Acarina 31(2): 283–288

THE COMPLETE MITOCHONDRIAL GENOME OF *NEOSEIULUS CALIFORNICUS* (MESOSTIGMATA, PHYTOSEIIDAE) AND CONTROL REGION POLYMORPHISM

L.A. Uroshlev[1], O.V. Vasilenko[2], Y.I. Meshkov[3] and B.V. Andrianov[1]*

[1] Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia
[2] All-Russian Collection of Microorganisms (VKM), G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences, Russia
[3] All-Russian Scientific Research Institute of Phytopathology, Moskovskaya Oblast, Odintsovsky Rayon, Bolshiye Vyazyomy, Russia
*corresponding author; e-mail: andrianovb@mail.ru

ABSTRACT. *Neoseiulus californicus* is widely used as an effective biocontrol agent of spider mites. In this study, the complete mitochondrial genome sequence of *N. californicus* was determined using Oxford Nanopore sequencing technology. The complete mitochondrial genome is 21,318 bp in length and contains 13 protein-coding genes, 2 ribosomal rRNA genes and 22 transfer RNA genes. Its AT content is 78.4%. All start and stop codons of the protein-coding genes are canonical, except for the missing stop codon for the cox3 gene. The control region was polymorphic in length between the sublines of *N. californicus* due to variable number of direct repeats. The mitogenome presented in this paper contributes to the study of the genetic structure of *N. californicus* biocontrol populations.

KEY WORDS: mites, predatory mites, mitochondrion, D-loop, phylogenetic analysis

DOI: 10.21684/0132-8077-2023-31-2-283-288

INTRODUCTION

Neoseiulus californicus McGregor, 1954 was recorded for the first time from California and described as *Typhlodromus californicus* McGregor, 1954. It inhabits regions with tropical and subtropical climate all over the world. *Neoseiulus californicus* is widely used as an effective biocontrol agent for the control of spider mites, especially against *Tetranychus urticae* Koch, 1836, *Tetranychus evansi* Baker and Pritchard, 1960, and to a lesser extent for the control of thrips, whiteflies and other minute pests inhabiting greenhouses and open fields (Sanchez *et al*. 2008; Akyazi and Liburd 2019).

Data on the comparative variability of the nucleotide sequences of mitochondrial genomes are actively used in the molecular genetic identification of biocontrol populations and the species of predatory mites of the family Phytoseiidae. *Metaseiulus occidentalis* Nesbitt, 1951 was the first phytoseiid mite with a characterized mitochondrial genome (Jeyaprakash and Hoy 2007). The unusually large mitochondrial genome of *M. occidentalis*, the presence of duplications of some genes and the absence of the ND3 and ND6 genes has stimulated interest in a comparative study of the mitochondrial genomes of phytoseiid mites. Long PCR and Sanger sequencing techniques were used to obtain mitochondria genome sequences from *Phytoseiulus persimilis* Athias-Henriot, 1957 (Dermauw *et al*. 2010) and *Euseius nicholsi* Ehara and Lee, 1971 (Xin *et al*. 2016). In these species, the mitochondrial gene composition was consistent with that expected for arthropod mitochondria. No duplications or deletions of mitochondrial genes were found in the mitochondrial genomes of *P. persimilis* and *E. nicholsi*.

In addition, a re-examination of the structure of the *M. occidentalis* mitochondrial genome did not confirm the absence of the ND3 gene (Dermauw *et al*. 2010). Unique gene order and extended intergenic spacers were observed in all mitochondrial genomes of these species. Illumina whole-genome sequencing technology was used to further investigate the variability of mitochondrial DNA in phytoseiid mites. The mitochondrial genomes of *Amblyseius tsugawai* Ehara, 1959, *Amblyseius swirskii* Athias-Henriot, 1962 and *Neoseiulus womersleyi* Schicha, 1975 have a standard set of mitochondrial genes, but differ in their order (Zhang *et al*. 2021). High variability in mitochondrial gene order within the family Phytoseiidae, and even within the genus *Amblyseius,* raises the question of the possible intraspecific mitochondrial gene order variability in the mites of the family Phytoseiidae. Recently, the mitogenome of *N. californicus* was obtained by Zhu, using Illu-

mina sequencing technology, and it is deposited in GenBank under accession numbers: NC_069213.1, ON262343.1. Due to the limitations of the Illumina sequencing technology, this mitogenome lacks the control region. The order of *N. californicus* mitochondrial genes is also species specific.

In the current paper we report two new complete mitochondrial genome sequences of two isofemale mite lines of *N. californicus* from the *N. californicus* biocontrol population that have been maintained at the Laboratory of Acarology and Entomology Collection (All-Russian Scientific Research Institute of Phytopatology) for several decades (Glinushkin *et al*. 2019). The mitochondrial genomes were obtained as a by-product of whole-genome sequencing of the mite genome using Oxford Nanopore sequencing technology. The previously known and two new *N. californicus* mitochondrial genomes have the same gene order and are very similar in nucleotide sequence but differ in the length of the control region due to different numbers of direct tandem repeats.

MATERIALS AND METHODS

Sample collection, DNA extraction and *N. californicus* genome sequencing

Mite lines were obtained from a single fertilized female taken from a mass culture of mites. The resulting lines were cultured on the prey mite *Tetranychus urticae*. Two lines of mites were selected for further study. The line called BioDefence was dominated by females. In the BioDefence2 line, the sex ratio was close to 1:1. Before the experiment, predatory mites were transferred to a culture flask with a bean leaf for moistening, but without prey mites, where they were starved for two days to remove DNA contamination from the prey mites. Adult mites: males and females were then collected in a lysis buffer. High molecular weight DNA for whole genome sequencing was isolated using the classical phenol-chloroform method. Most of the RNA was removed by treatment with RNase T1. The quality and purity of the isolated DNA was determined using a NanoDrop spectrophotometer (Thermo Scientific). DNA concentration was measured on a Qubit fluorometer (Invitrogen) using reagent kits (Qubit dsDNA HS Assay Kit, Invitrogen). Libraries for nanopore sequencing were prepared using an Oxford Nanopore kit SQK-LSK110, according to the manufacturer's instructions. 1 mkg of mite genomic DNA was taken at the start of the procedure. To obtain a library, the ends of the DNA molecules were repaired and then ligated with adapters. DNA repair and dA-terminal preparation was performed using a set of buffers and enzyme mixtures from the NEBNext® Companion Module for 5 minutes at room temperature, followed by incubation at 65 °C to inactivate the enzymes. Adapter ligation was performed according to the manufacturer's instructions (Ligation Sequencing Kit, SQK-LSK110 Oxford Nanopore). For final purification, DNA ligation products were purified using magnetic particles (SPRI-select, BeckmanCoulter, USA) in short fragment buffer (SFB). Loading solution (LS) was used to load the library (250–1,000 ng DNA) into the R9.4.1 cell. The resulting genomic libraries were sequenced on a 512 channel MinION nanopore sequencer (Oxford Nanopore Technologies). Sequencing was performed under the control of the MinKNOW v. 5.1.0 program without real-time basecalling. Reads that were longer than 200 bp were retained. Basecalling was performed on a server with a two GeForce RTX 3090 graphic cards and 12 Intel Xeon processors. Guppy v6.0.1+652ffd179 was used for basecalling, according to the super-neat sup model. After basecalling we got 3,507,265 reads for the BioDefence line and 2,853,344 reads for the BioDefence2 line. The complete genome sequence was then assembled from the reads using Canu v. 2.3 (Koren *et al*. 2017). As a result, we have obtained an assembly of the *N. californicus* genome from 419 contigs with a total length of 191,424,213 bp average coverage of 26.861 and N50 equal 13,147.

Mitochondrial genome assembly

The BLAST program was used to search for mitochondrial contigs. As a reference sequence for BLAST search, we used *N. californicus* mitochondrial genome NC_069213. The contig was recognized as mitochondrion if it had more than 90% correct alignment lengths in one BLAST hit. The resulting sequences were annotated using the MITOS program (Bernt *et al*. 2013). The mitochondrial genes were identified based on their structural features and by comparison with the mitochondrial genes of other Phytoseiidae species previously published in GenBank. The resulting mitochondrial genomes were deposited in GenBank under accession № OQ026345 for the BioDefence line and № OR195436 for the BioDefence2 line. Visualization of the circular mitochondrial genome was performed using the Chloroplot program (Zheng *et al*. 2020).

Bioinformatic analyses

For the phylogenetic analysis of the obtained mitochondrial genome, we used all available nucleotide sequences of the complete mitochondrial genomes of mites of the family Phytoseiidae deposited in GenBank. The maximum likelihood phylogeny of 6 species of the family Phytoseiidae based on the 13 concatenated nucleotide sequences of protein-coding genes (PCGs), using the GTR +G+I model, was performed using the MEGA 7.0 software with 10,000 bootstrap replicates (Kumar *et al*. 2018).

RESULTS AND DISCUSSION

Mitogenome organization

The complete mitochondrial genome of *N. californicus* BioDefence line is 21,318 bp in length. It has a strong AT bias (78.4%), which is in the range of typical mite mitochondrial genomes (Jeyaprakash and Hoy 2007). The mitochondial genome encodes 13 canonical protein-coding genes (PCGs), 2 ribosomal rRNA genes (rRNAs) and 22 transfer RNA genes (tRNAs). All 13 PCGs are initiated by ATN codons. Only the COIII gene has an incomplete stop codon. Large intergenic spacers are typical of the *N. californicus* mitochondrial genome. The longest one is between trnS and trnL genes and has the length of 4,846 bp. Another long intergenic spacer 1,400 bp is found between the trnD and trnP genes. The heavy strand (J-strand) contained 23 genes, including 6 PCGs, 2 rRNAs and 15 tRNAs. The remaining 14 genes are located on the light strand (N-strand), including 7 PCGs and 7 tRNAs (Fig. 1). The mitochondrial genomes of the BioDefence and BioDefence2 lines are almost identical, except for the intergenic spacer between trnS and trnL, which is larger in the BioDefence2 line, with the length of 6,645 bp.

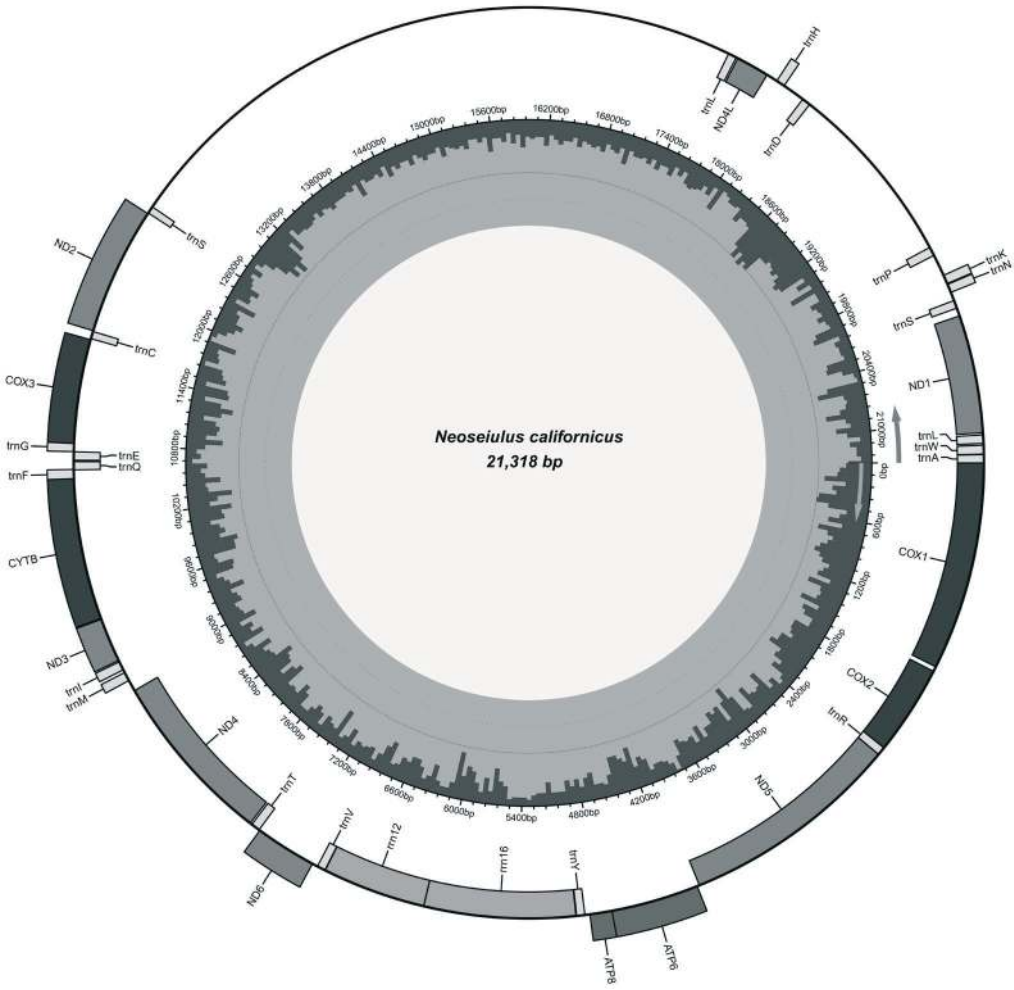

Fig. 1. The mitochondrial gene arrangements of the *N. californicus* BioDefence line. GenBank ID: OQ026345. Genes located on the J strand are shown inside the circle. Genes located on the N-strand are shown outside the circle. The control region is between trnS and trnL.

Phylogenetic analysis

At present, seven species of Phytoseiidae mites have known mitochondrial genomes: *M. occidentalis, A. tsugawai*, *A. swirskii*, *N. womersleyi*, *E. nicholsi*, *P. persimilis* and *N. californicus*. All these species have a unique mitochondrial gene order that differs from the canonical arthropod gene order (Zhang *et al*. 2021). To elucidate the phylogenetic relationships of these species, we constructed a concatenated data set of 13 mitochondrial PCGs (11,481 positions in the final data set), as well as a maximum likelihood tree. *Metaseiulus occidentalis* was not included due to duplicated genes in the mitochondrion of this species and the absence of the ND6 gene. The phylogenetic analysis showed that *N. californicus* is in the same clade as the *Amblyseius* species and in a different clade from *N. womersleyi* (Fig. 2).

Control region structure

Comparison of the mitochondrial nucleotide sequences of the BioDefence and BioDefence2 mite lines shows that the only difference is in the length of the large intergenic spacer between trnS and trnL. This region is rich of direct repeats that is typical of arthropod control regions or D-loops. The mitochondrial control region has only been mapped experimentally for a few arthropod species (Cameron *et al*. 2007) and there are no strict control region-specific markers. Typically, the mitochondrion has only one long non-coding region rich in poly(A/T) stretches, repeats and potential stem-loop structures (Garesse and Kaguni 2005; Saito *et al*. 2005). Rich in poly(A/T) stretches direct repeats are found in the longest non-coding region of the *N. californicus* mitochondrion (Fig. 3). The difference in the length of the putative control

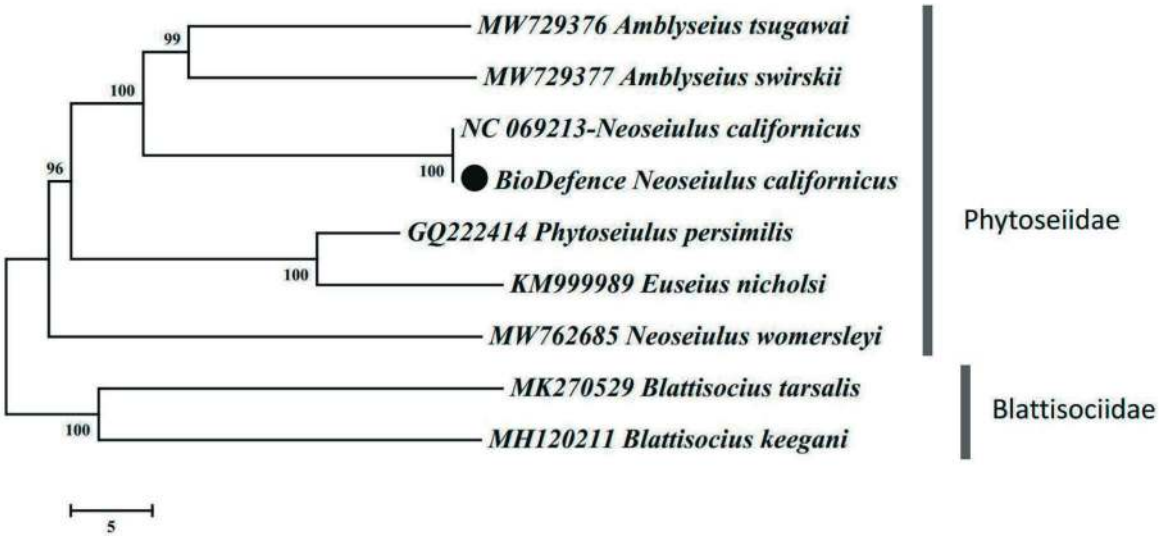

Fig. 2. A phylogenetic tree inferred from mitochondrial genome sequences using maximum likelihood method, based on 13 concatenated mitochondrial PCGs. The new *N. californicus* mitochondrial genome from the BioDefence mite line is denoted by a circle. The tree is drawn to scale with branch lengths in units of the number of base substitutions per site. Bootstrap support values are shown next to the branches (10,000 replicates). Two members of the family Blattisociidae, sister group of the Phytoseiidae, were used to root the phylogenetic tree.

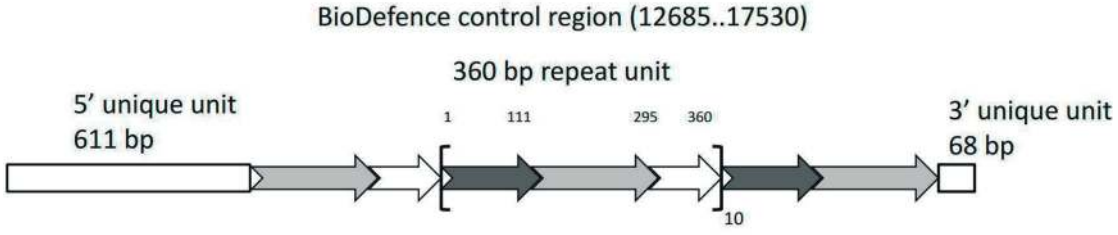

Fig. 3. The structure of the control region of the mitochondrial genome of *N. californicus* line BioDefence (GenBank ID: OQ026345). Numbers in brackets indicate the location of the control region in the mitochondrial genome. Numbers above the arrows indicate the boundaries of the structural regions of the 360 bp repeat unit. Square brackets indicate 10 repeats of the 360 bp repeat unit.

regions of the BioDefence and BioDefence2 mite lines is due to the number of direct repeats of 360 bp. The BioDefence mitochondrion has 10 repeats, whereas the BioDefence2 mitochondrion has 15 repeats.

CONCLUSION

Neoseiulus californicus is one of the most promising biocontrol agents for the control of spider mites and thrips. Obtaining genetically characterized lines of *N. californicus* is necessary to further expand its use as a biological control in greenhouse agriculture. We have sequenced the complete mitochondrial genomes of two lines of *N. californicus* using Oxford Nanopore sequencing technology. We have observed an interline polymorphism of the mitochondrial genomes of the two lines of *N. californicus* due to the length of the control region. This can be used as a line-specific DNA marker.

Data availability statement

The data that support the findings of this study are openly available in the GenBank of NCBI[1], reference Nos. OQ026345 and OR195436.

Disclosure statement

The authors report no conflicts of interest.

ACKNOWLEDGEMENTS

This work was financially supported by the Russian Science Foundation project № 22-24-00727.

REFERENCES

Akyazi, R. and Liburd, O.E. 2019. Biological Control of the Twospotted Spider Mite (Trombidiformes: Tetranychidae) with the Predatory Mite *Neoseiulus californicus* (Mesotigmata: Phytoseiidae) in Blackberries. *Florida Entomologist*, 102: 373–381. https://doi.org/10.1653/024.102.0217

Bernt, M., Donath, A. and Jühling, F. 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, 69: 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Cameron, S.L., Johnson, K.P. and Whiting, M.F. 2007. The mitochondrial genome of the screamer louse *Bothriometopus* (Phthiraptera: Ischnocera): effects of extensive gene rearrangements on the evolution of the genome. *Journal of Molecular Evolution*, 65: 589–604. https://doi.org/10.1007/s00239-007-9042-8

Dermauw, W., Vanholme, B., Tirry, L. and van Leeuwen, T. 2010. Mitochondrial genome analysis of the predatory mite *Phytoseiulus persimilis* and a revisit of the *Metaseiulus occidentalis* mitochondrial genome. *Genome*, 53: 285–301. https://doi.org/10.1139/G10-004

Emms, D.M. and Kelly, S. 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology*, 20: 238. https://doi.org/10.1186/s13059-019-1832-y

Garesse, R. and Kaguni, L.S. 2005. A drosophila model of mitochondrial DNA replication: proteins, genes and regulation. *IUBMB Life*, 57: 555–561. https://doi.org/10.1080/15216540500215572

Glinushkin, A.P., Yakovleva, I.N. and Meshkov, Y.I. 2019. The impact of pesticides used in greenhouses, on the predatory mite *Neoseiulus californicus* (Parasitiformes, Phytoseiidae). *Rossiiskaia Selskokhoziaistvennaia Nauka*, 3: 32–34. https://doi.org/10.31857/S2500-26272019332-34

Jeyaprakash, A. and Hoy, M.A. 2007. The mitochondrial genome of the predatory mite *Metaseiulus occidentalis* (Arthropoda: Chelicerata: Acari: Phytoseiidae) is unexpectedly large and contains several novel features. *Gene*, 391: 264–274. https://doi.org/10.1016/j.gene.2007.01.012

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H. and Phillippy, A.M. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Research*, 27: 722–736. https://doi.org/10.1101/gr.215087.116

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Manni, M., Berkeley, M.R., Seppey, M., Simao, F.A. and Zdobnov, E.M. 2021. BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. *Molecular Biology and Evolution*, 38: 4647–4654. https://doi.org/10.1093/molbev/msab199

McGregor, E.A. 1954. Two new mites in the genus *Typhlodromus* (Acarina: Phytoseiidae). *Bulletin of the Southern California Academy of Sciences*, 53: 89–92.

[1] https://www.ncbi.nlm.nih.gov

Saito, S., Tamura, K. and Aotsuka, T. 2005. Replication origin of mitochondrial DNA in insects. *Genetics*, 171: 1695–705. https://doi.org/10.1534/genetics.105.046243

Sanchez, N.E., Greco, N.M. and Cedola, C.V. 2008. Biological Control by *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). In: J.L. Capinera (Ed.). *Encyclopedia of Entomology*. Springer, Dordrecht, pp. 493–495. https://doi.org/10.1007/978-1-4020-6359-6_319

Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S. and Morgenstern, B. 2006. AUGUSTUS: *ab initio* prediction of alternative transcripts. *Nucleic Acids Research*, 34(suppl_2): W435–W439. https://doi.org/10.1093/nar/gkl200

Xin, T., Que, S., Zou, Z., Wang, J., Li, L. and Xia, B. 2016. Complete mitochondrial genome of *Euseius nicholsi* (Ehara et Lee) (Acari: Phytoseiidae). *Mitochondrial DNA*, 27: 2167–2168. https://doi.org/10.3109/19401736.2014.982609

Zhang, B., Havird, J.C., Wang, E., Lv, J. and Xu, X. 2021. Massive gene rearrangement in mitogenomes of phytoseiid mites. *International Journal of Biological Macromolecules*, 186: 33–39. https://doi.org/10.1016/j.ijbiomac.2021.07.011

Zheng, S., Poczai, P., Hyvönen, J., Tang, J. and Amiryousefi, A. 2020. Chloroplot: An Online Program for the Versatile Plotting of Organelle Genomes. *Frontiers in Genetics*, 11: 1–8. https://doi.org/10.3389/fgene.2020.576124