The Chlorophyceae is a late-diverging group of chlorophyte green algae that comprises the Chlamydomonadales (Volvocales) + Sphaeropleales (CS) clade and the Oedogoniales + Chaetophorales + Chaetopeltidales (OCC) clade (Turmel et al. 2008). Chlamydomonadales mitogenomes are characterized by derived traits, including a highly reduced gene content (12 genes), discontinuous tRNA genes (rns and rnl) occurring as multiple and dispersed fragments throughout the genome, a nonstandard genetic code, and high sequence divergence (Smith et al. 2013). Although their gene repertoire (37–42 genes) underwent less erosion (Nedelcu et al. 2000; Fucikova et al. 2014), sphaeroplean mitogenomes also show multiple sites of tRNA gene fragmentation and nonstandard genetic codes (Noutahi et al. 2019; Zihala and Elias 2019) and in addition encode derived tRNA genes. To gain better insight into how the mitogenome was transformed during evolution of the Chlorophyceae, we sequenced the mitogenomes of *Bulbochaete rectangularis* var. *hiloensis* (Oedogoniales) and *Stigeoclonium helveticum* (Chaetophorales).

*Bulbochaete rectangularis* (UTEX LB 954) and *S. helveticum* (UTEX 441) were obtained from the Culture Collection of Algae at the University of Texas at Austin. An organelle DNA fraction of the former alga was submitted to 454 pyrosequencing and the resulting reads were assembled using Newbler v2.5 (Margulies et al. 2005). Sanger sequencing of the *S. helveticum* mitogenome was performed using plasmid clones derived from an organelle DNA fraction as detailed in (Belanger et al. 2006). Sequence data were generated by the Genomic Analysis Platform of Laval University and analyzed as described in (Turmel et al. 2010).

The 70,191-bp mitogenome of *B. rectangularis* (GenBank MN810331) and the 46,765-bp mitogenome of *S. helveticum* (GenBank MN810332) contain the same set of 41 conserved genes, which includes *cox2a* (a partial version lacking about 100 codons) and 11 other respiratory protein-coding genes that are all shared with Sphaeropleales (only *atp6* is missing). In contrast, there exist notable differences in tRNA gene composition between the OCC clade and Sphaeropleales. The newly reported algal mitogenomes use a standard genetic code and their 27 tRNA genes all have counterparts in ancestral-type mitogenomes of chlorophytes (Turmel et al. 1999; Turmel, Otis, de Cambiaire, et al. 2020; Turmel, Otis, Lemieux 2020). While the *rns* genes of both algae and the *B. rectangularis rnl* are continuous, the *S. helveticum rnl* occurs as two separate pieces, with the breakpoint coinciding with a fragmentation site shared by Chlamydomonadales and Sphaeropleales (L2/L3 junction in Sphaeropleales). The *B. rectangularis* and *S. helveticum* mitogenomes exhibit 18 and 13 introns, respectively.

Phylogenetic analysis of concatenated proteins using RAxML v.8.2.3 (Stamatakis 2014) recovered the two newly
sampled chlorophyceans as a strongly supported clade with short branches at a position sister to the Sphaeropleales (Figure 1).

In conclusion, the mitogenome from the common ancestor of chlorophyceans possessed a reduced repertoire of protein-coding genes but its set of tRNA genes was complete, resembling those found in ancestral-type mitogenomes. Although its rnl gene was likely continuous, it might have encoded two distinct RNA species resulting from posttranscriptional cleavage at the sequence corresponding to the fragmentation site in \textit{S. helveticum} rnl.

**Disclosure statement**

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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**ORCID**

Monique Turmel \(\text{http://orcid.org/0000-0001-7060-035X}\)

Christian Otis \(\text{http://orcid.org/0000-0001-9680-5863}\)

Claude Lemieux \(\text{http://orcid.org/0000-0001-9580-8042}\)

**References**

Belanger AS, Brouard JS, Charlebois P, Otis C, Lemieux C, Turmel M. 2006. Distinctive architecture of the chloroplast genome in the chlorophycean green alga \textit{Stigeoclonium helveticum}. Mol Genet Genomics. 276(5):464–477.
Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 25(15):1972–1973.
Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5):1792–1797.
Fucikova K, Lewis PO, Gonzalez-Halphen D, Lewis LA. 2014. Gene arrangement convergence, diverse intron content, and genetic code modifications in mitochondrial genomes of Sphaeropleales (Chlorophyta). Genome Biol Evol. 6(8):2170–2180.
Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 437(7057):376–380.
Nedelcu AM, Lee RW, Lemieux C, Gray MW, Burger G. 2000. The complete mitochondrial DNA sequence of Scenedesmus obliquus reflects an intermediate stage in the evolution of the green algal mitochondrial genome. Genome Res. 10(6):819–831.
Noutahi E, Calderon V, Blanchette M, El-Mabrouk N, Lang BF. 2019. Rapid genetic code evolution in green algal mitochondrial genomes. Mol Biol Evol. 36(4):766–783.
Smith DR, Hamaji T, Olson BJ, Durand PM, Ferris P, Michod RE, Featherston J, Nozaki H, Keeling PJ. 2013. Organelle genome complexity scales positively with organism size in volvocine green algae. Mol Biol Evol. 30(4):793–797.
Smith SA, Dunn CW. 2008. Phyutility: a phyloinformatics tool for trees, alignments and molecular data. Bioinformatics. 24(5):715–716.
Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.
Turmel M, Brouard JS, Gagnon C, Otis C, Lemieux C. 2008. Deep division in the Chlorophyceae (Chlorophyta) revealed by chloroplast phylogenomic analyses. J Phycol. 44(3):739–750.
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(9):1717–1730.
Turmel M, Otis C, de Cambiaja JC, Lemieux C. 2020. Complete mitogenomes of the chlorophyte green algae Scherffelia dubia and Tetraselmis sp. CCMP 881 (Chlorodendrophyceae). Mitochondrial DNA B (in press). doi:10.1080/23802359.2019.1698349
Turmel M, Otis C, Lemieux C. 2010. A deviant genetic code in the reduced mitochondrial genome of the picoplanktonic green alga Pyrococcus provasolii. J Mol Evol. 70(2):203–214.
Turmel M, Otis C, Lemieux C. 2020. Complete mitogenomes of the marine picoplanktonic green algae Prasinoderma sp. MBIC 10622 and Prasinococcus capsulatus CCMP 1194 (Palmophyllophyceae). Mitochondrial DNA B. 5(1):166–168.
Zihala D, Elias M. 2019. Evolution and unprecedented variants of the mitochondrial genetic code in a lineage of green algae. Genome Biol Evol. 11(10):2992–3007.