




Complete chloroplast genomes of the *Chlamydomonas reinhardtii* nonphotosynthetic mutants CC-1375, CC-373, CC-4199, CC-2359 and CC-1051

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ABSTRACT

The chloroplast genomes (cpDNA) of five *Chlamydomonas reinhardtii* nonphotosynthetic mutants were sequenced. The architecture, gene content, and synteny of the cpDNAs from the five mutants are identical to the *C. reinhardtii* ‘wild-type’ plastome. A small number of differences at sequence level between coding regions of the reference genome and the cpDNAs of the mutants were detected. The vast majority of the sequence differences were synonymous and likely due to nucleotide substitutions preceding the generation of the mutant strains, but not caused by the erosion of the cpDNA following the loss of photosynthesis.

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Nonphotosynthetic algae; *Chlamydomonas*; colorless mutants; genome erosion; genome reduction



Chlamydomonas nonphotosynthetic mutants are suitable experimental models for studying the evolution of colourless algae (Figueroa-Martinez et al. 2015). To gain insights into the erosion and reduction processes of chloroplast genomes (cpDNA) following the loss of photosynthesis, we sequenced the cpDNAs of five *C. reinhardtii* mutants with nonphotosynthetic phenotypes. The group of analyzed strains included two plastome mutants, CC-1375 ac-u-lambda (*psbA*) and mt + CC-373 ac-u-c-2-21 mt + (*atpB*), and three variants with alterations in nuclear loci, CC-1051 M18 (locus ac9) mt+, CC-2359, *Its1-30* mt- (locus PSY1), and CC-4199 *Its1-204* (PSY1) (Figure 1). Mutant strains were acquired from the *Chlamydomonas Resource Center* (www.chlamycollection.org).

Total DNA from each strain was purified using phenol-chloroform extraction methods (Figueroa-Martinez et al. 2017). Paired-end libraries were prepared and sequenced at Genome Quebec (McGill University, Montreal, Quebec) using Illumina technology (HiSeq2500; Illumina; San Diego, CA). Sequence quality ($\sim 60.6 \times 10^6$ reads per strain) was evaluated with FastQC v0.11.5 (Andrews 2016). High-quality reads (Phred score 28, length >70 bp) were assembled with Ray v2.2.0 (Boisvert et al. 2010) using 21 and 31 kmers. Gap bridging by read coverage was performed with Geneious v8 (Kearse et al. 2012). Coding regions were annotated using the 203.8 kb sequence of the ‘wild-type’ *C. reinhardtii* cpDNA (Maul et al. 2002) as reference; tRNA’s were predicted using tRNAscan-SE v1.21 (Lowe and Eddy 1997) and rRNA’s using RNAsesal (Centre Robert-Cedergren Bio-informatique et Génomique, Université de Montréal 2014). The new cpDNA

sequences are deposited in DDBJ/EMBL/GenBank under accession numbers MF083688 to MF083692.

The five cpDNAs of the mutants present the same quadripartite structure of the reference genome. Gene content (66 unique protein-coding genes, 5 rRNA, 27 tRNAs), introns, and synteny are conserved. Besides the expected reduced length of the CC-373 and CC-1375 cpDNAs, the plastomes from the other three mutants are also slightly smaller (<199 kb) than the reference. If we exclude the mutant loci, all 64 plastid protein-coding genes are identical among the mutants. In contrast, 17 coding regions from the reference cpDNA presented at least one (at most nine) nucleotide substitution with respect to the mutant sequences. Most detected substitutions were synonymous and therefore likely fixed independently in the different strains used to assemble the reference cpDNA (Maul et al. 2002), which are not the same used to produce the five nonphotosynthetic mutants (Chemerilova 1978; Shepherd et al. 1979; Girard et al. 1980; Myers et al. 1982; McCarthy et al. 2004).

Deletions of the plastid loci *atpB* (CC-373) and *psbA* (CC-1375) were corroborated. Coincidentally, CC-1375 presents a point deletion in *atpB* that causes a frame shift and a premature stop codon. It was not possible to discern if this mutation arose as consequence of genome erosion following the loss of photosynthesis, or if it was produced by the same mutagenic method that caused the *psbA* deletion. No additional mutations were identified in the cpDNAs of the three nuclear mutants. The lack of evident erosion in the cpDNAs of the mutants is likely due to the relatively low

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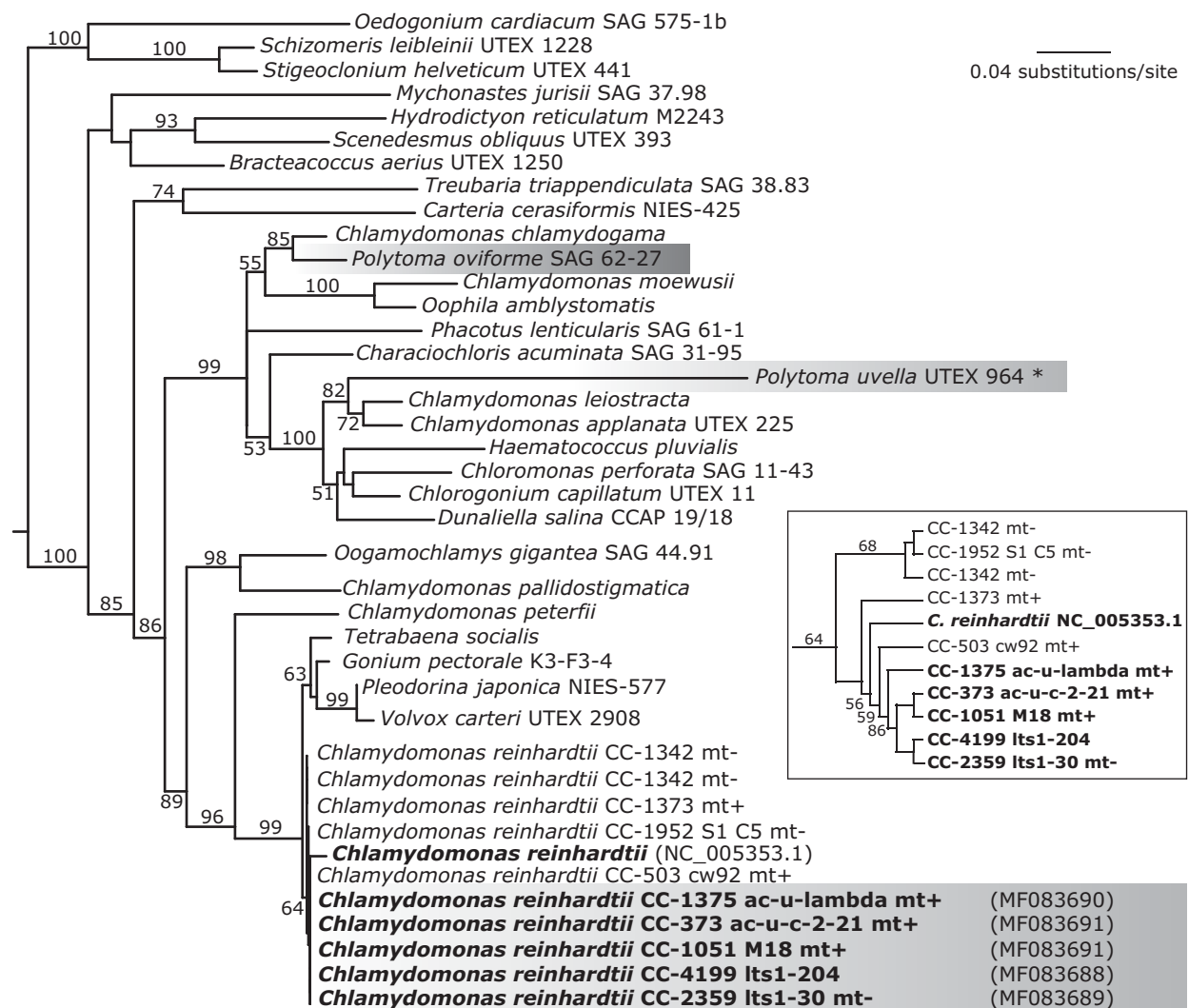


Figure 1. Maximum likelihood tree of diverse algae of the order Chlamydomonadales estimated using sequences of the chloroplast 16S ribosomal RNA (*rrs* gene). Nucleotide sequences were aligned with MAFFT v7 (Katoh and Standley 2013) and manually refined. Maximum likelihood (ML) tree was estimated with the RAXML v8.2 (Stamatakis 2014) considering the GTR + G substitution model. Branch support was assessed with 500 bootstrap replicates. Numbers near nodes indicate ML bootstrap support (only values >50% are shown). Branch lengths are proportional to the number of substitutions per site indicated by the scale bar. Gray boxes highlight nonphotosynthetic species and strains. The inset tree shows a cladogram detailing the *Chlamydomonas reinhardtii* lineage, which includes the five nonphotosynthetic mutants (bold font) analyzed in the present work. The DDBJ/EMBL/GenBank accession numbers of the complete chloroplast genomes are in parenthesis.

number of generations that have passed since the loss of photosynthesis and the mild selective conditions under which the cultures are maintained.

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Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article. The authors alone are responsible for the content and writing of the paper.

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References

- Andrews S. 2016. FastQC A Quality Control tool for High Throughput Sequence Data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Boisvert S, Laviolette F, Corbeil J. 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. *J Comput Biol.* 17:1519–1533.
- Centre Robert-Cedergren Bio-informatique et Génomique, Université de Montréal. 2014. RNAweasel. <http://megasun.bch.umontreal.ca/cgi-bin/RNAweasel/RNAweaselInterface.pl>
- Chemerilova VI. 1978. Investigation of pigmentation modifying mutations in *Chlamydomonas reinhardtii* strains of differing ploidy II. The lts1 mutation compounds and their use in obtaining triploid cultures. *Genetika.* 14:154–162.

- Figuroa-Martinez F, Nedelcu AM, Smith DR, Adrian R-P. 2015. When the lights go out: the evolutionary fate of free-living colorless green algae. *New Phytol.* 206:972–982.
- Figuroa-Martinez F, Nedelcu AM, Smith DR, Reyes-Prieto A. 2017. The Plastid Genome of *Polytoma uvella* Is the Largest Known among Colorless Algae and Plants and Reflects Contrasting Evolutionary Paths to Nonphotosynthetic Lifestyles. *Plant Physiol.* 173:932–943.
- Girard J, Chua NH, Bennoun P, Schmidt G, Delosme M. 1980. Studies on mutants deficient in the photosystem I reaction centers in *Chlamydomonas reinhardtii*. *Curr Genet.* 2:215–221.
- Katoh K, Standley DM. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol Biol Evol.* 30:772–780.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics.* 28:1647–1649.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
- Maul JE, Lilly JW, Cui L, dePamphilis CW, Miller W, Harris EH, Stern DB. 2002. The *Chlamydomonas reinhardtii* plastid chromosome: islands of genes in a sea of repeats. *Plant Cell.* 14:2659–2679.
- McCarthy SS, Kobayashi MC, Niyogi KK. 2004. White Mutants of *Chlamydomonas reinhardtii* Are Defective in Phytoene Synthase. *Genetics.* 168:1249–1257.
- Myers AM, Grant DM, Rabert DK, Harris EH, Boynton JE, Gillham NW. 1982. Mutants of *Chlamydomonas reinhardtii* with physical alterations in their chloroplast DNA. *Plasmid.* 7:133–151.
- Shepherd HS, Boynton JE, Gillham NW. 1979. Mutations in nine chloroplast loci of *Chlamydomonas* affecting different photosynthetic functions. *Proc Natl Acad Sci USA.* 76:1353–1357.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 30:1312–1313.