The complete mitochondrial genome of a marine triclad Miroplana shenzhensis (Platyhelminthes, Tricladida, Maricola)

Jia-Jie Huang, Yuan-Yuan Liao, Wei-Xuan Li, Jun-Yu Li, An-Tai Wang and Yu Zhang

ABSTRACT
The complete mitochondrial genome (mitogenome) of Miroplana shenzhensis Yu & Wang, 2013 is reported in the present study, representing the second mitogenome recorded in the suborder Maricola. The circular mitogenome is 14,344 bp in length, containing 12 protein-coding genes, 2 ribosomal RNAs and 22 transfer RNAs. Comparative analysis on mitochondrial gene order reveals a rearrangement in the suborder Maricola, indicating that mitochondrial gene order is conserved only in Continenticola, and is divergent across Tricladida. Phylogenetic analysis shows M. shenzhensis is clustered with another marine triclad, forming a well-supported monophyletic group of Maricolan.

MITOGENOME ANNOUNCEMENT
The complete mitochondrial genome of a marine triclad Miroplana shenzhensis (Platyhelminthes, Tricladida, Maricola)

Jia-Jie Huang, Yuan-Yuan Liao, Wei-Xuan Li, Jun-Yu Li, An-Tai Wang and Yu Zhang

ABSTRACT
The complete mitochondrial genome (mitogenome) of Miroplana shenzhensis Yu & Wang, 2013 is reported in the present study, representing the second mitogenome recorded in the suborder Maricola. The circular mitogenome is 14,344 bp in length, containing 12 protein-coding genes, 2 ribosomal RNAs and 22 transfer RNAs. Comparative analysis on mitochondrial gene order reveals a rearrangement in the suborder Maricola, indicating that mitochondrial gene order is conserved only in Continenticola, and is divergent across Tricladida. Phylogenetic analysis shows M. shenzhensis is clustered with another marine triclad, forming a well-supported monophyletic group of Maricolan.

CONTACT Yu Zhang biozy@szu.edu.cn
College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, Guangdong, P. R. China

ARTICLE HISTORY
Received 19 October 2021
Accepted 12 May 2022

KEYWORDS
Mitogenome; gene order; molecular phylogeny

Miroplana shenzhensis Yu & Wang, 2013 was classified into the genus Miroplana (Platyhelminthes, Tricladida, Maricola) according to its morphological features (Yu et al. 2013), while a more recent 18S and 28S rDNA based phylogenetic study demonstrated the phylogenetic position of the genus Miroplana (Li et al. 2019). Intriguingly, Miroplana exhibits good adaptation to both brackish water and freshwater habitats, which is similar to Sluysia triapertura (Souza et al. 2018) and species of the genus Paucumara (Li et al. 2021, Chen et al. 2019, Sluys et al. 2019) and Pentacoelum (Sluys et al. 2015), but stands out of the majority of suborder Maricola species. Therefore, it will be important to further resolve the phylogenetic position of M. shenzhensis among triclad species using molecular markers other than 18S and 28S rDNA. However, complete mitogenome is only available for one marine triclad, namely Obrimopisthia wandeli (Yang et al. 2019). In this study, we present the mitogenome of M. shenzhensis, representing the second mitogenome belonging to the suborder Maricola.

The specimen of M. shenzhensis was collected on 20 May, 2018 from mangrove wetlands of Shenzhen Waterfront Ecological Park, Guangdong, China (22°52.27’N, 114°00.34’E). The genomic DNA was extracted by Li et al (2019) and currently deposited at College of Life Sciences and Oceanography, Shenzhen University (Zhang Yu, biozy@szu.edu.cn) under the voucher number SW001. We used the REPLi-g Midi Kit (QIAGEN, Hilden, Germany) to amplify the genomic DNA. Paired-end sequencing was conducted on the Illumina Hiseq 2500 platform (Novogene, Beijing, China). The mitogenome sequences were assembled using MitoFlex v0.2.9 (Li et al. 2021). MITOS web server was used for gene annotation (Bernt et al. 2013), while the start and stop codons and the functional regions of the genes were verified by BLASTN (Altschul et al. 1997) with the query sequences from the closely related species O. wandeli (Yang et al. 2019). Multiple sequences alignment (MSA) was performed using MACSE v2.03 (Ranwez et al. 2018). MSAs were subsequently trimmed using Gblocks v0.91b (Talavera and Castresana 2007). Substitution saturation test (Xia et al. 2003; Xia and Lemey 2009) for each protein-coding gene (PCG) was carried out in DAMBE6 (Xia 2017), while the third positions of all PCGs were excluded for downstream analysis due to nucleotide substitution saturation. Best-fit evolution model for each PCG was selected by PartitionFinder2 (Lanfear et al. 2017).

The phylogenetic trees were constructed by Maximum Likelihood (ML) and Bayesian Inference (BI) methods, respectively. For ML, standard bootstrap analysis with 10,000 replications was performed by IQ-TREE v2.1.2 (Chernomor et al. 2016; Minh et al. 2020). While for BI, MrBayes v3.2.6 (Ronquist et al. 2012) was applied with 5,000,000 generations, sampling every 5,000 generations.

The circular mitogenome of M. shenzhensis is 14,344 bp in length and contains 12 PCGs, two rRNAs and 22 tRNAs. The nucleotide base composition is 28.7% A, 10.8% C, 15.8% G, and 44.7% T, with a total A + T content of 73.4%. ATP8 gene, through automatic annotation, was noted as missing in the mitogenome of M. shenzhensis. rrnL is situated at 5′ upstream of rrnS in the mitogenome of M. shenzhensis and O. wandeli, which is opposite to the unique arrangement of ribosomal genes in the suborder Continenticola (Solà et al. 2015).
Unexpectedly, except a few gene groups, namely ND4-ND4L and ND3-ND2, which are conserved in their arrangement in mitogenome, the PCG arrangement of *M. shenzhensis* differs from those of other known triclad species. The current data show that the gene order is highly conserved across the suborder Continentica while being divergent not only within Maricola, but also between Continentica and Maricola. As such, our data provides a valuable addition to the previous perspective that gene order is strikingly conserved among Tricladida (cf. Ross et al. 2016).

The phylogeny was inferred from 12 concatenated PCG sequences of *M. shenzhensis* and 12 species of Tricladida, while two species of Polycladida were included as outgroups. The phylogenetic trees obtained from both BI and ML analysis share identical topologies, and being well-supported in most nodes (Figure 1). *M. shenzhensis* is more closely related to a marine triclad *O. wandeli* than other freshwater and terrestrial Continenticans, forming a monophyletic group consists of Maricolans with high supporting values.

In conclusion, our study provides baseline information for future research regarding the origin and evolution in the suborder Maricola, suggesting the possible existence of the divergence of mitochondrial gene order in Tricladida. To better understand the phylogenetic relationships among species of Tricladida, it will be important to expand the mitogenome analysis within the order.

**Acknowledgement**

We thank Ms. Xiao-Yu Song and Ms. Ying Yang for their constructive comments on this work.

**Ethical approval**

This study does not involve Endangered or protected species according to IUZN (2021). The approval of sample collection is not required according to the Animal Ethical and Welfare Committee of Shenzhen University (+86-755-86671906).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This study was supported by Special Funds for the Cultivation of Guangdong College Students’ Scientific and Technological Innovation (“Climbing Program” Special Funds; [grant no. pdjh2021b0429, pdjh2021b0430]; a China Undergraduate Training Program for Innovation and Entrepreneurship [grant no. S202010590038], as well as grants from Scientific and Technical Innovation Council of Shenzhen and Guangdong Natural Science Foundation [JCYJ20210324093412035 and 2020A1515011117].

**Author contributions**

YZ and ATW conceived the project. JJH, WXL and YZ designed the experiments. JJH, YYL and WXL collected the samples. JJH and YYL extracted the DNA. JJH, YYL and JYL performed the bioinformatics analysis. JJH and YZ drafted the paper. All of the authors have read and approved the paper and approved its submission.

**ORCID**

Jia-Jie Huang [http://orcid.org/0000-0001-9209-9754](http://orcid.org/0000-0001-9209-9754)
Yuan-Yuan Liao [http://orcid.org/0000-0003-4002-7294](http://orcid.org/0000-0003-4002-7294)
Wei-Xuan Li [http://orcid.org/0000-0001-8853-3589](http://orcid.org/0000-0001-8853-3589)
Yu Zhang [http://orcid.org/0000-0001-7378-6946](http://orcid.org/0000-0001-7378-6946)

**Data availability statement**

The genome sequence data that support the findings of this study are openly available in Genbank of NCBI at [https://www.ncbi.nih.gov](https://www.ncbi.nih.gov) under the accession no. MW703985. The associated BioProject, SRA, and BioSample number are PRJNA777831, SRR16770661, and SAMN22883523 respectively.
References

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25(17):927–302.

Bernt M, Donath A, Ju¨hling F, Externbrink F, Florentz C, Fritzsch G, Putz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylgenet Evol. 69(2):313–319.

Chen JJ, Li WX, Sluys R, Wu MQ, Wang L, Li SF, Wang AT. 2019. Two new species of marine flatworm from southern China facilitate determination of the phylogenetic position of the genus Nerpa Marcus, 1948 and the histochemical structure of the nervous system in the genus Paucumara Sluys, 1989 (Platyhelminthes. Triclaidida, Maricola). Zootaxa. 4568(1):zootaxa.4568.1.9–167.

Chernomor O, Haeseler AV, Minh BQ. 2016. Terrace aware data structure for phylogenetic inference from supermatrices. Syst Biol. 65(6):997–1008.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic Analyses. Mol Biol Evol. 34(3):772–773.

Li JY, Li WX, Wang AT, Zhang Y. 2021. MitoFlex: an efficient, high-performance toolkit for animal mitogenome assembly, annotation and visualization. Bioinformatics. 37(18):3001–3003.

Li M-Y, Ma X-Y, Li W-X, Yang Y, Sluys R, Chen J-J, Li S-F, Wang A-T. 2021. A new species of Pacificides from the Western Pacific Coast and the first fully freshwater species of the Maricolan planarian genus Paucumara (Platyhelminthes, Triclaidida, Maricola). Syst Biodivers. 19(5):488–506.

Li W-X, Sluys R, Vila-Farré M, Chen J-J, Yang Y, Li S-F, Wang A-T. 2019. A new continent in the geographic distribution of the genus oregoniplana (Platyhelminthes: Triclaidida: Maricola), its rediscovery in South Africa and its molecular phylogenetic position. Zool J Linn Soc. 187(1):92–110.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Haeseler A, Von Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 37(5):1530–1534.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61(3):539–542.

Ross E, Blair D, Guerrero-Hernandez C, Alvarado AS. 2016. Comparative and transcriptome analyses uncover key aspects of coding- and long noncoding RNAs in flatworm mitochondrial genomes. G3 (Bethesda). 6(5):1191–1200.

Sluys R. 1989. In: A. A. Balkema, editor. A monograph of the marine triclad. Rotterdam & Brookfield.

Sluys R, Vila-Farré M, Alvarez-Presas M, Riutort M, Kawakatsu M, Tulp AS. 2015. The diet and distribution of Pentacoelem kazukolinda (Kawakatsu & Mitchell, 1984), a Maricolan planarian with a freshwater ecology. Zool Scr. 44(1):72–91.

Souza S, Riutort M, Ferreira RL, Leal-Zanchet A. 2018. An integrative taxonomic approach reveals the first marine triclad (Platyhelminthes) trapped in a cave from a semiarid neotropical environment. Invert Systematics. 32(3):627–638.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 56(4):564–577.

Xia X. 2017. DAMBE6: new tools for microbial genomics, phylogenetics, and molecular evolution. J Hered. 108(4):431–437.

Xia X, Lemey P. 2009. Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme A, editors. The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing. Cambridge: University Press; p. 615–630.

Xia X, Zheng X, Salemi M, Chen L, Wang Y. 2003. An index of substitution saturation and its application. Mol Phylogenet Evol. 26(1):1–7.

Yang HM, Ji SJ, Min GS. 2019. The complete mitochondrial genome of the Antarctic marine triclad, Obrimoposthia wandeli (platyhelminthes, triclaidida, maricola). Mitochondrial DNA B Resour. 4(2):2515–2516.

Yu AQ, AT W, Lai XT. 2013. A first recorded family Uteriporidae with a new species of the genus Miraplanus (Platyhelminthes, Triclaidida) from China. Acta Zootaxonomica Sinica. 38(2):257–266.