

ORIGINAL ARTICLE

First record of *Liposcelis entomophila* (Enderlein) (Psocodea: Liposcelididae) from Sri Lanka based on morphological and molecular data

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Abstract The booklice genus, *Liposcelis* (Psocodea: Liposcelididae), owns some stored product pests and have economic importance. In this study, 6 booklice specimens from Sri Lanka were collected and identified based on morphological and molecular data. According to the morphological and phylogenetic analyses, the samples were identified as *Liposcelis entomophila*. The mitochondrial gene cytochrome *c* oxidase I (*COI*) and second internal transcribed spacer (*ITS2*) were sequenced for phylogenetic work. The phylogenetic trees show that the samples assemble with *L. entomophila* together and could be distinguished with other *Liposcelis* spp. apparently. The genus *Liposcelis* and the species *L. entomophila* are firstly reported in Sri Lanka.

Key words Booklice, morphological characteristics, *COI*, *ITS2*.

1 Introduction

The booklice genus, *Liposcelis*, belong to the family Liposcelididae, is a kind of cosmopolitan wingless insects (Nayak *et al.*, 2014). So far, a total of 126 species have been found worldwide (Wang *et al.*, 2006; Johnson & Smith, 2019).

The booklice have a mixed feeding habit, but prefer powdery food, and can eat more than 46 kinds of substances. It has a wide range of temperature and humidity adaptability, and reproduces faster along with the rising of temperature and humidity (Opit & Throne, 2008; Nayak *et al.*, 2014).

The booklice can be roughly divided into wild type and indoor type according to its living habits (Turner, 1994; Athanassiou *et al.*, 2012; Kučerová *et al.*, 2014). In the genus *Liposcelis*, more than ten species, including *L. entomophila*, *L. bostrychophila*, *L. brunnea*, *L. pearmani*, *L. rufa*, *L. decolor*, *L. tricolor*, *L. mendax*, *L. corrodens* and *L. paeta*, are regarded as common stored products pests (Yang *et al.*, 2012; Zhao *et al.*, 2016). These species have important economic significance because they could cause huge loss of storage products such as grain, book and wood (Turner, 1994). In recent years, as the development of international trade, booklice are often found in the warehouses or intercepted in ports (Opit & Throne, 2008;), attentions on these species are gradually increasing.

Because of the small size (less than 1.5 mm in general), the booklice is usually hard to identified only by the morphological data (Mockford, 2009). Recently, molecular technologies were also used to identify insects, *e.g.*, Random Amplified Polymorphic DNA (RAPD) (Williams *et al.*, 1990), real-time PCR and DNA barcoding (Hebert *et al.*, 2003). Use a part of one specific gene sequence as a marker, if the sequences show there is a gap between intra- and inter- Kimura 2-parameter (K2-P) genetic distances in a group through the DNA gap analysis based on K2-P distance algorithm (Kimura, 1980), the gene can be used for phylogenetic analysis. If the samples are clustered on the same branch of phylogenetic tree, they can be proved to be the same species. (Kumar *et al.*, 2016). The 658bp sequences of mitochondrial gene cytochrome *c*

oxidase I (*COI*) is a common DNA barcode for the species identification (Hebert *et al.*, 2003). In previous study, *COI* can be used to identify *L. entomophila* (Yang *et al.*, 2012). However, researches on the genus *Liposcelis* showed that the DNA gap based on *COI* of different populations are not obvious in *L. bostrychophila* and *L. paeta* (Liu *et al.*, 2017). Compared with *COI*, nuclear 5.8 ribosomal RNA gene and internal transcribed spacer 2 (*ITS2*) have stronger variability and can be used to identify species with similar relatives (Young & Coleman, 2004; Wei *et al.*, 2011). Zhao *et al.* (2016) analyzed the Kimura 2-parameter genetic distances of *ITS2* sequences and found it could identify above ten species of common stored booklice. Based on *ITS2* sequences, Liu *et al.* (2017) developed gene chip to identify the ten species of those common stored booklice.

In previous studies, the genus *Liposcelis* has been reported in a wide range of the world. *L. entomophila*, one of the stored-products booklice, has been reported in Cyprus, Czechoslovakia (former), Finland, Germany, Great Britain, Israel, Italy, Spain, Switzerland, Turkey, Yugoslavia (former), Azores, Cape Verde Islands, Colombia, USA, Lesser Antilles, Cuba, Mexico, Nicaragua, Chile, Angola, Congo, Guinea-Bissau, Madagascar, Mozambique, Senegal, Tanzania, Togo, Zimbabwe, Mascarene Islands, China, India, Indonesia, Japan, Korea, Philippines, Singapore, Thailand, Vietnam, Australia, Galapagos, New Hebrides (Lienhard & Smithers, 2002; Johnson & Smith, 2019). *L. entomophila* belongs to Section I, Group IA of the genus *Liposcelis* (Lienhard, 1990). The *L. entomophila* is different from other *Liposcelis* spp. by the obvious brown transverse strip on the abdominal tergum. The main characteristics of this species are as following (Lienhard, 1990): length of body 1.28–1.40 mm, basic body color yellowish, abdominal terga 3–4 and 6–9 usually each with a brown transverse; brown transverse strip on abdominal terga 6–9 usually interrupted medially; pronotal setae (PNS) usually 3–4 and in row; prosternal setae usually 5–6 and in anterior.

In this study, six booklice specimens were sampled in the rainforest of central Sri Lanka. They were identified as *L. entomophila* by morphological and molecular data based on *COI* and *ITS2*, respectively. The genus *Liposcelis* and the species *L. entomophila* is reported in Sri Lanka for the first time.

2 Materials and methods

2.1 Specimen collection and morphological identification

Six samples (three female adults, one male adult and two nymphs) were collected from a handmade wooden box of local souvenirs in the rainforest of Udawalawe in central Sri Lanka (6.42561°N, 80.816594°E) in October 2018. After taken living photographs, the samples were preserved in 100% ethanol and -20°C refrigerator at the Plant Quarantine and Invasion Biology Laboratory of China Agricultural University.

The morphological identification follows the morphological description by Lienhard (1990). One female adult was used to make slide specimen. The morphological characteristics were observed by OLYMPUS CX31 optical microscope. Photos of main morphological characteristics were taken by Canon EOS500D and measured by the software ImageJ i.51J8.

2.2 DNA extraction, *COI* and *ITS2* amplification and sequencing

Total genomic DNA was extracted from each of other five samples (two female adults, one male adult and two nymphs), following TIANamp Micro DNA Kit protocol (TIANGEN, China).

For two genes, the Polymerase Chain Reaction (PCR) was completed in two final 25 µl volume. Containing 1 µl DNA as a template, 12.5 µl 2×Taq PCR MasterMix, 9.5 µl sterilized distilled water, 1 µl forward and reverse primer (10 µM), respectively. The primers selected for *COI* were LCO1490 (forward, 5'-GGTCAATCATAAAGATATATTGG-3') and HCO2198 (reverse, 5'-TAAACTTCAGGGTGAAAAATCA-3') (Folmer *et al.*, 1994). The primers selected for *ITS2* were ITSF1 (forward, 5'-TGAAGTGCAGGACATG-3') and ITS2 (reverse, 5'-GTCTTGTCATCTGAG-3') (Zhao *et al.*, 2016; Liu *et al.*, 2017). PCR was completed in Veriti TM 96-well Thermal Cycler (ABI USA), the system was set to 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 30 sec, then 72°C for 10 min. The PCR products were separated on 1.5% (w/v) agarose gel (1×Tris Acetate-EDTA buffer), stained with Gene Green Nucleic Acid Dye (TIANGEN, China) and checked under Gel Logic 212 PRO UV lights. The PCR products were amplified with the same primers and sent to Beijing Liuhe Huada Gene Technology Company for bi-directional sequencing.

2.3 Genetic distance and phylogenetic analyses

The obtained sequences were firstly checked by Chromas (Goodstadt & Ponting, 2001) for their quality, then aligned

with other booklice sequences in GenBank (Table 1) by MEGA 7.0 (Kumar *et al.*, 2016). The sequences were then upload to GenBank for accession numbers (Table 2). The pairwise genetic distance of *COI* and *ITS2* were calculated using Kimura 2-Parameter method in MEGA 7.0 (Kimura, 1980; Kumar *et al.*, 2016), respectively.



Figure 1. Habitats of the samples from the wooden box in Sri Lanka.

The maximum, minimum and mean genetic distances of two genes were calculated by Excel. According to the distances, a DNA gap analysis was performed to prove both *COI* and *ITS2* can be used to identify the *L. entomophila*.

Phylogenetic analysis was also computed using MEGA 7.0. Neighbor-joining (NJ) and maximum likelihood (ML) trees were constructed based on the two genes, respectively. For NJ trees, the percentage of replicate trees of the associated taxa clustered together in the bootstrap test (1000 replications) were shown next to the branches. For ML trees, we chose the Kimura 2-parameter model to construct the tree. The bootstrapping test was set to 1000 and the percentage of replicate is shown next to the branches as well.

3 Results

3.1 Morphological characteristics of *L. entomophila*

The sample of slide specimen was identified as *L. entomophila* according to its related morphological characteristics. 1) The brown transverse strips on the abdominal tergum could be clearly seen in the living photos (Fig. 1); 2) under 10 times microscopy, it was clear that the brown transverse on abdominal terga 6–9 is interrupted medially (Fig. 2); 3) the number of PNS is 3 (Fig. 3); 4) the number of prosternal setae is 5 and distribute in the forepart (Fig. 4).



Figures 2–4. *Liposcelis entomophila*, female. 2. Adult. 3. Pronotal setae (PNS, 3). 4. Prosternal setae (5). Scale bars: 2 = 1.0 mm; 3–4 = 0.1 mm.

3.2 Genetic distance and DNA gap

Five sequences of *COI* and *ITS2* were obtained and submitted to GenBank, respectively. The pairwise genetic distances of *COI* were presented in Table 3. The Kimura 2-Parameter genetic distances between the collected samples ranged as 0.002–0.014. The range distances between known *L. entomophila* and the collected samples was 0.002–0.019. It was relative lower than the range of collected samples and other *Liposcelis* species (0.329–0.488). The pairwise genetic distances of *ITS2* were given in Table 4. It presented the similar results. The distances between collected samples ranged as 0.000–0.013. The range distances between known *L. entomophila* and collected samples was 0.010–0.023, which is also relative lower than the range of collected samples and other *Liposcelis* species (0.475–0.641).

Table 1. Genes and GenBank Accession number of different species and populations used in the calculation of genetic distance and phylogenetic analysis.

Population	Location	Gene	GenBank Accession number
<i>L. entomophila</i> UK	Wales, UK	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910133
<i>L. entomophila</i> HB	Hubei, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	HQ658136
<i>L. entomophila</i> YN	Yunnan, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910135
<i>L. entomophila</i> BJ	Beijing, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707588
<i>L. entomophila</i> HB	Hubei, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707590
<i>L. entomophila</i> GX	Guangxi, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707589
<i>L. brunnea</i> CB	Central Boemia, Czech	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910140
<i>L. brunnea</i> KS	Kansas, USA	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910139
<i>L. brunnea</i> USA	USA	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707626
<i>L. rufa</i> KS	Kansas, USA	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910141
<i>L. rufa</i> USA	USA	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707627
<i>L. corrodens</i> UK	UK	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910137
<i>L. corrodens</i> KS	Kansas, USA	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	HQ658142
<i>L. corrodens</i> DMK	Danmark	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707621
<i>L. corrodens</i> USA	USA	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707624
<i>L. pearmani</i> KS	Kansas, USA	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JQ708202
<i>L. pearmani</i> USA	USA	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707628
<i>L. tricolor</i> HZ	Heze, Shandong, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JQ708201
<i>L. tricolor</i> SD	Shandong, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707632
<i>L. bostrychophila</i> CQ	Chongqing, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910136
<i>L. bostrychophila</i> GX	Guangxi, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	HQ018875
<i>L. bostrychophila</i> HN	Henan, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	JQ966097
<i>L. bostrychophila</i> GER	Berlin, GER	5.8ribosomal RNA gene and internal transcribed spacer 2	JQ966098
<i>L. decolor</i> CQ	Chongqing, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JN211064

Table 1 (continued)

Population	Location	Gene	GenBank Accession number
<i>L. decolor</i> KS	Kansas, USA	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JN211062
<i>L. decolor</i> CZ	Prague. CZ	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707594
<i>L. decolor</i> USA	USA	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707599
<i>L. paeta</i> ZJ	Zhejiang, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910138
<i>L. paeta</i> RC	Republic of Croatia	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	KJ680317
<i>L. paeta</i> HB	Hubei, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707614
<i>L. paeta</i> CZ	Prague. CZ	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707603
<i>L. mendax</i> JS	Yancheng, Jiangsu, P.R. China	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	JF910142
<i>L. mendax</i> JS	Jiangsu, P.R. China	5.8ribosomal RNA gene and internal transcribed spacer 2	KC707629
<i>Dorypteryx domestica</i>	Czech Republic	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	MK189489
<i>Dorypteryx domestica</i>	Czech Republic	5.8ribosomal RNA gene and internal transcribed spacer 2	MK189495

Table 2. Collection information and accession number of specimens in this study.

Specimen No.	Stage and sex	Collection locality	Date	Gene	GenBank Accession number
Specimen 1	Adult, Female	Nuwaragala, Udawalawa, Sri Lanka	2018.10	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	MK165486
Specimen 1	Adult, Female	Nuwaragala, Udawalawa, Sri Lanka	2018.10	5.8ribosomal RNA gene and internal transcribed spacer 2	MK189490
Specimen 2	Larva	Nuwaragala, Udawalawa, Sri Lanka	2018.10	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	MK165487
Specimen 2	Larva	Nuwaragala, Udawalawa, Sri Lanka	2018.10	5.8ribosomal RNA gene and internal transcribed spacer 2	MK189491
Specimen 3	Larva	Nuwaragala, Udawalawa, Sri Lanka	2018.10	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	MK165488
Specimen 3	Larva	Nuwaragala, Udawalawa, Sri Lanka	2018.10	5.8ribosomal RNA gene and internal transcribed spacer 2	MK189492
Specimen 4	Adult, Male	Nuwaragala, Udawalawa, Sri Lanka	2018.10	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	MK165489
Specimen 4	Adult, Male	Nuwaragala, Udawalawa, Sri Lanka	2018.10	5.8ribosomal RNA gene and internal transcribed spacer 2	MK189493
Specimen 5	Adult, Female	Nuwaragala, Udawalawa, Sri Lanka	2018.10	Mitochondrial DNA cytochrome <i>c</i> oxidase I gene	MK165490
Specimen 5	Adult, Female	Nuwaragala, Udawalawa, Sri Lanka	2018.10	5.8ribosomal RNA gene and internal transcribed spacer 2	MK189494

Table 3. Genetic distance of *COI* based on Kimura 2-parameter.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 <i>Dorypteryx domestica</i>																							
2 <i>L. brunnea</i> KS	0.511																						
3 <i>L. bostrychophila</i> GX	0.492	0.435																					
4 <i>L. bostrychophila</i> CQ	0.492	0.435	0.000																				
5 <i>L. decolor</i> CQ	0.541	0.345	0.446	0.446																			
6 <i>L. decolor</i> KS	0.541	0.345	0.446	0.446	0.002																		
7 <i>L. paeta</i> ZJ	0.473	0.497	0.324	0.324	0.401	0.401																	
8 <i>L. paeta</i> RC	0.471	0.514	0.365	0.365	0.437	0.437	0.102																
9 <i>L. mendax</i> JS	0.488	0.451	0.462	0.462	0.423	0.423	0.427	0.427															
10 <i>L. brunnea</i> CB	0.511	0.000	0.435	0.435	0.345	0.345	0.497	0.514	0.451														
11 <i>L. rufa</i> KS	0.546	0.313	0.439	0.439	0.222	0.222	0.433	0.442	0.422	0.313													
12 <i>L. corrodens</i> UK	0.467	0.417	0.336	0.336	0.361	0.363	0.323	0.341	0.355	0.417	0.376												
13 <i>L. corrodens</i> KS	0.461	0.422	0.334	0.334	0.363	0.366	0.325	0.344	0.363	0.422	0.358	0.017											
14 <i>L. pearmani</i> KS	0.476	0.321	0.484	0.484	0.321	0.321	0.462	0.474	0.456	0.321	0.320	0.419	0.416										
15 <i>L. tricolor</i> HZ JQ	0.468	0.406	0.368	0.368	0.428	0.425	0.364	0.369	0.380	0.406	0.405	0.303	0.303	0.426									
16 <i>L. entomophila</i> UK	0.518	0.452	0.370	0.370	0.400	0.398	0.347	0.381	0.390	0.452	0.377	0.333	0.328	0.485	0.378								
17 <i>L. entomophila</i> YN	0.515	0.454	0.371	0.371	0.400	0.398	0.353	0.387	0.396	0.454	0.377	0.333	0.328	0.482	0.378	0.008							
18 <i>L. entomophila</i> HB	0.512	0.452	0.370	0.370	0.395	0.392	0.345	0.378	0.388	0.452	0.374	0.333	0.328	0.482	0.373	0.006	0.009						
19 Specimen1	0.515	0.449	0.368	0.368	0.398	0.395	0.345	0.378	0.388	0.449	0.374	0.330	0.326	0.482	0.375	0.002	0.006	0.005					
20 Specimen2	0.503	0.452	0.371	0.371	0.406	0.404	0.343	0.376	0.393	0.452	0.383	0.336	0.331	0.477	0.374	0.016	0.019	0.016	0.014				
21 Specimen3	0.516	0.450	0.363	0.363	0.396	0.393	0.343	0.377	0.386	0.450	0.378	0.329	0.324	0.480	0.374	0.003	0.008	0.006	0.002	0.016			
22 Specimen4	0.518	0.455	0.373	0.373	0.406	0.403	0.348	0.381	0.394	0.455	0.385	0.336	0.331	0.483	0.373	0.008	0.012	0.011	0.006	0.012	0.005		
23 Specimen5	0.518	0.456	0.372	0.372	0.402	0.399	0.349	0.377	0.397	0.456	0.383	0.336	0.332	0.488	0.383	0.006	0.011	0.009	0.005	0.019	0.006	0.011	

Table 4. Genetic distance of *ITS2* based on Kimura 2-parameter.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 <i>Dorypteryx domestica</i>																						
2 <i>L. brunnea</i> USA	1.019																					
3 <i>L. rufa</i> USA	0.985	0.703																				
4 <i>L. corrodens</i> DMK	0.852	0.640	0.707																			
5 <i>L. corrodens</i> USA	0.852	0.633	0.707	0.003																		
6 <i>L. pearmani</i> USA	0.915	0.686	0.643	0.712	0.712																	
7 <i>L. tricolor</i> SD	0.892	0.656	0.719	0.406	0.406	0.690																
8 <i>L. bostrychophila</i> HN	0.863	0.660	0.676	0.447	0.440	0.670	0.444															
9 <i>L. bostrychophila</i> GER	0.817	0.569	0.667	0.405	0.398	0.682	0.454	0.103														
10 <i>L. decolor</i> CZ	0.715	0.716	0.584	0.573	0.573	0.573	0.686	0.686	0.675													
11 <i>L. decolor</i> USA	0.745	0.723	0.580	0.573	0.573	0.613	0.686	0.695	0.683	0.031												
12 <i>L. paeta</i> HB	0.896	0.769	0.807	0.474	0.474	0.688	0.494	0.596	0.600	0.777	0.795											
13 <i>L. paeta</i> CZ	0.867	0.799	0.849	0.530	0.530	0.682	0.560	0.524	0.546	0.741	0.770	0.098										
14 <i>L. mendax</i> JS	0.685	0.525	0.636	0.580	0.570	0.633	0.621	0.656	0.666	0.673	0.651	0.625	0.627									
15 <i>L. entomophila</i> BJ	0.809	0.612	0.660	0.541	0.549	0.588	0.566	0.487	0.482	0.628	0.646	0.578	0.594	0.580								
16 <i>L. entomophila</i> HB	0.780	0.614	0.653	0.520	0.527	0.597	0.574	0.488	0.483	0.603	0.621	0.577	0.584	0.572	0.010							
17 <i>L. entomophila</i> GX	0.794	0.605	0.644	0.527	0.535	0.589	0.574	0.497	0.491	0.612	0.630	0.577	0.593	0.572	0.006	0.003						
18 specimen1	0.782	0.584	0.629	0.526	0.534	0.577	0.563	0.479	0.475	0.623	0.641	0.577	0.584	0.562	0.023	0.020	0.016					
19 specimen2	0.777	0.604	0.627	0.519	0.526	0.574	0.563	0.480	0.475	0.612	0.630	0.577	0.584	0.562	0.016	0.013	0.010	0.006				
20 specimen3	0.777	0.621	0.627	0.519	0.526	0.574	0.563	0.480	0.475	0.621	0.639	0.577	0.593	0.582	0.023	0.019	0.016	0.013	0.006			
21 specimen4	0.782	0.584	0.629	0.526	0.534	0.577	0.563	0.479	0.475	0.623	0.641	0.577	0.584	0.562	0.023	0.020	0.016	0.000	0.006	0.013		
22 specimen5	0.782	0.584	0.629	0.526	0.534	0.577	0.563	0.479	0.475	0.623	0.641	0.577	0.584	0.562	0.023	0.020	0.016	0.000	0.006	0.013	0.000	

According to the Kimura 2-parameter genetic distance for two genes between collecting samples and known *Liposcelis* species, the minimum inter-specific genetic distance of each species was significantly higher than the maximum intra-specific genetic distance for each gene. Based on the data in Table 5, there was a DNA gap between the intra- and inter-specific divergence in both two genes (Fig. 5). This result shows that the species *L. entomophila* could be identified based on phylogenetic trees constructing by either *COI* or *ITS2*.

Table 5. The intra- and inter-specific Kimura 2-parameter divergence values (%) of *COI* and *ITS2*.

Genes	Intra Mean	Intra Min	Intra Max	Inter Mean	Inter Min	Inter Max
<i>COI</i>	0.0090	0.0015	0.0187	0.3899	0.3240	0.4875
<i>ITS2 rDNA</i>	0.0127	0.0000	0.0228	0.5688	0.4747	0.6602

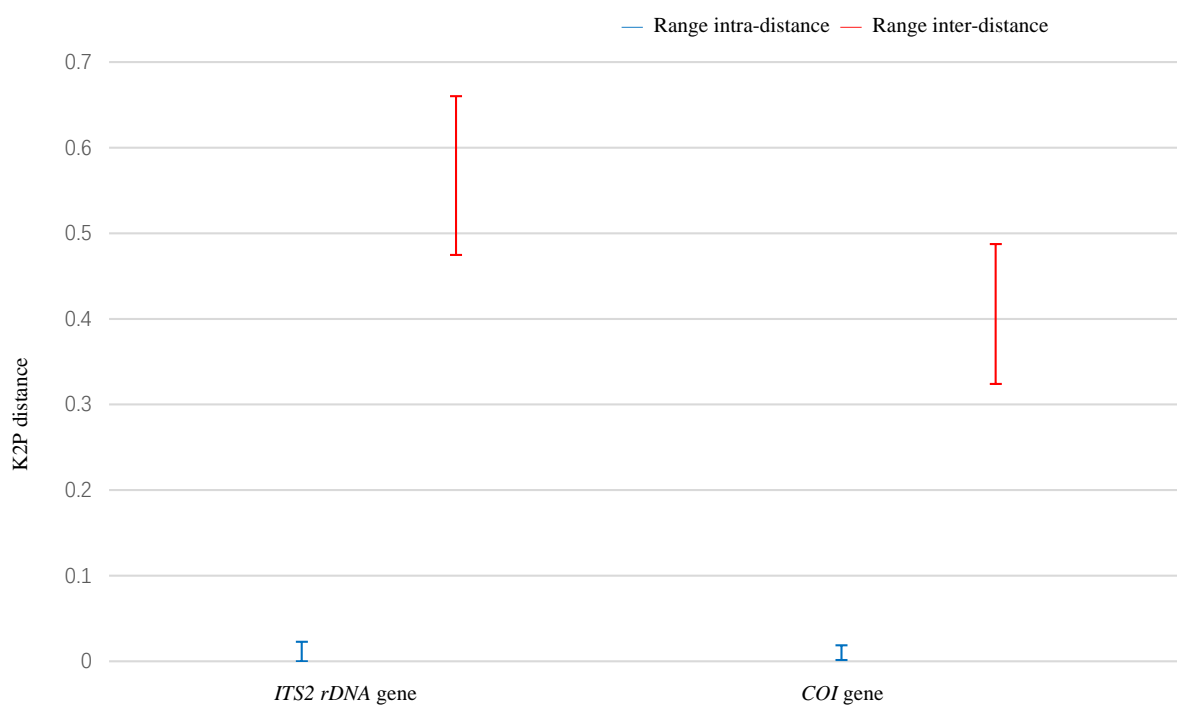


Figure 5. The barcoding gap analysis of *COI* and *ITS2* for *L. entomophila*.

3.3 Phylogenetic analysis

For the phylogenetic tree of *COI*, both NJ trees and ML trees (Fig. 6) showed that all the samples collected from Sri Lanka were clustered on the same branch with known individuals of *L. entomophila*, which was distinct from other species obviously. The NJ trees and ML trees based on *ITS2* (Fig. 7) showed the same result. All unknown samples were clustered on the same branch with the *L. entomophila*, and were clearly distinguished from other species. The results of all the 4 trees were consistent. The collected samples could be identified as *L. entomophila*.

4 Discussion

The molecular techniques present a rapid identification of the common species of *Liposcelis* (Yang *et al.*, 2012; Zhao *et al.*, 2016; Liu *et al.*, 2017). In this study, based on *COI* and *ITS2*, we identified *L. entomophila* by the calculation of genetic distance, DNA gap analysis and construction of phylogenetic tree. The result is in accord with the morphological result. Moreover, the molecular work can also be done on the immature stages of the booklice, which is useful when the samples are not adults. As the morphology of the genus *Liposcelis* is confusing sometimes, we suggest that the related molecular work should be done when identifying *Liposcelis* species considering its importance for phytosanitary.

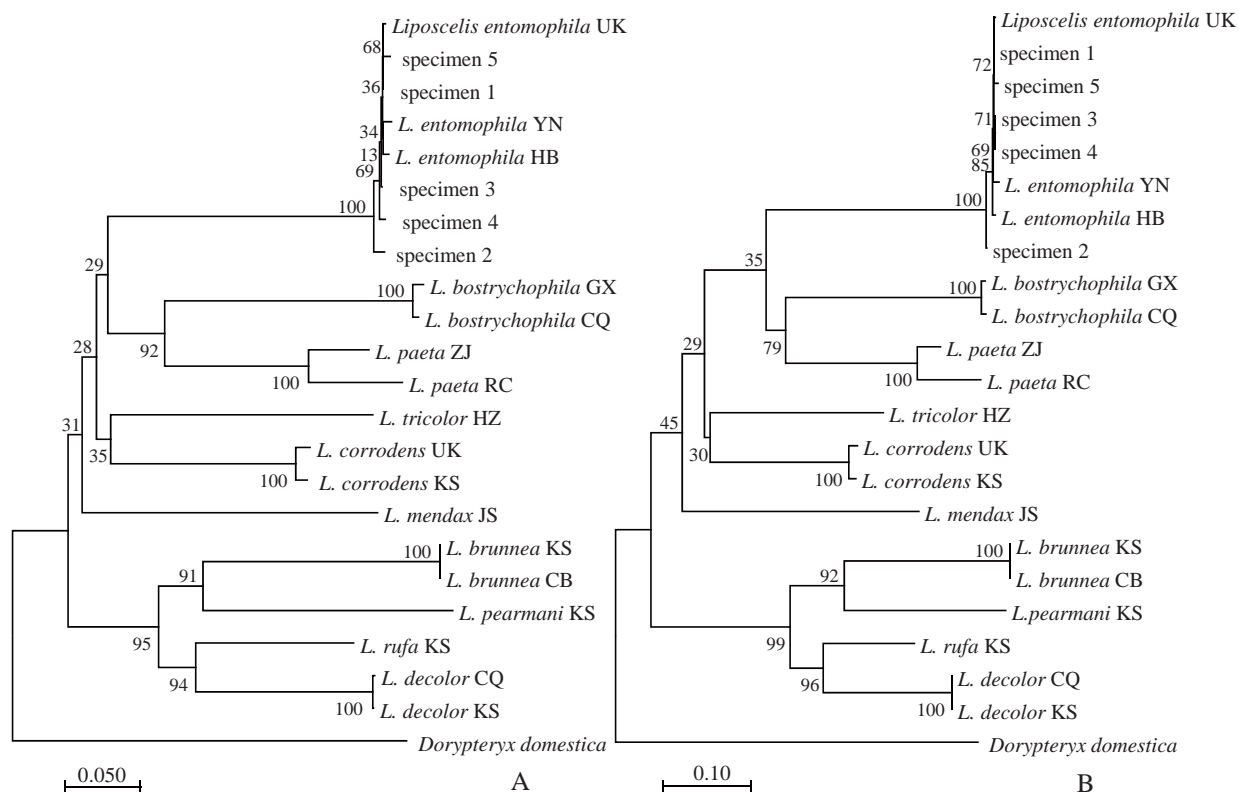


Figure 6. Neighbor-joining and maximum likelihood phylogenetic trees constructed based on *COI*. The number at each branch showed the percentage supported by bootstrap. A. Neighbor-joining tree. B. Maximum likelihood tree.

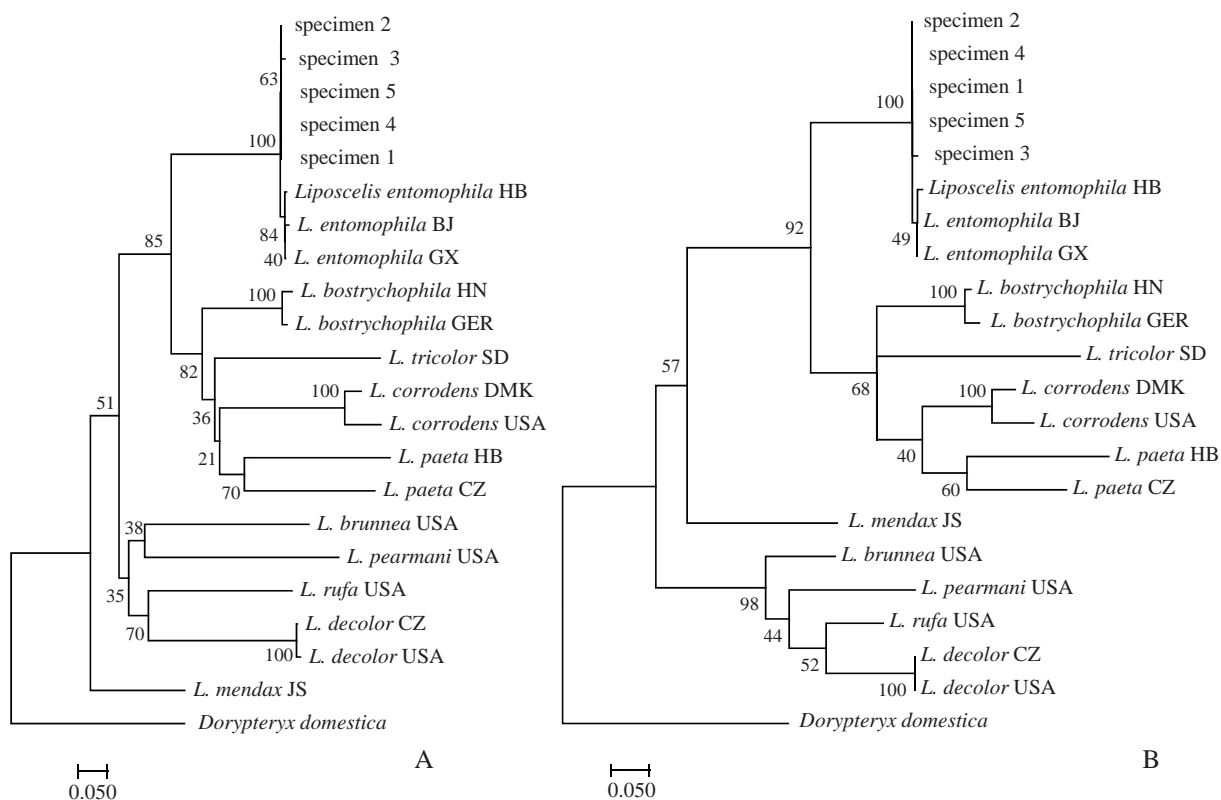


Figure 7. Neighbor-joining and maximum likelihood phylogenetic trees constructed based on *ITS2*. The number at each branch showed the percentage supported by bootstrap. A. Neighbor-joining tree. B. Maximum likelihood tree.

The samples were collected from handmade wooden boxes in the rainforest of Udawalawe, Sri Lanka. Before this study, the genus *Liposcelis* was not reported in Sri Lanka. This study proved the existence of *L. entomophila* in Sri Lanka. It was also a new record of Sri Lanka for the genus. Sri Lanka is an island with relatively isolated ecological environment (Miriya & Rowan, 2008; Gunawardene *et al.*, 2010). Considering the biosafety, the *Liposcelis* from Sri Lanka should pay more attention from now on.

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