

Table 3. Sequence divergence of COI gene (K2P) for *Laccotrephes* species and out groups

Genebank No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
KY320485	<i>Laccotrephes japonensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KF966560	<i>Laccotrephes japonensis</i>	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KY320469	<i>Laccotrephes aff. pfeifferiae</i>	0.15	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KP274069	<i>Laccotrephes maculatus</i>	0.16	0.15	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-
OR056392	<i>Laccotrephes maculatus</i>	0.20	0.19	0.18	0.15	-	-	-	-	-	-	-	-	-	-	-	-
PP409484	<i>Laccotrephes maculatus</i>	0.20	0.19	0.18	0.15	0.00	-	-	-	-	-	-	-	-	-	-	-
PV041420	<i>Laccotrephes ruber</i>	0.16	0.15	0.15	0.02	0.14	0.14	-	-	-	-	-	-	-	-	-	-
PV60380	<i>Laccotrephes griseus</i>	0.18	0.18	0.18	0.16	0.15	0.15	0.17	-	-	-	-	-	-	-	-	-
PP032035	<i>Laccotrephes griseus</i>	0.18	0.18	0.19	0.16	0.15	0.15	0.17	0.01	-	-	-	-	-	-	-	-
PQ436053	<i>Diplonychus annulatus</i>	0.20	0.19	0.20	0.19	0.20	0.20	0.18	0.19	0.19	-	-	-	-	-	-	-
PV041409	<i>Diplonychus molestus</i>	0.18	0.19	0.20	0.18	0.19	0.19	0.19	0.17	0.18	0.19	-	-	-	-	-	-
PP885728	<i>Diplonychus rusticus</i>	0.22	0.22	0.20	0.17	0.18	0.18	0.18	0.21	0.22	0.17	0.15	-	-	-	-	-
PV041413	<i>Diplonychus rusticus</i>	0.21	0.21	0.19	0.17	0.17	0.17	0.17	0.21	0.21	0.17	0.15	0.01	-	-	-	-
MN053270	<i>Diplonychus esakii</i>	0.20	0.20	0.22	0.21	0.19	0.19	0.21	0.21	0.21	0.19	0.15	0.09	0.09	-	-	-
MN053261	<i>Diplonychus esakii</i>	0.19	0.20	0.22	0.21	0.20	0.20	0.21	0.20	0.20	0.19	0.15	0.10	0.09	0.01	-	-
MN053268	<i>Diplonychus esakii</i>	0.19	0.20	0.22	0.21	0.20	0.20	0.21	0.21	0.21	0.19	0.15	0.10	0.09	0.01	0.01	-
PV041414	<i>Metrocoris communis</i>	0.26	0.26	0.25	0.22	0.23	0.23	0.23	0.28	0.29	0.24	0.23	0.22	0.21	0.23	0.23	0.23

species throughout India and occurs at the edges of water bodies.

B. *Laccotrephes ruber* (Linnaeus, 1764)

1764. *Nepa ruber* Linnaeus, *Mus. Lud. Ulr.*, 165.

1906. *Laccotrephes ruber* (Linnaeus): Distant, *Fauna of Brit. India*, 3: 18.

Material examined: 1 ♂, FBRC/ZSI/INS/2471, 22.xii.2020, Gambheeram Gadda reservoir; 1 ♂ 1 ♀, FBRC/ZSI/INS/2478, 15.vii.2021, Koyalagudem; 1 ♀, FBRC/ZSI/INS/2482, 16.viii.2021, Rollapadu Wildlife Sanctuary; 1 ♀, FBRC/ZSI/INS/2837, 18.i.2022, Octopus view point; 1 ♂, FBRC/ZSI/INS/3011, 6.xii.2021, Muran dam; 1 ♂, FBRC/ZSI/INS/3013, 5.xii.2021, Kalab dam; 1 ♂, FBRC/ZSI/INS/3024, 7.xii.2021, Saradapur; 2 ♀ 2 ♂, FBRC/ZSI/INS/3143, 27.vii.2022, Musurumilli reservoir; 2 ♂ 3 ♀, FBRC/ZSI/INS/3377, 19.vii.2022, Kondakarla lake; 1 ♂ 2 ♀, FBRC/ZSI/INS/3597, 26.ii.2023, Thatipudi reservoir, Coll. Deepa J and Somesh B.

Diagnostic characters: Medium sized species

with body length 27.41-31.23mm in males and 28.79-33.33mm in females. Respiratory siphon longer than the body length, 29.36-33.83mm in males and 29.76-35.37mm in females. Prosternum convex in the middle. Pygophore elongate than wide, posterior margin deeply emarginate, lateral angles prominent. Parameres stout and strongly curved inward, apex distinctly hooked and broadened (claw-like), inner margin with subapical swelling, basal half thick, apical half abruptly narrowed. In females, subgenital plate broadly rounded posteriorly. Gonapophyses shorter and stouter, apex blunt rather than needle-like.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Odisha, Punjab, Sikkim, Tamil Nadu, Telangana, Tripura, Uttar Pradesh and West Bengal (Jaiswal and Somesh, 2023).

Elsewhere: China, Japan, Nepal, Pakistan and Taiwan (Jaiswal and Somesh, 2023).

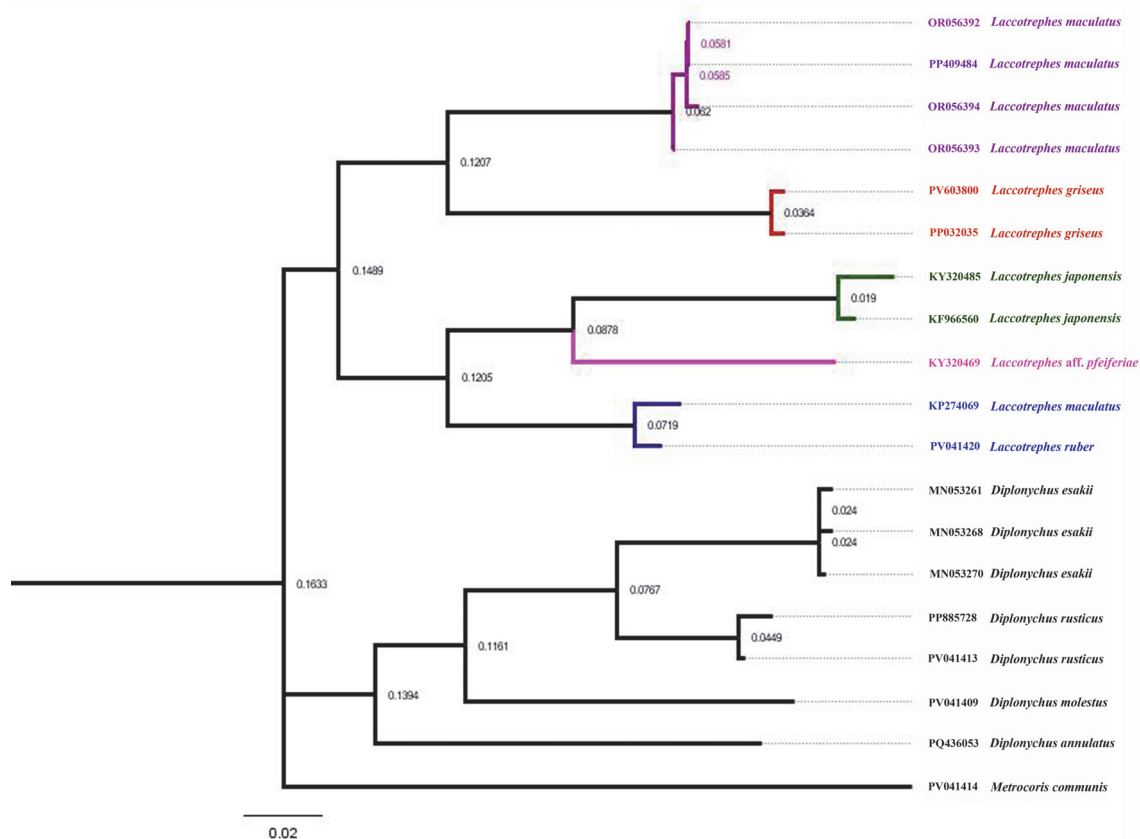


Fig. 2 Maximum likelihood phylogenetic tree for *Laccotrephes* species through mtCOI gene in the present study

Note: It is a common species with wide distribution range throughout the Indo-Australian region and found all most all sorts of freshwater bodies with stagnant or low flowing water entangled with aquatic weeds.

Molecular identification: Pairwise genetic distances were estimated using the mitochondrial COI gene to aid in species identification and the evaluation of genetic divergence within and among *Laccotrephes* species. Table 3 shows the matrix of interspecific and intraspecific distances. Sequences from both GenBank and freshly generated data are included, with GenBank accession numbers and associated species names provided.

The genetic distance matrix shows minimal intraspecific divergence between conspecific sequences but substantial interspecific divergence. For example, two sequences of *L. griseus* (Sl. No.

3 and 4; GenBank No. PV603800 and PP032035) have a minimal divergence of 0.0073, indicating their conspecific identity. In comparison, the divergence between *L. griseus* and *L. ruber* or *L. maculatus* varies from 0.155 to 0.168, indicating a clear species-level distinction.

The morphological and molecular distinction of the genus *Laccotrephes* was evaluated for the first time from the Eastern Ghats. IQTREE and MEGA 11 software were used to examine the conventional mitochondrial DNA barcode (COI) and build a maximum-likelihood tree based on the Kimura 2-parameter (K2P) model. The number labels on internal branches represent K2P genetic distances (substitutions per site) calculated by the ML analysis. The scale bar (0.02) indicates that branch lengths are expressed in terms of predicted substitutions (2% divergence per 0.02). This study's morphological and genetic investigation revealed the identification of two species. Previous

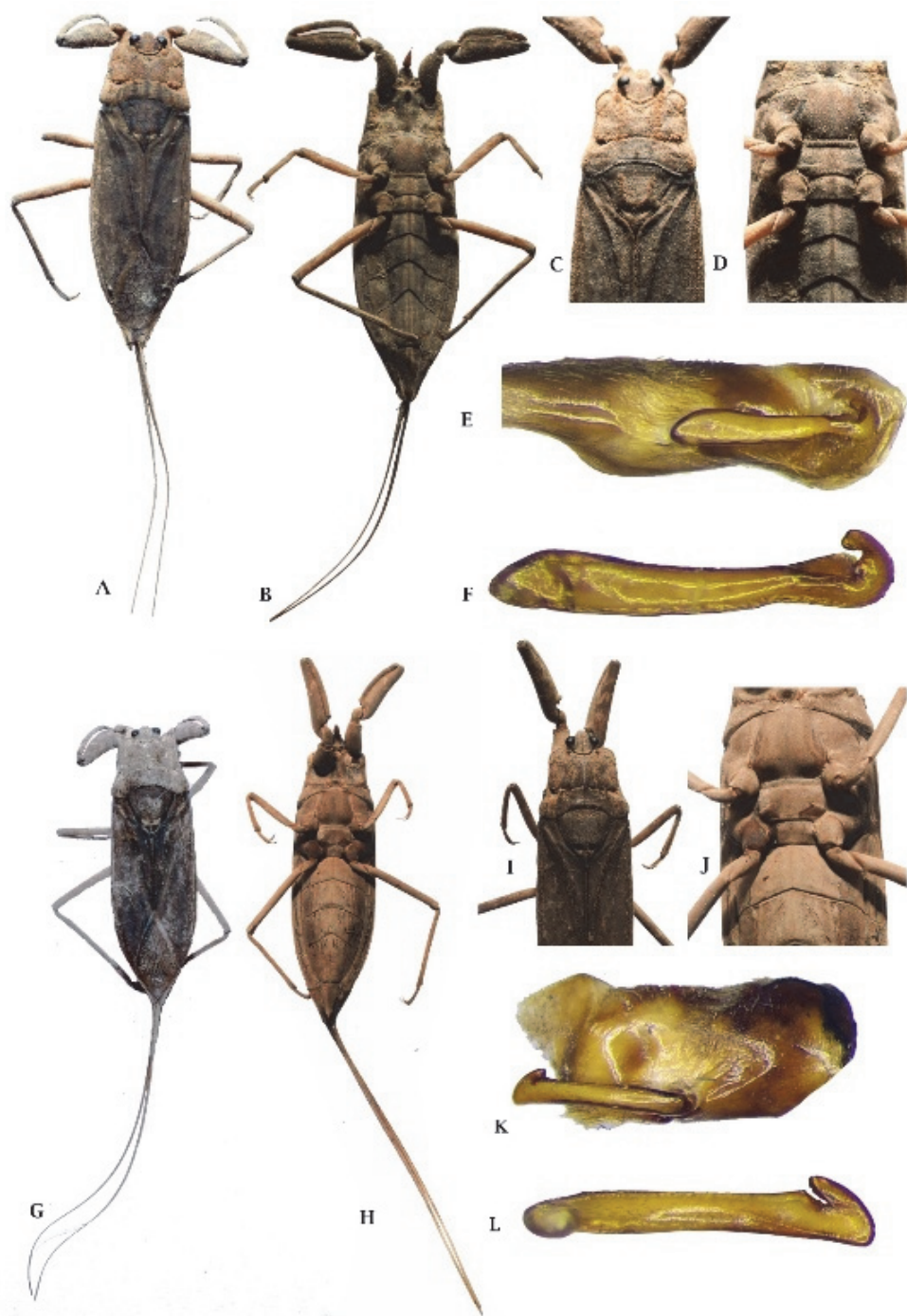


Fig. 3 *Laccotrephes griseus* (Guérin-Méneville, 1835). A – habitus (dorsal view), B – habitus (ventral view), C – head and pronotum, D – metasternum, E – pygophore with paramere, F – right paramere (lateral view); (G -L) *Laccotrephes ruber* (Linnaeus, 1764). G – habitus (dorsal view), H – habitus (ventral view), I – head and pronotum, J – metasternum, K – pygophore with paramere, L – left paramere (lateral view)

morphological investigations focused mostly on male genitalia (Polhemus and Keffer, 1999; Keffer, 2004). This time, molecular data is included to discriminate between the two sister species, *L. griseus* and *L. ruber*.

The tree (Fig. 2, Table 2) mostly supports the current species boundaries, but some changes may be required. *L. maculatus*, *L. japonensis*, and *L. griseus* form monophyletic clades, indicating that these species are genetically separate. In contrast, the nesting of the *L. aff. pfeifferiae* specimen within *L. japonensis* suggests that it may not be a distinct taxon; it could be a cryptic morph or just mislabeled. Similarly, the merger of *L. ruber* and *L. maculatus* indicates that their species borders are ambiguous and should be reconsidered. *Laccotrephes maculatus* (KP274069) does not form its own clade and clusters with *L. ruber* (PV041420) at a modest divergence (~0.0719). This shows that some specimens may have been miss-assigned. Based on partial mitochondrial (COI) sequences, the molecular data provided here show very low genetic divergence (p-distance = 0.0166) between *L. ruber* (PV041420) and *L. maculatus* (KP274069). This degree of divergence, around 1.66%, is well within the range of intraspecific variation seen in aquatic Hemiptera and other insect species (Hebert et al., 2003; Zhou et al., 2009). Interspecific divergence values within the genus *Laccotrephes*, on the other hand, usually approach 15%, as seen in other taxa studied. These discordant placements show areas where morphology and genetics disagree and require further exploration. In contrast, the apparent separation of each *Diplonychus* species in the tree suggests that there is no obvious taxonomic conflict within the genus.

Laccotrephes griseus can be distinguished from its congeners by its larger body size, shorter respiratory siphon, and fine granulation on the pronotum and hemelytra. It is most morphologically like *L. ruber*, but can be distinguished by its stronger forelegs and changes in male genitalia, and the body length with respiratory siphon (Jaiswal and Somesh, 2023). Molecular data indicate *L. griseus* in a distinct clade (blue, Fig. 2), with a pairwise divergence of 0.17-0.18 from *L. ruber*, confirming

its status as a different species. The clade that includes *L. griseus* (blue in Fig. 2) is well-supported and distinct from *L. japonensis* and *L. ruber*. Genetic distances indicate species-level separation, with values above normal intraspecific thresholds. Morphological distinctions remain consistent with prior taxonomic treatments, while subsequent investigations may investigate intraspecific variation across a wider range.

Overall, the COI phylogeny highlights potential cryptic diversity and suggests that an integrative approach (using both morphology along with molecular data) is needed to resolve species limits in these water bugs. These findings demonstrate the utility of DNA barcoding for uncovering unexpected relationships and guiding future revisionary work. The identified specimen mentioned in the publication is deposited at the National Zoological Collection of Freshwater Biology Regional Center, Zoological Survey of India and available on request.

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