ACADEMIA JOURNAL OF BIOLOGY 2024, 46(3): 73–84
DOI: 10.15625/2615-9023/21146

THE *Laccotrephes robustus* STÅL, 1871 (Hemiptera: Nepidae: Nepinae) IN VIETNAM, WITH NOTES ON ITS' MORPHOLOGICAL AND DNA ANALYSES

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Received 23 July 2024; accepted 13 September 2024

ABSTRACTS

The genus *Laccotrephes* Stål, 1865 has been known in Vietnam with four species, *Laccotrephes grossus* (Fabricius, 1787), *Laccotrephes longicaudatus* Nieser, Zettel & Chen, 2009, *Laccotrephes pfeiferiae* (Ferrari, 1888) and *Laccotrephes simulatus* Montandon, 1913. The species *Laccotrephes robustus* Stal, 1871 is a species endemic to the Philippines, first recorded in Vietnam from specimens collected in Cao Bang, Bac Kan and Nghe An provinces. The occurrence of *L. robustus* in Vietnam was confirmed by morphological and molecular data.

Keywords: Nepidae, Nepinae, *Laccotrephes*, taxonomy, first record, Vietnam.

Citation: Dai Dac Nguyen, Phan Thi Giang, Ha Ngoc Linh, Nguyen Quang Cuong, Nguyen Thi Hanh, Nguyen Thanh Manh, Ryndevich Sergey, Truong Xuan Lam, 2024. The *Laccotrephes robustus* Stål, 1871 (Hemiptera: Nepidae: Nepinae) in Vietnam, with notes on its' morphological and DNA analyses. *Academia Journal of Biology*, 46(3): 73–84. https://doi.org/10.15625/2615-9023/21146

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INTRODUCTION

The genus *Laccotrephes* Stål, 1866, is a large-sized genus of water scorpions or water stick insects assigned to the subfamily Nepinae, belonging to the family Nepidae, with seventy-one valid species and one fossil species (Keffer, 2004; Polhemus & Polhemus, 2008; BioLib.cz, 2024). *Laccotrephes* species are characterized by their elongated bodies ranging from a few millimeters to several centimeters in length, adapted for life in aquatic habitats, with prominent raptorial forelegs specialized for capturing prey. They exhibit remarkable diversity in size, colouration, and morphology, reflecting their adaptation to various environmental niches. Predatory near the water surface, they are predominantly found in freshwater bodies such as ponds, lakes, streams, marshes, and shallow, stagnant, or slow-moving waters in warm regions of Africa, Asia, and Australia (Keffer, 2004; Polhemus & Polhemus, 2008, 2013; Polhemus & Keffer, 1999). However, the genus *Laccotrephes* remains relatively understudied compared to other aquatic insects. Further research into their taxonomy, biology, and ecological roles is essential for a comprehensive understanding of freshwater ecosystems and the conservation of biodiversity.

In Vietnam, four species of *Laccotrephes* have been recorded, i.e., *Laccotrephes grossus* (Fabricius, 1787), *Laccotrephes longicaudatus* Nieser, Zettel & Chen, 2009, *Laccotrephes pfeiferiae* (Ferrari, 1888) and *Laccotrephes simulatus* Montandon, 1913 (Nieser et al., 2005, 2009; Polhemus et al., 2013; Thi et al., 2022). In this study, the species *Laccotrephes robustus* Stål, 1871 is recorded from Vietnam for the first time. The species *L. robustus* has been found only in the Philippines and is currently restricted to the Philippines (Nieser et al., 2009; Polhemus & Keffer, 1999). We also provided a new morphological description of this species based on the Vietnamese specimens with notes on the DNA molecular data.

MATERIALS AND METHODS

Laccotrephes specimens were collected from ponds and lakes in Vietnam (Cao Bang, Bac Kan, and Nghe An provinces) (Appendix 1). A total of 11 specimens of adult male (♂) and female (♀) from the genus *Laccotrephes* were collected by the Institute of Ecology and Biological Resources (IEBR) and 10 sequences of *Laccotrephes* species obtained from GenBank were analyzed as ingroups (Table 1). Additionally, three specimens of *Nepa hoffmanni* Esaki, 1925 from GenBank were included in the analyses as outgroups (Table 1).

Table 1. The data of specimens used in this study

Morphospecies	Specimen code	Collecting date	Locality	Accession numbers	Depository/ Reference
Laccotrephes (ingroups)					
Laccotrephes robustus Stal, 1871	TXL IV 1	10.vii.2024	Nghe An province, Vietnam	PQ319868	IEBR
Laccotrephes robustus Stal, 1871	TXL IV 2	10.v.2021	Cao Bang province, Vietnam	PQ319866	IEBR
Laccotrephes robustus Stal, 1871	TXL IV 3	26.x.2021	Bac Kan province, Vietnam	PQ319867	IEBR
Laccotrephes sp.	TXL II 4	03.viii.2024	Nghe An province, Vietnam	PQ319876	IEBR
Laccotrephes sp.	TXL II 5	10.v.2021	Cao Bang province, Vietnam	PQ319875	IEBR
Laccotrephes sp.	TXL II 6	12.v.2021	Cao Bang province, Vietnam	PQ319874	IEBR
Laccotrephes sp.	TXL II 7	16.vii.2024	Nghe An province, Vietnam	PQ319873	IEBR

Morphospecies	Specimen code	Collecting date	Locality	Accession numbers	Depository/ Reference
Laccotrephes sp.	TXL II 8	26.x.2021	Bac Kan province, Vietnam	PQ319872	IEBR
Laccotrephes sp.	TXL II 10	31.x.2021	Bac Kan province, Vietnam	PQ319871	IEBR
Laccotrephes sp.	TXL II 11	31.x.2021	Bac Kan province, Vietnam	PQ319870	IEBR
Laccotrephes sp.	TXL II 12	31.x.2021	Bac Kan province, Vietnam	PQ319869	IEBR
Laccotrephes (ingroups) - Genbank					
Laccotrephes griseus (Gúerin-Méneville, 1835)			India	KX365491	Basu & Venkatesan, 2016
Laccotrephes pfeiferiae (Ferrari, 1888)	LEBIP-SN		CHINA: Tianjin (Wuqing Country)	KY320469	Ribeiro et al., 2018
Laccotrephes robustus Stal, 1871				FJ456948	Hua et al., 2009
Laccotrephes japonensis Scott, 1874	SOKN034		South Korea	KF966560	Park & Oh, 2014
Laccotrephes japonensis Scott, 1874	NSMK-IN-1503A0218			OL664504	Kim, 2021
Laccotrephes japonensis Scott, 1874	NSMK-IN-1503A0219			OL664505	Kim, 2021
Laccotrephes japonensis Scott, 1874	NSMK-IN-1503A0220			OL664506	Kim, 2021
Laccotrephes maculatus (Fabricius, 1775)	ANMB15			OR056394	Mwaura & Kamiri, 2023
Laccotrephes maculatus (Fabricius, 1775)	ANMB14			OR056393	Mwaura & Kamiri, 2023
Laccotrephes maculatus (Fabricius, 1775)	ANMB13			OR056392	Mwaura & Kamiri, 2023
Outgroup (Genbank)					
Nepa hoffmanni Esaki, 1925	NSMK-IN-1603A0053			OL663292	Kim, 2021
Nepa hoffmanni Esaki, 1925	NSMK-IN-1603A0052			OL663291	Kim, 2021
Nepa hoffmanni Esaki, 1925	NSMK-IN-1603A0051			OL663290	Kim, 2021

All specimens were deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, located in Ha Noi, Vietnam.

Morphological terminology followed that of Keffer (1996) and Nieser et al. (2009). The following parts of the bodies were measured for *Laccotrephes* samples following Polhemus & Keffer (1999), using the software Image-J (http://imageJ.nih.gov/ij/) based on the direct stacking pictures designed as stated above, all dimensions were given in mm. Specimens were labelled with their identification numbers and locality information and individually preserved in vials containing 99% ethanol. For DNA extraction, the right hind leg or right mid-leg of each specimen was removed for subsequent molecular phylogenetic analysis and DNA barcoding. The remaining body was pinned for morphological study.

External morphological characteristics were examined in specimens using a Nikon SMZ1270 stereomicroscope. The preparation of genitalia for examination was conducted as follows. Each male specimen was relaxed by soaking it in 70% ethanol for three days. The genitalia were then detached and soaked in hot 10% KOH for five minutes to remove body fat and muscle. The endosoma was extracted from the phallosoma using fine tweezers after the phallus was removed from the pygophore. All parts of the male genitalia were preserved in a vial filled with propylene glycol and associated with the pinned specimens. The male genital morphology was examined using the Nikon SMZ1270 stereomicroscope.

Focus-stacked images were produced by using Helicon Focus Pro 7.5.8 (Helicon Soft Ltd., Ukraine), and then improved using the retouching function of Helicon Focus. Finally, the color balance, contrast, and sharpness were adjusted using GIMP 2.8.22 (available at http://www.gimp.org).

Total DNA was isolated from each specimen's left leg/legs using the Chelex-TE-ProK protocol (Satria et al., 2015). Mitochondrial COI gene fragments were analyzed using the primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGT GACCAAAAAATCA-3'). Polymerase chain reaction (PCR) amplification, cycle sequencing reaction, sequencing using ABI PRISM 3130xl (Applied Biosystems), and sequence assembly using ChromasPro 1.7.6 (Technelysium Pty Ltd., Australia) were performed following the methods outlined by Satria et al. (2015). The PCR thermal profile for the COI gene fragment included initial denaturation at 94 ^{o}C for 2 minutes, followed by denaturation at 94 ^{o}C for 30 seconds, annealing at 48.5 ^{o}C for 30 seconds, and extension at 72 ^{o}C for 45 seconds for 35 cycles, with a final extension step at 72 ^{o}C for 7 minutes. The obtained COI sequences were aligned using ClustalW (Thompson et al., 1994) built-in MEGA X (Kumar et al., 2018; Folmer et al., 1994). Successful assembly of each COI sequence was confirmed by translating it to the amino acid sequence and checking the absence of frameshifts caused by erroneous indels. Finally, a sequence dataset (567 bp) was obtained and used for phylogenetic analyses. All nucleotide sequences were deposited in GenBank (Table 1).

Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were performed based on the COI dataset. ML analyses were performed by using IQ-TREE 2.1.2 (Nguyen et al., 2015), with 1,000 replicates of ultrafast bootstrap (Hoang et al., 2018). The optimal substitution model was selected based on BIC for the dataset by using ModelFinder (Kalyaanamoorthy et al., 2017): GTR+I+G. BI analyses were performed by using MrBayes v.3.2.7 (Ronquist & Huelsenbeck, 2003) for 10,000,000 generations, with default parameter setting (GTR+G model; sampling every 500 generations; tuning parameters every 100 generations; with a burn-in of 25%). The effective sampling size (ESS) of each parameter was confirmed to be $\geq$ 200 for all runs by using Tracer 1.7.1 (Rambaut et al., 2018).

RESULTS

Based on the COI dataset comprising sequences from 11 Vietnamese *Laccotrephes* specimens in this study and 10 COI sequences

of *Laccotrephes* specimens obtained from GenBank (Table 1, Fig. 1), the monophyly of the genus *Laccotrephes* was confirmed with maximum posterior probability and support values (PP = 1; UFB = 100) (Fig. 1). On the other hand, both BI and ML trees identified six species with high supporting values (PP ≥ 0.96; UFB ≥ 95) and long basal branches (Fig. 1). These species are *Laccotrephes pfeiferiae* (PP = 0.93/UFB = 81), *Laccotrephes japonensis* (1/100), *L.* sp. (1/100), *Laccotrephes robustus* (1/100), *Laccotrephes griseus* (0.85/58), and *Laccotrephes maculatus* (1/99) (Fig. 1). Among the 11 *Laccotrephes* specimens from Vietnam, 8 belong to one group of OTUs, and 3 belong to the same group of OTUs as the species *L. robustus*.

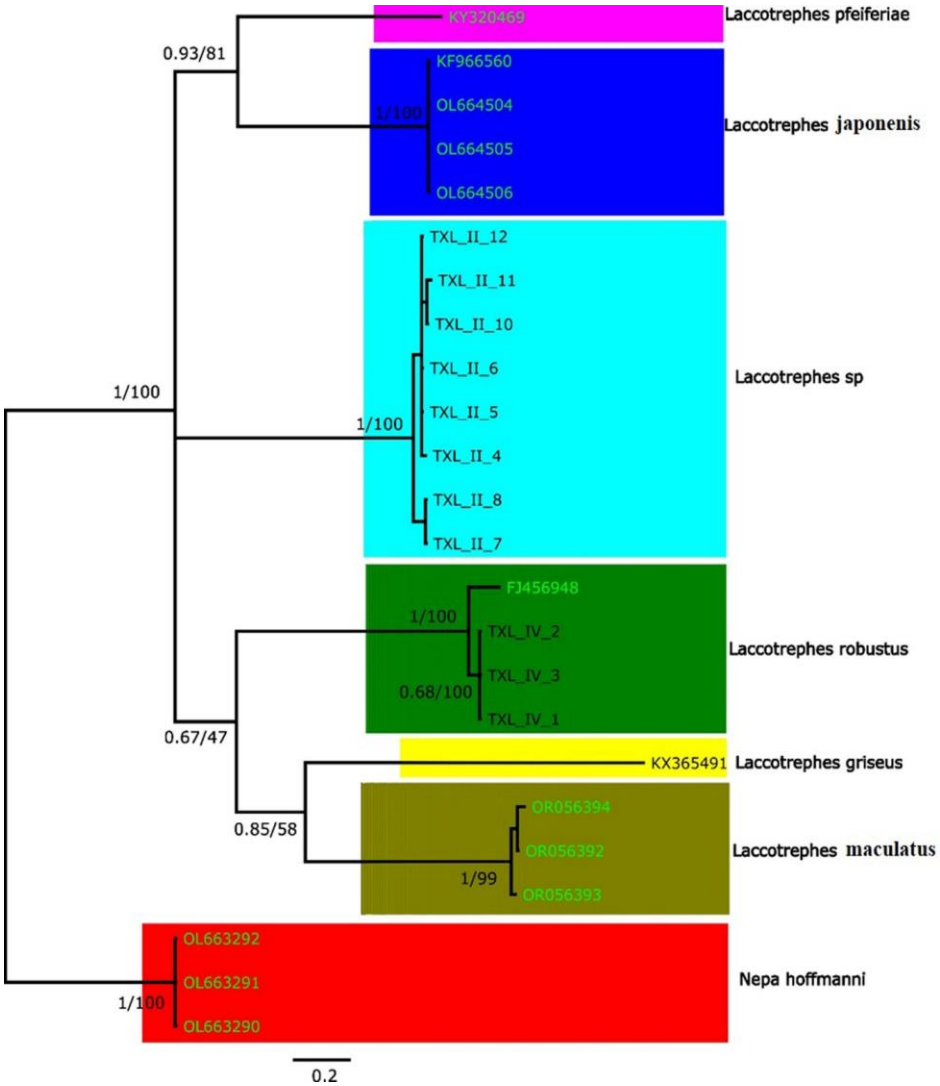

Figure 1. Bayesian inference phylogenetic tree was constructed based on a COI dataset (567 bp) of *Laccotrephes* species collected from Vietnam and COI sequences of *Laccotrephes* species obtained from GenBank. Posterior probability values and bootstrap values (in %) are indicated beside the basal nodes. Tips of the tree are labelled with voucher specimen IDs. Voucher specimen IDs in black represent specimens from the IEBR collection, while those in green represent specimens from GenBank

Consequently, in this study, we recorded *Laccotrephes robustus* from Vietnam based on molecular data.

Taxonomic account

***Laccotrephes robustus* Stål, 1871 (Figs. 2–4)**

Laccotrephes robustus Stål, 1871: 706.

Nepa robusta Ferrari 1888: 182.

Laccotrephes robustus Polhemus & Keffer 1999: 2–3.

Material examined. VIETNAM: Nghe An province: 1 ♂, 1 ♀, Quy Hop, H = 312 m, 10.vii.2024, Truong Xuan Lam. Cao Bang province: 1 ♂, Nguyen Binh, H = 879 m, 10.v.2021, Truong Xuan Lam. Bac Kan province: 2 ♂, 1 ♀, Nam Xuan Lac, H = 889 m, 26.x.2021, Truong Xuan Lam.

Diagnosis (male). Dimensions (n = 4 ♂, 2 ♀, Appendix 2): Length of body 43.7–45.88 mm, maximum width of body 12.88–13.65 mm, length of siphon 40.85–45.35 mm, median length of head 3.76–4.02 mm, width of head 3.98–4.18 mm, width across eyes 4.04–4.21 mm, length of eye 1.61–1.73 mm, width of eye 1.35–1.42 mm, distance between eyes 1.71–1.89 mm, length of posterior eyes of head 1.10–1.22 mm, median length of pronotum 6.61–6.98 mm, maximum width of anterior lobe pronotum 9.89–10.02 mm, maximum width of posterior lobe pronotum 11.37–12.51 mm, posterior width of abdomen 12.87–13.35 mm.

Colour (Fig. 2): Light to dark brown. Eyes are blackish. The dorsum of the abdomen below the wings is light to medium brick red, except for the apex, which is blackish. Legs are light brown, with the fore femur exhibiting a distinct light to pale yellow zigzag band just distally of the middle, and the fore tibia having a pale spot in the proximal third. The middle and hind legs also have the fore femur with a distinct light to pale yellow zigzag band just distally of the middle, and the fore tibia with a pale yellow straight line.

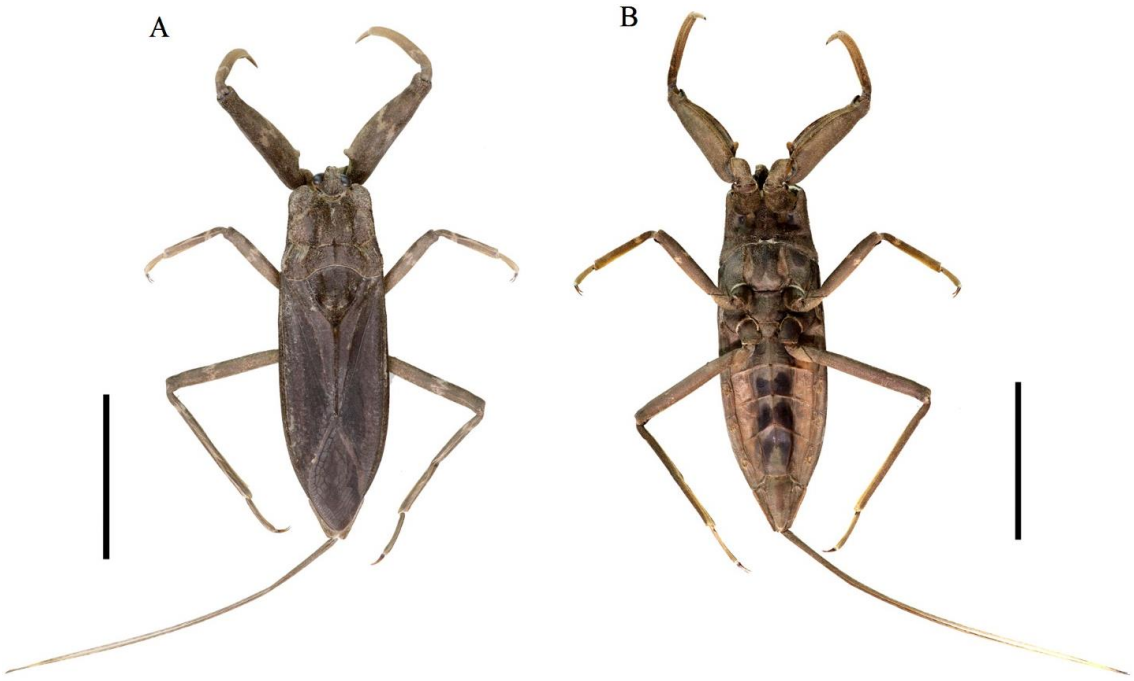

Figure 2. Laccotrephes robustus Stal, 1871 (male): A- habitus, dorsal aspect; B- habitus, thoracic and abdominal. Scale bars: 20 mm for Figs. A, B

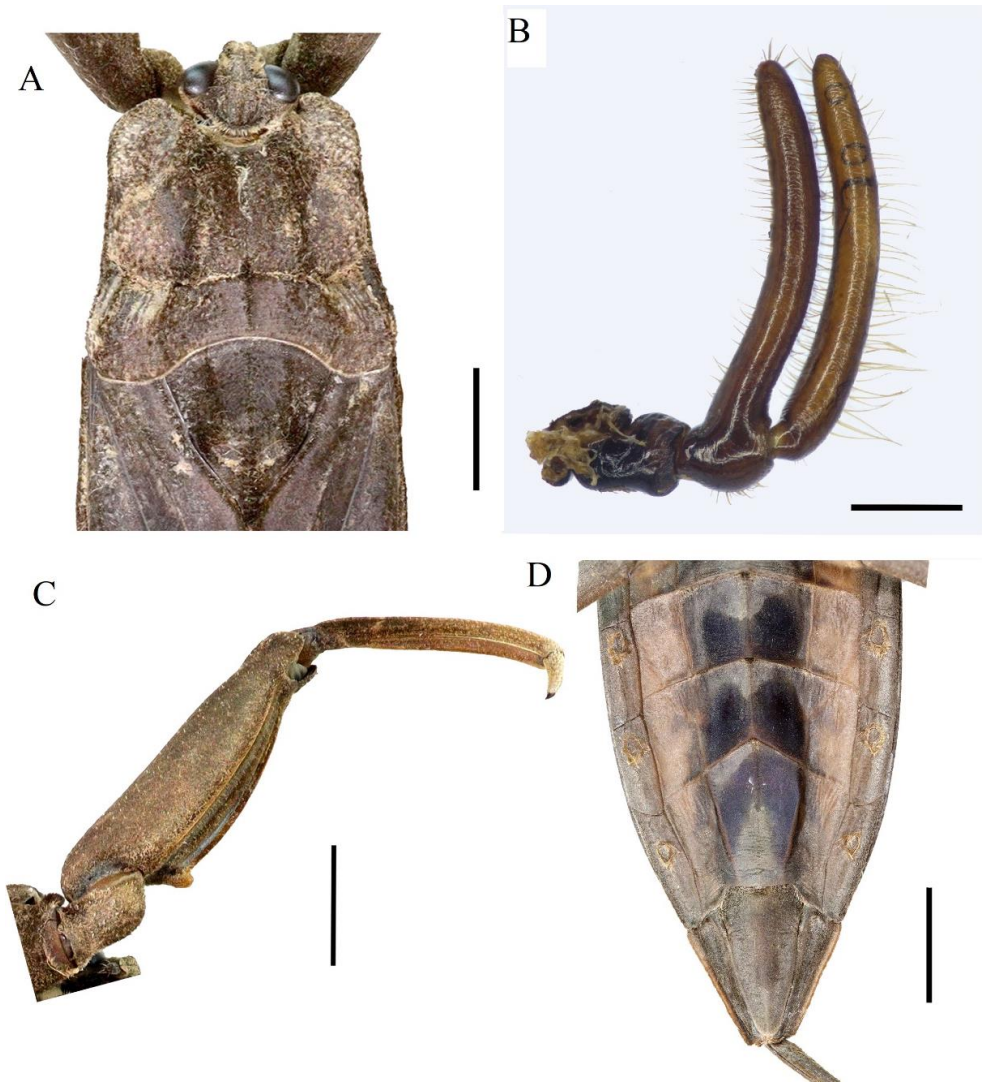

Figure 3. Laccotrephes robustus Stal, 1871: A- pronotum; B- antenna; C- foreleg; D- terminal abdominal habitus. Scale bars: 5 mm for Figs. C, B; 2.5 mm for Fig. A; 0.5 mm for Fig. B

Structural characteristics (Figs. 2, 3): By general large body (Fig. 2). Head, pronotum, scutellum, proximal fifth of hemielytron, and proximal half of its costal margin are beset with tufts of short bristles. The head is small, with a length slightly less than its width across the widest part (1.3 times). Clypeus and lora well recognizable, maxillary plates large, meeting in front of clypeus; median carina on vertex well developed, with distinct transverse depression at level of anterior third of eyes, anterior part of carina higher than posterior part. Eyes in lateral view about even with the level of the median carina. The eyes are comparatively small, with a length/with = 1.19–1.21. The minimum distance between the eyes is 1.89 mm, which equals 0.4 times the width of the head; the length of the posterior eyes of the head 1.16. Antennal segment III is slightly shorter than segment II (III/II = 0.92) (Fig. 3B). The outer margin is adorned with long setae, and segment III distally bears approximately 44–48 long hairs, arranged in a single or more or less double row, measuring about 0.85–1.23 times as long as the width of the segment and ranging from 0.17 to 0.26 in length. The width of

segment III is between 0.20 and 0.22. Additionally, shorter bristle-like hairs, about half as long as the width of the segment, are evenly dispersed over the ventral surface of segments II and III. Pronotum (Fig. 3A), the anterior part of pronotum is 1.8 times longer than the posterior part of pronotum; anterior submedian tubercles are present, blunt; submedian carinae are broad and dorsally blunt; the transverse groove is medially interrupted, laterally deep and wide alongside the submedian carinae, becoming shallow and narrow medially. Lateral margins of the pronotum are nearly straight, with humeral angles slightly rounded and projecting; humeral width is less than the maximal width (1:0.86). The prosternal carina is slightly raised posteriorly with a distinct blunt tooth anteriorly; a fringe of setae is only present posterolaterally, not meeting posteriorly. Scutellum (Fig. 3A) with hayfork-shaped carinae are well developed except for the medioanterior part. Abdomen (Fig. 3D) parallel-sided in the anterior two-thirds, posteriorly convergent in males. Fore leg (Fig. 3C) with profemur large and strongly incrassate, a length about 3.1 times its width. It has a distinct and comparatively acute proximal tooth, while the femoral groove of the male bears a small but distinct distal tooth. The lengths of the segments of the foreleg are as follows: profemur/protibia/protarsus = 13.18/ 9.681/1.91. The protibia (Fig. 3C) features a strong tooth and a concavity near the base on the flexor side. The lengths of the segments of the middle leg are: mesofemur/mesotibia/ mesotarsus = 10.72/7.78/3.12. The lengths of the segments of the hind leg are: metafemur/ metatibia/metatarsus = 14.11/14.78/5.41. Forewings are sparsely set with long, fine setae, except on the clavus. The apex exhibits clear network venation. The abdomen is nearly parallel-sided anteriorly and slightly convex posteriorly. Sternite 7 (genital operculum; Fig. 3D) is narrow and shield-shaped.

Male genitalia (Fig. 4) typical for the *Laccotrephes ater* group as described by Keffer (2004). The pygophore (Fig. 4A) is elongated, with the anterior part (ahead of paramere insertion) longer than the posterior part. The proctiger appears sharply acuminate in the lateral aspect, with a densely pilose anal cone. The paramere (Fig. 4G) is elongated, featuring a triangular, recurved apex, without teeth, closely resembling that of *Laccotrephes archipelagi*. The phallus slightly longer than the genital capsule, with the anterior diverticulum longer than the posterior diverticulum (4.27/3.27 mm), its paired apical laminae rounded and relatively long; the free part of the gonoporus is short and round.

Distribution. Philippines (Nieser et al., 2009); Vietnam (Nghe An, Cao Bang, Bac Kan).

DISCUSSION

Based on the COI dataset comprising sequences from 3 Vietnamese *Laccotrephes* specimens (TXL IV 1, TXL IV 2 and TXL IV 3) in this study belong to the same group of OTUs as the species *L. robustus* COI sequences obtained from GenBank with accession numbers FJ456948 (Hua et al., 2009). The male of *L. robustus* (in Vietnam) is also similar to the species *L. robustus* from the Philippines **(**Nieser et al., 2009) in the following characters: with the ratio of the length of antennal segments III:II = 1:0.92; the antennal segment III has about 40–50 medium setae; the prosternal carina from anterior to the right varying is slightly elevated in anterior to blunt and low tooth posteriorly; the scutellum is hayfork-shaped with carinae well developed except the medioanterior part. Maximum width of body 10.4–13.65 mm; Length of siphon 33.0–42.8 mm; Humeral width of pronotum 10.8 mm; male genitalia with genital capsule and paramere (Figs. 4A, 4B, 4C, 4G) with Fig. 17 of Nieser et al. (2009).

However, male of *L. robustus* from Vietnam are larger than males of *L. robustus* from the Philippines **(**Nieser et al., 2009) in length of body 43.7–45.88 mm, median length of head 3.76–4.02 mm, width across eyes 4.04–4.21 mm, length of eye 1.61–1.73 mm, distance between eyes 1.71–1.89 mm, median length of anterior lobe of pronotum 4.31–4.62 mm, median length of posterior lobe of pronotum 2.21–2.37 mm, maximum width of anterior lobe pronotum 9.89−10.02 mm, maximum width of posterior lobe pronotum

11.37–12.51 mm, max width of pronotum 12.04–12.24 mm, length of prosternum 5.32–6.01 mm, length of metasternum 5.91–6.12 mm, length of posternum 3.06–3.78 mm (Appendix 2). Male genitalia of *L. robustus* from Vietnam including dorsal deep articulatory and phallic structures, lateral deep articulatory and phallic structures and ventral deep articulatory and phallic structures are illustrated (Figs. 4D, 4E, 4F).

Figure 4. Genitalia of *Laccotrephes robustus* Stal, 1871: A- Dorsal genital capsule; B- lateral genital capsule; C- ventral genital capsule; D-dorsal deep articulatory and phallic structures; E- lateral deep articulatory and phallic structures; F-ventral deep articulatory and phallic structures; G- Paramere. Scale bars: 1 mm for Figs. A, B, C, D, E, F; 0.5 mm for Fig. G

Acknowledgements: This research was supported by a grant from the Vietnam Academy of Science and Technology (NVCC09.08/24–24) for the senior researcher, Truong Xuan Lam.

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Appendix 1. Habitat of sampling locations. (a) Nam Xuan Lac in Bac Kan province; (b) Nguyen Binh in Cao Bang province; (c) Quy Hop in Nghe An province

Appendix 2. The measurement of the species *Laccotrephes robustus* Stål, 1871 in Vietnam

Measurement (mm)	*Laccotrephes robustus* from Philippine (Nieser et al., 2009)	*Laccotrephes robustus* from Vietnam (in this study)
n	20	4
Length of body	32.5–39.6	43.7–45.88
Maximum width of body	10.4–13.2	12.88–13.65
Length of siphon	33.0–42.8	40.85–45.35
Inner hemelytral commissure	-	23.89–25.03
Median length of head	3.69	3.76–4.02
Width of head	-	3.98–4.18
Width across eyes	3.99	4.04–4.21
Length of eye	-	1.61–1.73
width of eye	-	1.35–1.42
Distance between eyes	-	1.71–1.89
Length of posterior eyes of head	-	1.10–1.22
Length of II segments antenna	1.57	1.56–1.64
length of III segments antenna	1.41	1.42–1.46
The number long hairs in III segments antenna	40-50	43–55
Median length of pronotum	-	6.61–6.98
Median length of anterior lobe of pronotum	-	4.31–4.62
Median length of posterior lobe of pronotum	-	2.21–2.37
maximum width of anterior lobe pronotum	-	9.89–10.02
maximum width of posterior lobe pronotum	-	11.37–12.51
Humeral width of pronotum	10.8	10.6–11.12
Max width of pronotum	11.7	12.04–12.24
Posterior width of abdomen		12.87–13.35
Length of femur of fore leg	14.10	14.28–14.65
Width of femur of fore leg	3.33	3.42–4.06
Length of segments of foreleg: femur/tibia/tarsus	-	14.08–14.52/11.02–11.40/1.89–2.03/0.24–0.26
Length of segments of middleleg: femur/tibia/tarsus	-	11.01–21.12/ 7.13–7.98/3.01–3.26
Length of segments of hindleg: femur/tibia/tarsus	-	15.01–15.75/ 15.03–15.23/3.97–4.58
Length of prosternum	-	5.32–6.01
Length of metasternum	-	5.91–6.12
Length of posternum	-	3.06–3.78
Length of operculum	-	5.92–6.20