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

Shiqian Feng[1,a], Qianqian Yang[1,a], Hu Li[1], Fan Song[1], Václav Stejskal[3] , George P. Opit[4], Wanzhi Cai[1], Zhihong Li[1,*], Renfu Shao[2,*]

[1]Department of Entomology, College of Plant Protection, China Agricultural University, Beijing 100193, China
[2]GeneCology Research Centre, Centre for Animal Health Innovation, School of Science and Engineering, University of the Sunshine Coast, Maroochydore DC, Queensland 4556, Australia.
[3]Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic
[4]Department of Entomology and Plant Pathology, Oklahoma State University, Oklahoma 74078, USA.
[a]These authors contributed equally.
*Corresponding authors: Zhihong Li: lizh@cau.edu.cn, Renfu Shao: rshao@usc.edu.au
DOI 10.5073/jka.2018.463.250

Abstract

The booklouse, *Liposcelis bostrychophila* is an important stored pest worldwide. The mt genome of an asexual strain (Beibei, China) of the booklouse, *L. bostrychophila*, comprises two chromosomes; each chromosome contains approximate half of the 37 genes typically found in animals. The mt genomes of two sexual strains of *L. bostrychophila*, however, comprise five and seven chromosomes respectively; each chromosome contains one to six genes. To understand mt genome evolution in *L. bostrychophila*, we sequenced the mt genomes of six strains of asexual *L. bostrychophila* collected from different locations in China, Croatia and USA. The mt genomes of all of the six asexual strains of *L.* bostrychophila collected in China, Croatia and USA have two chromosomes. Phylogenetic analysis of mt genome sequences divided nine strains of *L. bostrychophila* into four groups. Each group has a distinct mt genome organization and substantial sequence divergence (48.7-87.4%) from other groups. Furthermore, the seven asexual strains of *L.* bostrychophila including the published Beibei strain are more closely related to two other species of booklice, *L. paeta* and *L. sculptilis*, than to the sexual strains of *L. bostrychophila*. Our results revealed highly divergent mt genomes in the booklouse, *L. bostrychophila*, and indicated that *L. bostrychophila* is a cryptic species.

Keywords: Mitochondrial genome, *Liposcelis bostrychophila*, intraspecific variation, cryptic species, evolution

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. sculptilis* (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. sculptilis*, than to the sexual strains of *L. bostrychophila*. The two sexual strains formed the monophyly.

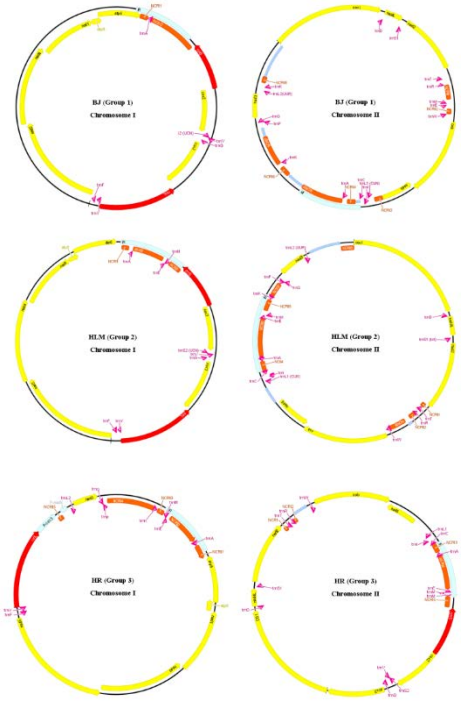

Figure 1. The mitochondrial genome organizations of three groups of *L. bostrychophila*. The transcriptional direction is indicated with arrows. Coding genes are shown in grey, non-coding regions in black, the identical region between the two chromosomes in white. Abbreviations of gene names are: cox1–3 for cytochrome oxidase subunits 1–3, cob for cytochrome b, nad1–6 and nad4L for NADH dehydrogenase subunits 1–6 and 4L, rrnL and rrnS 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.

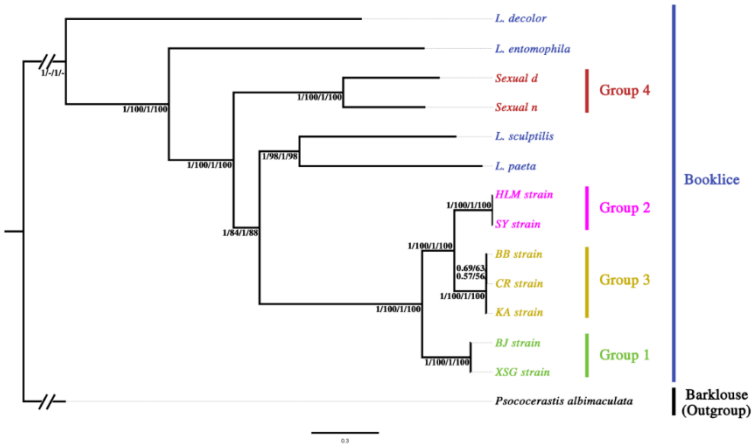

Figure 2. Bayesian inference (BI) and Maximum likehood (ML) phylogenetic trees inferred from mitochondrial genomes of booklice. Numbers above the branches show support for tree nodes from nucleotide sequences of the two datasets: Bayesian posterior probability of PCG12, ML bootstrap support values of 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 *L. bostrychophila* and indicated that *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.

Acknowledgements

We thank Charles Lienhard, Fasheng Li, Zuzana Kučerová for identifying the species of the *L. bostrychophila*. This work was supported by the National Natural Science Foundation of China (Nos. 31372230, 31420103902, 31401991), the Beijing Natural Science Foundation (No. 6144027).

Note: All related content in this research was published online on January 19, 2018 with the title "The highly divergent mitochondrial genomes indicate that the booklouse, *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) is a cryptic species" on *G3-Genes Genomes Genetics*, 2018, 8(3): 1039-1047.

References

CHEN, S., WEI, D., SHAO, R., DOU, W. AND J. WANG, 2014: The Complete Mitochondrial Genome of the Booklouse, *Liposcelis decolor*: Insights into Gene Arrangement and Genome Organization within the Genus *Liposcelis*. PLoS one **9**, e91902.

CHEN, S., WEI, D., SHAO, R., SHI, J., DOU, W. AND J. WANG, 2014: Evolution of multipartite mitochondrial genomes in the booklice of the genus *Liposcelis* (Psocoptera). BMC Genomics **15**, 861.

FENG, S., YANG, Q., LI, H., SONG, F., STEJSKAL, V., OPIT, G., CAI, W., LI, Z. AND R. SHAO, 2018: The highly divergent mitochondrial genomes indicate that the booklouse, *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) is a cryptic species. G3: Genes|Genomes|Genetics **8**, 1039-1047.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. AND R. VRIJENHOEK, 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3**, 294-299.

KAMBHAMPATI, S. AND P.T. SMITH, 1995: PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Molecular Biology **4**, 233-236.

MOCKFORD, E.L. AND P.D. KRUSHELNYCKY, 2008: New species and records of *Liposcelis Motschulsky* (Psocoptera: Liposcelididae) from Hawaii with first description of the male of Liposcelis bostrychophila Badonnel. Zootaxa, 53-68.

NAYAK, M.K., COLLINS, P.J., THRONE, J.E. AND J. WANG, 2014: Biology and Management of Psocids Infesting Stored Products. Annual Review of Entomology **59**, 279-297.

PERLMAN, S.J., HODSON, C.N., HAMILTON, P.T., OPIT, G.P. AND B.E. GOWEN, 2015: Maternal transmission, sex ratio distortion, and mitochondria. Proceedings of the National Academy of Sciences of the United States of America **112**, 10162-10168.

RONQUIST, F. AND J.P. HUELSENBECK, 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**, 1572-1574.

SHI, Y., CHU, Q., WEI, D., QIU, Y., SHANG, F., DOU, W. AND J. WANG, 2016: The mitochondrial genome of booklouse, *Liposcelis sculptilis* (Psocoptera: Liposcelididae) and the evolutionary timescale of *Liposcelis*. Scientific Reports **6**: 30660.

STAMATAKIS, A., 2006: RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22**, 2688-2690.

WEI, D., SHAO, R., YUAN, M., DOU, W., BARKER, S.C. AND J. WANG, 2012: The Multipartite Mitochondrial Genome of Liposcelis bostrychophila: Insights into the Evolution of Mitochondrial Genomes in Bilateral Animals. PLoS one **7**: e33973.

YANG, Q., KUCEROVA, Z., PERLMAN, S.J., OPIT, G.P., MOCKFORD, E.L., BEHAR, A., ROBINSON, W.E., STEJSKAL, V., LI, Z. AND R. SHAO, 2015: Morphological and molecular characterization of a sexually reproducing colony of the booklouse *Liposcelis bostrychophila* (Psocodea: Liposcelididae) found in Arizona. Scientific Reports **5**, 10429.

YANG, Q., ZHAO, S., KUCEROVA, Z., STEJSKAL, V., OPIT, G., QIN, M., CAO, Y., LI, F. AND Z. LI, 2013: Validation of the 16S rDNA and COI DNA Barcoding Technique for Rapid Molecular Identification of Stored Product Psocids (Insecta: Psocodea: Liposcelididae). Journal of Economic Entomology **106**, 419-425.