

RESEARCH ARTICLE

Conserved gene arrangement in the mitochondrial genomes of barklouse families Stenopsocidae and Psocidae

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Abstract Substantial variation in gene organization and arrangement has been reported for sequenced mitochondrial (mt) genomes from the suborders of the insect order Psocoptera. In this study we sequenced the complete mt genome of *Stenopsocus immaculatus*, the first representative of the family Stenopsocidae from the suborder Psocomorpha. Relative to the ancestral pattern, rearrangements of a protein-coding gene (*nad3*) and five tRNA genes (*trnQ*, *trnC*, *trnN*, *trnS1*, *trnE*) were found. This pattern was similar to that of two barklice from the family Psocidae, with the exception of the translocation of *trnS1*, *trnE* and *trnI*. Based on comparisons of pairwise breakpoint distances of gene rearrangements, gene number and chromosome number, it was concluded that mt genomes of Stenopsocidae and Psocidae share a relatively conserved pattern of gene rearrangements; mt genomes within the Psocomorpha have been generally stable over long evolutionary history; and mt gene rearrangement has been substantially faster in the booklice (suborder Troctomorpha) than in the barklice (suborders Trogiomorpha and Psocomorpha). It is speculated that the change of life history and persistence of unusual reproductive systems with maternal inheritance contributed to the contrasting rates in mt genome evolution between the barklice and booklice.

Keywords gene rearrangement, mitochondrial genome, Psocoptera, Stenopsocidae, TDRL model

1 Introduction

The animal mitochondrial (mt) genome is important in cell metabolism, apoptosis, disease and aging^[1]. The mt genome is the most extensively studied genomic system

in insects, with sequenced representatives from all insect orders available in GenBank^[2]. Sequence data from the mt genome has been used widely for phylogenetic analyses across broad taxonomic levels^[3–8] due to the abundance of mt genomes in tissues, their greater rate of evolution than the nuclear genome and their evolutionarily conserved transcription products^[2,9,10]

The insect mt genome is a compact circular molecule and typically encodes 13 protein-coding genes (PCGs), two rRNA (rRNA) genes and 22 tRNA (tRNA) genes. In addition to this set of 37 genes, there are a variety of non-coding regions, the largest of which is usually termed the control region (CR) as it contains both an origin of replication and transcription^[11]. In general, the arrangement of genes in insect mt genomes is highly conserved^[2,12,13], with notable exceptions in the Hymenoptera, Thysanoptera and Psocodea (Phthiraptera and Psocoptera)^[14–19].

The insect order Psocoptera contains 39 extant families and more than 5000 described species in three suborders: Trogiomorpha (barklice), Psocomorpha (barklice) and Troctomorpha (booklice)^[20]. To date, complete mt genomes have been published for three barklice and five booklice^[18,19,21–24]. The mt genomes of the five booklice (all from the genus *Liposcelis*) differed significantly in gene arrangement from the generally-accepted ancestral insect mt genome, and even from each other^[18], e.g., no commonalities were observed between the ancestral gene arrangement and the arrangement in *Liposcelis entomophila*^[21], and only one commonality (*atp8-atp6*) was seen between the ancestral gene arrangement and the other four *Liposcelis* species^[22]. *Liposcelis decolor* and *Liposcelis sculpitilis*, like most other insects, have the typical single-chromosome mt genome; the other three *Liposcelis* species, however, possess multipartite mt genomes comprising two chromosomes^[22,23]. The mt genomes of the barklice were much less rearranged than those of the booklice. The genome of the lepidopsocid (suborder

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Trogiomorpha) had rearrangements of eight genes, including a PCG^[24]. Two genomes of the other two barklice, *Psococerastis albimaculata* and *Longivalvus hyalospilus* (family Psocidae, suborder Psocomorpha), had the least rearrangements, with the transposition of one PCG and five tRNA genes, explained by the mechanism of tandem duplication-random loss (TDRL)^[19]. The variation observed within the currently-available barklice and booklice mt genomes greatly limits their use in reliably inferring evolutionary patterns within the Psocoptera, and additional mt genomic data from other families are needed.

To further understand the evolution of the mt genome organization in the Psocoptera, we sequenced the complete mt genome of *Stenopsocus immaculatus*, the first representative of the family Stenopsocidae. The conserved pattern of gene arrangement in the mt genomes of two barklice families, Stenopsocidae and Psocidae suggested that mt genomes within the suborder Psocomorpha have been stable over long evolutionary timescales after some early gene rearrangements.

2 Materials and methods

2.1 Samples collection and DNA extraction

Specimens of the narrow barklouse, *S. immaculatus*, were collected from Ashoro Washippu, Hokkaido, Japan in 2013 and kept in 100% ethanol before being transferred to -20°C for long-term preservation at the Entomological Museum of China Agricultural University, Beijing, China. Genomic DNA was extracted from six adult whole bodies excluding the abdomen with a DNeasy DNA Extraction kit (Qiagen, Valencia, CA, USA).

2.2 Mitochondrial genome sequencing, assembly and annotation

Two fragments of mtDNA (*cox1* and *rrnS*) were amplified using PCR and Sanger sequencing following Li et al.^[25] (Table S1). A library was prepared from the genomic DNA with an insert size of 450 bp and was sequenced on the Illumina HiSeq 2500 platform at Berry Genomics, Beijing. 4 Gb of clean data (250 bp paired-end reads) were used to assemble the mt genome using the method map to reference in Geneious v9.0.4^[26] with the sequences of *cox1* and *rrnS* as the reference. The assembly parameters were: minimum overlap identity 98%, no gaps, maximum mismatches per read 2%, maximum ambiguity 2, and minimum overlap 100 bp. PCGs and rRNAs were identified using BLAST searches in GenBank and subsequently by alignment with sequences of other barklice^[19,24]. tRNA genes were identified by tRNAscan-SE Search Server v1.21^[27] and checked manually. tRNA genes that could not be determined by tRNAscan-SE were

determined in the unannotated regions by sequence similarity to tRNAs of other barklice. The annotated entire mt genome sequences of *S. immaculatus* have been deposited in GenBank under accession number KX187004.

2.3 Nucleotide composition, substitution rate and gene rearrangement analyses

The nucleotide composition of the mt genomes was calculated using MEGA 6.0^[28]. The nonsynonymous (*Ka*) and synonymous (*Ks*) substitution rate was calculated using DnaSP v.5.0^[29]. To compare the rates in mt gene rearrangement among major lineages of Psocoptera, pairwise breakpoint distances were calculated with the CREx web server^[30].

2.4 Sequence alignment and phylogenetic analyses

Four barklice and five booklice from Psocoptera were included in phylogenetic analyses (Table 1). The true bug, *Halyomorpha halys*, was used as an outgroup. Sequences of 12 PCGs (without *nad4L*) along with two rRNA genes were used for the phylogenetic analyses. Each PCG was aligned separately based on multiple codon alignment with the MAFFT algorithm in the TranslatorX online platform^[32]. The two rRNA genes were aligned with the MAFFT 7.0 online server^[33].

The individual gene alignments were assembled into a concatenated data set which was partitioned by genes using both Bayesian inference (BI) and maximum likelihood (ML) analyses with MrBayes 3.23^[34] and RAxML-HPC2 8.1.11^[35]. The best-fitting model (GTR + I + G) for the nucleotide data set was determined with jModelTest 0.1.1^[36]. For the ML analysis, the reliability of the inferred topology was assessed by performing 1000 rapid bootstrap replicates. For Bayesian analysis, two simultaneous runs of 10 million generations were performed for the data set and trees were sampled every 1000 generations, with the first 25% discarded as burn-in. The analysis was considered to have reached stationarity when the average standard deviation of split frequencies decreased to 0.01^[37].

3 Results and discussion

3.1 The mitochondrial genome of the narrow barklouse, *Stenopsocus immaculatus*

The mt genome of *S. immaculatus* was determined to be a double-strand circular DNA molecule 16991 bp in length, including the 37 typical coding genes (13 PCGs, 22 tRNAs and two rRNAs) and a 1720 bp CR (Fig. 1). Twenty-three genes are encoded on the major strand (J-strand) and other 14 genes are encoded on the minor strand (N-strand). In

Table 1 Species phylogenetically analyzed in this study

Order/Suborder	Family	Species	Accession number	Reference
Psocoptera				
Psocomorpha	Psocidae	<i>Psococerastis albimaculata</i>	JQ910986	[19]
		<i>Longivalvus hyalospilus</i>	JQ910989	[19]
	Stenopsocidae	<i>Stenopsocus immaculatus</i>	KX187004	Present study
Trogiomorpha	Lepidopsocidae	Unidentified species	NC_004816	[24]
Troctomorpha	Liposcelidae	<i>L. decolor</i>	JX870621	[23]
		<i>L. bostrychophila</i>	JN645275-76	[22]
		<i>L. paeta</i>	NC_025505-06	[21]
		<i>L. entomophila</i>	NC_025503-04	[21]
		<i>L. sculptilis</i>	KX171073	[18]
Hemiptera				
Heteroptera	Pentatomidae	<i>Halyomorpha halys</i>	NC_013272	[31]

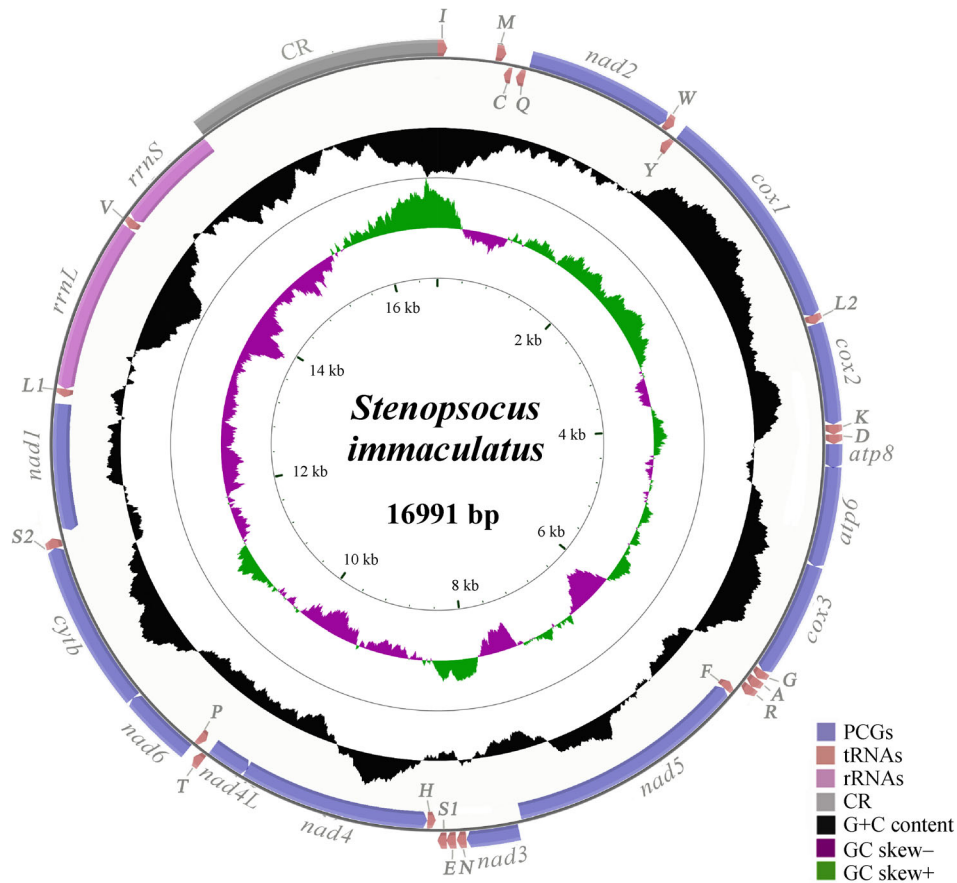


Fig. 1 The mitochondrial genome of the narrow barklouse, *Stenopsocus immaculatus*. Arrows indicate the orientation of gene transcription. PCGs are shown as blue arrows, rRNA genes as purple arrows, tRNA genes as brown arrows and the control region as gray rectangle. Abbreviations of gene names are: *atp6* and *atp8* for ATP synthase subunits 6 and 8, *cox1–3* for cytochrome oxidase subunits 1–3, *cytb* for Cytochrome *b*, *nad1–6* and *nad4L* for NADH dehydrogenase subunits 1–6 and 4L, *rrnL* and *rrnS* for large and small rRNA subunits. tRNA genes are shown with their one-letter corresponding amino acids; the two tRNA genes for leucine and serine have different anticodons: L1 (TAG), L2 (TAA), S1 (TCT) and S2 (TGA). The GC content is plotted using a black sliding window, as the deviation from the average GC content of the entire sequence. GC-skew is plotted as the deviation from the average GC-skew of the entire sequence. The inner cycle indicates the location of genes in the mt genome.

addition to the CR, another 14 non-coding regions were observed with the longest one (332 bp) flanked by *trnI* and *trnM* (Table S2). There were also 13 overlaps identified, ranging from 1 to 7 bp in length.

The nucleotide composition of the *S. immaculatus* mt genome had a bias toward A and T, with an A + T content of 78.3%; the highest A + T content was found in the third codon position of PCGs (88.9%) and the CR (84.8%) (Table 2). The whole mt genome was found to have a slight T skew and C skew, and the PCGs had an obvious T skew and moderate G skew. The codon usage of PCGs also contributed to the genome-wide bias toward AT and there was a strong bias to A or T at the third codon position of PCGs (Table S3). All of the PCGs had the standard ATN start codon. Eleven PCGs had the complete stop codon, either TAG or TAA, whereas *cox3* and *nad5* have an incomplete stop codon T, which is presumed to be subsequently completed by the post-transcriptional polyadenylation process^[38].

The typical 22 tRNAs found in insect mt genomes were found in *S. immaculatus*, with lengths varying from 61 to 69 bp. Most of the tRNAs could be folded as classic cloverleaf structures, with the exception of *trnSI*, in which its dihydrouridine arm simply forms a loop, instead of a stem-loop (Fig. S1). Based on the secondary structure, a total of 22 unmatched base pairs were found in the tRNAs. Eighteen of them were G-U pairs, which form a weak bond. The remaining four pairs included one A-G, one C-U, and two A-C mismatches.

The putative CR (1720 bp) was flanked by *rrnS* and *trnI* and is highly AT-rich (84.8%). Two tandem mirror repeats, unit I (683 bp) and unit II (686 bp), were identified in this region (Fig. 2). The sequence of mirror-repeat unit II was reversed with respect to unit I and showed high sequence similarity (99.4%) to unit I, with only four point mutations. Mirror repeats are known to form triplex H-DNA which are intrinsically mutagenic in mammalian cells^[39]. They are known to induce large scale mutations such as deletions and/or rearrangements at a higher frequency^[40]. Most of the repetitions found in the CRs of insect mt genomes, to date, are short repeat units similar to minisatellites with high copy numbers^[41]. The occurrence of a large mirror repeats, with few mutations, found in the CR of the *S. immaculatus* mt genome is a rare event deserving the attention of future studies.

3.2 Gene rearrangements in the mitochondrial genome of *Stenopsocus immaculatus*

Compared with the generally-accepted ancestral mt gene arrangement of insects, rearrangements of a PCG (*nad3*) and five tRNAs (*trnM*, *trnC*, *trnN*, *trnSI* and *trnE*) were found in two regions of the *S. immaculatus* mt genome: (1) between *cox3* and *trnH*, and (2) between CR and *trnY* (Fig. 3). These two regions are considered as the active regions for mt gene rearrangement in hymenopterans^[15], flat bugs^[3] and barklice from the family Psocidae^[19].

Three TDRL events could explain the gene rearrangements observed in the mt genome of *S. immaculatus*. In the first hypothesized TDRL event, the gene cluster of *trnI-trnQ-trnM-nad2-trnW-trnC-trnY* was duplicated in tandem and one copy of each duplicated gene was randomly deleted, leading to the new gene arrangement *trnI-trnM-trnC-trnQ-nad2-trnW-trnY*. The gene rearrangement in the second active region could be derived from another hypothesized TDRL event (Fig. 4). The mt gene rearrangements in *S. immaculatus* is slightly more complex than those in two barklice from the Psocidae (suborder Psocomorpha) with a third hypothesized TDRL event changing *trnSI-trnE* to *trnE-trnSI*.

3.3 Evolution of mitochondrial genome organization in the Psocoptera

To investigate the evolution of mt genome organization in the Psocoptera, we inferred phylogenetic relationships between the four barklice and five booklice, representing all three suborders, for which complete mt genome sequences are available. From ML and BI analyses trees were inferred with an identical topology with strong support for most of the clades (Fig. 5). Within the suborder Psocomorpha, *S. immaculatus* (Stenopsocidae) was recovered as the sister to a clade comprising *P. albimaculata* and *L. hyalospilus* (Psocidae). The close relationship between these two Psocomorpha families was also supported by their highly similar gene arrangement, sharing the same gene boundaries, with the exception of the translocation of *trnSI* and *trnE* and *trnI*. This result indicated a relatively conserved pattern of gene rearrangements in the mt genomes of Stenopsocidae and Psocidae. A phylogenetic study had previously suggested that these two families

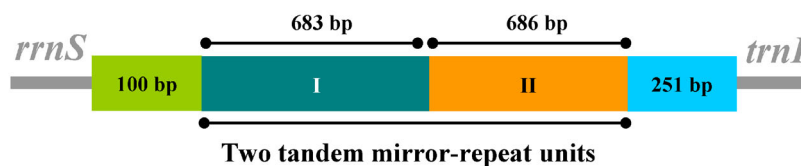


Fig. 2 The control region of the narrow barklouse *Stenopsocus immaculatus*. I and II indicate two mirror-repeat units.

Table 2 Nucleotide composition of the *Stenopsocus immaculatus* mitochondrial genome

Feature	T (U)	C	A	G	A% + T%	AT-skew	GC-skew
Whole genome	39.9	11.8	38.4	9.8	78.3	– 0.019	– 0.093
Control region	42.8	7.5	41.9	7.7	84.8	– 0.011	0.015
Protein-coding genes	43.6	11.5	32.1	12.8	75.7	– 0.151	0.053
First codon position	35.0	10.7	35.7	18.4	70.9	0.006	0.263
Second codon position	46.0	18.3	21.3	14.3	67.4	– 0.367	– 0.123
Third codon position	49.0	5.5	39.5	5.6	88.9	– 0.112	0.017
tRNA genes	39.7	8.3	40.1	11.9	79.8	0.005	0.175
rRNA genes	43.5	6.7	39.0	10.8	82.5	– 0.055	0.234

Note: AT-skew = $(A\% - T\%)/(A\% + T\%)$; GC-skew = $(G\% - C\%)/(G\% + C\%)$.

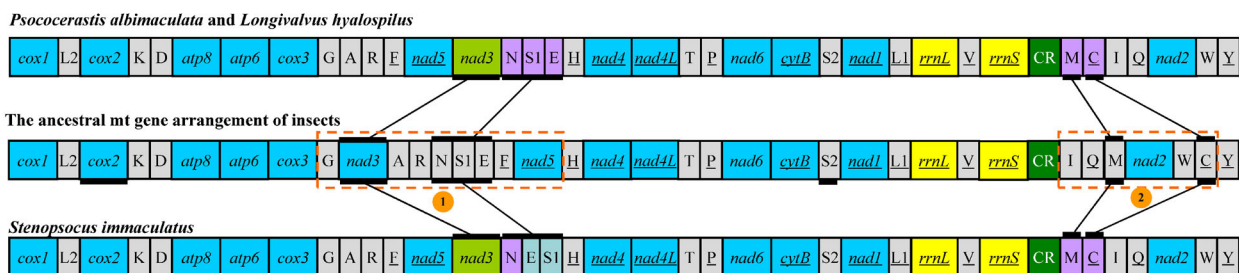


Fig. 3 Comparison of mitochondrial gene arrangement between Psocomorpha (Stenopsocidae and Psocidae) and the hypothetical ancestor of insects. Abbreviations of gene names follow Fig. 1. Genes are transcribed from left to right except those underlined, which have the opposite transcriptional orientation. Orange frames show two active regions of gene rearrangements.

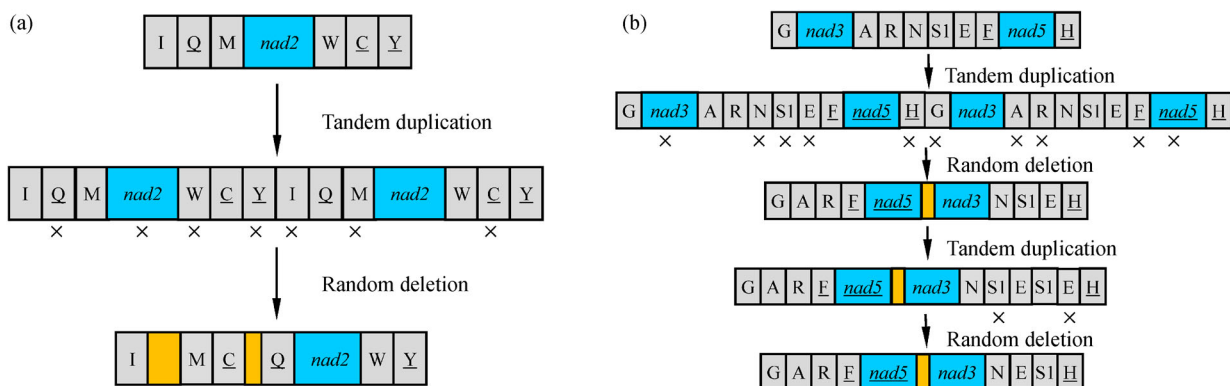


Fig. 4 Inferred TDRL events that account for the mitochondrial gene rearrangements in the narrow barklouse *Stenopsocus immaculatus*. (a) Genes between CR and *cox1*; (b) genes between *cox3* and *nad4*. Genes with crosses below were eliminated. Two longer non-coding sequences are highlighted in orange.

(Stenopsocidae and Psocidae) represent the most widely divergent clades within the suborder Psocomorpha^[42]. This suggests that mt genomes within Psocomorpha have been stable over a long period of evolutionary history following some early gene rearrangements.

To compare the evolutionary rates of mt gene rearrangement among major lineages of the Psocoptera, the pairwise breakpoint distances of gene rearrangement, gene number and chromosome number, and nonsynonymous (K_a) and

synonymous (K_s) substitution rates were calculated, and are given adjacent to the phylogenetic tree (Fig. 5). The mt genomes of the Psocoptera showed accelerated evolutionary rates with breakpoint distances of gene rearrangements, relative to the ancestral insect genome, ranging from 12 to 36. There were substantial differences found between the three suborders of the Psocoptera. The five booklice from the Troctomorpha have had much faster evolutionary rates than the three barklice from the other

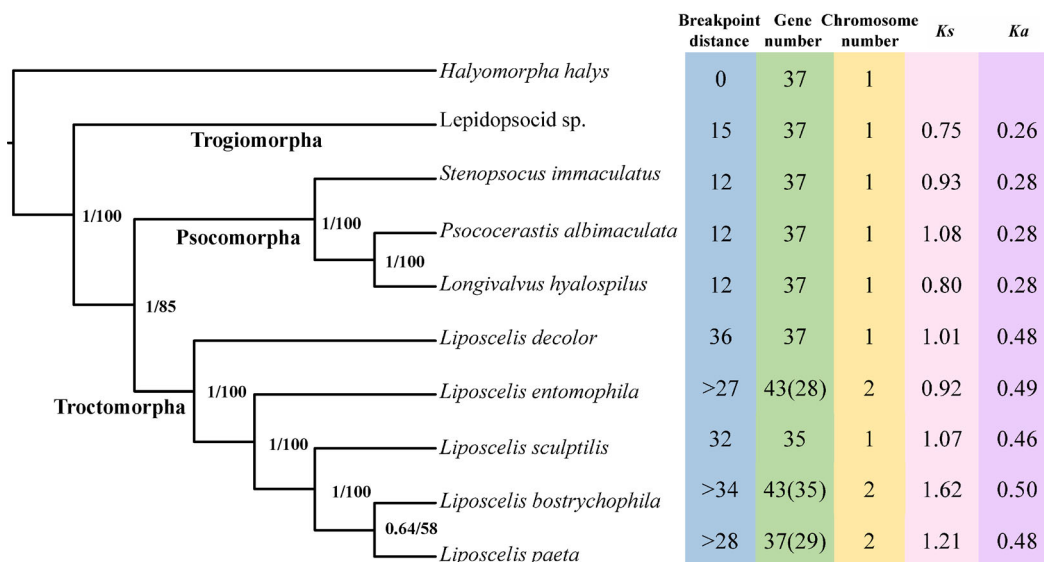


Fig. 5 Phylogenetic relationships among major lineages of the Psocoptera inferred from mitochondrial genome sequences. Numbers close to the branching points are ML bootstrap support values (right) and Bayesian posterior probabilities (left) in percentages. Breakpoint distances are relative to the ancestral arranging pattern. Gene number in the bracket calculates gene without repetitions.

two suborders, with notably higher breakpoint distances and nucleotide substitution rates. The five booklice also showed more variation in terms of missing/copied genes and genome fragmentation (i.e., two chromosomes) (Fig. 5).

Differences in the mt genomes among the three suborders may be attributable to the obviously different ecologies of barklice and booklice. Barklice are entirely free-living insects, whereas booklice are closely associated with the nests of birds and mammals as well as human households. Our previous study indicated that ecological changes in booklice and parasitic lice (Phthiraptera) appears to be associated with an increased rate of mt gene rearrangement^[19]. This finding is supported by a recent study which associated an extraordinarily divergent mitochondrial karyotype with maternally transmitted sex ratio distortion in *Liposcelis bostrychophila*^[43]. When considering that parthenogenetic reproduction is common in booklice, we speculate that the change of life history and persistence of unusual reproductive systems with maternal inheritance may have contributed to the contrasting rates of evolution in mt genomes between the barklice and booklice.

4 Conclusions

We present the complete mt genome of *Stenopsocus immaculatus*, the first representative of the family Stenopsocidae from the suborder Psocomorpha. Comparative mt genome analyses reveal conserved pattern of gene rearrangements in Stenopsocidae and Psocidae, and mt gene rearrangement has been substantially faster in the

booklice (suborder Troctomorpha) than in the barklice (suborders Trogiomorpha and Psocomorpha). The change of life history and persistence of unusual reproductive systems with maternal inheritance is likely to contribute to the contrasting rates in mt genome evolution between the barklice and booklice.

Supplementary materials The online version of this article at <http://dx.doi.org/10.15302/J-FASE-2017158> contains supplementary materials (Tables S1–S3; Fig. S1).

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