Complete mitochondrial genome of Coscinodiscus granii (Coscinodiscophyceae, Bacillariophyta)

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\section*{ABSTRACT}
Coscinodiscus is a genus common in marine phytoplankton, with some species thought to have a significant negative ecological impact. However, the availability of their genome sequences is rather limited. Here, we assembled and annotated the first complete mitochondrial genome (mtDNA) of the species Coscinodiscus granii L.F.Gough 1905, as part of our efforts to gain a better understanding of the genetic characteristics of Coscinodiscus taxa at a genomic level. The circular mtDNA was 34,970 bp in length and encoded 60 genes, including 32 protein-coding genes (PCGs), 24 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and two conserved open reading frames (orf's). The overall GC content of \textit{C. granii} mtDNA was 24.30\%, which was slightly lower than that of \textit{C. wailesii} (25.00\%), the first species in the genus Coscinodiscus whose mtDNA has been reported, and higher than that of \textit{Melosira undulata} (21.60\%), the first species in the class Coscinodiscophyceae whose mtDNA has been reported. As expected for congeneric species, phylogenetic analysis using concatenated amino acid sequences of 27 shared PCGs suggested that \textit{C. granii} has a closer evolutionary relationship with \textit{C. wailesii}. Coscinodiscus was found to be monophyletic in the phylogeny. The complete mtDNAs of more Coscinodiscus species will facilitate the exploration of the evolutionary relationships of species in the Class Coscinodiscophyceae.

Coscinodiscus is a species-rich genus among diatoms, with 174 taxonomically accepted species in AlgaeBase (Guiry and Guiry 2021), among which 49 species have been described in China (Chen et al. 2021; Chen and Chen 2021; Chen and Huang 2021; Chen and Zhang 2021). Some of these Coscinodiscus species have been found to form blooms that can cause serious damage to the aquaculture of macro rhodophyta (Nishikawa et al. 2010). Some Coscinodiscus species can be relatively large with valves up to 500 \(\mu\)m in diameter (Kühn and Raven 2008). The species Coscinodiscus granii L.F. Gough 1905 can be up to 300 \(\mu\)m in diameter (Boalch 1971; Yan 2017) and has a worldwide distribution. Coscinodiscus granii has been reported to form characteristic autumn blooms in the Baltic Sea and presented throughout the year in ‘low to moderate’ abundance in the North European Seas (Wasmund et al. 2003; Kraberg et al. 2010). Coscinodiscus granii poses an impact on the whole carbon pool and plays a role in the marine ecosystem, due to its relatively large cell sizes and high carbon content (Zhang et al. 2007).

The strain CNS00554 analyzed in this study was isolated in water samples collected during an expedition to the Jiaozhou Bay, China (36\(^{\circ}\)08.031'N, 120\(^{\circ}\)11.309'E) in August 2020 onboard the research vehicle ‘Innovation,’ using same sampling protocol as previously reported in Wang et al. (2021). The strain CNS00554 was annotated as \textit{C. granii} based on its morphological features (Hasle and Lange 1992; Goessler et al. 2016) (Figure 1(B,C)) and molecular characterization based on common molecular markers including full-length 18S rDNA (Damsté et al. 2004) and \textit{rbcL} (Theriot et al. 2010) (Figure S1). The \textit{C. granii} culture is maintained in the collection of marine algae in KLMEES of IOCAS (Nansheng Chen, chenn@qdio.ac.cn) under the voucher number CNS00554.

The total DNA of \textit{C. granii} was extracted by using DNasecure Plant Kit (Tiangen Biotech, Beijing, China). The DNA library was prepared by using the NEB Next\textsuperscript{TM} DNA Library Prep Kit for Illumina (NEB, USA). The PCR products were purified using AMPure XP system (Beckman Coulter, Beverly, USA), and libraries were analyzed for size

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Supplemental data for this article is available online at https://doi.org/10.1080/23802359.2021.1951135.
distribution by NGS3K/Caliper and quantified using real-time PCR (Qubit® 3.0 Flurometer, Invitrogen, USA). After qualification, the library was sequenced using a 2/C150 bp Illumina NovaSeq 6000 platform (Illumina, USA). Paired-end reads of C. granii were assembled using SPAdes (Bankevich et al. 2012). Scaffolds of target mtDNAs were selected from the assembly results using BLASTN (Altschul et al. 1990). The mtDNA sequence was examined using DOTTER (Sonnhammer and Durbin 1995) and validated using the MEM algorithm of BWA (Li and Durbin 2010). The annotation of PCGs, tRNA genes, rRNA genes, and orfs was conducted using Open Reading Frame Finder (ORF finder) with SmartBLAST and BLASTP, tRNAscan-SE (Chan and Lowe 2019) and MFannot.

The circular mtDNA of C. granii was 34,970 bp in size (GenBank accession number: MW435847). The mtDNA encoded a set of 60 genes, including 32 PCGs, two rRNA genes, 24 tRNA genes, and two orfs. Genes rps2, rps10, and rpl5 that are found in mtDNAs of many other diatom species (Wang et al. 2021) were absent from the mtDNA of C. granii.

The two orfs identified in C. granii shared no similarity with these three genes. The GC content of the mtDNA of C. granii was 24.30%, which was essentially the same as that of C. walesii (25.00%), and substantially higher than that of M. undulata (21.60%) (Pogoda et al. 2019). The 32 PCGs included atp6, 8, 9; cob; cox1, 2, 3; nad1-7, 4L, 9; rpl2, 13, 14, 19; rps2, 4, 8; and tatC from 34 publicly available diatom mitochondrial genomes. Two species of Ochrophyta, Sargassum fusiforme (KJ946428) and Sargassum muticum (KJ938301), were used as out-group taxa. The numbers beside branch nodes are the percentage of 1000 bootstrap replications.

To build the maximum likelihood (ML) phylogenetic tree (Figure 1(A)), all of the 27 shared PCGs from 34 publicly available diatom mtDNAs, were first individually aligned using MAFFT (Katoh and Standley 2013) and then trimmed using trimAL (Capella-Gutierrez et al. 2009) with default parameters: gt = 1, and all amino acid sequences were concatenated using Phyutility (Smith and Dunn 2008). The phylogenetic tree was constructed using IQ-TREE webserver with 1000 bootstrap replications (Trifinopoulos et al. 2016). Two species of Ochrophyta, Sargassum fusiforme (KJ946428) and Sargassum muticum (KJ938301), were used as out-group taxa. The phylogenetic tree showed that the mtDNAs of species in Bacillariophyceae formed a single clade. The mtDNAs of Mediophyceae and Coscinodiscophyceae each formed a paraphyletic assemblage (Figure 1(A)). Coscinodiscus granii (MW435847) was found sister to C. walesii (Huang et al. 2021) in a highly supported clade (Figure 1(A)). Comparative analysis of mtDNAs revealed many rearrangement events. While the order of genes in the mtDNAs of C. granii and C. walesii was generally similar, except for the rearrangements of a few gene blocks, including tmnPl(ugg)-tmnTrGl(gua)-rps11, extensive genome rearrangement events were found among mtDNAs of two Coscinodiscus species and that of M. undulata, including translocation and inversion events. These results suggested that mtDNAs of many more species of
the class Coscinodiscophyceae are needed for in-depth understanding of their evolutionary dynamics.

Acknowledgments

We are thankful to all staff members of the Marine Ecological Environment Genomics Research Group at the Institute of Oceanology, the Chinese Academy of Sciences. We are grateful to colleagues from the Jiaozhou Bay Marine Ecosystem Research Station for their help with field sampling.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was supported by the Strategic Priority Research Program of Chinese Academy of Sciences [Grant No. XDB42000000], the Chinese Academy of Sciences Pioneer Hundred Talents Program (to Nansheng Chen), the Taishan Scholar Project Special Fund (to Nansheng Chen), the Qingdao Innovation and Creation Plan (Talent Development Program – 5th Annual Pioneer and Innovator Leadership Award to Nansheng Chen, 19-3-2-16-2ch), the Key Research Program of Frontier Sciences, Chinese Academy of Sciences [No. QYZDB-SSW-DQC023] (to Feng Liu), and the Major Scientific and Technological Innovation Project of Shandong Province [No. 2019JZZY020706] (to Feng Liu), the Post-doctoral Program of Qingdao 2020 [Grant No. E0KY061] (to Hailong Huang), the Natural Science Foundation of Shandong Province, China [Grant No. ZR2020QD095] (to Hailong Huang).

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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 number MW435847. The associated BioProject, SRA and Bio-Sample numbers are PRJNA689860, SRR13363725 and SAMN17220988, respectively.

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