Variation in mitochondrial minichromosome composition among *Hoplopleura* lice (Phthiraptera: Hoplopleuridae) from rats

Yi-Tian Fu
Hunan Agricultural University
Yu Nie
Hunan Agricultural University
De-Yong Duan
Hunan Agricultural University
Guo-Hua Liu (liuguohua5202008@163.com)
Hunan Agricultural University https://orcid.org/0000-0002-8434-899X

Short report

Keywords: Rat louse, Fragmented mt genome, Phylogenetic analyses

Posted Date: September 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-42350/v3

Version of Record: A version of this preprint was published at Parasites & Vectors on October 6th, 2020.
See the published version at https://doi.org/10.1186/s13071-020-04381-y.

Abstract

Background The family Hoplopleuridae contains at least 183 species of blood-sucking lice, which widely parasitize both mice and rats. Fragmented mitochondrial (mt) genomes have been reported in two rat lice (Hoplopleura kitti and H. akanezumi) from this family, but some minichromosomes were unidentified in their mt genomes. Methods We sequenced the mt genome of rat louse Hoplopleura sp. with an Illumina Hiseq platform and compared its mt genome organization with H. kitti and H. akanezumi . Results Fragmented mt genome of the rat louse Hoplopleura sp. contains 37 genes which are on 12 circular mt minichromosomes. Each mt minichromosome is 1.8-2.7 kb long, which contains 1-5 genes and one large non-coding region. The gene content and arrangement of three mt minichromosomes of Hoplopleura sp. and H. kitti are different from that of the three mt minichromosomes of H. akanezumi . Phylogenetic analyses based on the deduced amino acid sequences of the eight protein-coding genes showed that the Hoplopleura sp. was more closely related to H. akanezumi than to H. kitti , and then they form a monophyletic group. Conclusions Comparison among the three rat lice revealed variation in the composition of mt minichromosomes within the genus Hoplopleura . Hoplopleura sp. is the first species from the family Hoplopleuridae for which a complete fragmented mt genome has been sequenced. The new data provides useful genetic markers for studying the population genetics, molecular systematics and phylogenetics of blood-sucking lice.

Background

Blood-sucking lice are known vectors and transmit various disease agents and cause significant vector-borne diseases in humans, domestic and wild mammals [1]. The family Hoplopleuridae contains at least 183 described species of blood-sucking lice and is currently classified into eight genera [2]. Of the eight genera, *Hoplopleura* Enderlein, 1904 is the most species-rich (165 described species) found on rodents [3]. The *Hoplopleura* spp*.* are common ectoparasites of both mice and rats, causing pruritus, alopecia, dermal irritation and even anemia.

Metazoan mitochondrial (mt) genomes are usually circular DNA molecules (13-20 kb) with 36-37 genes that contain 12-13 protein-coding genes, two rRNA genes and 22 tRNA genes [4]. Some parasitic lice have an unusual, fragmented mt genome organization, but not all species of parasitic lice have been shown to have a fragmented genome. Fragmentation of the mt genome was first found in the human body louse, *Pediculus humanus corporis* (suborder Anoplura) [5]. Since then, 11 other blood-sucking lice, *P. humanus capitis*, *P. schaeffi*, *Pthirus pubis*, *Haematopinus suis, H. apri, H. asini*, *H. akanezumi, H. kitti, Polyplax asiatica, P. spinulosa and Microthoracius praelongiceps* (suborder Anoplura); eight avian feather lice, *Bovicola bovis*, *B. ovis*, *B. caprae*, *Trichodectes canis, Columbicola columbae*, *C. macrourae*, *C. passerinae* 1 and 2 (suborder Ischnocera), and the elephant louse, *Haematomyzus elephantis* (suborder Rhynchophthirina), have been found with fragmented mt genomes [6-15]. While seven feather lice *Colpocephalum griffoneae*, *Amyrsidea minuta*, and *Heterodoxus macropus* (suborder Amblycera), *Ibidoecus bisignatus*, *Campanulotes compar*, *Bothriometopus macrocnemis* and *Falcolipeurus quadripustulatus* (suborder Ischnocera) do not have fragmented mt genomes [15]. To date, the complete

mt genomes of 12 blood-sucking lice have been sequenced and deposited in GenBank, but the complete mt genomes have been only reported for two rat lice (*H. kitti* and *H. akanezumi*) from this family Hoplopleuridae [7]. In addition, three genes (*nad*1, *nad*3 and *nad*5) or minichromosomes were unidentified in the mt genomes of two *Hoplopleura* species [7]. Interestingly, gene rearrangement has been reported in fragmented mt genome of two *Hoplopleura* species [7]. Therefore, *Hoplopleura* mt genomes may represent one of the most frequently rearranged/fragmentations mt genomes within the family Hoplopleuridae.

To understand the composition of mt minichromosomes among species of the same genus *Hoplopleura*, we sequenced the complete mt genome of rat louse *Hoplopleura* sp. and compared its mt genome organization with other two *Hoplopleura* species, and to re-construct its phylogenetic relationships within the suborder Anoplura using protein sequences derived from coding genes.

Methods

Sample collection and DNA extraction

Adult specimens of *Hoplopleura* sp. were collected from the Edward's long-tailed rats *Leopoldamys edwardsi* in Chongqing, China. The specific identity of the examined wild rats was determined by PCR-based sequencing of the mitochondrial (mt) *cox*1 gene using an established method [16]. These rat lice were washed five times in physiological saline solution, identified preliminarily to the genus level (as *Hoplopleura* sp.) based on morphological features [2], and stored in 70% (v/v) ethanol at -20 °C. Whole genomic DNA including nuclear and mt DNA was extracted from 50 single rat lice (25 females and 25 males) using the DNeasy Tissue Kit (Promega Corporation, Madison, USA) according to the manufacturer's recommendations. The identities of these specimens were further confirmed by polymerase chain reaction (PCR) amplification and subsequent sequencing of the mt *cox*1 and *rrn*S genes using primer pairs L6625 (5'-CCGGATCCTTYTGRTTYTTYGGNCAYCC-3') - H7005 (5'-CCGGATCCACNACRTARTANGTRTCRTG-3') and 12SA (5'-TACTATGTTACGACTTAT-3') - 12SB (5'-AAACTAGGATTAGATACCC-3'), respectively.

Sequencing and assembling

The purity of the extracted whole genomic DNA was assessed by agarose-gel electrophoresis [17]. The DNA concentration was determined using a Quantus Fluorometer (Invitrogen, UK). A paired-end genomic DNA library (350 bp inserts) was constructed for high throughput sequencing with Miseq PE300 (Illumina, San Diego, CA, USA) and collected raw reads were exported in the FASTQ format. The raw reads were filtered by removing adaptor reads, redundant reads and 'N'-rich reads. Finally, 2 Gb clean data (256 bp pair-end reads) was produced for this rat louse. Illumina sequence reads were assembled into contigs with *de novo* using Geneious 11.1.5 [18] based on *cox*1 and *rrn*S relatively conserved sequences. The assembly parameters were minimum overlap identity 99% and minimum overlap 150 bp. When the two

ends of the contig overlapped, indicating circular organization of the minichromosome. We observed in previous studies that each mt minichromosome has a distinct coding region but a well-conserved noncoding region [10-13]. The conserved non-coding region sequences were identified between the *cox*1 and *rrn*S minichromosomes and were used as references to align the Illumina sequence dataset. We assembled these minichromosomes individually in full length using the same method stated above for *cox*1 and *rrn*S minichromosome assembly.

Annotation

Sequences were aligned against the mt minichromosome sequences of rat louse *H. kitti* [7] available using the MAFFT 7.122 software [19] to identify gene boundaries. Protein-coding genes and rRNA genes were identified with BLAST searches of NCBI database. Amino acid sequences of each protein-coding genes were inferred using MEGA 6.0 [20]. tRNA genes were identified using ARWEN [21] and the program tRNAscan-SE [22] with manual adjustment.

Verification of mt minichromosomes

The size of each mt minichromosome of *Hoplopleura* sp. were verified by PCR using specific primers (Table 1). The forward primer and reverse primer in each pair were next to each other with a small gap in between (10-50 bp). PCR with these primers amplified each circular minichromosome in full length (Fig. 1). To obtain full-length sequences of the non-coding regions of the minichromosomes, these positive amplicons were also sequenced with high throughput sequencing as described above.

Phylogenetic analysis

Phylogenetic relationship among representing the blood-sucking lice of suborder Anoplura was performed based on concatenated amino acid sequences (Table 2), using one elephant louse, *H. elephantis* (GenBank accession numbers: KF933032-41) as an outgroup [10]. Eight amino acid sequences (except for *nad*1, *nad*2, *nad*3, *nad*4 and *nad*5 because these genes were unidentified in some blood-sucking lice) were aligned individually using MAFFT 7.122 and were then concatenated to form a single dataset; ambiguously aligned regions were excluded using Gblocks 0.91b using default parameters [23]. The MtArt + I + G + F was selected as the most appropriate evolutionary model by ProtTest 2.4 based on the Akaike information criterion (AIC) [24]. Phylogenetic analyses were conducted with maximum likelihood (ML) using PhyML 3.0 with a BioNJ starting tree, and tree topology search was set from the subtree pruning and regrafting (SPR) method [25]. Bootstrap value was calculated using 100 bootstrap replicates. Phylograms were drawn using FigTree v.1.31.

Results And Discussion

Identity of the rat louse *Hoplopleura* sp.

Two blood-sucking louse species (*H. kitti* and *P. insulsa*) parasitize in *L. edwardsi* (http://phthiraptera.info/category/mammal-wilson-reeder/mammals/rodentia/muridae/murinae/leopoldamys/leopoldamys-edwardsi). The *Hoplopleura* sp. has close morphological and morphometric similarities with *H. kitti* recovered from the same host (*L. edwardsi*). The mt *cox*1 and *rrn*S genes of *Hoplopleura* sp. shared 76% and 77.6% identity with previously published sequences of *H. kitti* (KJ648943) from *Berylmys bowersi* and *H. akanezumi* (KJ648928) from *Apodemus chevrieri* in China, respectively.

General features of the mt genome of the rat louse *Hoplopleura* sp.

We sequenced the *Hoplopleura* sp. genome and produced 3 Gb of Illumina short-read sequence data and obtained a total of 6,526,349×2 raw reads from adults of *Hoplopleura* sp.. After quality filtration, 3,937,826×2 clean reads (2 Gb) were generated for assembly of the mt genome. We assembled these sequence-reads into contigs and identified 37 mt genes typical of bilateral animals (Fig. 2; Table 3). These genes are on 12 minichromosomal; each minichromosome is 1.8-2.7 kb in size and consists of a coding region and a non-coding region (NCR) in a circular organization (Table 3). The coding regions have 1-5 genes each and vary in size from 675 bp to 1,760 bp (Table 3). All genes are transcribed in the same direction except for *nad*1 gene. The nucleotide sequences of the mt minichromosomes of *Hoplopleura* sp. were deposited in GenBank under accession numbers MT792483-94.

We sequenced the full-length non-coding regions of all of the 12 mt minichromosomes of the *Hoplopleura* sp., which range from 935 (H-*nad*5-F minichromosome) to 1,305 bp (C-*nad*6-W-L_2 minichromosome) (Table 3). The longest non-coding region of *Hoplopleura* sp. was shorter than the longest non-coding region of other sucking lice known, such as pig lice (2,370 bp) [6] and horse lice (3,276 bp) [13]. As in the human lice [12], rat lice [7] and pig lice [6], each coding region of *Hoplopleura* sp. is flanked by a conserved non-coding AT-rich motif (88 bp,71.6%) upstream and a GC-rich motif (39 bp, 79.5%) downstream, indicating functional significance of these motifs in the mt genomes of blood-sucking lice.

Annotation

The boundaries between protein-coding genes of the mt genome of *Hoplopleura* sp. were determined by aligning its sequence and identifying translation initiation and termination codons with those of *H. kitti* and *H. akanezumi* [7]. *Hoplopleura* sp. mt genome encoded 13 protein-encoding genes, which had four initiation codons (ATT, ATG, TTG, GTG). Among them, both ATT (*nad*2, *nad*4L, *nad*5, *cox*3 and *cyt*b) and

ATG (*nad*3, *nad*4, *nad*6, *atp*6 and *atp*8) are the highest frequency of being used as initiation codons. Moreover, TTG (*nad*1 and *cox*2) and GTG (*cox*1) are used in the mt genome. This mt genome has three termination codons (TAA, TAG, T). Among them, TAG is the most frequently used with five times altogether, by *cox*1, *nad*2, *nad*3, *nad*4L and *cyt*b. TAA with secondary high rate of recurrence (four times) as termination codons, *cox*2, *atp*6, *atp*8 and *nad*4, used it in the mt genome of *Hoplopleura* sp.. Furthermore, *cox*3, *nad*1, *nad*5 and *nad*6 genes use T as termination codons. Incomplete terminations (TA and T) of protein-coding genes are commonly found in other mt genomes of blood-sucking lice, including *H. suis [6], H. apri* [6], *H. asini [13], H. kitti* [7], *P. asiatica* [8], *P. spinulosa* [8], *P. schaeffi* [9], *M. praelongiceps* [11] and *P. pubis* [12]. In the mt genome of *Hoplopleura* sp., the sizes of the *rrn*L and *rrn*S genes were 1,125 bp and 675 bp, respectively. The 22 tRNA genes ranged from 59 to 71 bp in size. The secondary structures predicted (not shown) were similar to those of *H. kitti* and *H. akanezumi* [7].

Variation in mt minichromosome composition among three rat lice

The complete mt genome sequences of *Hoplopleura* sp. fragmented into 12 circular minichromosomes. The incomplete mt genomes of *H. kitti* and *H. akanezumi* have identified 11 circular minichromosomes [7]. Eleven minichromosomes of the rat louse, *Hoplopleura* sp., have the same gene content and gene arrangement as their counterparts of the rat louse, *H. kitti*. Eight of these minichromosomes of the rat lice, *Hoplopleura* sp. and *H. kitti*, have the same gene content and gene arrangement as their counterparts of the rat louse, *H. akanezumi* [7]. The other two minichromosomes of the rat louse *Hoplopleura* sp., however, are not present in the rat louse *H. akanezumi* [7]. In the *Hoplopleura* sp., one of the minichromosomes has four genes, D-Y-*cox*2-T (Fig. 2). In the *H. akanezumi*, however, this minichromosome has only three genes, D-Y-*cox*2. Similarly, another minichromosome of the *Hoplopleura* sp. has five genes, R-*nad*4L-P-*cox*3-A (Fig. 2). In the *H. akanezumi*, however, this minichromosome has six genes, R-*nad*4L-P-*cox*3-A-T. Interestingly, a chimeric minichromosome has found in the *H. akanezumi* which contains parts of the two rRNA genes, p*rrn*L and p*rrn*S, which are only 5% (51 bp) and 24% (172 bp) of the full-length *rrn*L and *rrn*S, respectively [7]. However, this case has unidentified in the *H. kitti* and *Hoplopleura* sp..

Comparative mt genomic analyses of *Hoplopleura* sp. with *H. kitti* and *H. akanezumi*

A comparison of the nucleotide and the amino acid sequences of each protein-encoding gene (except for *nad*1, *nad*3 and *nad*5) of the three *Hoplopleura* species is given in Table 4. Pairwise comparisons of the nucleotide and amino acid sequences revealed identities of 50.6-77.2% and 37.5-90.2% among them, respectively. The greatest nucleotide variation was in the *atp*8 gene (49.4%), whereas least differences (22.8%) was detected in the *cox*1 gene (Table 4). The difference across both concatenated nucleotide and amino acid sequences of the ten protein-coding genes was 37.5% and 36.8% between *Hoplopleura* sp.

and *H. kitti*, 36.7% and 34.7% between *Hoplopleura* sp. and *H. akanezumi*, and 34.6% and 33.4% between *H. kitti* and *H. akanezumi*.

Phylogenetic relationships

In the present study, phylogenetic analysis of the concatenated amino acid sequence datasets for eight mt protein-coding genes (Fig. 3) showed that the family Hoplopleuridae (*Hoplopleura* sp., *H. kitti* and *H. akanezumi*) clustered to the exclusion of representatives of the families Polyplacidae (*P. asiatica* and *P. spinulosa*), Haematopinidae (*H. apri*, *H. asini* and *H. suis*), Pediculidae (*P. humanus corporis*, *P. humanus capitis* and *P. schaeffi*), Pthiridae (*P. pubis*), and the family Microthoraciidae (*M. praelongiceps*) clustered separately with strong nodal support (Bootstrap = 100). Within the family Hoplopleuridae, *Hoplopleura* sp. and *H. akanezumi* clustered together with moderate support (Bootstrap value = 73), to the exclusion of *H. kitti*, and then they formed a monophyletic group (Bootstrap value = 100). The result was also strongly supported by RAxML analysis (Bootstrap value = 100) (Additional file 1: Fig. S1)

The work of Johnson et al. (2018) created robustness and stability in higher systematics within the order Phthiraptera based on analyses of 1,107 single-copy orthologous genes from sequenced genomes of 46 species of lice [26]. Their result has indicated that the genera *Hoplopleura* and *Haematopinus* were more closely related than to the genus *Pediculus* with strong bootstrap value [26]. However, mt genomic phylogenetic relationships deviated from phylogenies derived from the nuclear genome. Shao et al. (2017) performed a phylogenetic analysis with mt genomes, indicating that the genera *Haematopinus* and *Pediculus* were more closely related than to the genus *Hoplopleura* with strong bootstrap value [11]. Our result also showed the genera *Haematopinus* and *Pediculus* were more closely related than to the genus *Hoplopleura*, but was weak bootstrap value (Bootstrap value = 55) (Fig. 3). Although the number of sucking lice mt genome sequences is increasing, so far, mt genomes of many lineages of sucking lice are underrepresented or not represented. Insufficient taxon sampling for the suborder Anoplura mt genomes might be the cause of the discordance between the mt and nuclear phylogenies.

Many studies have indicated that the mt genome sequence is a valuable genetic marker for phylogenetic studies at various taxonomic levels of different organisms [27,28], including lice [14,15]. The fragmentation of the mt genome may have arisen independently in multiple louse clades. Therefore, the mt genome sequences of rat louse *Hoplopleura* sp. could promote to reassess the systematic relationships of lice within suborder Anoplura using mt genomic datasets. No species from the other genera (*Ancistroplax*, *Ferrisella*, *Haematopinoides*, *Paradoxophthirus*, *Pterophthirus*, *Schizophthirus* and *Typhlomyophthirus*) within family Hoplopleuridae was included in our analyses. Therefore, more expanding taxa sampling is necessary for future phylogenetic studies of family Hoplopleuridae using mt genomic dataset.

Conclusions

Comparison among the three rat lice revealed variation in the composition of mt minichromosomes among species of the genus *Hoplopleura*. *Hoplopleura* sp. is the first species from the family Hoplopleuridae for which a complete fragmented mt genome has been sequenced. The new data provides useful genetic markers for studying the population genetics, molecular systematics and phylogenetics of blood-sucking lice.

Abbreviations

mt: mitochondrial; rDNA: ribosomal DNA; *nad*1: NADH dehydrogenase subunit 1; *nad*3: NADH dehydrogenase subunit 3; *nad*5: NADH dehydrogenase subunit 5; *cox*1: cytochrome c oxidase subunit 1; *rrn*S: small subunit of rRNA; *rrn*L: large subunit of rRNA; tRNA: transfer RNA; *nad*2: NADH dehydrogenase subunit 2; *nad*4: NADH dehydrogenase subunit 4; *nad*4L: NADH dehydrogenase subunit 4L; *cox*3: cytochrome c oxidase subunit 3; *cyt*b: cytochrome b; *nad*6: NADH dehydrogenase subunit 6; *atp*6: ATP synthase F0 subunit 6; *atp*8: ATP synthase F0 subunit 8; *cox*2: cytochrome c oxidase subunit 2.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Y-TF, G-HL conceived and designed the study, and critically revised the manuscript, and Y-TF performed the experiments. Y-TF, G-HL analyzed the data. D-YD and G-HL drafted the manuscript. YN helped in study design, study implementation, and manuscript preparation. All authors read and approved the final manuscript.

Funding

This study was supported by the Planned Programme of Hunan Province Science and Technology Innovation (Grant no. 2018RS3085) and the Training Programme for Excellent Young Innovators of Changsha (Grant No. KQ1905013).

Availability of data and materials

The fragmented mitochondrial genome sequences of *Hoplopleura* sp. from the Edward's long-tailed rats have been deposited in the GenBank database under the accession numbers MT792483-94.

Ethics approval and consent to participate

All procedures involving animals in the present study were approved and this study was approved by the Animal Ethics Committee of Hunan Agricultural University (No. 43321503).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 PCR primers used to amplify and sequence the mitochondrial genome of the rat lice, *Hoplopleura* sp.

Primer	Sequence (5′ to 3′)	Minichromosome
1F	AGCACTTGTTCTGATTCTTCGGTC	I-*cox*1
1R	TCGTGATACCCCCTGCCAAAACTG	I-*cox*1
2F	CTTTCAAGAGACACAAGGGGTTCA	*rrn*S
2R	TATTTTCCCAGTCCTACAGAGAGC	*rrn*S
3F	TGTCCTTGTCCCGAAAGAGAGTGAT	M-L1-*rrn*L-V
3R	CTATTCCACCCTCCCTGATACAAAA	M-L1-*rrn*L-V
4F	TGAGTAAGGGGGATACATCACGCTA	Q-*nad*1-G-*nad*3
4R	CAGCGAACTCTGCGTATTCCTCCAT	Q-*nad*1-G-*nad*3
5F	TAAGGTTATCGGGCATCAGTGGTA	D-Y-*cox*2-T
5R	AGAGGGGATGGCGAGGACAAAAAG	D-Y-*cox*2-T
6F	CGCCAACTATCAGAACTTTCCAAC	*atp*8-*atp*6-N
6R	TCGTGGATAACAGTCACAAAGATG	*atp*8-*atp*6-N
7F	GCATTTACAGTGCTCAGTCTTCGC	*nad*2
7R	ACAAAGACAAAGGGGGAAACGGGA	*nad*2
8F	TTAGCGGTAAGCGGGACTGAGGTA	C-*nad*6-W-L2
8R	AACTCTATTTCCCCCGTTTCCCAA	C-*nad*6-W-L2
9F	GTTCCTCTCGGTTTTCCATCCCTCA	R-*nad*4L-P-*cox*3-A
9R	TCTATCGCTACCAGAGAGATTGTTA	R-*nad*4L-P-*cox*3-A
10F	GGGAAAACTCCGACAAGGTCACATT	E-*cyt*b-S1-S2
10R	CCTAAGGGATTTGAACTTCCTGTCG	E-*cyt*b-S1-S2
11F	GGTATTGCTAAAGTTTGGAGGTATC	K -*nad*4
11R	CAGCCAAGAGTATTCTCCCCAACAT	K -*nad*4
12F	GGGGATTACCTCCTTCCTTCTCATT	H-*nad*5-F
12R	AAGCAATGAAGAGCAACAAGGACAC	H-*nad*5-F

Table 2 The blood-sucking lice included in the phylogenetic analyses in this study

Species	Hosts	GenBank accession numbers	References
Haematopinus apri	Wild pig	KC814611-19	Jiang et al., 2013
Haematopinus asini	Horse	KF939318, KF939322, KF939324, KF939326, KJ434034-38	Song et al., 2014
Haematopinus suis	Domestic pig	KC814602-10	Jiang et al., 2013
Hoplopleura akanezumi	Rat	KJ648922-32	Dong et al., 2014a
Hoplopleura kitti	Rat	KJ648933-43	Dong et al., 2014a
Microthoradus praelongiceps	Guanacos	KX090378-KX090389	Shao et al., 2017
Pediculus humanus corporis	Human	FJ499473-90	Shao et al., 2009
Pediculus humanus capitis	Human	JX080388-407	Shao et al., 2012
Pediculus schaeffi	Chimpanzee	KC241882-97, KR706168-69	Herd et al., 2015
Pthirus pubis	Human	JQ976018, EU219987-95, HM241895-8	Shao et al., 2012
Polyplax asiatica	Rat	KF647751-61	Dong et al., 2014b
Polyplax spinulosa	Rat	KF647762-72	Dong et al., 2014b
Hoplopleura sp.	**Rat**	**MT792483-94**	**Present study**

Table 3 Mitochondrial minichromosomes of the rat louse *Hoplopleura* sp., identified by Illumina sequencing

Minichromosome	Size (bp)	Size of coding region (bp)	Size of non-coding region (bp)	Intergenic region (bp)
I-*cox*1	2,531	1,549	975	7
*rrn*S	1,869	675	1,194	0
M-L1-*rrn*L-V	2,257	1,323	934	0
Q-*nad*1-G-*nad*3	2,525	1,445	1,063	17
D-Y-*cox*2-T	2,087	880	1,129	78
*atp*8-*atp*6-N	2,023	896	1,118	9
*nad*2	2,141	981	1,160	0
C-*nad*6-W-L2	1,979	673	1,305	1
R-*nad*4L-P-*cox*3-A	2,311	1,251	1,057	3
E-*cyt*b-S1-S2	2,417	1,304	1,113	0
K -*nad*4	2,289	1,313	975	1
H-*nad*5-F	2,695	1,759	935	1
Total	27,124	14,049	12,958	117

Table 4 Nucleotide (nt) and/or predicted amino acid (aa) sequence differences in mitochondrial genes among Hoplopleura sp. (Hs), *H. kitti* (Hk) and H. akanezumi (Ha) upon pairwise comparison

Gene/region	Nt sequence length			Nt difference (%)			Number of aa			aa difference (%)		
	Hs	Hk	Ha	Hs / Hk	Hs /Ha	Hk / Ha	Hs	Hk	Ha	Hs / Hk	Hs / Ha	Hk / Ha
atp6	651	651	654	36.34	36.54	33.49	216	216	217	32.26	33.64	27.65
atp8	174	195	177	47.50	49.44	46.97	57	64	58	62.50	59.02	54.69
nad2	981	990	984	48.44	44.18	43.40	326	329	327	55.15	53.19	54.85
nad4	1,248	1,242	1,254	40.38	40.49	41.21	415	413	417	44.84	45.56	44.84
nad4L	273	273	270	42.34	39.56	42.12	90	90	89	48.89	44.44	50.00
nad6	478	483	474	43.83	44.49	41.74	158	160	157	47.20	50.63	48.75
cox1	1,485	1,530	1,530	29.15	29.26	22.80	494	509	509	17.68	15.32	9.80
cox2	687	681	684	38.24	34.40	33.77	228	226	227	41.30	32.02	31.00
cox3	787	787	789	34.18	34.60	32.57	261	261	262	27.48	29.77	30.92
cytb	1,104	1,102	1,107	34.30	33.97	32.13	367	367	368	29.70	25.27	25.00
rrnS	675	737	690	31.80	25.32	33.73						
rrnL	1,125	1,107	1,131	28.48	27.55	29.86						

Figures

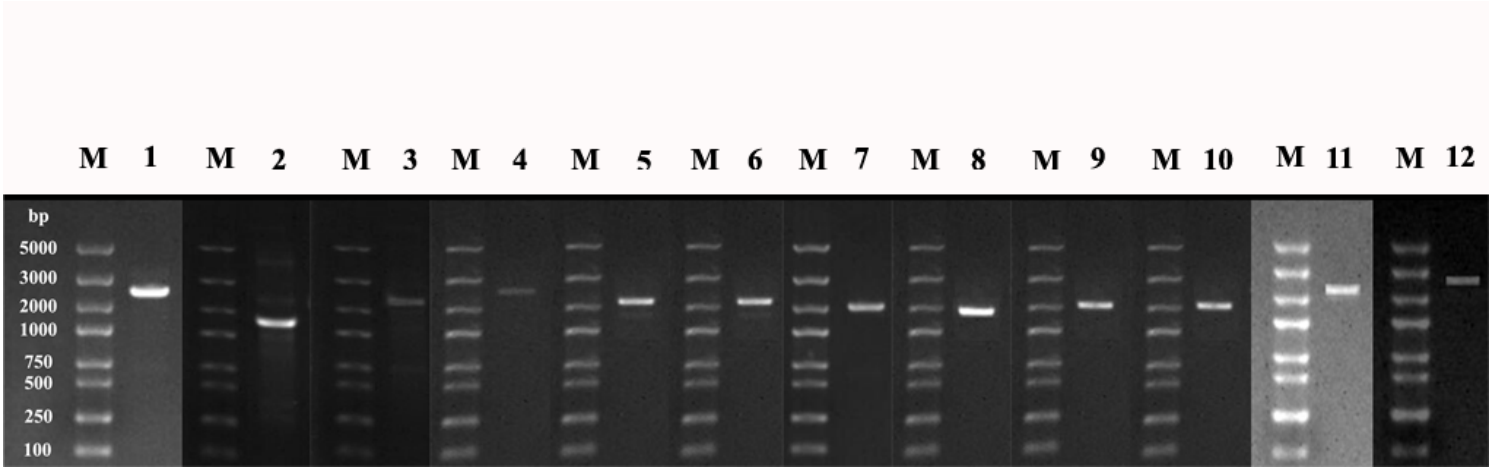

Figure 1

PCR verification of the 12 mt minichromosomes of the rat louse, Hoplopleura sp.. M: DL2000 DNA marker; Lane 1-12: I-cox1, rrnS, M-L1-rrnL-V, Q-nad1-G-nad3, D-Y-cox2-T, atp8-atp6-N, nad2, C-nad6-W-L2, R-nad4L-P-cox3-A, E-cytb-S1-S2, K -nad4 and H-nad5-F.

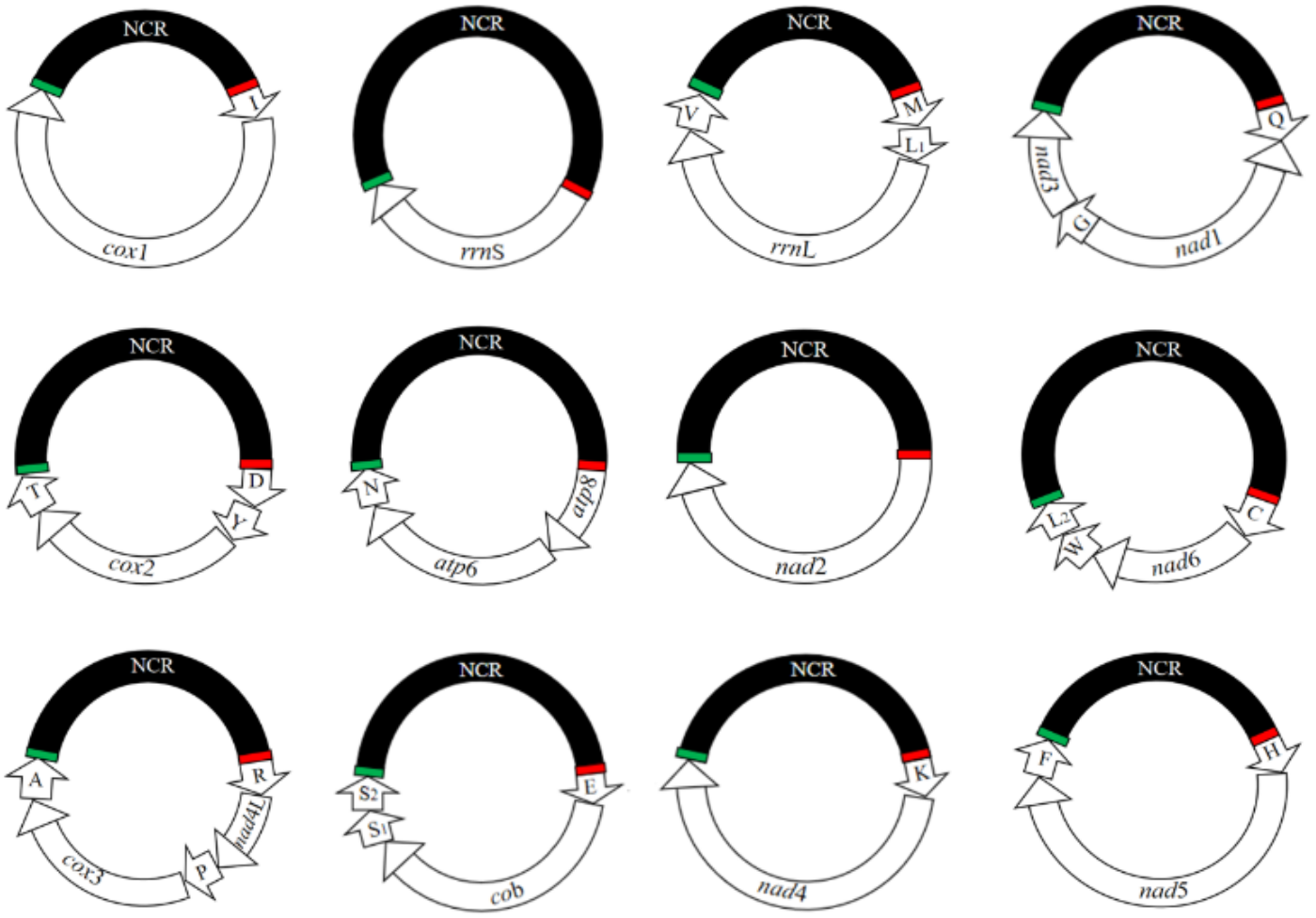

Figure 2

The complete mitochondrial genome of rat louse, Hoplopleura sp.. Each minichromosome has a coding region and a non-coding region (NCR, in black). The names and transcript orientation of genes are indicated in the coding region and the minichromosomes are placed in alphabetical order of protein-coding genes and rRNA genes. Gene names are all in Abbreviation: atp6 and atp8 for ATP synthase subunits 6 and 8; cob for cytochrome b; cox1-3 for cytochrome coxidase subunits 1–3, nad1-6 and nad4L for NADH dehydrogenase subunits 1–6 and 4L; rrnS and rrnL for small and large subunits of ribosomal RNA. tRNA genes are indicated with their single-letter abbreviations of the corresponding amino acids.

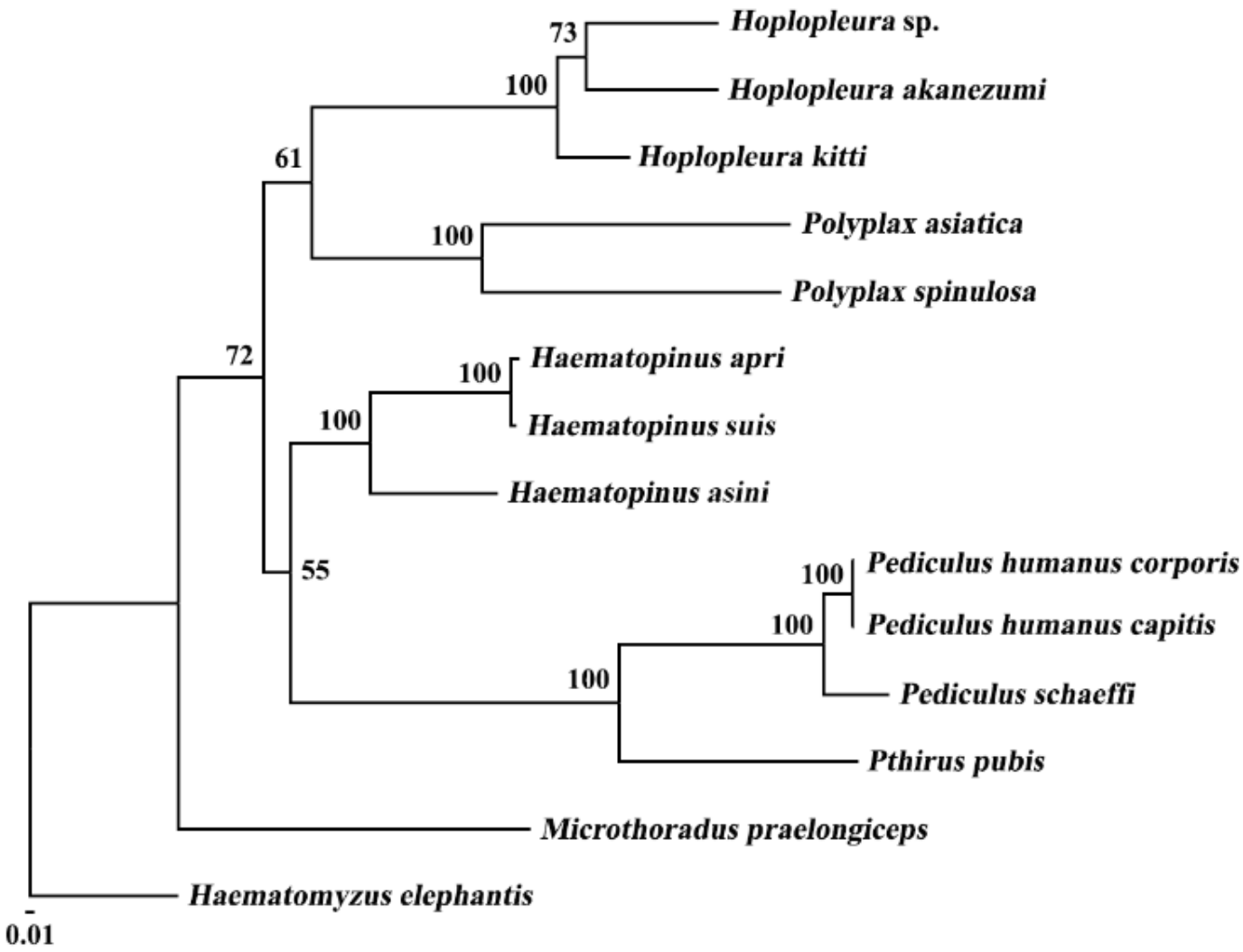

Figure 3

Phylogenetic relationships among 13 species of the suborder Anoplura inferred from maximum likelihood of deduced amino acid sequences of eight mitochondrial proteins using PhyML. One elephant louse, Haematomyzus elephantis was used as the outgroup. Bootstrap values were indicated at nodes.

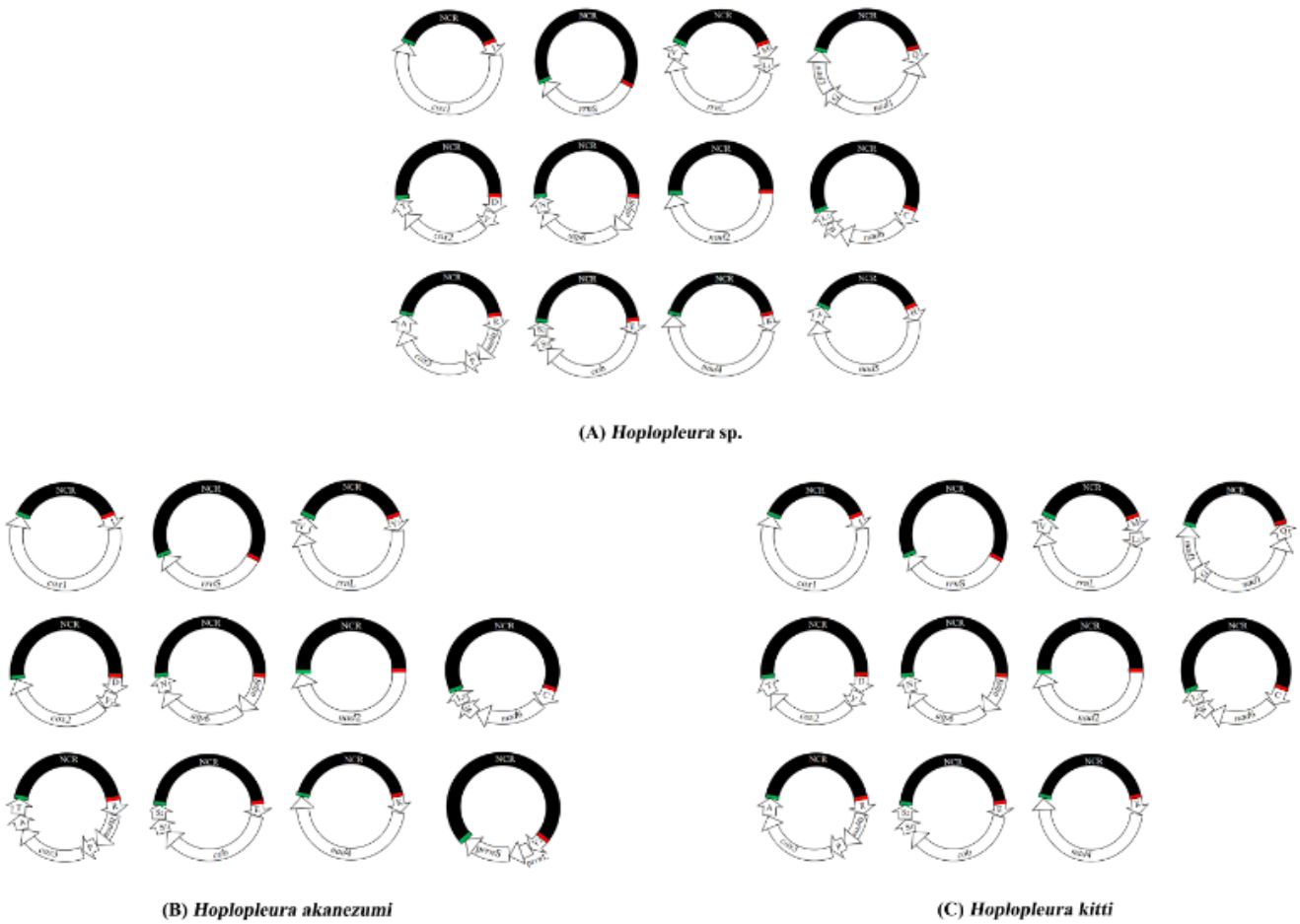

Figure 4

The differences among all minichromosomes of three Hoplopleura lice.

Supplementary Files

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- Fig.S1.tif