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Morphometrical and molecular survey of some water beetles of Manipur

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Abstract

The genus *Laccophilus* from different wetlands were collected during the period 2012 to 2017 in nine districts of Manipur. The specimens comprised of five species viz., *Laccophilus parvulus*, *Laccophilus flexuosus*, *Laccophilus inefficient*, *Laccophilus anticatus* and *Laccophilus chinensis*. Taxonomic study of *Laccophilus* species was more or less a challenging task due to varying degree of variation such as elytral pattern, genitalia and quite difficult to identify the species only by morphological taxonomy. To solve the problems an attempt to confirm the identities of the beetles mitochondrial COX I genes were sequenced and phylogenetic tree was constructed with sequences from databases. The sequences of *Laccophilus anticatus* and *Laccophilus chinensis* reported here were distinct from the available congeneric sequences in databases. They formed two haplotypes, *L. anticatus* was near to *L. comes* and *L. alluaudi* while *L. chinensis* was quite distant from *L. anticatus*.

Keywords: Manipur, *Laccophilus*, Morphology, Molecular, COX I, Phylogeny

1. Introduction

Manipur is one of the tiny hilly states in the North-Eastern (NE) region of India. The state is one of the Indo-Burma Hotspots of the world ^[1]. A high endemism for including in the World hottest hotspots categories for this region might be due to the edge effect of the two continents: India and Euro-Asian plates that collided in the early Eocene epoch 50 to 55 million years ago, ^[2-3], besides other plausible reasons. Still many endemic species has been reporting from the region. Above all the invertebrates and fish were not included in the study for determining the hotspots. In order to fill some of the lacunae of biodiversity assessment of Manipur, the present studies have been taken up.

The genus *Laccophilus* (1817) is one of the largest and most widespread genera of the family Dytiscidae. *Laccophilus* occur in all zoogeographical regions except Antarctica but mostly distributed in tropics. From the recent world catalogue ^[4] lists 282 valid species out of which 60 species occur in oriental region. In India 30 species of genus *Laccophilus* was recorded by ^[5]. However, from the Manipur State Fauna Series No. 10 (Zoological Survey of India) by Mukhopadhyay *et al* ^[6] recorded 10 species from Manipur. The taxonomic work of Sharp ^[7] treated the genus into the global level. The comprehensive revisions of the taxonomy of Oriental *Laccophilus* are comparatively well known – mainly due to the excellent revision of Palaearctic, Oriental and Australian species by Brancucci ^[8], who redescribed all the known species and added several new ones. In India Fabricius ^[9] was the first pioneer in contribution to the taxonomy of Indian aquatic beetles. Later Sharp, D'Orchymout ^[10] and Vazirani ^[11-12], work on the some aquatic beetles species including *Laccophilus* species from Central India. However, the genus *Laccophilus* from Manipur state was not known since the State Fauna Series No. 10 (Zoological Survey of India) by Mukhopadhyay *et al* ^[6]. Hence the present work is undertaken to prepare a list of taxon on the *Laccophilus* species of Manipur.

The molecular studies of *Laccophilus* are scanty from Manipur and taken as promising methods for confirmation of species and identification of the specimens. Besides such study differentiates the con-species and impart much valuable information on the specimens.

To understand *Laccophilus* species diversity, we used an integrated taxonomic approach combining morphological taxonomy and molecular taxonomy for species-level identifications of taxa. The present study reported and confirmed the presence of *Laccophilus anticatus* and *Laccophilus chinensis* among the five species. The sequences of *Laccophilus anticatus* and

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Laccophilus chinensis reported here were distinct from the available congeneric sequences in databases.

2. Materials and Methods

2.1 Morphotaxonomy

A total of 81 specimens (Table 1) were collected from five districts of Manipur for last 5 years (2012 to 2016) and were preserved in the Laboratory of Entomology, P.G. Department of Zoology, D.M. College of Science, Imphal with appropriate voucher numbers. Identification of the specimens was carried out morphologically such as elytra pattern, prosternal process and male external genitalia. Male genitalia

were removed from specimens that were first relaxed in lightly boiling water for 10 minute ^[13]. An insect pin with a bent apex was inserted into the abdominal cavity to hook the base of the genital capsule. The entire capsule was then removed from the abdomen and placed in alcohol to dissect and examine. To clean and clear the genitalia, they were kept in 10% KOH for a few hours and washed in water with little acetic acid added (about one drop per 50cc of water), then examined on microscope slides in glycerol and male genitalia were then glued to a point and placed on the pin beneath the specimen. The illustrations and photographs were based on the examination of the material collected in this study.

Table 1: Study materials for the present studies from four districts of Manipur, number of specimens and date of collection.

S. No	Species	Collection site	Districts of Manipur	Date of collection	Numbers of specimens
1	<i>Laccophilus parvulus</i>	8 exs. Ngaikhong sites of Loktak Lake, 5 exs. Ukhrlul; 7 exs. Tamenglong	Bishnupur Ukhrlul Tamenglong	31.iii.2015 6.xii.2015 6.viii.2016	20 specimens
2	<i>Laccophilus flexuosus</i>	3 exs. Takmupat; 5 exs. Porompat; 2 exs. Churachandpur	Bishnupur Imphal East Churachandpur	31.iii.2015 6.ii.2016 6.iii.2015	10 specimen
3	<i>Laccophilus inefficiens</i>	5exs. Ngaikhong; 5 exs. Longum 3 exs. Thünungei 2 exs. Phubala on	Bishnupur	4.xii.2012 2.ix.2012 6.ii.2013 6.iii.2013	15 specimen
4	<i>Laccophilus chinensis</i>	5exs. Ngaikhong 5 exs. Longum; 3 exs. Thünungei; 2 exs. Phubala	Bishnupur	4.xii.2012 2.ix.2012 6.ii.2013 6.iii.2013	15 specimen
5	<i>Laccophilus anticatus</i>	8exs. Ngaikhong; 6 exs. Khordak; 3 exs. Thünungei; 4 exs. Phubala	Bishnupur	4. xii. 2013 2.ix.2012 6.ii.2013 6.iii.2013	21 specimen

Table 2: Details of sequences and source, species names and codes of the sequences in the present studies.

S. No.	Species	Accession no	Source	COI partial/complete
1	<i>Agabus bipustulatus</i>	JQ355038.1	NCBI	Partial
2	<i>Laccophilus chinensis</i>	Present study	BOLD	Partial
3	<i>Laccophilus anticatus</i>	Present study	BOLD	Partial
4	<i>Laccophilus anticatus</i> (2)	Present study	BOLD	Partial
5	<i>Laccophilus alluaudi</i>	FJ819665	BOLD	Partial
6	<i>Laccophilus alluaudi</i>	FJ819664	BOLD	Partial
7	<i>Laccophilus alluaudi</i>	FJ819663	BOLD	Partial
8	<i>Laccophilus alluaudi</i>	FJ819654	BOLD	Partial
9	<i>Laccophilus alluaudi</i>	FJ819653	BOLD	Partial
10	<i>Laccophilus alluaudi</i>	FJ819652	BOLD	Partial
11	<i>Laccophilus alluaudi</i>	FJ819651	BOLD	Partial
12	<i>Laccophilus alluaudi</i>	FJ819644	BOLD	Partial
13	<i>Laccophilus biguttatus</i>	JF888317	BOLD	
14	<i>Laccophilus biguttatus</i>	KC017198	BOLD	Partial
15	<i>Laccophilus biguttatus</i>	KC017197	BOLD	Partial
16	<i>Laccophilus biguttatus</i>	KC017196	BOLD	Partial
17	<i>Laccophilus biguttatus</i>	KC017202	BOLD	Partial
18	<i>Laccophilus biguttatus</i>	KC017203	BOLD	Partial
19	<i>Laccophilus biguttatus</i>	KC017204	BOLD	Partial
20	<i>Laccophilus comes</i>	FJ819671	BOLD	Partial
21	<i>Laccophilus comes</i>	FJ819670	BOLD	Partial
22	<i>Laccophilus comes</i>	FJ819669	BOLD	Partial
23	<i>Laccophilus comes</i>	FJ819668	BOLD	Partial
24	<i>Laccophilus hyalinus</i>	KJ966346	BOLD	Partial
25	<i>Laccophilus hyalinus</i>	KU913415	BOLD	Partial
26	<i>Laccophilus hyalinus</i>	KU914868	BOLD	Partial
27	<i>Laccophilus hyalinus</i>	KU907720	BOLD	Partial
28	<i>Laccophilus hyalinus</i>	KU909340	BOLD	Partial
29	<i>Laccophilus hyalinus</i>	KU913477	BOLD	Partial
30	<i>Laccophilus hyalinus</i>	KU915605	BOLD	Partial
31	<i>Laccophilus hyalinus</i>	KU906463	BOLD	Partial

32	<i>Laccophilus hyalinus</i>	KU916189	BOLD	Partial
33	<i>Laccophilus hyalinus</i>	KU907230	BOLD	Partial
34	<i>Laccophilus minutus</i>	KU906692	BOLD	Partial
35	<i>Laccophilus minutus</i>	KU911853	BOLD	Partial
36	<i>Laccophilus minutus</i>	KU908057	BOLD	Partial
37	<i>Laccophilus minutus</i>	KU910820	BOLD	Partial
38	<i>Laccophilus Pallescens</i>	FJ819681	BOLD	Partial
39	<i>Laccophilus pallescens</i>	FJ819682	BOLD	Partial
40	<i>Laccophilus pallescens</i>	FJ819681	BOLD	Partial
41	<i>Laccophilus poecilus</i>	HM401334	BOLD	Partial
42	<i>Laccophilus poecilus</i>	JF889434	BOLD	Partial
43	<i>Laccophilus ponticus</i>	KU909423	BOLD	Partial
44	<i>Laccophilus ponticus</i>	KU918984	BOLD	Partial
45	<i>Laccophilus ponticus</i>	KU916848	BOLD	Partial
46	<i>Laccophilus ponticus</i>	KU913910	BOLD	Partial
47	<i>Laccophilus posticus</i>	FJ819680	BOLD	Partial
48	<i>Laccophilus posticus</i>	FJ819679	BOLD	Partial
49	<i>Laccophilus posticus</i>	FJ819678	BOLD	Partial
50	<i>Laccophilus Posticus</i>	FJ819677	BOLD	Partial
51	<i>Laccophilus posticus</i>	FJ819676	BOLD	Partial
52	<i>Laccophilus posticus</i>	FJ819675	BOLD	Partial

2.2 Taxonomical Characteristics

(i) Morphological Characters

Adults are distinguished by the small size (2.5-6.0mm) hidden scutellum; lanceolate prosternal process; 5 segmented fore and mid tarsus; apically bifid or notched spines on hind tibiae; hind tarsus with single straight claw and basal abdominal sternite with longitudinal striae.

(ii) Molecular aspects

The representatives of the different species were preserved in 90% ethanol and DNA was extracted for the molecular studies. Twenty-five beetle specimens representing 8 species were used for DNA extraction and sequencing. But unfortunately only three sequences could be in our hand. That might be due to transportation defects or imperfect PCR products.

(iii) DNA Extraction and PCR Amplification

DNA was extracted from the two hind legs of specimens by using DNA Sure Tissue Mini Kit (Nucleo- pore, Genetix) as per manufacturer's protocol [14] and stored at -20°C. The genomic DNA were amplified by targeting mitochondrial cytochrome oxidase 1 (mtCO1) after a PCR reaction with universal primers (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'; HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The final volume of the PCR mixture was 25 µl consisting of 12.5 µl of Thermo Scientific maxima hot start PCR master mix, 8.5 µl of molecular grade water, 1 µl each forward primer LCO1490 and reverse primer HCO2198 and 2 µl of genomic DNA. Ventri® 96- well thermal cycler (Applied Biosystems® Life Technologies) was used for the amplification of the samples. The amplified products were resolved in 1% Agarose gel, stained by Ethidium Bromide and visualized in a gel documentation system (DNr, Bio-Imaging systems, MiniLumi). The amplified products were purified using sequenced at Sci Genome Pvt. Ltd. (Cochin, India).

Polymerase chain reactions consisted of the following cycling steps: initial denaturation for 4 min s at 95 °C; 40 cycles of denaturation at 30 s for 95 °C, annealing for 30s at 47 °C, and extension for 1.5 min at 72 °C; final extension for 10 min at 72 °C [15].

a. Phylogenetic Analysis

The mitochondrial COI (mtCOI) sequences in FASTA format

were imported into the sequence alignment application of MEGA 5 software package and multiple sequence alignments were accomplished with the Clustal W algorithm using default parameters [16]. The Basic Local Alignment Search Tool (BLAST) was used to query the National Center for Biotechnology Information (NCBI) non-redundant nucleotide database with other Dytiscidae species mtCOI sequence data in blastn suite searches. Sequence divergences between selected Dytiscidae species were calculated using the Kimura 2-Parameter distance model and graphically displayed in a neighbour-joining (NJ) tree by the program MEGA 5. Tree robustness was evaluated by bootstrapping with 1,000 replicates with the *Agabus bipustulatus* sequence as outgroups.

b. Statistical analysis

All the sequences were aligned in clustal W, and divergence at population, species and genus levels was analysed by K2P model of base substitution. Phylogenetic analysis was carried out using Neighbour Joining (NJ, Fig. 2) approaches in MEGA 5 software [16].

3. Results and Discussion

The morphological taxonomy of the five species of the genus *Laccophilus* was described and key to the species were given below.

Key to the species of genus *Laccophilus*

- (a) Elytra brownish black with marking consisting of yellow fascia. Length 3.0-3.2 mm.....
L. anticatus Sharp, 1890
- (b) Elytra brownish yellow to reddish brown with marking consisting of brown /black zigzag undulating line.....2
- (a) Elytra testaceous with solid brown/ black irritations---
-----3
- (b) Elytra testaceous with undulating single or double lines, sometimes single lines reduced to irritation of small, then, curved lines-----3
- (a) A yellowish rounded patches near the apex of each elytron-----*L. chinensis* Boheman, 1858
- (B) Without a yellowish rounded patches near the apex of each elytron -----
-----*L. inefficiens* Walker, 1859

4. (a) Elytra marking flexuosus, excessively irregular and covering the entire surface excepting lateral margins-----
L. flexuosus Aube, 1938
 (b) Elytra marking consisting of zig zag double marking, generally thick and coalescent-----
L. parvulus Aube, 1838

Out of these, the sequencing was done only in two species such as *Laccophilus anticatus* and *Laccophilus chinensis*. Morphologically these two species differed from one another in various structures such as their elytral pattern and their male external genitalia. *Laccophilus anticatus* elytra is black with 5 yellow marking and lack of undulating zig zag pattern and median lobe of adeagus moderately curved, twisted on itself in the apical half and progressively narrowed anteriorly and somewhat pointed at the apex. Whereas *Laccophilus chinensis* elytra consist of undulating zig zag pattern and median lobe of adeagus sufficiently curved in the anterior one third portion and nearly uniformly wide, being truncated at the apex.

Genus: *Laccophilus*

Laccophilus Leach 1817

Laccophilus Leach, 1815:84; type species: *Dytiscus minutus* Linnaeus, 1758:412.

Laccophilus parvulus Aube, 1838

Laccophilus parvulus Aube, 1838, In Dejean's species Coleopteres, paris, 6:429; Sharp, 1882, Sci. Trans. R. Dublin Soc., 2; 312; Regimbart, 1899, Ann. Soc. Ent. France, 68:258-259; Zimmermann, 1920, Coleopterorum Catalogus, 4(71): 25; Guignot, 1954, Ark. Zool., (N.S.), 6:565; Guignot, 1954, Opusc. Ent. 19:222.

Laccophilus undulifer Molschusky, 1859, Etud Ent., 8: 44

Laccophilus obtusus Sharp, 1882, Sci. Trans. R. Dublin Soc., 2; 311.

Laccophilus derasus Sharp, 1882, Sci. Trans. R. Dublin Soc, 2:311

Material Examined: 20 specimens, 8 exs. Ngaikhong on 31.iii.2015 sites of Loktak Lake, Bishnupur district, 5 exs. Ukhrul on 6.xii.2015; Ukhrul district, 7 exs. Tamenglong on 6.viii.2016; Tamenglong, Manipur.

Diagnosis

Body elongated oval, elytral outline is smoothly curved from base to apex, size 3.27 mm long and 1.69 mm wide. Antennae filiform and 11 segmented eyes continuous with outline of head, testaceous with indistinct darker spots. Pronotum with thin basal blackish bands and two sub equal blackish spots. Scutellum hidden and lanceolate prosternal process, Elytra dark without trace of irroration lines with sub basal transverse sinuous band, mediolateral spots, sub apical transverse sinuous band and apical spots. Legs 5 segmented fore and mid tarsi; apically notches spines on hind tibia, with single straight claw, basal abdominal sternite with longitudinal striae.

Distribution: India; China, Vietnam, Laos, Thailand, Indonesia, Nepal, Bangladesh.

Laccophilus flexuosus Aube, 1938

Laccophilus flexuosus Aube, 1938, In Dejean's Species Coleopteres, Paris, 6:430; Sharp, 1882, Sci. Trans. R. Dublin Soc., 2:1; Regimbart, 1899, Ann. Soc. Ent. France, 68:256; Zimmermann, 1920, Coleopterorum Catalogus,

4;(71):18; Guignot, 1952, opusc. Ent., 19: 222; 1954, Bull. Soc. Linn. Lyon, 28:11; Fernando, 1961, Ceylon J. Sci. (Biol. Sci.) 4 (1): 46, 53.

Laccophilus cognatus Sharp, 1882, Sci. Trans. R. Dublin Soc., 2; 316.

Laccophilus solutes indicus Gschwendtner, 1936, Rec. Indian Mus., 37(1935):367.

Material examined

10 specimens, 3 exs. Takmupat on 31.iii.2015; Bishnupur district, 5 exs. Porompat on 6.ii.2016 ; Imphal East District, 2 exs. Churachandpur on 6.iii.2015; Churachandpur District, Manipur.

Diagnosis

Body subglobose body length 3.50 mm long and 1.78 mm wide, antennae filiform, 11 segmented with uniformly yellowish brown. Pronotum testaceous with two sub equal blackish spots, scutellum quite concealed, and prosternal process acute behind, metasternum slender arcuate, elytra with irregular sub basal transverse sinuous band with zig zag pattern on each elytron. Legs 5 segmented fore and mid tarsi apically notches spines on hind tibia with single straight claw.

Distribution: India; China, Vietnam, Laos, Thailand, Indonesia, Nepal, Bangladesh, Hong Kong, Japan, Taiwan, Bhutan.

Laccophilus inefficiens Walker, 1859

Laccophilus inefficiens Walker, 1859, Ann. Mag. Nat. Hist., (3)3:51.

Material Examined

15 specimens, 2 exs. Thünungei on 6.ii.2013; 2 exs. Phubala on 6.iii.2013; 2 exs. Oaksoipat on 9.xi.2012; 2 exs. Tongbram 8.ix.2012; Bishnupur District 4 exs. Porompat on 6.ii.2016; Imphal East District, 4 exs. Churachandpur on 6.iii.2015; Churachandpur District, Manipur

Diagnosis

Body elongated oval, body length 3.43 mm long and 1.84 mm width. Antennae thread like 11 segmented uniformly yellowish brown. Eyes continuous with outline of head, testaceous with indistinct darker spots. Pronotum testaceous, scutellum hidden and lanceolate prosternal process. Elytra with sub basal transverse sinuous band comprising each elytron with small sinuous dark marks separated by yellow one of about same size. Legs 5 segmented fore and mid tarsi apically notches spine on hind tibia with single straight claw.

Distribution: India; China, Vietnam, Laos, Thailand, Indonesia, Nepal, Bangladesh, Hong Kong, Japan, Taiwan, Bhutan.

Laccophilus chinensis Boheman, 1858

Laccophilus chinensis Boheman, 1858, In Konliga svenska Fregatten Eugenies resa Zoologi, I, Insecta: 21; *Laccophilus chinensis* var. *inefficiens*, Regimbart, 1899, Ann. Soc. Ent. France, 68:260; Zimmermann, 1920, Coleopterorum Catalogue, 4 (71):17. *Laccophilus chinensis inefficiens*, Guignot, 1954, Ark. Zool., (N.S.), 6:565; Fernando, 1959, Ceylon J. Sci. (Biol. Sci.), 2(1):1,3; 1961, Ceylon J. Sci. (Biol. Sci.), 4(1):46,53.

Material Examined: 15 specimens, 5 exs. Ngaikhong on 4.xii.2012; 5 exs. Longum on 2.ix.2012; 3 exs. Thünungei on

6.ii.2013; 2 exs. Phubala on 6.iii.2013; Bishnupur district, Manipur.

Diagnosis

Body elongate oval, body length 3.36 mm long and 1.91 mm wide. Antennae thread like with 11 segmented with uniformly yellowish brown. Head short and broad usually testaceous in colour, pronotum yellowish with thin blackish band and two sub equal blackish spots, Scutellum hidden, elytra yellowish brown with sub basal transverse sinuous band, mediolateral spots, sub apical transverse sinuous band and apical spots comprising each elytron with small sinuous dark marks separated by yellow one of about same size. Legs 5 segmented fore and mid tarsi; apically notches spines on hind tibia with single straight claw.

Distribution: India; China, Vietnam, Laos, Thailand, Indonesia, Nepal, Bangladesh, Hong Kong, Japan, Taiwan, Bhutan.

Laccophilus anticatus Sharp, 1890.

Laccophilus anticatus Sharp, 1890, Trans. ent. Soc. Lond. p. 341.

Regimbart, 1899, Ann.Soc.Ent. France, 68:262-263.

Vazirani, 1968, Orient. Ins., 2 (3-4): 240-241.

Material Examined: 21 specimens, 8exs. Ngaikhong on 4. xii. 2013; 6 exs. Khordak on 2.ix.2012; 3 exs. Thinungei on 6.ii.2013; 4 exs. Phubala on 6.iii.2013; Bishnupur district, Manipur.

Diagnosis

Body elongated oval and sub depressed, body length 3.00 mm long and 1.70 mm wide. Head short brownish yellow with faint brownish marking without punctuation. Pronotum testaceous and almost concolourous with head. Elytra brownish black with yellow patches under lateral borders, small median, sublateral spot, postbasal transverse irregular and dendate band, largely confluent with the border, small post median transverse spot but not touching the suture and punctuation indistinct. Prominent punctures present on sutural margin along anterior one fourth of elytra. Legs 5 segmented fore and mid tarsi; apically notches spines on hind tibia with single straight claw. Ventral surface brownish yellow and abdominal sternites slightly darker.

Distribution: Assam, Bihar, Manipur, Orissa and West Bengal.

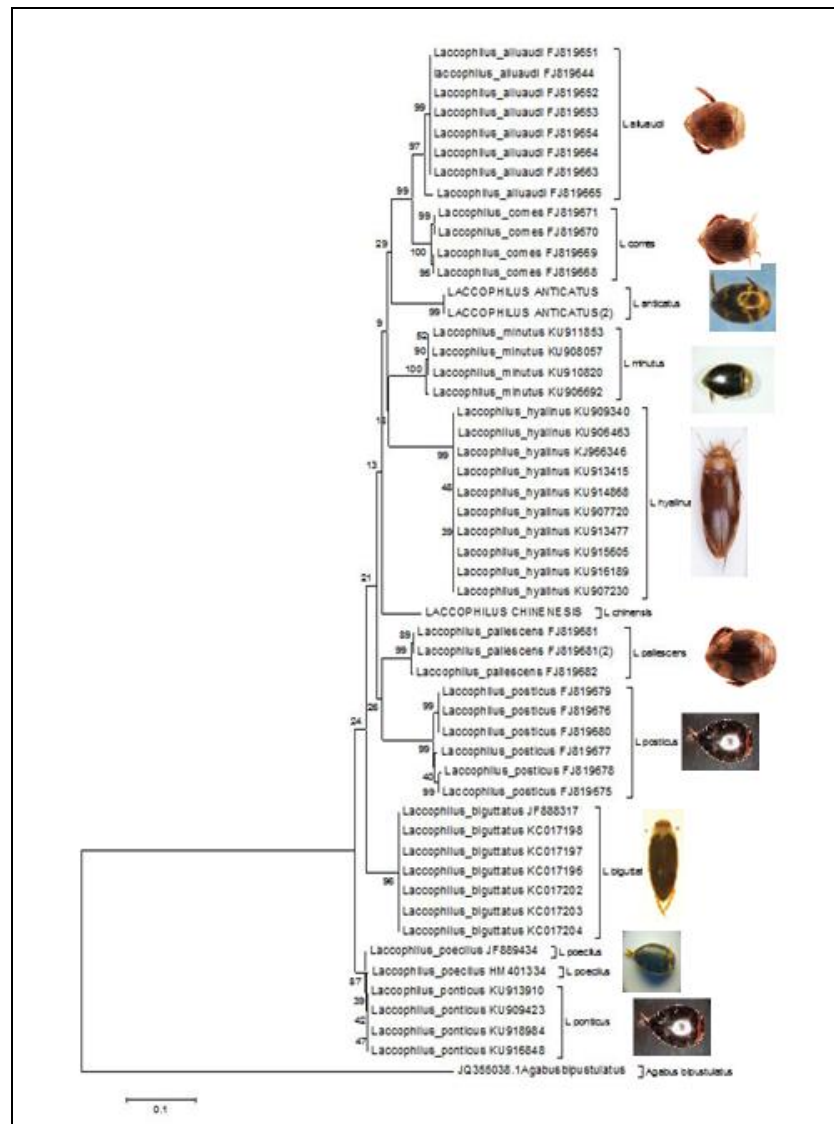


Fig 2: Phylogram of the analysed genera *Laccophilus*, showing relationships of the *Agabus bipustulatus* using by neighbouring-joining (NJ) tree method and Kimura 2 –parameter distances mitochondrial COI sequences. Numbers at nodes indicate bootstrap values by the NJ and ML methods (1000 replicates, > 50%). The taxon names correspond to those in Table 2. Bar represent time of evolution.

Fifty two COI sequences representing 51 species of *Laccophilus* species including the three species in the present studies and other procured from Gene Banks particularly the BOLD System and NCBI (Table 2). The data were analysed for sequence divergence at different taxonomic levels. Interpopulation divergence 2.147 with S. E. was -17.4. Among all living organisms, insects are the most numerous group in terms of the number of species. Taxonomic study of aquatic insects is more or less a challenging task. The most challenges faced by every taxonomist who working with insects is identification of the species type due to varying degrees of variation, it is quite difficult to diagnose insect species^[17-19]. In this study also we faced same problem in identification of *Laccophilus* species due to the lack of sufficient literature in Manipur. In addition, sex differences, sibling species and etc. are the frequently encountered problems. To solve all these difficulties, molecular taxonomy studies with advancing technology, continues to increase every day^[20-22]. In the present study *Laccophilus anticatus* elytra is black with 5 yellow marking and lack of undulating zig zag pattern and median lobe of aedeagus moderately curved, twisted on itself in the apical half and progressively narrowed anteriorly and somewhat pointed at the apex. Whereas *Laccophilus chinensis* elytra consist of undulating zig zag pattern and median lobe of aedeagus sufficiently curved in the anterior one third portion and nearly uniformly wide, being truncated at the apex. Besides solving many of the problems mentioned above, molecular systematics also provides some additional conveniences related insect groups that are worked by researchers. In these cases, many researchers have begun to use the remedy of molecular systematic^[23-25].

In present study the morphological features of five species are in accordance with the literature published so far. The five species in present study: *Laccophilus parvulus*, *Laccophilus flexuosus*, *Laccophilus inefficiens*, *Laccophilus chinensis* and *Laccophilus anticatus* could be divided into two groups. The two groups are *Laccophilus anticatus* group with elytra without undulating zig zag pattern and *Laccophilus chinensis* group with elytra consist of undulating zig zag pattern. The latter group consists of *Laccophilus parvulus*, *Laccophilus flexuosus*, *Laccophilus inefficiens*, and *Laccophilus chinensis*. This particular feature is also manifested in molecular studies, though the available sequences for the study are only three-two sequences for *L. anticatus* and lone sequence of *L. chinensis*, shows two distinct haplotype (Fig. 2). In the phylogram *L. anticatus* falls within the clades of *L. chinensis*. In future the collection of large numbers of specimens and much higher sequences to compare will give meaningful results.

The comparison of present available sequences viz., *L. chinensis* and two sequences of *L. anticatus* with sequences from the databanks the interpopulation divergence is 2.147 with S. E. was -17.4 means that the sequences are novel and it is wealthy to be submitted. However, Hausmann *et al.*^[26] found out it with the mean of 0.73% (SE=0.033) in family Geometridae while Rougerie *et al.*^[27] gave the intraspecific distance in Australian Sphingids ranging from 0.0% to 2.19% (mean=0.3%, SE=0.007). At present scarcity of the published papers on these two species both in molecular and morphotaxonomy is a hindrance on the comparative studies of the present species. It is wealthy to note here that the molecular and morphotaxonomy of the present species should be reexamined when the species are published from rest of the world.

4. Conclusion

In the present study five species were reporting out of which two species were studied through the COI sequences. The morphometric studies of these five species were in accordance with the published data so far. But the available COI studies of the two species viz., *Laccophilus chinensis* and *L. anticatus* could not be compared with the available data since the COI sequences of these species could not be found. Hence the two species could be compared when the sequences of these two species were available in near future to compare with the rest of the world.

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