Mitochondrial genome organization varies among different groups of the booklouse, *Liposcelis bostrychophila*

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**Abstract**

The booklouse, *Liposcelis bostrychophila* Badonnel is an important pest of stored products around the world (Nayak et al. 2014) and has two types of reproductive mode: parthenogenesis and sexual reproduction (Mockford et al. 2008). Its interception number at a number of entry points into China is increasing with the development of international trade. DNA barcode was used to identify different species of booklice. However, it cannot distinguish *L. bostrychophila* as different strains for this species differs greatly in *cox1* gene fragment sequences. The *cox1* gene belongs to the mitochondrial (mt) genome which included normally 13 protein coding genes, two ribosome genes and 22 transfer RNA genes. The sequence diversity in *cox1* gene of different strains of *L. bostrychophila* implied there may be divergence in mt genome sequences in intra-specific level (Yang et al. 2013). The mt genome of *L. bostrychophila* was reported to split into two minichromosomes (Wei et al. 2012). Every minichromosome accounted for a half the length and the gene number of regular mt genomes. However, the sexual *L. bostrychophila* collected outdoors was reported to have five or seven minichromosomes in their mitochondrial genomes which added the complexity of this species (Perlman et al. 2015; Yang et al. 2015). Subsequently, to explore further the mt genome variations in *L. bostrychophila*, we sequenced the mt genomes of six strains of asexual *L. bostrychophila* collected from different locations in China, Croatia and USA.

**Extended abstract**

The booklouse, *Liposcelis bostrychophila* Badonnel is an important pest of stored products around the world (Nayak et al. 2014) and has two types of reproductive mode: parthenogenesis and sexual reproduction (Mockford et al. 2008). Its interception number at a number of entry points into China is increasing with the development of international trade. DNA barcode was used to identify different species of booklice. However, it cannot distinguish *L. bostrychophila* as different strains for this species differs greatly in *cox1* gene fragment sequences. The *cox1* gene belongs to the mitochondrial (mt) genome which included normally 13 protein coding genes, two ribosome genes and 22 transfer RNA genes. The sequence diversity in *cox1* gene of different strains of *L. bostrychophila* implied there may be divergence in mt genome sequences in intra-specific level (Yang et al. 2013). The mt genome of *L. bostrychophila* was reported to split into two minichromosomes (Wei et al. 2012). Every minichromosome accounted for a half the length and the gene number of regular mt genomes. However, the sexual *L. bostrychophila* collected outdoors was reported to have five or seven minichromosomes in their mitochondrial genomes which added the complexity of this species (Perlman et al. 2015; Yang et al. 2015). Subsequently, to explore further the mt genome variations in *L. bostrychophila*, we sequenced the mt genomes of six strains of asexual *L. bostrychophila* collected from different locations in China, Croatia and USA.
To reconstruct the mitochondrial genomes of the six strains of *L. bostrychophila*, *cox1*, *rrnS* and *rrnL* gene fragments were chosen as “anchors” to get the mitochondrial genome sequences. We firstly sequenced the *cox1*, *rrnS* and *rrnL* gene fragments by using universal primer pairs (Folmer et al. 1994; Kambhampati et al. 1995). Then, Long PCR primers were designed to amplify the chromosomes where the gene fragments located. The prepared libraries were then sent to the BGI company for next generation sequencing by using an Illumina sequencer. The mt genomes of all six asexual strains of *L. bostrychophila* collected in China, Croatia and USA have two chromosomes (Figure 1). The six newly sequenced mt genomes could be divided into three groups based on their mt genome rearrangements and sequence similarities. Each group has a distinct mt genome organization and substantial sequence divergence (48.7-87.4%) from other groups. Furthermore, all published mt genomes in *Liposcelis* genus, including one published asexual strain in China (Wei et al. 2012) and two published sexual strains of *L. bostrychophila*, *L. entomophila* (Chen et al. 2014), *L. paeta*, *L. decolor* (Chen et al. 2014) and *L. scultilis* (Shi et al. 2016) together with data in this research were included in the phylogenetic analysis. After fundamental bioinformatic analysis and annotation, phylogeny of the genus *Liposcelis* was inferred by using MrBayes (Ronquist et al. 2003) and RAxML (Stamatakis et al. 2006) softwares with two concatenated datasets. Phylogenetic analysis of mt genome sequences divided nine strains of *L. bostrychophila* into four groups. The seven asexual strains of *L. bostrychophila* are more closely related to *L. paeta* and *L. scultilis*, than to the sexual strains of *L. bostrychophila*. The two sexual strains formed the monophyly.

**Figure 1.** The mitochondrial genome organizations of three groups of *L. bostrychophila*. The transcriptional direction is indicated with arrows. Coding genes are shown in grey, non-coding regions in black, the identical region between the two chromosomes in white. Abbreviations of gene names are: cox1–3 for cytochrome oxidase subunits 1–3, cob for cytochrome b, nad1–6 and nad4L for NADH dehydrogenase subunits 1–6 and 4L, rrmL and rmS for large and small rRNA subunits, atp6 and atp8 for ATP synthase subunits 6 and 8. tRNA genes are indicated with their one-letter corresponding amino acids.
Figure 2. Bayesian inference (BI) and Maximum likelihood (ML) phylogenetic trees inferred from mitochondrial genomes of booklice. Numbers above the branches show support for tree nodes from nucleotide sequences of the two datasets: Bayesian posterior probability of PCG12, ML bootstrap support values of PCG123, Bayesian posterior probability of PCG12, ML bootstrap support values of PCG123. Group 1 is in green, Group 2 in pink, Group 3 in brown, Group 4 in red, other species of booklice in blue, the outgroup in black.

Our results revealed highly divergent mt genomes in \textit{L. bostrychophila} and indicated that \textit{L. bostrychophila} is a cryptic species. Cryptic species is a common question in plant quarantine, mt genome sequencing and phylogenetic analysis maybe as one way to resolve it.

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References


