

## MITOGENOME ANNOUNCEMENT

**Complete genome sequence of mitochondrial DNA (mtDNA) of *Chlorella sorokiniana***Massimiliano Orsini<sup>1</sup>, Cristina Costelli<sup>2</sup>, Veronica Malavasi<sup>2</sup>, Roberto Cusano<sup>1</sup>, Alessandro Concas<sup>1</sup>, Andrea Angius<sup>1,3</sup>, and Giacomo Cao<sup>1,2,4</sup><sup>1</sup>Center for Advanced Studies, Research and Development in Sardinia (CRS4), Loc Piscina Manna, Building 1, Pula (CA), Italy, <sup>2</sup>Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Cagliari (CA), Italy, <sup>3</sup>Institute of Genetic and Biomedical Research (IRGB), National Research Council (CNR), Monserrato (CA), Italy, and <sup>4</sup>Department of Mechanical, Chemical and Materials Engineering, Cagliari (CA), Italy**Abstract**

The complete sequence of mitochondrial genome of the *Chlorella sorokiniana* strain (SAG 111-8k) is presented in this work. Within the *Chlorella* genus, it represents the second species with a complete sequenced and annotated mitochondrial genome (GenBank accession no. KM241869). The genome consists of circular chromosomes of 52,528 bp and encodes a total of 31 protein coding genes, 3 rRNAs and 26 tRNAs. The overall AT contents of the *C. sorokiniana* mtDNA is 70.89%, while the coding sequence is of 97.4%.

**Keywords**Biofuels, *Chlorella sorokiniana*, DNA sequencing, mitochondrial genome**History**Received 5 August 2014  
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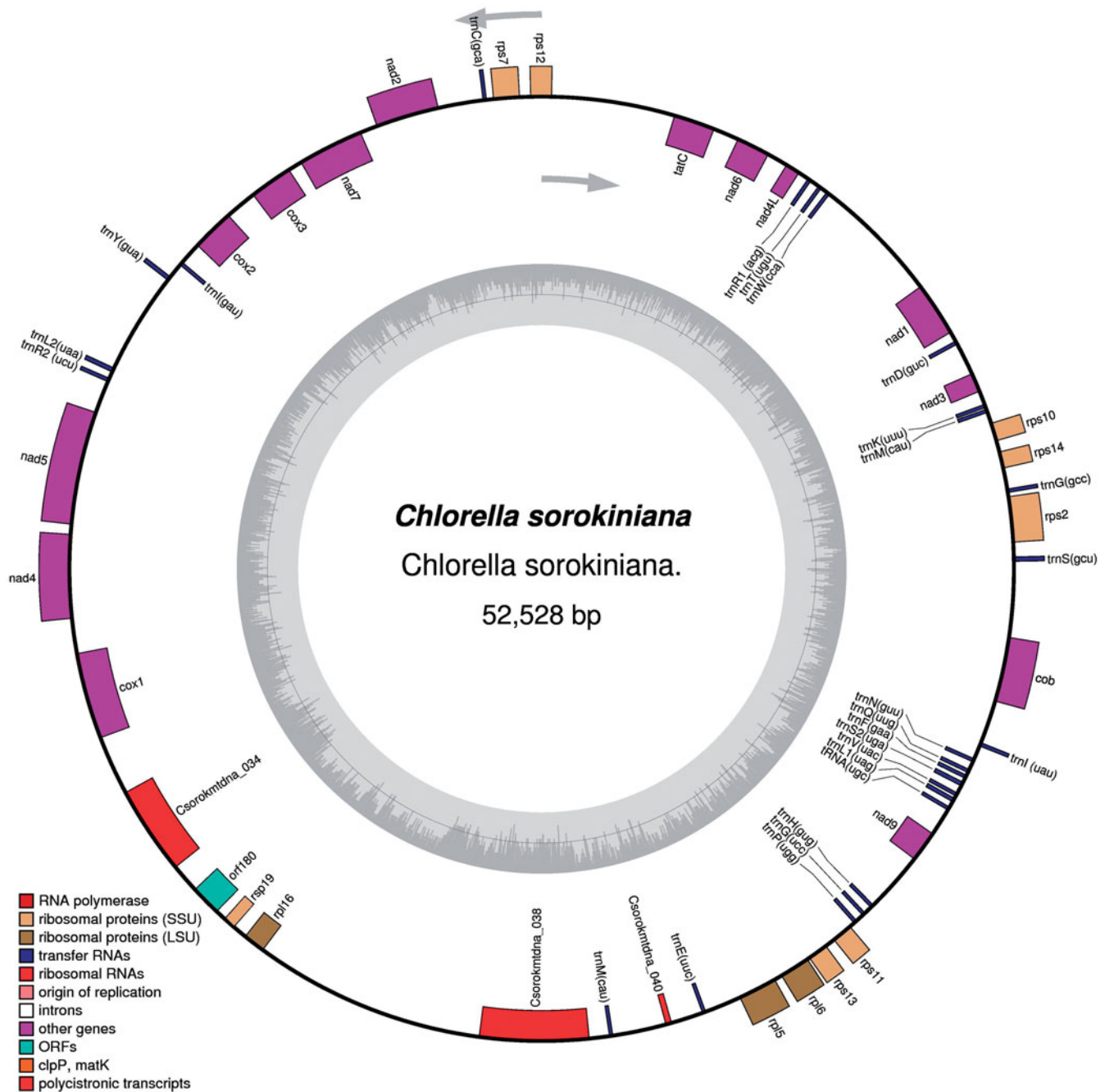
Mitochondria and plastids contain their own distinct genome DNA and their origin is due to the acquisition of an endosymbiont  $\alpha$ -proteobacteria. The evolutionary coherence of land plants and green algae is apparent at the levels of both the nuclear and chloroplast genomes. However, at the level of mitochondrial genome, this affiliation breaks down (Gray et al., 1989; Turmel et al., 1999). To date, complete mitochondrial DNA (mtDNA) sequences have been determined for five Trebouxiophyceae, i.e. *Prototheca wickerhamii* (Wolff et al., 1994); *Helicosporidium* sp. (Pombert et al., 2010); *Coccomyxa* sp. C169 (Smith et al., 2011); *Trebouxiophyceae* sp. MX-AZ01 (Servín-Garcidueñas & Martínez-Romero, 2012) and *Chlorella* sp. ArM0029B (Jeong et al., 2014). *Chlorella* is a genus of green cells photosynthetic algae, belonging to the Phylum Chlorophyta. Within this genus, the *C. Sorokiniana* strain can take advantage of organic carbon sources, in addition to carbon dioxide, to grow under mixotrophic conditions with higher biomass yields than the corresponding ones observed under photoautotrophic conditions (Wan et al., 2011). Moreover, a recent work by Rosenberg et al. (2014) investigated the effects of heterotrophic and mixotrophic growth on lipid biochemistry of *Chlorella* species, thus demonstrating that different lipid compositions can be achieved by cultivating *C. sorokiniana* under the trophic conditions above. Ultimately, the desired lipid composition and content might be varied by suitably tuning the cultivation conditions. For these reasons, this strain

might represent a promising feedstock for the production of nutritional oils or biofuels in a biorefinery framework. In spite of this, the commercial exploitation of *C. sorokiniana* is still not widespread since its large scale production might be affected by technical constraints mainly arising from the still low lipid productivity achievable through the current cultivation technologies (Concas et al., 2012, 2014). In this regard, the knowledge of *C. sorokiniana* genome represents the first step towards the identification of suitable genetic engineering strategies aimed to increase its lipid productivity and thus to overcome the limitations described above.

While Orsini et al. (2014) have recently presented the complete chloroplast genome sequence of *C. sorokiniana* strain (SAG 111-8k), in this work the knowledge of organelles genomes is completed through the sequencing of the mitochondrial genome of this strain. Within the *Chlorella* genus, this is the second species with a complete sequenced and annotated mitochondrial genome (GenBank accession no. KM241869).

Whole-genome sequencing of the *C. sorokiniana* strain was performed using Illumina HiSeq2000 platform (Pula, CA, Italy), after quality trimming reads were assembled by a denovo approach under the Orione framework (Cuccuru et al., 2014). The mtDNA sequence of *C. sorokiniana* assembles as a circular map of 52,528 bp (with an AT of 70.89%) which encodes a total of 31 protein-coding genes, 3 rRNAs and 26 tRNAs (cf. Figure 1), while accounting for 97.4% of the total genome. The comparison of the *C. sorokiniana* mt genome with respect to the Trebouxiophyceae one mentioned above revealed substantial rearrangements among strains including large inversion. In addition, a basic phylogenetic analysis placed *C. sorokiniana* mtDNA in a specific clade distinct from that one of *Chlorella* sp. ArM0029B (which, in turn, resulted in a separate clade itself) and the other Trebouxiophyceae.

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Figure 1. Gene map of the mitochondrial genome of *C. Sorokiniana*.

## Declaration of interest

The authors report no conflicts of interest. The financial support of Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, CRS4 and Sardegna Ricerche is gratefully acknowledged. Two of us (C. C. and V. M.) gratefully acknowledge the Sardinia Regional Government for the financial support of the Scholarship awarded for the International Environmental Science and Engineering PhD program at the University of Cagliari and the awarded grant Master&Back, respectively received in the framework of POR Sardegna F.S.E. Operational Program 2007-2013. The financial support of the INNOVARE project is also gratefully acknowledged.

## References

Concas A, Lutz GA, Pisu M, Cao G. (2012). Experimental analysis and novel modeling of semi-batch photobioreactors operated with

*Chlorella vulgaris* and fed with 100% (v/v) CO<sub>2</sub>. Chem Eng J 213: 203–13.

Concas A, Steriti A, Pisu M, Cao G. (2014). Comprehensive modeling and investigation of the effect of iron on the growth rate and lipid accumulation of *Chlorella vulgaris* cultured in batch photobioreactors. Bioresource Technol 153:340–50.

Cuccuru G, Orsini M, Pinna A, Sbardellati A, Soranzo N, Travaglione A, Uva P, et al. (2014). Orione, a web-based framework for NGS analysis in microbiology. Bioinformatics 30:1928–9.

Gray MW, Cedergren R, Abel Y, Sankoff D. (1989). On the evolutionary origin of the plant mitochondrion and its genome. Proc Natl Acad Sci USA 86:2267–71.

Jeong H, Lim JM, Park J, Sim YM, Choi HG, Lee J, Jeong WJ. (2014). Plastid and mitochondrion genomic sequences from Arctic *Chlorella* sp. ArM0029B. BMC Genomics 15:286.

Orsini M, Cusano R, Costelli C, Malavasi V, Concas A, Angius A, Cao G. (2014). Complete genome sequence of chloroplast DNA (cpDNA) of

- Chlorella sorokiniana*. Mitochondrial DNA. [Epub ahead of print]. doi: 10.3109/19401736.2014.919466.
- Pombert JF, Keeling PJ. (2010). The mitochondrial genome of the entomoparasitic green alga *Helicosporidium*. PLoS One 5:e8954.
- Rosenberg JN, Kobayashi N, Barnes A, Noel EA, Betenbaugh MJ, Oyler GA. (2014). Comparative analyses of three chlorella species in response to light and sugar reveal distinctive lipid accumulation patterns in the Microalga *C. sorokiniana*. PLoS One 9:e92460 doi:10.1371/journal.pone.0092460.
- Servín-Garcidueñas LE, Martínez-Romero E. (2012). Complete mitochondrial and plastid genomes of the green microalga Trebouxiophyceae sp. strain MX-AZ01 isolated from a highly acidic geothermal lake. Eukaryotic Cell 11:1417–18.
- Smith DR, Burki F, Yamada T, Grimwood J, Grigoriev IV, van Etten JL, Keeling PJ. (2011). The GC-rich mitochondrial and plastid genomes of the green alga coccomyxa give insight into the evolution of organelle DNA nucleotide landscape. PLoS One 6:e23624.
- Turmel M, Lemieux C, Burger G, Lang BF, Otis C, Plante I, Gray MW. (1999). The complete mitochondrial DNA sequences of *Nephroselmis olivacea* and *Pedinomonas minor*. Two radically different evolutionary patterns within green algae. Plant Cell 11:1717–30.
- Wan MX, Liu P, Xia J, Rosenberg JN, Oyler GA, Betenbaugh MJ, Nie Z, Qiu G. (2011). The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in *Chlorella sorokiniana*. Appl Microbiol Biotechnol 91:835–44.
- Wolff G, Plante I, Lang BF, Kück U, Burger G. (1994). Complete sequence of the mitochondrial DNA of the chlorophyte alga *Prototheca wickerhamii*. Gene content and genome organization. J Mol Biol 237: 75–86.