



MITOGENOME ANNOUNCEMENT



The complete mitochondrial genome and phylogenetic analysis of *Lepidozona coreanica* (Reeve, 1847)

Donghui Sun^{a*}, Zhongyu Lin^{a*}, Xindong Teng^b, Li Xu^c, Lijia Qian^a, Xinyue Yu^a, Huafang Wu^a, Ziyi Wang^a, Liming Jin^d, Xiumei Liu^a, Lijun Wang^a , Jiangyong Qu^a, Xumin Wang^a and Zhikai Xing^a 

^aCollege of Life Science, Yantai University, Yantai, China; ^bQingdao International Travel Healthcare Center, Qingdao, China; ^cQingdao Dagang Customs, Qingdao, China; ^dKey Laboratory of Biotechnology and Bioresources Utilization, Dalian Minzu University, Dalian, China

ABSTRACT

In the present study, the complete mitochondrial genome of *Lepidozona coreanica* was sequenced and described. The complete mitogenome sequence of *L. coreanica* is 16,572 bp long and contains 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and two ribosomal RNA (rRNA) genes. The base composition was AT biased (70.1%). The 13 PCGs of *L. coreanica* and the other 15 species of Polyplacophora were used for phylogenetic analysis using maximum-likelihood methods. The results showed that *L. coreanica*, *Ischnochiton hakodadensis*, and *Chaetopleura apiculata* are sister groups of the three lineages.

ARTICLE HISTORY

Received 1 August 2022
Accepted 17 February 2023

KEYWORDS

Chiton; *Lepidozona coreanica*; mitochondrial genome; phylogenetic analysis

Introduction

Lepidozona coreanica (Reeve, 1847) (Polyplacophora, Chitonida, Ischnochitonidae) (Figure 1) is an oblong and oval-shaped marine mollusk averaging 22 mm in length and 15 mm in width, which is morphologically composed of a large muscular foot that allows it to adapt conform to irregular surfaces (Garcia-Ibanez et al. 2013; Owada 2018). In China, it is commonly found in the intertidal zone along the coast, particularly on the north coast; however, it is uncommon on the south coast. It has also been spotted in the Korean Peninsula and Japan (from Hokkaido to south of Kyushu) and plays an important role in maintaining community structure and dynamics on rocky shores (Xu et al. 2020). Most chitons are edible and have medicinal properties. In particular, *L. coreanica*, has been used as a research material for biomineralization and natural nanomaterials containing large amounts of magnetite in the form of nanoparticles (Kirschvink and Lowenstam 1979). The taxonomy of *L. coreanica* has not yet been clarified at the molecular biological level; thus, it has only provisionally been assigned to the Ischnochitonidae family (Higo 1999). Therefore, it is important to elucidate the taxonomy of *L. coreanica* based on the complete mitochondrial genome while considering the threat to population genetic diversity from coastal land reclamation and commercial exploitation.

Materials and methods

Samples used for sequencing were collected from Jingouzhai, Yantai, Shandong Province, China (37°31'52"N, 121°26'16"E). The samples were stored in 70% ethanol immediately after collection (Pu et al. 2017). A specimen has been deposited in the marine specimen room of Yantai University (<https://lsc.ytu.edu.cn/index.htm>, Jiangyong Qu, qjy@ytu.edu.cn) under the specimen number YTU-LSC-201900370015. The mitochondrial genome sequence of *L. coreanica* has been stored in GenBank under accession number NC_046935. Genomic DNA was extracted from animals using a mitochondrial DNA column extraction kit (Sequencing Grade, BioRab, Beijing, China), and quality control was performed on the purified DNA samples. The mitochondrial genome was sequenced using the Illumina NovaSeq 6000 sequencing platform (Shanghai BIOZERON Co., Ltd., Shanghai, China). Readings were filtered using Trimmomatic 0.39 (<http://www.usadellab.org>). The Q30 of the clean data was 94.3%, which could be used for subsequent compilation and analysis. The clean data were compiled using SPAdes v3.10.1 (<http://bioinf.spbau.ru/spades>). The MITOS software (<http://mitos.bioinf.uni-leipzig.de/index.py>) was used to identify protein-coding genes (PCGs), transfer RNA (tRNA), and ribosomal RNA (rRNA) genes in the mitochondrial genome. The mitochondrial genome was obtained using OrganellarGenomeDRAW (Greiner et al. 2019). The phylogenetic tree was constructed by maximum-likelihood (ML) in MEGA v11 (Tamura et al. 2021), using 13

CONTACT Zhikai Xing  xingzhk@ytu.edu.cn; Xumin Wang  wangxm@ytu.edu.cn; Jiangyong Qu  qjy@ytu.edu.cn; Lijun Wang  wanglijun@ytu.edu.cn
 College of Life Science, Yantai University, No. 30 Qingquan Road, Laishan District, Yantai, Shandong 264005, China

*Both authors contributed equally to this work.

 Supplemental data for this article is available online at <https://doi.org/10.1080/23802359.2023.2183723>.

© 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.



Figure 1. *L. coreanica* reference image.

PCGs from 17 mitogenomes (*L. coreanica*, other 15 species from Polyplacophora and one outgroup *Haliotis discus han-nai*). Bootstrap values are evaluated using the bootstrap method with 1000 repetitions.

Results

The complete mitochondrial genome of *L. coreanica* was 16,572 bp in length (GenBank accession number: NC_046935) and comprised 13 PCGs, 22 tRNA genes, and two rRNA genes (Figure 2). The overall base composition was A 36.1%, T 34.1%, G 14.5%, and C 15.4%, with a GC content of 29.9%. Similar to the mitogenomes of other Polyplacophora species, the GC content was almost 30% (Cui et al. 2019; Guo et al. 2019). Eight of the 13 PCGs started with ATG (*cox1*, *cox2*, *cox3*, *atp6*, *atp8*, *nad1*, *nad3*, and *nad5*), two genes started with ATT (*nad2* and *cytb*), two genes started with ATA (*nad4L*, and *nad6*), and one gene started with GTG (*nad4*).

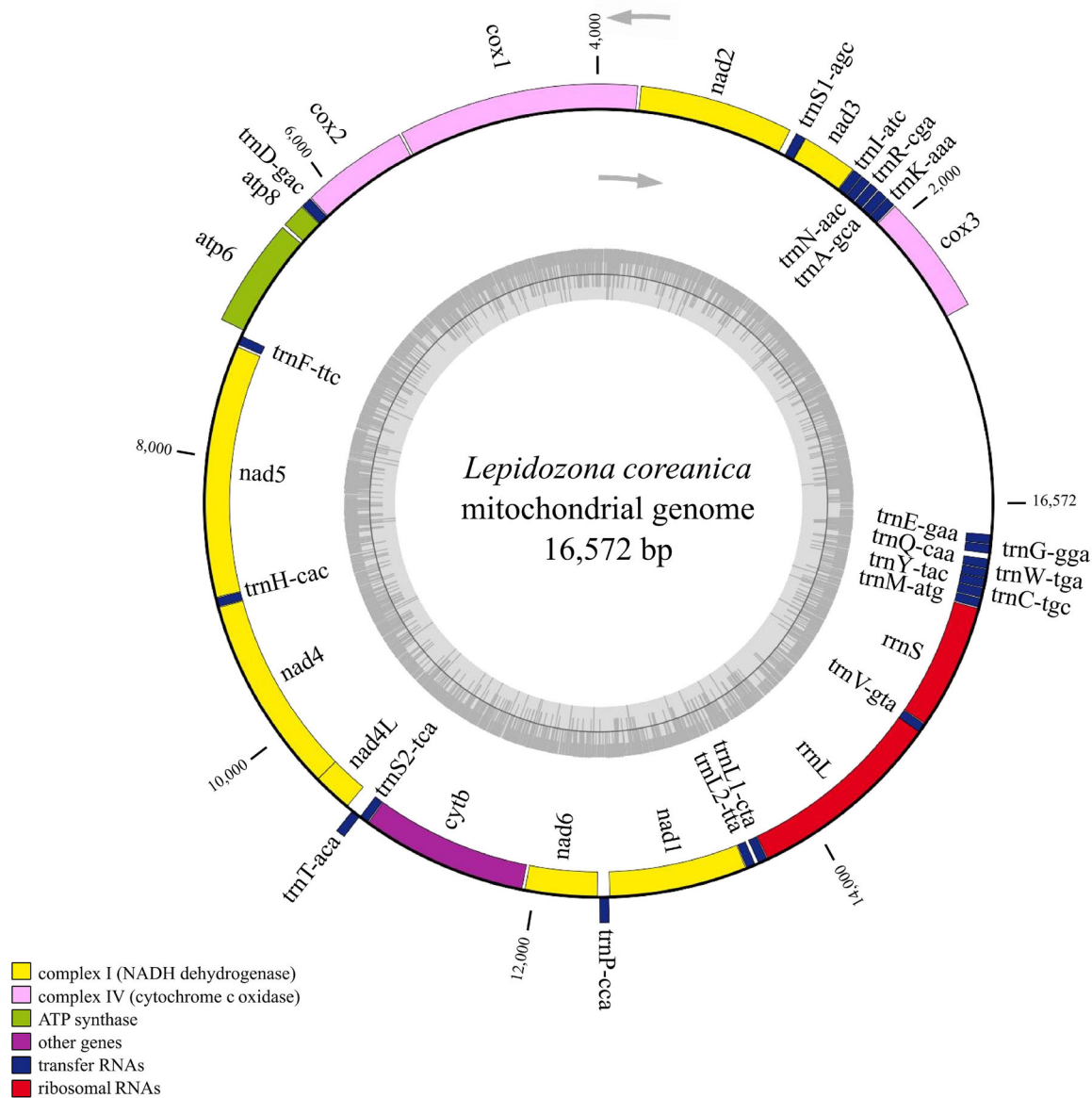


Figure 2. Gene maps of the mitochondrial genome of *L. coreanica*.

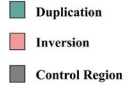


Figure 3. Mitochondrial gene orders of Polyplacophora main lineages: genes encoded in the major and minor strands are shown in the top and bottom lines, respectively. The rearrangement of genes is colored.



Figure 4. Phylogenetic position of *L. coreunica* based on 13 mitochondrial protein-coding genes. Sixteen ingroups (Polyplacophora) and one outgroup. Numbers next to each node indicate bootstrap support values. The content in parenthesis after the species name is the GenBank accession number. The following sequences were used: NC_047421 (Irisarri et al. 2020), NC_047422 (Irisarri et al. 2020), NC_047423 (Irisarri et al. 2020), KY824658 (Guerra et al. 2018), KY827038 (Cui et al. 2019), NC_047425 (Irisarri et al. 2020), NC_024173 (Veale et al. 2016), NC_024174 (Veale et al. 2016), KY827039 (Guo et al. 2019), NC_047426 (Irisarri et al. 2020), NC_001636 (Boore and Brown 1994), NC_026850 (Irisarri et al. 2020), NC_047424 (Irisarri et al. 2020), NC_026849 (Irisarri et al. 2020), NC_026848 (Irisarri et al. 2020), and KU310896 (Pu et al. 2020).

Seven of the 13 PCGs had stop codons TAA (*nad1*, *nad2*, *nad3*, *atp6*, *cox1*, *cox3*, and *cytb*), five had TAG (*cox2*, *nad4L*, *nad5*, *nad6*, and *atp8*), and *nad4* ended with an incomplete stop codon T. Two rRNA genes, *12S rRNA* and *16S rRNA*, were isolated by *tRNA-Val* (GTA). The 22 tRNA genes were between 64 bp and 71 bp in length.

Discussion and conclusions

These mitochondrial genomes share the same gene order in terms of the relative position of PCGs; however, some tRNAs are rearranged (Figure 3). The main strand encoded seven PCGs (*cox1*, *cox2*, *atp6*, *atp8*, *cox3*, *nad3*, and *nad2*) and 10

tRNAs (*trnD*, *trnK*, *trnT*, *trnP*, *trnK*, *trnA*, *trnR*, *trnN*, *trnI*, and *trnS1*). The remaining PCGs (*nad5*, *nad4*, *nad4L*, *cytb*, and *nad1*) and 12 tRNAs (*trnF*, *trnH*, *trnS1*, *trnP*, *trnL1*, *trnL2*, *trnV*, *trnM*, *trnY*, *trnC*, *trnW*, and *trnQ*) were located in the minus strand. The *trnF*, *trnS2*, *trnV*, *trnW*, and *trnT* tRNAs are arranged in either the minus strand or main strand. Both rRNA genes are transcribed from the minus strand, with the 16S rRNA being flanked by *trnL* and *trnV*; the 12S rRNA is located between *trnV* and *trnM*. The genome structure and gene composition were comparable to those of species of the Polyplacophora class, with the exception of Figure 3, indicating that the control region was relatively stable. We interrupted the non-coding control region between *trnE* and *cox3* in the circle mitochondrial genome and placed it in the middle of a determined linear sequence for BWA alignment, and calculated the coverage depth at each position (Additional Figure 1).

The results of the constructed phylogenetic tree showed with strong support that *L. coreanica* and *Chaetopleura apiculata* are sister groups (SH-aLRT support = 99%, Figure 4). It also showed with strong support that *I. hakodadensis* (Ischnochitonidae) was sister to *L. coreanica* (Ischnochitonidae) and *Chaetopleura apiculata* (Chaetopleuridae) (SH-aLRT support = 99%, Figure 4). Furthermore, the phylogenetic tree showed that Ischnochitonidae (Chitonida) was relatively distant from Nierstraszellidae (Lepidopleurida) and that the Ischnochitonidae (Chitonida), Chitonidae (Chitonida), and Chaetopleuridae (Chitonida) families were closer, which is consistent with other studies (Irisarri et al. 2020). These results provide new insights into the diversity of the Polyplacophora mitogenome and the evolution of chitons.

Acknowledgements

Geolocation information: The location of sample collection was Jingouzhai, Yantai City, Shandong Province, China (37.5311 N, 121.4378 E).

Author contributions

Conceived and designed the experiments: Lijun Wang, Jiangyong Qu, Xumin Wang, and Zhikai Xing. Performed the experiments: Donghui Sun and Zhongyu Lin. Analyzed the data: Donghui Sun, Xindong Teng, Li Xu, Lijia Qian, Xinyue Yu, Huafang Wu, Ziyi Wang, and Liming Jin. Contributed reagents/materials/analysis tools: Donghui Sun, Zhongyu Lin, and Xiumei Liu. Wrote the paper: Donghui Sun and Zhongyu Lin. Final approval of the version to be published: Lijun Wang, Jiangyong Qu, Xumin Wang, and Zhikai Xing. All authors agree to be accountable for all aspects of the work.

Ethical approval

All animal protocols have been reviewed and approved by the Experimental Animal Welfare and Ethics Review Committee of Yantai University.

Disclosure statement

The authors report there are no competing interests to declare.

Funding

This work was supported by the Key Funded with the MNR Key Laboratory of Eco-Environmental Science and Technology, China [MEEST-2021-05]; Open Fund of Key Laboratory of Biotechnology and Bioresources Utilization (Dalian Minzu University), Ministry of Education, China [No. KF2022006]; Research and Development Program of Shandong Province, China, (Major Science and Technology Innovation Project) [Grant No. 2021CXGC011306]; Natural Science Foundation Shandong Province [Grant No. ZR2020MD002]; National Key Research and Development Program of China [Grant No. 2021YFC2401005]; The National Natural Science Foundation of China. [Grant No. 31460562]; The Doctoral Science Research Foundation of Yantai University under [Grant Nos. SM15B01, SM19B70, and SM19B28]; Yantai "Double-Hundred Project" [2320004-SM20RC02].

ORCID

Lijun Wang  <http://orcid.org/0000-0002-2763-1211>
Zhikai Xing  <http://orcid.org/0000-0002-9722-606X>

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.nlm.nih.gov/> under the accession no. NC_046935. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA781747, SRR16979199, and SAMN21370456, respectively.

References

- Boore JL, Brown WM. 1994. Complete DNA sequence of the mitochondrial genome of the black chiton, *Katharina tunicata*. *Genetics*. 138(2): 423–443.
- Cui YT, Guo XY, Wang SS, Xu YR, Xiaoyue S, Li RR, Wang YH, Qu JY, Wang XM, Liu XM. 2019. The complete mitochondrial genome and phylogenetic analysis of *Ischnochiton hakodadensis* (Carpenter, 1893). *Mitochondrial DNA B Resour*. 4(2):2619–2621.
- Garcia-Ibanez S, Flores-Garza R, Flores-Rodriguez P, Violante-Gonzalez J, Valdes-Gonzalez A, Olea-de la Cruz FG. 2013. Fisheries diagnostic of *Chiton articulatus* (Mollusca: Polyplacophora) at Acapulco, Mexico. *Rev Biol Mar Oceanogr*. 48(2):293–302.
- Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res*. 47(1):59–64.
- Guerra D, Bouvet K, Breton S. 2018. Mitochondrial gene order evolution in Mollusca: inference of the ancestral state from the mtDNA of *Chaetopleura apiculata* (Polyplacophora, Chaetopleuridae). *Mol Phylogenet Evol*. 120:233–239.
- Guo XY, Cui YT, Wang SS, Xu YR, Sun XY, Li RR, Wang YH, Qu JY, Wang XM, Liu XM. 2019. The complete mitochondrial genome and phylogenetic analysis of *Acanthochiton rubrolineatus* (Lischke, 1873). *Mitochondrial DNA B Resour*. 4(2):2622–2624.
- Higo S, Callomon P, Goto Y. 1999. Catalogue and bibliography of the marine shell-bearing Mollusca of Japan. Osaka, Japan: Elle Scientific Publications, p. 749.
- Irisarri I, Uribe JE, Eernisse DJ, Zardoya R. 2020. A mitogenomic phylogeny of chitons (Mollusca: Polyplacophora). *BMC Evol Biol*. 20(1):22–36.
- Kirschvink JL, Lowenstam HA. 1979. Mineralization and magnetization of chiton teeth: paleomagnetic, sedimentologic, and biologic implications of organic magnetite. *Earth Planet Sci Lett*. 44(2):193–204.
- Owada M. 2018. Phylogenetic relationships among Japanese species of the genus *Ischnochiton* (Polyplacophora: Ischnochitonidae), including a new species. *Zoolog Sci*. 35(3):281–291.
- Pu DQ, Liu HL, Gong YY, Ji PC, Li YJ, Mou FS, Wei SJ. 2017. Mitochondrial genomes of the hoverflies *Episyrphus balteatus* and *Eupeodes corollae* (Diptera: Syrphidae), with a phylogenetic analysis of Muscomorpha. *Sci Rep*. 7:44300.

- Pu SW, Lu F, Huang B. 2020. The complete mitochondrial genome of *Haliotis ovina*. Mitochondrial DNA B. 5(3):2312–2313.
- Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 38(7):3022–3027.
- Veale AJ, Williams L, Tsai P, Thakur V, Lavery S. 2016. The complete mitochondrial genomes of two chiton species (*Sypharochiton pelliserpentis* and *Sypharochiton sinclairi*) obtained using Illumina next generation sequencing. Mitochondrial DNA A DNA Mapp Seq Anal. 27(1):537–538.
- Xu HW, Chu ZL, Zhang J, Jing MD, Huang L. 2020. Genetic diversity and population structure of *Acanthochiton rubrolineatus* (Polyplacophora) based on mitochondrial and nuclear gene markers. Diversity. 12(4): 159–172.