

## TITLE

Intron-rich plastid genomes of monostromatic foliose green seaweeds *Monostroma grevillei* and *Gayralia oxysperma* (Ulotrichales, Ulvophyceae).

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## ABSTRACT

Despite recent developments, organellar genomes are still badly lacking in many algal groups. One such case is the green algal order Ulotrichales, where only six chloroplast genomes are currently available. Here, we present the complete chloroplast genomes of foliose *Monostroma grevillei* and *Gayralia oxysperma*, bringing the total number of available plastid genomes in this order to eight. Sequenced genomes are large (188 981 bp and 221 027 bp, respectively) and remarkably rich in introns – *G. oxysperma* is the most intron-rich plastome from Ulotrichales yet reported. Our results show that increasing taxon coverage in algal genomic resources keeps uncovering novel and interesting features in algal genome evolution and hope to spark wider efforts in generating genomic data in green algae.

## INTRODUCTION

Despite recent developments, organellar genomes are still badly lacking in many algal groups. One such case is the green algal order Ulotrichales, where only six chloroplast genomes are currently available (Table 1) out of the c. 200 species recognised in the order (Guiry and Guiry 2026). Here, we present the complete chloroplast genomes of foliose *Monostroma grevillei* and *Gayralia oxysperma*, bringing the total number of available plastid genomes in this order to eight.

Ulotrichalean algae are morphologically diverse, ranging from microscopic coccoid and sarcinoid forms to filamentous and foliose macroscopic seaweeds (Škaloud et al. 2018). This diversity establishes Ulotrichales as an interesting model taxon for the evolution of functional traits and growth forms in algae. Increasing genomic taxon sampling allows us to generate a clearer picture of the landscape of genomic evolution in Ulotrichales, facilitating future work in the evolution of algal form and function. Additionally, previously published ulotrichalean plastomes show very interesting and unusual features, such as a high presence of introns (Pombert et al. 2005, Turmel et al. 2016), gene transfers between plastids and mitochondria (Turmel et al. 2016) and plastome rearrangements, shedding light to the evolutionary origins of the inverted repeat (IR) region (Turmel et al. 2017, Kim et al. 2019).

## MATERIALS AND METHODS

Specimens of *Monostroma grevillei* (SV\_97) and *Gayralia oxysperma* (SV\_434) were collected from near Tjörnö, and Grimsholmen, respectively, from the Swedish west coast at the Atlantic-Baltic Sea boundary in 2019. The specimens were photographed, identified using light microscopy, pressed into herbarium sheets and permanently stored at the University Museum of Bergen herbarium. From fresh material, c. 2 cm<sup>2</sup> piece of the thallus, clear from epiphytes, was separated and flash-dried in silica gel for DNA extraction.

DNA was extracted using the Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany) according to the manufacturer's instructions. DNA aliquots were sent for sequencing to an external sequencing facility (Macrogen, Amsterdam, The Netherlands). Here, samples went through initial quality control, library preparation with the Illumina TruSeq Nano (350 bp) paired-end library preparation kit and Illumina NovaSeq sequencing (2x150 bp). The identity of the specimens was verified separately by DNA barcoding the *tufA* region.

### *Plastid genome assembly*

Produced raw reads were error-corrected and normalised separately for forward and reverse reads in Geneious Prime v2025.0.3 (GraphPad Software LLC, MA, USA) using the error correct & normalise -function with default settings. GetOrganelle v1.7. 5 (Jin et al. 2020) was used on these normalised reads to generate plastid genome assemblies. GetOrganelle was run with the kmer array of 21, 45, 65, 85 and 105 base pairs. Word size was auto-estimated and maximum extension rounds were kept at default 15. The target organelle database used was “others\_pt” consisting of non-embryophyte chloroplast reference genomes. Produced contigs were examined in Bandage v0.8.1 (Wick et al. 2015) to select a single produced circular contig, which was separated from contaminant contigs of typically bacterial origin. The quality of produced plastid contigs was increased by re-mapping the raw reads to the GetOrganelle circular contig using the “Map to reference” -function in Geneious Prime with medium sensitivity and 10 iterations. Produced high-coverage plastid genomes were

annotated with the GeSeq v2.03 (Tillich et al. 2017) using published annotated chloroplast genomes in Ulotrichales and Ulvales in the NCBI RefSeq database as references. Additionally, tRNAs were secondarily annotated with ARAGORN v1.2.38 (Laslett & Canback 2004) and tRNAscan-SE v2.0.7 (Chan & Lowe 2019). Annotations were manually checked; duplications were removed and annotations visualised with OGDRAW v1.3.1 (Greiner et al. 2019). The raw reads and assembled genomes are submitted to GenBank and available from the authors upon request until given accession codes by NCBI staff.

### *Phylogenomic analysis*

A total of 13 chloroplast genomes were used in the phylogenomic analysis, including the newly generated genomes, previously available Ulotrichales genomes (Table 1) as well as four outgroup taxa: *Oltmannsiellopsis viridis* NC\_008099 (Oltmannsiellopsidales, Pombert et al. 2006), *Sykidion marinum* NC\_034710 (Sykidiales, Turmel et al. 2017, Darienko et al. 2021), *Ulva flexuosa* NC\_035823 (Ulvales, Cai et al. 2017) and *Gloeotilopsis sterilis* NC\_025538 (Lemieux et al. 2014). Nomenclaturally *G. sterilis* is classified in Ulotrichales (Guiry & Guiry 2026), but according to the phylogenies by Lemieux et al. 2014 this taxon lies in the *Geminella* clade in core Trebouxiophyceae, indicating that this taxon does not belong to Ulotrichales.

The sampling of protein coding genes from the plastids followed the conservative approach by Crowell et al. 2026. Protein coding genes present in 13 or 12 of the 13 target genomes were extracted, concatenated into a single matrix and aligned using MUSCLE 5.1 (Edgar 2004) in Geneious Prime. Introns were removed, and alignments checked visually to drop genes with obvious alignment issues. In total 69 protein coding genes were used in the concatenated alignment of 49 174 bp. Maximum likelihood phylogenies were constructed using IQ-TREE2 (Minh et al. 2020) with a partition model (Chernomor et al. 2016) using *a priori* partitions by gene and by codon position within each gene. The best substitution model was chosen by using ModelFinder (Kalyaanamoorthy et al. 2017) with the -MERGE option on to collapse *a priori* partitions to meaningful groups within IQTREE. Node support was estimated using UFBoot2 (1000 replicates, Hoang et al. 2018). The phylogeny was visualised with TreeViewer (Bianchini & Sánchez-Baracaldo 2024).

## RESULTS AND DISCUSSION

Sequencing produced 14 085 710 reads (2,13 Gb) with a GC content of 56,1 % and Q30 of 90,7 % for *Monostroma grevillei* SV\_92, and 13 735 358 reads (2,07 Gb) with a GC content of 49,5 % and Q30 of 83,1 % for *Gayralia oxyasperma* SV\_434.

The circular chloroplast genome of *Monostroma grevillei* (average base coverage 420.2) is 188 981 bp long, with a GC content of 32.2 % (Figure 1). The *Gayralia oxyasperma* chloroplast (average base coverage 228.7) is similarly circular, 221 027 bp long with a GC content of 34.3 % (Figure 2). The genome sizes are large and GC content comparatively high, as expected from other Ulotrichales plastomes available (Table 1). The phylogenetic treatment confirms the placement of the newly sequenced taxa to Ulotrichales (Figure 3). *Capsosiphon fulvescens* does not cluster with other Ulotrichales, confirming the observation by Kim et al. 2019 that the phylogenetic placement of this taxon requires further attention. The quadripartite architecture typical to chloroplast genomes of many vascular plants and other chlorophytes is lost in *G. oxyasperma* but retained in *M. grevillei*, illustrating the lability

of chloroplast genome structure in Ulotrichales (Turmel et al. 2016, Kim et al. 2019). The loss of inverted repeats has occurred at least twice in Ulotrichales, in lineages leading to *Gayralia* and *Rhexinema* (Figure 3).

Both *Monostroma grevillei* and *Gayralia oxysperma* chloroplast genomes contain 73 annotated protein coding genes (Table 1, Table 2, Figures 1-2). For instance, both feature the *trnI(CAU)-tilS* module, coding for a tRNA<sup>Ile</sup>-lysine synthetase which modifies the *trnI(CAU)* anticodon to maintain translation fidelity (Nakanishi et al. 2009). The retention/loss dynamics of this module has gained recent attention in green algae (Barcytė et al. 2025, Lee et al. 2026), our results adding evidence that in Ulotrichales this module is typically retained in the plastid genome.

The large size of both genomes is seemingly due to a large number of introns, especially in the *Gayralia oxysperma* genome. The annotation of this genome contains 46 candidate intronic regions – most reported from any Ulotrichales genome up to date. A high prevalence of Group I introns has been previously reported from *Tupiella akineta* (Pombert et al. 2005), whereas Group II introns are dominant in *Rhexinema* plastids (Turmel et al. 2016). Preliminary analyses show that both Group I and Group II introns are present in both *M. grevillei* and *G. oxysperma* genomes. Further detailed work is necessary to verify and classify these intronic regions to understand their role in the evolution of plastid genomes in Ulotrichales.

Our results show that increasing taxon coverage in algal genomic resources keeps uncovering novel and interesting features in algal genomic evolution. Wide taxon sampling forms the basis for comparative genomic studies (Crowell et al. 2026). For these comparative analyses to be powerful and informative, it is essential that the taxa compared showcase the true diversity of the lineage being studied. The plastid genomes of *Monostroma grevillei* and *Gayralia oxysperma* help close this gap in macroalgae in the Ulotrichales and hope to spark wider efforts in generating genomic data in green algae.

Table 1. Features of published and newly generated Ulotrichales plastid genomes.

| Taxon                                                                                 | Growth form                                       | Size (bp) | GC%  | protein-coding genes <sup>a</sup> | tRNAs | rRNAs | Intron candidates | Accession | Reference                             |
|---------------------------------------------------------------------------------------|---------------------------------------------------|-----------|------|-----------------------------------|-------|-------|-------------------|-----------|---------------------------------------|
| <i>Monostroma grevillei</i> (Thuret) Wittrock                                         | Macroscopic foliose seaweed                       | 188 981   | 32,2 | 73                                | 31    | 3     | 23                | TBC       | This study                            |
| <i>Gayralia oxysperma</i> (Kützing) K.L. Vinogradova ex Scagel et al.                 | Macroscopic foliose seaweed                       | 221 027   | 34,3 | 73                                | 28    | 3     | 46                | TBC       | This study                            |
| <i>Tupiella akineta</i> (Tupa) Darienko & Pröschold <sup>1</sup>                      | Short filaments forming prostrate thalli          | 195 867   | 31,5 | 73                                | 31    | 6     | 27                | NC_008114 | Pombert et al. 2005                   |
| <i>Rhexinema paucicellulare</i> (Vischer) Geitler <sup>2</sup>                        | Very short filaments or cell packages in mucilage | 221 431   | 31,5 | 73                                | 28    | 3     | 31                | KX306821  | Turmel et al. 2016                    |
| <i>Rhexinema sarcinoideum</i> (Groover & H.C. Bold) Darienko & Pröschold <sup>3</sup> | Very short filaments or cell packages in mucilage | 262 888   | 31,5 | 73                                | 28    | 4     | 27                | KX306824  | Turmel et al. 2016                    |
| <i>Sarcinofilum mucosum</i> (Broady) Darienko & Pröschold <sup>4</sup>                | Filamentous forming sarcinoid structures          | 227 181   | 37,2 | 73                                | 29    | 6     | 17                | NC_034709 | Turmel et al. 2017                    |
| <i>Hazenella capsulata</i> (Tupa) Škaloud & Leliaert <sup>5</sup>                     | Filaments in mucilage                             | 189 599   | 30,8 | 73                                | 31    | 9     | 16                | NC_034714 | Turmel et al. 2017                    |
| <i>Capsosiphon fulvescens</i> (C.Agardh) Setchell & N.L.Gardner <sup>6</sup>          | Macroscopic tubular seaweed                       | 111 561   | 31,3 | 73                                | 27    | 6     | 18                | NC_039920 | Kim et al. 2019                       |
| <i>Gayralia brasiliensis</i> Pellizzari, M.C.Oliviera & N.S.Yokoya <sup>7</sup>       | Macroscopic foliose seaweed                       | 237 223   | 34,1 | 74                                | 27    | 3     | NA                | NC_072923 | Lin direct submission to GenBank 2022 |

<sup>1</sup>Sequenced and reported in Pombert et al. 2005 as *Pseudendoclonium akinetum* (UTEX 1912), later combined to *Tupiella akineta* by Darienko and Pröschold 2017.

<sup>2</sup>Sequenced and reported in Turmel et al. 2016 under the name *Gloeotilopsis planctonica* (SAG:29.93). Later this strain was re-identified and combined to the genus *Rhexinema* as *R. paucicellularis* by Darienko and Pröschold 2017.

<sup>3</sup>Sequenced and reported in Turmel et al. 2016 under the name *Gloeotilopsis sarcinoidea* (UTEX 1710). Later combined to *Rhexinema* by Darienko and Pröschold 2017.

<sup>4</sup>Sequenced and reported in Turmel et al. 2017 under the name *Trichosarcina mucosa* (SAG:4.90). Later combined to *Sarcinofilum* as *S. mucosum* by Darienko and Pröschold 2017.

<sup>5</sup>Sequenced and reported in Turmel et al. 2017 under the name *Chamaetrichon capsulatum* (UTEX 1918). Later combined to *Hazenella* as *H. capsulata* by Škaloud and Leliaert 2017.

<sup>6</sup>Inclusion to Ulotrichales *s. stricto* unclear (Kim et al. 2019).

<sup>7</sup>Submitted as an annotated, complete chloroplast genome directly to GenBank by Lin K., Guangdong Ocean University, China based on Chinese material, not published or discussed in a publication, including some misleading metadata. The sequence is identical to a second entry NC\_072924 labelled *Monostroma nitidum* by the same author, including identical metadata. We treat this data preliminary. If correct, these two taxa may be conspecific.

<sup>a</sup>This number excludes annotations labelled as ORFs without further gene classification. These often lie inside introns and may represent intron-encoded proteins.

Table 2 Gene content in *Monostroma grevillei* and *Gayralia oxysperma* chloroplast genomes. Genels labelled with asterisk (\*) contain candidate introns.

| Gene group                               | <i>Monostroma grevillei</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | <i>Gayralia oxysperma</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Photosystem I</b>                     | <i>psaA, psaB*, psaC, psaI, psaJ, psaM</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <i>psaA*, psaB*, psaC, psaI, psaJ, psaM*</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Photosystem II</b>                    | <i>psbA*, psbB, psbC*, psbD*, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbN, psbM, psbT, psbZ</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <i>psbA, psbB*, psbC*, psbD*, psbE*, psbF, psbH*, psbI, psbJ, psbK, psbL, psbN, psbM, psbT, psbZ</i>                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Cytochrome b/f complex</b>            | <i>petA, petB, petD, petG, petL</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <i>petA*, petB, petD*, petG*, petL</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>ATP synthase</b>                      | <i>atpA, atpB, atpE, atpF, atpH, atpI</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <i>atpA*, atpB*, atpE, atpF, atpH*, atpI</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Rubisco</b>                           | <i>rbcL</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | <i>rbcL*</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>RNA polymerases</b>                   | <i>rpoA, rpoB*, rpoC1*, rpoC2*</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <i>rpoA*, rpoB*, rpoC1*, rpoC2*</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Ribosomal proteins, small subunit</b> | <i>rps2, rps3, rps4, rps7, rps8, rps9, rps11*, rps12, rps14, rps18, rps19</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <i>rps2, rps3, rps4, rps7, rps8, rps9, rps11, rps12, rps14, rps18, rps19</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Ribosomal proteins, large subunit</b> | <i>rpl2, rpl5, rpl12, rpl14, rpl16, rpl19, rpl20, rpl23*, rpl32, rpl36</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <i>rpl2, rpl5, rpl12, rpl14, rpl16, rpl19, rpl20, rpl23, rpl32, rpl36</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Transfer RNAs</b>                     | <p><i>trnA-UGC (2 copies), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnG-UCC, trnH-GUG, trnI-GAU (2 copies^), trnK-UUU, trnL-CAA, trnL-UAA, trnL-UAG, trnMe-CAU (3 copies^), trnN-GUU, trnP-UGG, trnQ-UUG, trnR-CCG, trnR-CCU, trnR-UCU, trnS-GCU, trnS-UGA, trnT-UGU, trnV-UAC, trnW-CCA, trnY-GUA</i></p> <p>^the multiple copies of <i>trnI-GAU</i> &amp; <i>trnM-CAU</i> -annotations could be functionally different and participating in the <i>tilS-trnI(CAU)</i> -module similar to Lee et al. 2026. Further characterisation of these tRNAs is needed.</p> | <p><i>trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnG-UCC, trnH-GUG, trnI-GAU, trnK-UUU, trnL-UUA, trnL-UAA, trnL-UAG, trnM-CAU (4 copies^), trnN-GUU, trnP-UGG, trnQ-UUG, trnR-UCU, trnR-ACG, trnR-CCG, trnS-GCU, trnS-UGA, trnT-UGU, trnV-UAC, trnW-CCA, trnY-GUA</i></p> <p>^the multiple copies of <i>trnM-CAU</i> -annotations could be functionally different and participating in the <i>tilS-trnI(CAU)</i> -module similar to Lee et al. 2026. Further characterisation of these tRNAs is needed.</p> |
| <b>Ribosomal RNAs</b>                    | <i>rrf, rrl, rrs</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <i>rrf, rrl*, rrs*</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Other genes</b>                       | <i>accD*, ccsA, cemaA, chlI, clpP, ftsH*, infA, minD, tilS*, tufA, ycf1, ycf3, ycf4, ycf12, ycf20</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <i>accD, ccsA, cemaA, chlI, clpP, ftsH*, infA*, minD, tilS*, tufA*, ycf1, ycf3*, ycf4 ycf12, ycf20</i>                                                                                                                                                                                                                                                                                                                                                                                                                         |

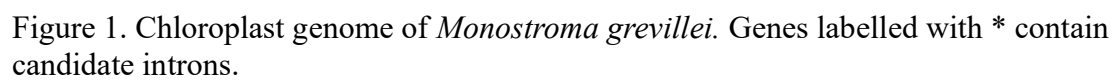


Figure 1. Chloroplast genome of *Monostroma grevillei*. Genes labelled with \* contain candidate introns.

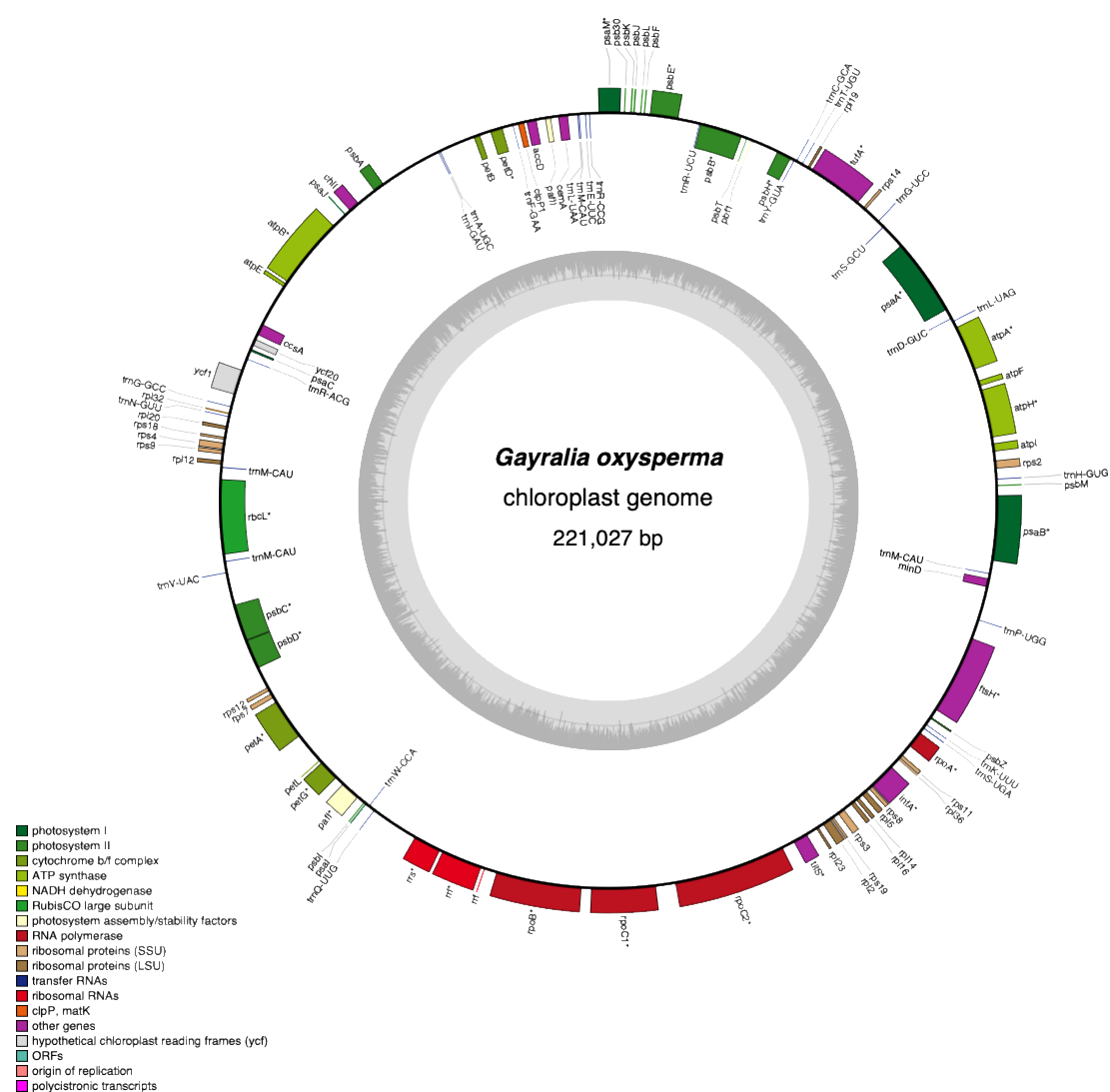


Figure 2. Chloroplast genome of *Gayralia oxysperma*. Genes labelled with \* contain candidate introns.

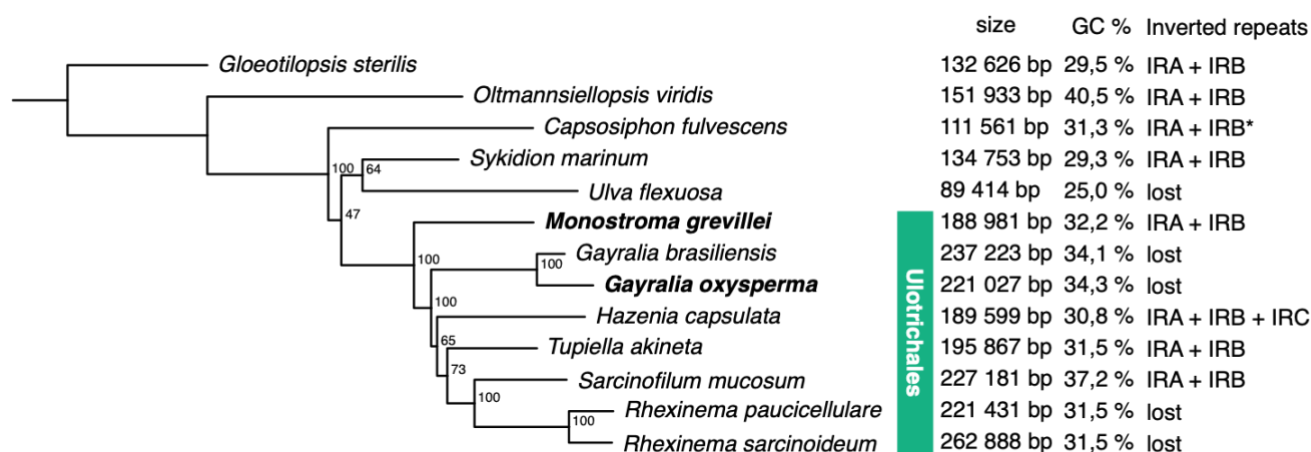


Figure 3. Maximum likelihood phylogeny based on 69 protein coding genes in green algal chloroplast genomes in Ulotrichales and related orders. Newly sequenced genomes in bold. UFBoot2 support values are shown as node labels (1000 replicates). Genomic features shown on the right (size, GC%, inverted repeat structure). \*In *Capsosiphon fulvescens* the inverted repeat structure features different isoforms of IRA and IRB, see Kim et al. 2019.

### Conflicts of interest

The authors report no conflict of interest. A part of this research is based on the Msc dissertation of Niko R. Johansson (Johansson 2024).

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### Data availability

The raw reads and assembled genomes are submitted to GenBank and available from the authors upon request until given accession codes by NCBI staff. Voucher specimens are stored in the University Museum at the University of Bergen.

### CRedit roles:

**Niko R. Johansson:** conceptualization (equal); laboratory work (supporting); analysis (equal); writing – original draft preparation (lead); writing – review & editing (lead). **Jaanika Blomster:** conceptualization (equal); supervision (equal); writing – review & editing (equal). **Péter Poczai:** analysis (equal); writing – review & editing (equal). **Sophie Steinhagen:** conceptualization (equal); field sampling (lead); laboratory work (lead); supervision (equal); writing – review & editing (equal).

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