Mitochondrial genomes of two Barklice, Psococerastis albimaculata and Longivalvus hyalospilus (Psocoptera: Psocomorpha): contrasting rates in mitochondrial gene rearrangement between major lineages of Psocodea

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Mitochondrial Genomes of Two Barklice, *Psococerastis albimaculata* and *Longivalvus hyalospilus* (Psocoptera: Psocomorpha): Contrasting Rates in Mitochondrial Gene Rearrangement between Major Lineages of Psocodea

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Abstract

The superorder Psocodea has ~10,000 described species in two orders: Pscoptera (barklice and booklice) and Phthiraptera (parasitic lice). One booklouse, *Liposcelis bostrychophila* and six species of parasitic lice have been sequenced for complete mitochondrial (mt) genomes; these seven species have the most rearranged mt genomes seen in insects. The mt genome of a barklouse, lepidopsocid sp., has also been sequenced and is much less rearranged than those of the booklouse and the parasitic lice. To further understand mt gene rearrangements in the Psocodea, we sequenced the mt genomes of two barklice, *Psococerastis albimaculata* and *Longivalvus hyalospilus*, the first representatives from the suborder Psocomorpha, which is the most species-rich suborder of the Psocodea. We found that these two barklice have the least rearranged mt genomes seen in the Psocodea to date: a protein-coding gene (*nad3*) and five tRNAs (*trnN, trnS1, trnE, trnM* and *trnC*) have translocated. Rearrangements of mt genes in these two barklice can be accounted for by two events of tandem duplication followed by random deletions. Phylogenetic analyses of the mt genome sequences support the view that Pscoptera is paraphyletic whereas Phthiraptera is monophyletic. The booklouse, *L. bostrychophila* (suborder Troctomorpha) is most closely related to the parasitic lice. The barklouse (suborders Trogiomorpha and Psocomorpha) are closely related and form a monophyletic group. We conclude that mt gene rearrangement has been substantially faster in the lineage leading to the booklouse and the parasitic lice than in the lineage leading to the barklouse. Lifestyle change appears to be associated with the contrasting rates in mt gene rearrangements between the two lineages of the Psocodea.

Introduction

The organization of mitochondrial (mt) genome is highly conserved in insects, as in most other bilateral animals [1,2]. With few exceptions, the mt genomes of insects consist of 13 protein-coding genes, two rRNA genes, 22 tRNA genes and a large non-coding region (also called the control region) on a single circular chromosome [1–3]. Arrangement of genes in mt genomes is usually stable; most insects known retained exactly the ancestral pattern of mt gene arrangement or have minor changes from the ancestral pattern of mt gene arrangement [1,4–8].

One group of insects that stands out and has major changes in the organization of mt genome is the superorder Psocodea. Psocodea has ~10,000 described species in two orders: Pscoptera (barklice and booklice) and Phthiraptera (parasitic lice) [9–12]. Complete mt genomes have been sequenced for two species of the Pscoptera and six species of the Phthiraptera [13–20]. Compared to other insects, species of the Psocodea have three unusual features in their mt genomes that have not been found in any other insects. First, all of the eight species that have been sequenced have rearranged mt genomes. The booklouse, *Liposcelis bostrychophila*, and the six species of parasitic lice have the most rearranged mt genomes seen in insects - they differ at nearly every gene boundary from the putative ancestor of insects. Second, the booklouse, *L. bostrychophila* (Pscoptera: suborder Troctomorpha) and the parasitic lice in the suborder Anoplura have multipartite mt genomes. The mt genome of the booklouse, *L. bostrychophila*, has two mt chromosomes; each chromosome is 7–9 kb and has 16 to 22 genes [20]. The mt genomes of the human body louse, *Pediculus humanus*, and the human head louse, *P. capitis*, have 20 minichromosomes; each minichromosome is 3–4 kb in size and contains one to three genes [14,16]. The mt genome of the human pubic louse, *Pthirus pubis*, has at least 14 minichromosomes; each minichromosome is 1.8–2.7 kb in size and contains one to five genes [16].
Table 1. Species of insects used in the phylogenetic analyses in the present study.

| Order/suborder | Family          | Species                          | Accession number | Reference |
|----------------|----------------|----------------------------------|------------------|-----------|
| Pscoptera      | Lepidopsocidae | lepidopsocid sp.                 | NC_004816        | [13]      |
| Pscomorpha     | Psocidae       | Psocerastis albimaculata         | JQ910989         | present study |
|                |                | Longivalvus hyalosilus           | JQ910986         | present study |
| Troctomorpha   | Liposcelidae   | Liposcelis bastrychophila        | JN645275, JN645276 | [20]      |
| Phthiraptera   | Philopteridae  | Bothriometopus macrocnemis       | NC_009983        | [18]      |
|                |                | Campanulotes bidentatus compar   | NC_007884        | [17]      |
|                |                | Ibitoceras bissignatus           | NC_015999        | [19]      |
|                | Boopidae       | Heterodoxus macropus             | NC_002651        | [15]      |
|                | Pediculidae    | Pediculus humanus                | EU219987−95, HM241895−8 | [16]      |
| Hemiptera      | Nabidae        | Alloearurhynchus baveri           | NC_016432        | [28]      |
|                | Pentatomidae   | Halyomorpha halys                | NC_013272        | [27]      |
| Neuroptera     | Chrysopidae    | Chrysoperla nipponensis          | NC_015093        | [29]      |
| Coleoptera     | Carabidae      | Calosoma sp.                     | NC_018339        | [30]      |

Figure 1. Mitochondrial genomes of the barklice, *Psocerastis albimaculata* and *Longivalvus hyalosilus*. Circular maps were drawn with CGView [42]. Arrows indicate the orientation of gene transcription. Protein-coding genes are shown as blue arrows, rRNA genes as purple arrows, tRNA genes as brown arrows and large non-coding regions (>100 bp) as grey 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, *rml* and *rms* for large and small rRNA subunits. tRNA genes are indicated with their one-letter corresponding amino acids; the two tRNA genes for leucine and serine have different anticodons: *L1* (anticodon 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.

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Table 2. Codon usage of the protein-coding genes in the mitochondrial genomes of Psococerastis albimaculata and Longivalvus hyalospilus.

| Codon                   | P. albimaculata count | P. albimaculata % | L. hyalospilus count | L. hyalospilus % |
|-------------------------|-----------------------|-------------------|----------------------|------------------|
| A+T-rich codons         | 1482                  | 40.0              | 1509                 | 41.0             |
| G+C-rich codons         | 473                   | 12.8              | 480                  | 13.0             |
| Codon ratio             | 0.13                  |                   | 0.13                 |                  |

* A+T-rich codons are those encoding amino acids Asn, lle, Lys, Met, Phe, and Tyr.
* G+C-rich codons are those encoding amino acids Ala, Arg, Gly, and Pro.

Third, in the screamer louse, Bothriometopus macrocnemis (suborder Ischnocera), all mt genes are encoded by the same strand [18]. In the pigeon louse, Campanulotes bidentatus compar (suborder Ischnocera), all mt genes except trnQ are on the same strand [17].

The order Psocoptera has three suborders: Troctomorpha, Trogiornomorpha and Psocomorpha. In addition to the booklouse, L. bostycrophila (suborder Troctomorpha), the complete mt genome of a barklouse, lepidopsocid sp. (suborder Trogiornomorpha), has also been sequenced. The mt genome of the lepidopsocid sp. is much less rearranged than those of the booklouse and the parasitic lice; nevertheless, eight genes including a protein-coding gene have rearranged [13]. Prior to the present study, nothing is known about the mt genomes for species in the suborder Psocomorpha, which is the largest suborder of the Psocoptera, containing 25 of the 39 extant families and about 5,000 described species of the Psocoptera [10,21,22]. To further understand mt gene rearrangements and changes in mt genome organization in the Psocidea, we sequenced the mt genomes of two barklice, Psococerastis albimaculata and Longivalvus hyalospilus, the first representatives from the suborder Psocomorpha. We found that these barklice have the least rearranged mt genomes seen in the Psocidea to date. We show that there are contrasting rates in mt gene rearrangement between the two major lineages of the Psocidea.

Materials and Methods

Ethics Statement

No specific permits were required for the insects collected for this study. The insect specimens were collected from roadside vegetation by sweeping. The field collections did not involve endangered or protected species. The species in the family of Psocidae are common insects and are not included in the “List of Protected Animals in China”.

Samples and DNA Extraction

Specimens of P. albimaculata and L. hyalospilus were collected in Kuankuoshui, Suiyang, Guizhou, China, in June 2010. Specimens were initially preserved in 95% ethanol in the field, and transferred to −20°C for long-term storage at the China Agricultural University (CAU). For each species, the genomic DNA was extracted from one male adult’s muscle tissues of the thorax using the DNeasy DNA Extraction kit (Qiagen).

PCR Amplification and Sequencing

The mt genome was amplified by PCR in overlapping fragments with universal insect mt primers [23], and species-specific primers designed from sequenced fragments (Table S1). Short PCRs (<1.5 kb) were with Taq DNA polymerase (Qiagen); the cycling conditions were: 5 min at 94°C, followed by 35 cycles of 50 s at 94°C, 50 s at 48–55°C, 1–2 min at 72°C depending on the size of amplicons, and a final elongation step at 72°C for 10 min. Long PCRs (>1.5 kb) were with Long Taq DNA polymerase (New England BioLabs); the cycling conditions were: 30 s at 95°C, followed by 40 cycles of 10 s at 95°C, 50 s at 48–55°C, 3–6 min at 68°C depending on the size of amplicons, and a final elongation step at 68°C for 10 min. The concentration and size of PCR products were measured by spectrophotometry and agarose gel electrophoresis. PCR fragments were ligated into the pGEM-T Easy Vector (Promega); the resulting plasmid DNAs were isolated using the TIANprep Midi Plasmid Kit (Qiagen). All fragments were sequenced in both directions with an ABI 3730XL Genetic Analyzer, using the BigDye Terminator Sequencing Kit (Applied Biosystems) with two vector-specific primers and internal primers for primer walking.

Assembly, Annotation and Bioinformatics Analysis

Sequence reads from the mt genome of each barklouse species were assembled into contigs with Sequencher (Gene Codes). Protein-coding genes and tRNA genes were identified by BLAST searches in GenBank and then confirmed by alignment with homologous genes from other insects. tRNA genes were identified with tRNAscan-SE v.1.21 [24]. trnR and trnSI, which could not be identified by tRNAscan-SE, were determined by sequence similarity comparison with tRNA genes of other insects. The base composition, codon usage, and nucleotide substitution were analyzed with Mega 5.0 [25]. Secondary structures of stem-loop composition, codon usage, and nucleotide substitution were analyzed with Mega 5.0 [25]. Secondary structures of stem-loop composition, codon usage, and nucleotide substitution were analyzed with Mega 5.0 [25]. Secondary structures of stem-loop composition, codon usage, and nucleotide substitution were analyzed with Mega 5.0 [25]. Secondary structures of stem-loop composition, codon usage, and nucleotide substitution were analyzed with Mega 5.0 [25]. Secondary structures of stem-loop composition, codon usage, and nucleotide substitution were analyzed with Mega 5.0 [25].

Table 3. Nucleotide composition at each codon position of the protein-coding genes in the mitochondrial genomes of Psococerastis albimaculata and Longivalvus hyalospilus.

| Codon | AT % | GC % | AT-skew | GC-skew |
|-------|------|------|---------|---------|
| Pa    | Lh   | Pa   | Lh      | Pa      | Lh      |
| All   | 75.0 | 72.3 | 25.0    | 27.7    | −0.14   | −0.14   | −0.03   | −0.03   |
| 1st position | 69.8 | 68.2 | 30.2    | 31.8    | −0.09   | 0.005   | 0.20    | 0.16    |
| 2nd position | 68.3 | 68.0 | 31.7    | 32.0    | −0.39   | −0.40   | −0.11   | −0.13   |
| 3rd position | 87.0 | 80.6 | 13.0    | 19.4    | −0.05   | −0.03   | −0.35   | −0.25   |

Pa, P. albimaculata; Lh, L. hyalospilus; AT-skew = (A−T)/(A+T); GC-skew = (G−C)/(G+C).
Hyalospilus

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control region; B) sequences of the tandem-repeat units; C) predicated stem-loop structures.

Chrysoperla nipponensis
lacewing, Alloeorhynchus bakeri and doi:10.1371/journal.pone.0061685.g002
bonds. Green circle with red letter inside highlight variations of nucleotides in tRNA between two barklice.

Figure 3. The control region in the mitochondrial genome of the barklouse, Phthiraptera were included in our phylogenetic analyses (Table 1).

These species are: 1) three barklouse, Ps. albimaculata, L. hyalospilus and lepidopsocid sp. [13]; 2) a booklouse, L. bostrychophila [20]; 3) four chewing lice, Bothriometopus macroenemis [18], Campanulotes bidistatus cambar [17], Idiacoccus suisginnatus [19], and Heterodoxus macropus [15]; 4) the human body louse, Pediculus humanus [14]; and 5) the human pubic louse, Phthirus pubis [16]. Two true bugs, Alloorynchus bakeri and Halymorpha halys (Hemiptera) [27,28], the lacewing, Chrysoperla nipponensis (Neuroptera) [29], and the ground beetle, Calosoma sp. (Coleoptera) [30], were used as outgroups.

Sequences of all mt protein-coding genes and rRNA genes except nad4 were used in phylogenetic analyses; nad4 was excluded because it was not identified in the human pubic louse, P. pubis [16]. Segments of identical sequences (26–127 bp long) shared between five pairs of mt genes in the human body louse, P. humanus, and the human pubic louse, P. pubis [14,16], were also excluded to ensure only homologous regions of the mt genes were aligned and used in subsequent phylogenetic analyses. Alignment of the nucleotide sequences of each protein-coding gene and its putative amino acid sequence was with MUSCLE [31], adjusted to preserve the reading frame. Sequences of each tRNA gene were aligned with the GUIDANCE algorithm [32,33], adjusted to its RNA secondary structure [34]. The alignments of individual genes were concatenated after removing poorly aligned sites using Gblocks 0.91 [35].

Phylogenetic Analysis

Three alignments were used for phylogenetic analyses: 1) a concatenated nucleotide sequence alignment of protein-coding genes and two rRNA genes (PCG123R); 2) a concatenated nucleotide sequence alignment of the first and the second codon positions of protein-coding genes and two rRNA genes (PCG12R); and 3) a concatenated amino acid sequence alignment of protein-coding genes (AA). Partitioned ML and Bayesian analyses were run with PCG123R, PCG12R and AA matrix, using RAxML 7.0.3 [36] and MrBayes 3.2.1 [37]. The best-fit model for the amino acid sequence alignment was determined with ProtTest [38], and the jModelTest 0.1.1 [39] was used for the nucleotide sequence of each gene, according to the Akaike Information Criterion (AIC). For the ML analyses, GTRMIX option for nucleotide sequence and MrREV model for amino acid sequence were used to optimize the topology. For the combined dataset, 1,000 independent runs from random starting trees were performed to find the highest scoring replicate. Node support was calculated by acquiring bootstrap values from heuristic searches of 1000 resampled datasets, using the rapid bootstrap feature of RAxML [40].

For Bayesian analyses, the most appropriate substitution model was GTR+I+G model for each protein-coding gene, 1st and 2nd codon positions of protein-coding genes, and rrnL gene; GTR+G model for rrnS gene; and MrREV model for amino acid sequence of each protein-coding genes. Two simultaneous runs of 10 million generations were conducted for the matrix and trees were sampled every 1,000 generations, with the first 25% discarded as burn-in. Stationarity was considered to be reached when the average standard deviation of split frequencies was below 0.01 [41].

Results and Discussion

Mitochondrial Genomes of the Barklice, Psococerastis Albimaculata and Longivalvus Hyalospilus

The mt genome of P. albimaculata contains the entire set of 37 genes (13 protein-coding genes, 22 tRNA genes, and two rRNA genes; Figure 1 and Table S2) and a putative control region that are usually present in animal mt genomes [1,3]. We found the same set of mt genes in L. hyalospilus, except the control region and the adjacent trnM gene due to our unsuccess to amplify this region (Figure 1 and Table S3). These two barklice have the same arrangement of mt genes to each other but differs from the putative ancestor of insects: a protein-coding gene (nad3) and five tRNA genes (trnN, trnS1, trnE, trnM and trnC) have translocated. Genes are encoded by both strands in the mt genomes of these two barklice: 14 genes on one strand whereas the rest on the other strand (Table S2 and S3). Thirteen pairs of adjacent genes in the mt genomes of these two barklice overlap by 1 to 16 bp. All of the protein-coding genes of the two barklice start with ATN codons and stop with TAA/TAG codons or truncated codons TA or T.

The nucleotide compositions of the mt genomes of the two barklice are biased toward A and T. The nucleotide skew statistics for the entire majority strand indicate moderate A skew and obvious C skew, and the coding strand of protein-coding genes display a moderate T skew and slight C skew (Table 2). The A+T-

Figure 3. The control region in the mitochondrial genome of the barklouse, Psococerastis albimaculata. A) Structural elements in the control region; B) sequences of the tandem-repeat units; C) predicated stem-loop structures.
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richness of mt genomes of these two barklice is reflected further in the codon usage (Table S4). The overall ratio of G+C-rich codons to A+T-rich codons is 0.13 in the two barklice and there is a strong bias to A+T at the third codon positions of the protein-coding genes (Table 3).

The multiple alignment of 21 tRNA genes (excluded trnM) extends over 1,366 positions and contains 1,229 conserved nucleotides (90.0%) between the two barklice. Nucleotides at the stems and anticodon loops were conserved; variations were largely restricted to the loop of TΨC and DHU arm (Figure 2). The nucleotide sequences of the mt genomes of two barklice, *P. albimaculata* and *L. hyalospilus*, have been deposited in GenBank under accession numbers JQ910989 and JQ910986.

The Control Region in the Mitochondrial Genome of the Barklouse, *Psococerastis albimaculata*

The putative control region (911 bp) was flanked by *rrnS* and *trnM* in the mt genome of *P. albimaculata*. The control region is highly AT-rich (86.9%; majority strand) and can be divided into five parts (Figure 3A): 1) 59 bp leading sequence; 2) 180 bp tandem repeat sequences consisting of five 36 bp repeat units (Figure 3B); 3) 448 bp A+T-rich sequences (A+T 88.2%); 4) 180 bp tandem repeat sequences consisting of nine 20 bp repeat units (Figure 3B); and 5) 44 bp stem-loop structure (Figure 3C). Stem-loops are common in the control regions of animal mt genomes and may have roles in the initiation of gene transcription and DNA replication [43–47]. The pattern of two tandem repeated sequences with an A+T-rich sequence in between is also present in the control region of the other barklouse, lepidopsocid-RS (Trogiomorpha), but not in the booklouse, *L. bostrychophila* (Troctomorpha), nor in any parasitic lice.

Outside the control region, there are 146 bp non-coding sequences in 13 intergenic regions of *P. albimaculata*, and 178 bp non-coding sequences in 12 intergenic regions in *L. hyalospilus*. Most non-coding sequences are scattered in short runs (1–16 bp in *P. albimaculata* and 1–26 bp in *L. hyalospilus*). However, two of these non-coding sequences are longer in length and locate in the same intergenic regions in the two barklice: 1) between *trnQ* and *nad2* (29 bp in *P. albimaculata* and 38 bp in *L. hyalospilus*); and 2) between *nad5* and *nad3* (71 bp in *P. albimaculata* and 73 bp in *L. hyalospilus*). These two non-coding sequences are in the regions where gene rearrangement occurred and are likely generated from events of tandem duplication followed by random deletions (See below).

Phylogenetic Relationships among Major Lineages of the Psocodea Inferred from Mitochondrial Genome Sequences

The Psocoptera (booklice and barklice) and the Phthiraptera (parasitic lice) have traditionally been recognized as two separate orders [12,48,49]. The Psocoptera (booklouse and barklouse) are free-
lacking insects and consist of over 5,000 species with a world-wide distribution, and are divided into three suborders: Trogomorpha, Troctomorpha, and Psocomorpha [10,22]. Members of the order Phthiraptera (~4,900 species) are wingless insects, parasitic on birds and mammals. There are four recognized suborders in the Phthiraptera: Amblycera, Ischnocera, Anoplura, and Rhynchophthirina [9,11,50]. Both morphological and molecular analyses indicate a close relationship between parasitic lice (Phthiraptera) and booklouse (family Liposcelididae); the order Psocoptera is monophyletic with strong support; within the parasitic lice, the suborder Ischnocera, however, is paraphyletic.

**Contrasting Rates in Mitochondrial Gene Rearrangement between Two Major Lineages of the Psocodea**

Seven species from the Clade B above have been sequenced for complete mt genomes previously, i.e., the booklouse, *L. bostrychophila* (suborder Trogomorpha) [20] and six parasitic lice [14–19]. All of these seven species have extremely rearranged mt genomes, having little similarity in gene arrangement with each other, nor with any other insects. Both translocations and inversions occurred in these species relative to the ancestral gene arrangement of insects. Only four ancestral gene boundaries of insects (*trnM-nad3*, *trnl1-trnl*, *nad4-nad4L*, and *atp8-atp6*) were found in the Clade B species, of which only *atp8-atp6* was retained by all of the seven species in the Clade B. Apparently, mt gene rearrangement has been occurring much more often in the Clade B species than in other insects.

In contrast, species from the Clade A investigated to date have much less rearranged mt genomes and retained most of the ancestral gene arrangements of insects [13]. In particular, the two barklice we sequenced in the present study, *P. albimaculata* and *L. hyalospilus*, have the least rearranged mt genomes seen in the Psocodea: a protein-coding gene (*trnM*-*nad3*) and five tRNAs (*trnN*, *trnS1*, *trnE*, *trnM*, and *trnC*) have translocated relative to that of the ancestor of insects (Figure 5). Gene rearrangement in these two barklice occurred in the two “hot spot” regions for gene rearrangement [56]: 1) between *cox3* and *trnG*, and 2) between *CR* and *trnI* (Figure 5). Two events of tandem duplication followed by random deletions could account for, straightforwardly, the rearrangement of mt genes observed in *P. albimaculata* and *L. hyalospilus* (Figure 6). The number of mt genes that have rearranged in *P. albimaculata* and *L. hyalospilus* is even less than in the other barklouse, lepidopsocid-RS (*cox2* and seven tRNAs rearranged) [13]. Together, 20 gene boundaries (Figure 5, seven
gene blocks: A, B, D, E, F, G, and H) are shared and 32 ancestral gene boundaries are retained in these three barklice.

Tandem duplication followed by random deletion model has been proposed to account for mt gene rearrangement [8,57–59]. This model can explain the mt gene rearrangements observed in the two barklice, *P. albimaculata* and *L. hyalospilus* (Figure 6). The rearranged mt gene blocks of these two barklice, one from trnG to trnH and another from trnM to trnY can be generated by a single event of tandem duplication of the ancestral gene block from trnG to trnH (Figure 6A) and from trnI to trnY (Figure 6B), respectively, and followed by random deletion of excess genes. The non-coding sequences present in the two rearranged gene boundaries (between trnQ and nad2, and between nad5 and nad3) are likely traces of the random deletion. The mt gene rearrangement in the other barklouse lepidopsocid-RS (suborder Trogiomorpha) is more complicated than in *P. albimaculata* and *L. hyalospilus*; cox2 gene and four tRNA genes have translocated from long distance and cannot be accounted for alone by tandem duplication followed by random deletion model. The mt gene rearrangements in the booklice and parasitic lice are much more complicated than in the barklice; multiple mechanisms and frequent rearrangement events are likely involved [16,60,61].

What caused the substantial difference between the two clades of the Psocodea in the rates of mt gene rearrangement? An obvious difference between the two clades is the lifestyle. The barklice in the Clade A are entirely free-living insects, which often feed on fungal spores; whereas Clade B (booklice and parasitic lice) is a mixture of short-term commensal and parasitic. The booklouse, *L. bostrychophila*, is mainly an inhabitant of households and a major pest to stored grains world-wide; moreover, there are many records of various species of booklouse in the plumage of birds and the pelage of mammals, as well as in their nests [62,63]. This association is believed to be a short-term commensal (non-parasitic) relationship, which may have given rise to a parasitic and permanent association [64]. All parasitic lice (Phthiraptera) feed on the skin, skin debris or blood of their vertebrate hosts and spend their entire life cycle on the body of the host [12]. The lifestyle change in the Clade B appears to be associated with an increased rate of mt gene rearrangement. However, why they are linked and exactly what biological and lifestyle factors contributed to the contrasting rates in mt gene rearrangement between the two major
Table S3 Genes in the mitochondrial genome of the barklouse, Psococerastis albimaculata.

Table S2 Genes in the mitochondrial genome of the barklouse, Longivalvus hyaloisplius.

Table S4 Codon usage in the protein-coding genes of the mitochondrial genomes of the barklouse, Psococerastis albimaculata and Longivalvus hyaloisplius.

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Author Contributions
Conceived and designed the experiments: HL WZC. Performed the experiments: HI FS. Analyzed the data: HI FS. Contributed reagents/materials/analysis tools: RS WZC. Wrote the paper: HL RS XGZ QQY ZHL WZC.
47. Li H, Gao JY, Liu HY, Liu H, Liang AP, et al. (2011) The architecture and complete sequence of mitochondrial genome of an assassin bug Agrochilus dohrii (Hemiptera: Reduviidae). Int J Biol Sci 7: 792–804.

48. Hemig W (1966) Phylogenetic systematics. Urbana: University of Illinois Press.

49. Jamieson BGM, Dalai R, Adelis BA (1999) Insects: Their spermatozoa and phylogeny. Enfield: Science Publisher.

50. Clay T (1970) The Amblycera (Phthiraptera: Insecta). Bull Br Mus (Nat Hist) Entomol 25: 73–90.

51. Yoshizawa K, Johnson KP (2003) Phylogenetic position of Phthiraptera (Insecta: Paraneoptera) and elevated rate of evolution in mitochondrial 12S and 16S rDNA. Mol Phylogenet Evol 29: 102–114.

52. Yoshizawa K, Johnson KP (2006) Morphology of male genitalia in lice and their relatives and phylogenetic implications. Syst Entomol 31: 350–361.

53. Yoshizawa K, Johnson KP (2010) How stable is the ‘Polyphyly of Lice’ hypothesis (Insecta: Psocodea)? A comparison of phylogenetic signal in multiple genes. Mol Phylogenet Evol 55: 939–951.

54. Murrell A, Barker SC (2005) Multiple origins of parasitism in lice: phylogenetic analysis of SSU rDNA indicates that the Phthiraptera and Psocoptera are not monophyletic. Parasitol Res 97: 274–280.

55. Yoshizawa K, Lienhard C (2010) In search of the sister group of the true lice: a systematic review of booklice and their relatives, with an updated checklist of Liposcelididae (Insecta: Psocodea). Arthropod Syst Phylo 68: 181–195.

56. Dowton M, Austin AD (1999) Evolutionary dynamics of a mitochondrial rearrangement ‘Hot Spot’ in the Hymenoptera. Mol Biol Evol 16: 298–309.

57. Moritz C, Brown WM (1986) Tandem duplication of D-loop and ribosomal RNA sequences in lizard mitochondrial DNA. Science 300: 1423–1427.

58. Boore JL (2000) The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of deuterostome animal. In: Sankoff D, Nadeau JH, editor. Comparative genomics. Dordrecht: Kluwer Academic Press. p.133–147.

59. Shao R, Barker SC, Mitani H, Takahashi M, Fukunaga M (2006) Molecular mechanisms for the variation of mitochondrial gene content and gene arrangement among chigger mites of the genus Leptotrombidium (Acari: Acariformes). J Mol Evol 63: 251–261.

60. Dowton M, Campbell NJH (2001) Intramitochondrial recombination – is it why some mitochondrial genes sleep around? Trends Ecol Evol 16: 269–271.

61. Krzywinski Y, Schwartz M, Brown TA, Ebralidse K, Kunz WS, et al. (2004) Recombination of human mitochondrial DNA. Science 304: 981.

62. Pearlman JV (1960) Some African Psocoptera found on rats. Entomologist 93: 246–250.

63. Mockford EL (1967) Some Psocoptera from the plumage of birds. Proc Entomol Soc Wash 69: 307–309.

64. Hopkins GHE (1949) The host associations of the lice of mammals. Proc Zool Soc Lond 119: 387–404.