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Lee, Chanhee

Cooper, Joshua

Moroni, Francesca

et al.

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PLASTOME ANNOUNCEMENT

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Complete plastome of *Coelastrum microporum* Nägeli (Scenedesmaceae, Sphaeropleales)

Chanhee Lee[a], Joshua T. Cooper[b], Francesca Moroni[a], Ana M. Salim[a], Chaehee Lee[c], Trisha Spanbauer[d] and Edward C. Theriot[e]

[a]Plant Biology Graduate Program, University of Texas at Austin, Austin, Texas, USA; [b]Department of Biological Sciences, Northern Kentucky University, Highland Heights, Kentucky, USA; [c]Department of Plant Sciences, University of California Davis, Davis, California, USA; [d]Department of Environmental Sciences and Lake Erie Center, University of Toledo, Toledo, Ohio, USA; [e]Department of Integrative Biology, University of Texas at Austin, Austin, Texas, USA

ABSTRACT

The genus *Coelastrum* Nägeli (Sphaeropleales; Scenedesmaceae) is a diverse genus of green algae with potential biotechnical applications. A sound understanding of its phylogeny will be a useful tool for predicting the distribution of traits that may enhance its utility, and may lead to a better understanding of its evolution and ecology. Here we present the plastome of *Coelastrum microporum*. Our exemplar was isolated from Gull Lake, Michigan and the complete plastome as assembled was 169,961 bp in length. The plastome contained 104 genes of which 68 were protein-coding genes (CDSs), 27 tRNA genes and three rRNA genes. The GC content of the plastome was 31.2%. The maximum likelihood phylogeny suggested that *C. microporum* was the sister group to a clade of single exemplars of three other genera in the Scenedesmaceae (*Tetradesmus*, *Pectinodesmus* and *Coelastrella*).

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Coelastrum; plastome; Sphaeropleales; phylogeny

Introduction

Coelastrum microporum Nägeli, 1855 is a broadly distributed freshwater green alga with three-dimensional, spherical coenobia. This genus has recently been investigated for biotechnological applications such as bioremediation of wastewater, production of carotenoids, fatty acids, and biodiesel (Bhuyar et al. 2021; Liu et al. 2020; Maltsev et al. 2021). Understanding the plastome is known to be important to understanding synthesis and regulation of unsaturated fatty acids (He et al. 2020). Phylogenetic reconstructions have been attempted using marker genes such as ITS, and 18s rDNA regions (Goecke et al. 2020; Hegewald et al. 2010), with *Coelastrum* Nägeli itself recovered as monophyletic or not. The plastome of *C. microporum* has not been published previously. To help better understand evolutionary relationships of *Coelastrum*, we fully sequenced the plastome of *C. microporum* and compared it to available plastomes of other Sphaeropleales.

Materials and methods

Coelastrum microporum was collected from Gull Lake in southwest Michigan (42.403061 N; 85.414341 W) in July 2019. The isolated strain was cultured in WC artificial freshwater medium (Guillard 1975) at 14 °C (a temperature similar to lakes when samples were collected, which should be conducive for the maximum growth and survival rate of most species (Thomas et al. 2016)). The strain used is available from the UTEX Culture Collection of Algae (https://utex.org, Dr. David Nobles, Curator and Director: dnobles@austin.utexas.edu) as *Coelastrum microporum* UTEX LB 3178. Light microscopy using morphological criteria were used for initial species identification, with the isolate (Figure 1) corresponding to morphological descriptions of *C. microporum* (Komárek and Fott 1983).

Figure 1. Light Micrograph of *Coelastrum microporum*, taken by the authors, from the strain deposited as UTEX 3178.

CONTACT Edward C. Theriot etheriot@austin.utexas.edu Department of Integrative Biology, University of Texas at Austin, Austin, TX 78705, USA

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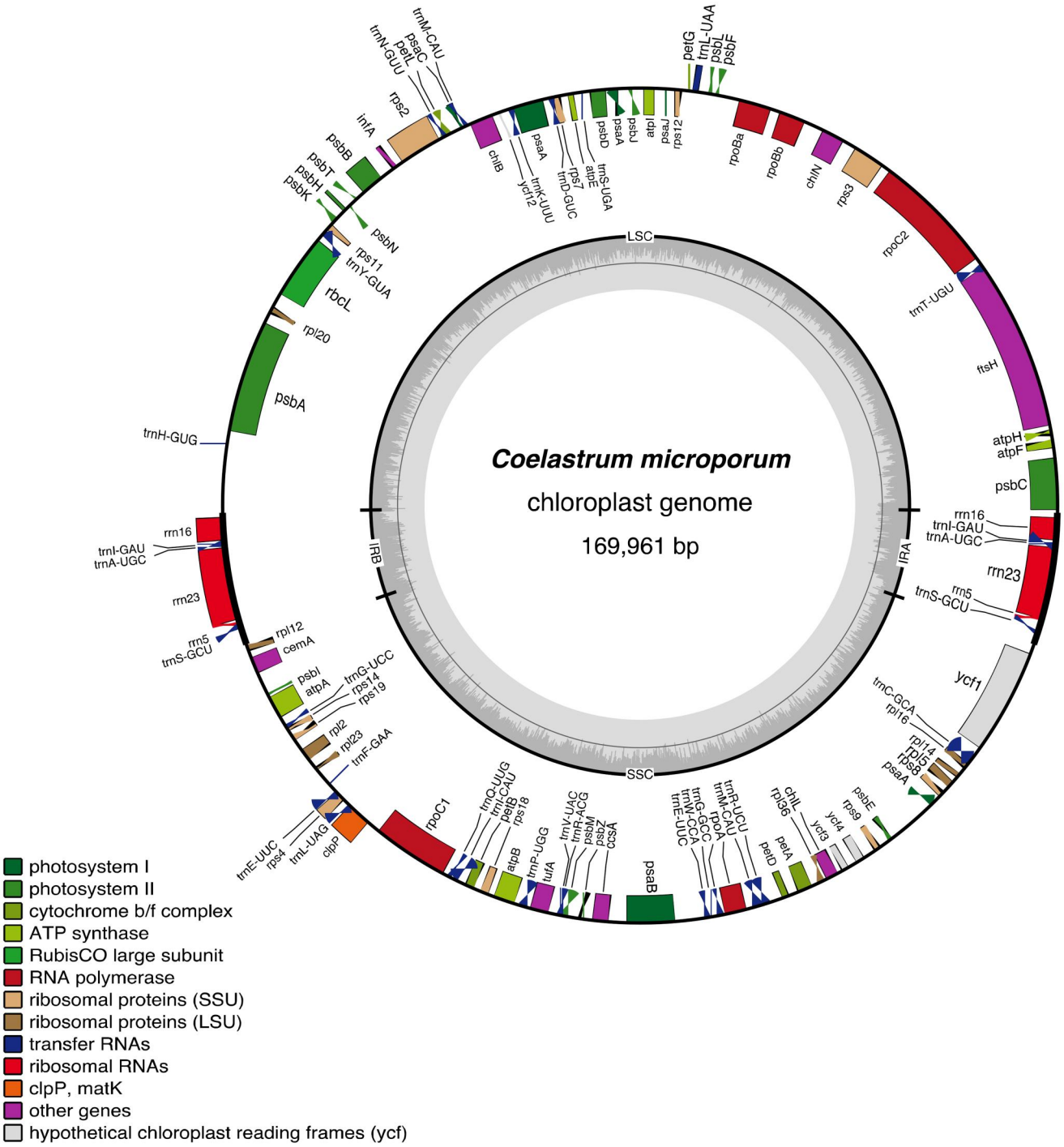

Figure 2. Gene map of *Coelastrum microporum* plastome. Inverted repeats (IRA and IRB) and two single-copy regions are indicated on the inner circle with G/C content (dark grey) and A/T content (light grey). colored gene boxes are indicated by functional group as shown in the key.

DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) following the kit's protocol. Monoclonal cultures were sampled in late exponential phase after visual inspection in the light microscope of the culture for fungal hyphae and bacterial abundance. Approximately 30 million 150 bp paired-end reads were sequenced using Illumina HiSeq 4000 platform (Illumina, San Diego, CA) at the Genome Sequencing and Analysis Facility (GSAF) at the University of Texas at Austin. The raw reads were trimmed using BBDuk from the BBTools software package (https://jgi.doe.gov/data-and-tools/bbtools/), and assembled with NOVOPlasty v. 4.2.1 (Dierckxsens et al. 2017). The assembled contig was imported into Geneious 2020.2.4 (Biomatters Ltd., http://www.geneious.com (Kearse et al. 2012)), and compared to vouchered sequences from the NCBI database using BLAST (Altschul et al. 1990) to check for any possible contaminants. The contig was annotated with available complete plastomes of closely related species in Geneious. The plastome was mapped using Bowtie2 v.2 (Langmead and Salzberg 2012) with trimmed reads to determine sequence coverage and potential mis-assemblies. Verification of protein-coding genes was manually performed in Geneious, and tRNA genes were verified using tRNAscan-SE

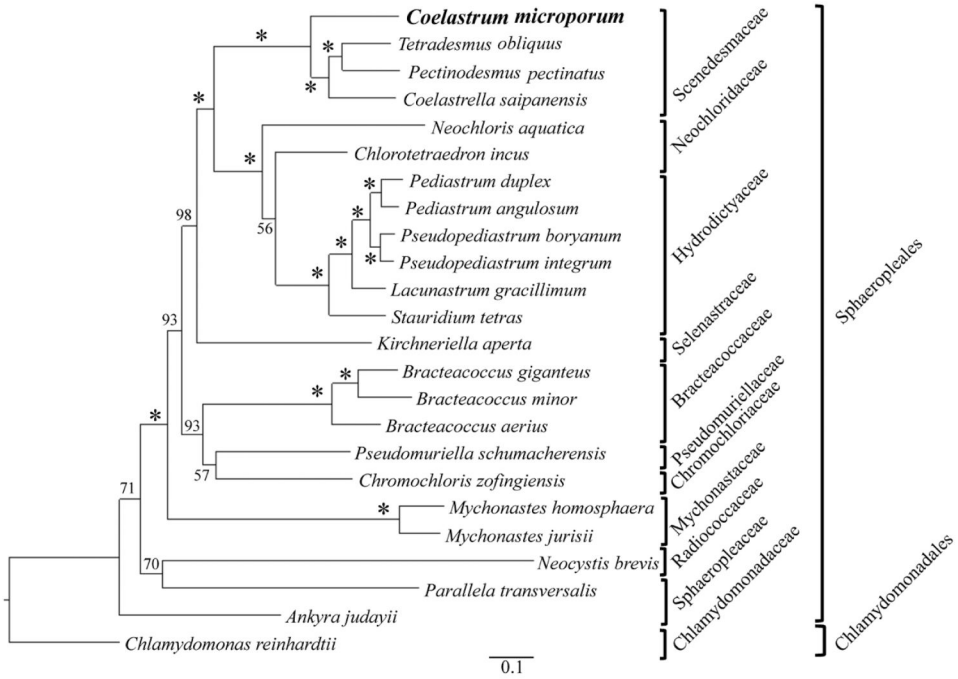

Figure 3. Maximum likelihood phylogeny of *Coelastrum microporum*, 22 species in Sphaeropleales and *Chlamydomonas reinhardtii* (outgroup) based on 58 CDSs shared by *C. microporum* and 23 publicly accessible plastomes in Sphaeropleales and chlamydomonadales. Numbers above branches are bootstrap values from 1000 bootstrap replicates in which asterisks represent bootstrap values of 100. The following sequences were used: *Tetradesmus obliquus* NC_008101 (de Cambiaire et al. 2006), *Pectinodesmus pectinatus* NC_036668 (unpublished), *Coelastrella saipanensis* NC_042181 (unpublished), *neochloris aquatica* NC_029670 (Fučíková et al. 2016), *chlorotetraedron incus* NC_029673 (Fučíková et al. 2016), *Pediastrum duplex* NC_034654 (McManus et al. 2017), *Pediastrum angulosum* NC_037919 (McManus et al. 2018), *Pseudopediastrum boryanum* NC_037920 (McManus et al. 2018), *Pseudopediastrum integrum* NC_037921 (McManus et al. 2018), *lacunastrum gracillimum* NC_037918 (McManus et al. 2018), *stauridium tetras* NC_037923 (McManus et al. 2018), *kirchneriella aperta* NC_029676 (Fučíková et al. 2016), *Bracteacoccus giganteus* NC_028586 (Lemieux et al. 2015), *Bracteacoccus minor* NC_029674 (Fučíková et al. 2016), *Bracteacoccus aerius* NC_029675 (Fučíková et al. 2016), *pseudomuriella schumacherensis* NC_029669 (Fučíková et al. 2016), *chromochloris zofingiensis* NC_029672 (Fučíková et al. 2016), *Mychonastes homosphaera* NC_029671 (Fučíková et al. 2016), *Mychonastes jurisii* NC_028579 (Lemieux et al. 2015), *neocystis brevis* NC_025535 (Lemieux et al. 2014), *parallela transversalis* NC_042241 (unpublished), *ankyra judayi* NC_029735 (Fučíková et al. 2016), *Chlamydomonas reinhardtii* NC_005353 (Maul et al. 2002).

v.2.0 (Chan and Lowe 2019). We extracted 58 CDS sequences shared by *C. microporum* and 22 publicly accessible plastomes from other Sphaeropleales, aligning them using MAFFT v.7.450 (Katoh and Standley 2013) presented in Table S1. ModelFinder (Kalyaanamoorthy et al. 2017) was used to find the best fitting model for maximum likelihood analysis. A maximum likelihood phylogeny was constructed using IQTree2 v. 1.6.12 (Minh et al. 2020) with 1000 bootstrap replicates.

Results

The complete plastome of *Coelastrum microporum* (GenBank accession number: NC_068582) was 169,961 bp in length. We annotated 104 genes which included 68 protein-coding genes (CDSs), 27 tRNA genes and three rRNA genes. The average coverage was 645.5 (ranging from 359 to 1337) mapped from 745,406 trimmed raw reads (Figure S1). The circularized plastome had a typical quadripartite construction (Figure 2), with a large single copy region (length 85,914 bp; GC content 30.2%), and a small single copy region (length 66,611 bp; GC content 29.2%), interrupted by the two inverted repeats, A and B (IRA and IRB, length of each 8,718 bp; GC content 43.7%). The overall plastome GC content was 31.2%. The best model for maximum likelihood analysis was GTR + F+R5. Our maximum likelihood tree (Figure 3) recovered *C. microporum* as sister to a clade containing *Pectinodesmus pectinatus* (Meyen) Hegewald et al. 2010, *Tetradesmus obliquus* (Turpin) M.J. Wynne, 2016, and *Coelastrella saipanensis* N. Hanagata, 2001.

Discussion and conclusions

Goecke et al. (2020), using ITS and 18s rDNA sequences, but much denser taxon sampling than our study, recovered similar relationships among Sphaeropleales genera, as did Hegewald et al. (2010), using only ITS2 sequences. We hesitate to argue for any one of these hypotheses of relationships without further analysis, because different taxon sampling strategies can change inferred phylogenetic relationships even when the same gene(s) and same optimality criterion are applied (Theriot et al. 2009). This description of the complete plastome of *C. microporum* provides additional support for its inclusion in the Sphaeropleales and valuable information for further research on phylogenetic relationships in this order, which in turn can help guide research on practical applications of these algae. We intend to expand our studies to better understand the evolution and classification of the genus *Coelastrum*.

Authors' contributions

Edward Theriot, Joshua Cooper, Chanhee Lee, and Trisha Spanbauer conceived and designed the experiments. Edward Theriot acquired and isolated the alga. Chanhee Lee, and Ana Salim grew the algae and

extracted DNA; Chanhee Lee, Ana Salim, Chaehee Lee, and Francesca Moroni made substantial contributions to the acquisition and analysis of data; Chanhee Lee and Edward Theriot contributed substantially to authorship; all authors contributed to revision of the manuscript, and all approved the final version to be published. All authors agree to be accountable for all aspects of the work.

Disclosure statement

The authors report that there is no conflict of interest involved.

Geolocation

Gull Lake, Kalamazoo County, Michigan. 42.403061 N; 85.414341 W.

Research statement

Phytoplankton sampling and cell isolation was done following all local, state, and US Federal regulations, in the publicly accessible Gull Lake. To our knowledge there is no convention on ethical treatment of microalgae.

Funding

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Data availability statement

The genome sequence data that supported the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. NC_068582. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA909268, PRJNA909268, and SAMN32062636, respectively. The strain used is available from the UTEX Culture Collection of Algae (https://utex.org, Dr. David Nobles, Curator and Director: dnobles@austin.utexas.edu) as *Coelastrum microporum* UTEX LB 3178.

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