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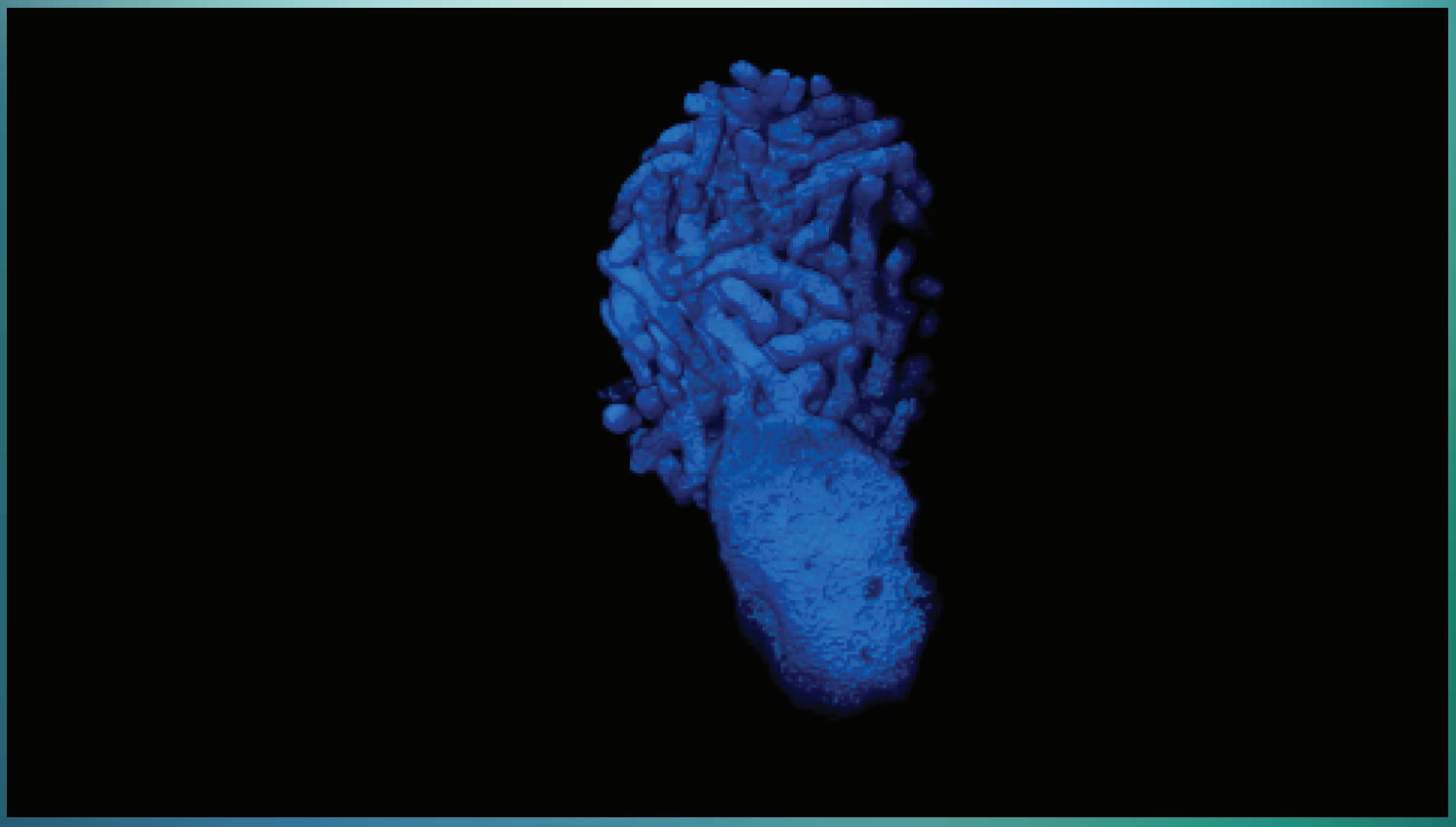

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The Organellar Genomes of *Melanthalia abscissa* and *Polyopes polyideoides* (Rhodophyta, Florideophyceae).

Los genomas organelares de *Melanthalia abscissa* y *Polyopes polyideoides* (Rhodophyta, Florideophyceae).

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ABSTRACT

Florideophyceae is the most species rich red algal class, including large numbers of well-studied, economically important species and lesser-known clades. We present the complete plastid and mitochondrial genome assemblies of two Florideophycean species, *Melanthalia abscissa* (Gracilariaceae) and *Polyopes polyideoides* (Halymeniaceae). We identified more large-scale rearrangements within the plastid genomes of Gracilariales than within Halymeniales. Maximum likelihood phylogenies using *rbc*L data support the placement of both *M. abscissa* and *P. polyideoides* samples in monophyletic genera. However, not all genera within Halymeniales were recovered as monophyletic. Sequences that appear to be derived from red algal plasmids were identified within the plastid genome of *M. abscissa*. Determining the presence or absence of plasmid-derived sequences in the *P. polyideoides* plastome is more difficult due to a lack of publicly available data for Halymeniaceae. The addition of the sequences produced by this study will support further phylogenetic and systematic research on these Rhodophytan genera and orders.

*Keywords: Florideophyceae, mitochondria, phylogenetics, plastid genome, rbc*L.

RESUMEN

Las Florideophyceae es la clase más numerosa en especies de las algas rojas, incluye un gran número de especies bien estudiadas económicamente importantes y algunos clados menos conocidos. En este trabajo presentamos los genomas completos plastidial y mitocondrial de dos especies de algas Florideophyceae, *Melanthalia abscissa* (Gracilariaceae) y *Polyopes polyideoides* (Halymeniaceae). Se identificaron más rearreglos a gran escala dentro de los genomas plastidiales de Gracilariales que dentro de las Halymeniales. Las filogenias por Máxima Probabilidad utilizando datos del *rbc*L apoyan la posición de ambas muestras, *M. abscissa* y *P. polyideoides*, dentro de géneros monofiléticos. Sin embargo, no todos los géneros dentro de Halymeniales fueron recuperados como monofiléticos. Secuencias que parecen ser derivadas de plásmidos de algas rojas se identificaron dentro del genoma plastidial de *M. abscissa*. La determinación de la presencia o ausencia de secuencias derivadas de

plásmidos en el plastoma de *P. polyideodies* es más difícil debido a la ausencia de datos públicamente disponibles para las Halymeniaceae. La adición de las secuencias producidas en este estudio apoyará futuras investigaciones filogenéticas y sistemáticas en estos géneros y ordenes de Rhodophyta.

*Palabras clave: filogenética, Florideophyceae, genoma plastidial, mitocondria, rbc*L.

INTRODUCTION

Rhodophyta is a monophyletic division of red algae that consists of seven classes. Florideophyceae, the most species rich class in the division, contains ~7,100 of the ~7,500 Rhodophytan species (Guiry 2017). The class has a considerable impact on multiple sectors of the human economy. The commercially used agarophytes, carrageenophytes, and several economically important edible seaweeds belong to Florideophyceae. Members of the Florideophyceae also hold major importance to ocean ecosystems. Corallinales, a resilient and widespread order of crustose Florideophyceans, are critical builders of coral reef frameworks in the global tropics (Bjork *et al.* 1995). Despite this economic and ecological importance, several branches of subordinate Florideophyceae taxonomy rely entirely on analyzing morphological features, especially the reproductive structures (De Clerck *et al.* 2012). The uncertain inter-ordinal relationships may be resolved by further implementing phylogenomic approaches.

Organellar genomes contain useful sequence data for developing reliable phylogenies. Compared to nuclear genomes, their high copy and short length make extraction, assembly, and analysis straight-forward. Plastids and mitochondria are typically inherited uniparentally, gene-dense, and highly conserved. Due to their conserved nature, organellar DNA can be particularly valuable in resolving deep-branching phylogenies (Palmer *et al.* 1988).

Less than three percent of Florideophyceans have plastid genomes available on RefSeq, NCBI's non-redundant database, with only 149 published (O'Leary *et al.* 2016). Using a matrix of mostly plastid coding and ribosomal loci, as well as a selection of nuclear coding and ribosomal loci, Verbruggen *et al.* (2010) identified five notable regions of low support in the Rhodophytan phylogeny. Substantial increases have been made in the availability of the loci used by Verbruggen *et al.* since the study's release in 2010. However, much of the red algal phylogeny remains unresolved (Díaz-Tapia *et al.* 2018). The addition of sequence data will provide better resolution of these regions of the phylogeny. In this study, we focus on the plastid and mitochondrial genomes of two species, *Melanthalia abscissa* (Turner) Hooker f. & Harvey and *Polyopes polyideoides* Okamura, from two Florideophycean orders, Gracilariales and Halymeniales, respectively.

Melanthalia Montagne is a genus of only four species within the family Gracilariaceae, which contains agarophytan genera such as *Gracilaria* Greville and *Gracilariopsis* E.Y. Dawson. Despite the variety of known applications for *Gracilaria*, *Melanthalia* is not used as extensively.

Currently, the genus has minimal usage in aquaculture. It is not used commercially for agar production (Furneaux *et al.* 1990) or for human consumption. Laboratory experiments with extracts from *M. abscissa* have increased larval settlement of *Perna canaliculus*, a commonly cultivated mussel in New Zealand aquaculture (Alfaro *et al.* 2006). *Melanthalia* has a small geographic range and limited distribution in the subtidal waters of New Zealand and Australia (Nelson *et al.* 2013). A plastid genome from one member of the genus, *Melanthalia obtusata* (Labillardière) J. Agardh is available on GenBank under the synonymous name, *Melanthalia intermedia*. The systematics of this genus and corresponding species within it have changed multiple times in recent years and remains elusive (Iha 2018, Lyra 2021, Nelson *et al.* 2013).

Polyopes J. Agardh is a small genus of eleven species within the family Halymeniaceae (Guiry 2017). It is native to East Asia and particularly prevalent in the coastal waters of Japan. However, it has recently appeared on European coasts, likely through accidental introduction from human activity (Mineur *et al.* 2010). Phylogenomic analyses of the genus have been limited, though it has gained some attention for the various pharmaceutical applications of *Polyopes affinis* (Harvey) Kawaguchi & Wang and *Polyopes lancifolius* (Harvey) Kawaguchi & Wang. Ethanol extracts from *P. affinis* have shown potential in treating airway inflammation in mice and human asthma models (Lee *et al.* 2011; Ha *et al.* 2022), as well as acting as photoprotection against ultraviolet-B light on human cells (Hyun *et al.* 2014). *P. lancifolius* has been used to treat high blood sugar in diabetic mice (Kim *et al.* 2010). At the date of writing, only eight unique plastid genomes from Halymeniales are available on GenBank. From these, three correspond to the family Halymeniaceae.

The goal of this study is to add to the limited knowledge base of the Florideophycean genera *Melanthalia* and *Polyopes*, by providing complete organellar genome sequences, and examining the

characteristics of these genomes. Publication of organellar genomes from clades underrepresented in databases will benefit future species identifications, systematic and ecological investigations, and conservation of this critical class. The Florideophyceae are crucial to the global ecosystem, acting as keystone reef builders and invasive species.

METHODOLOGY

M. abscissa specimens were collected by D. Wilson Freshwater in 1994 from a subtidal habitat near the Mataikona river in Wairarapa, New Zealand. The voucher specimen is stored at the Te Papa Tongarewa Museum of New Zealand Herbarium with the voucher number WELT A024150. DNA is in the algal DNA collection at The University of Alabama under the number UA 816. *P. polyideoides* specimens were collected by Suzanne Fredericq in Keelung City, Taiwan, in 1993. DNA is stored at the University of Alabama under the number UA 733. Sequencing was performed at Cold Spring Harbor Laboratory (Cold Spring Harbor, New York, USA) on the Illumina MiSeq Platform. For the *M. abscissa* sample, 2,159,473 paired-end reads (101 bp) were produced. For the *P. polyideoides* sample, 4,732,738 paired-end reads were produced.

Plastid genomes were assembled and subsequently analyzed on The University of Alabama High-Performance Computing (HPC) cluster using the following steps. Initial read quality was examined using FastQC v0.11.5 (Andrews 2010) before cleaning and trimming with Trimmomatic v0.4 (Bolger *et al.* 2014) with minimum leading and trailing quality scores of 20 and a minimum read length of 65. Following trimming, normalization to 100x read coverage to reduce any possible overrepresented sequences was done using BBnorm (Bushnell 2014). *De novo* assembly was performed by SPAdes v3.14 (Prjibelski *et al.* 2020) using the 'plasmid' and 'careful' options. A database of published Rhodophyta organellar genes and complete organellar genomes was created with BLASTn 2.9.0 (Altschul *et al.* 1990) to select the scaffolds containing the organellar genomes. Seed based microassembly tool, afin (Wilson 2016) was needed to extend and finish the plastome of *P. polyideoides*. Coverage analyses were performed by Fast-Plast v1.2.9 (McKain & Wilson 2017) to evaluate the assembly. Protein coding sequences were identified by Plastid Genome Annotator (Qu *et al.* 2019), and ribosomal genes and tRNAs within the plastid genome were detected by Chlorobox GeSeq (Tillich *et al.* 2019) and tRNAscan-SE 2.0 (Lowe & Chan 2016). Gene lengths and reading frames were manually checked by aligning each protein product to the closest homolog. Any necessary edits to the automated annotations were made in Geneious V.2023.0.4 (Geneious 2023) and UGENE V.47 (Okonechnikov *et al.* 2012).

Open reading frames (ORFs) of 25 amino acids or longer in intergenic space with BLAST matches to other members of Rhodophyta were retained in the annotation. BLASTp searches of the non-redundant database of Rhodophyta data were used. An E-value of 1e-5 and percent identity cut off of 60% were used to determine potential homology with ORFs in other Rhodophyta plastomes. ORFs were named based on the length of their potential amino acid product. Microsatellites, or simple sequence repeats (SSRs), were identified by the MIcroSAtellite (MISA) identification tool v.2.1 (Beier *et al.* 2017). The minimum number of repeats to detect microsatellites was set to ten for mononucleotide repeats, six for dinucleotide repeats, and five for trinucleotide repeats or anything larger. These settings are the default option on the MISA web server. These parameters have also been used in other studies analyzing microsatellites in algal plastid genomes (Kuntal *et al.* 2012). Visualization of complete organellar genomes was done by Chlorobox OGDRAW (Greiner *et al.* 2019).

Complete coding sequences of *rbc*L, extracted from plastid genome assemblies were aligned by MAFFT v7.313 (Katoh & Stanley 2013) with plastid genome data available on GenBank. To further assess the systematic position of the algal samples, two maximum likelihood phylogenies were constructed using this data, in IQ-Tree v2.2.0 (Minh *et al.* 2020). Substitution models were chosen using the "auto" option in IQ-Tree. The TIM2+F+I+G4 model was chosen for the Gracilariales phylogeny, while the TIM2+F+G4 model was chosen for the Halymeniales phylogeny. The Halymeniales phylogeny was constructed with all *rbc*L sequences from the order with a length over 1400 base pairs available on GenBank. Due to the small number of complete plastid genomes published on GenBank, the use of data solely from complete assemblies would not construct a sufficient phylogeny of the order.

Tree visualization was done using FigTree v1.4.4 (Rambaut 2010), Adobe Illustrator v28.2 and Acrobat v23 (Adobe Inc. 2024, Adobe Inc. 2024). Plastome rearrangements between both novel sequences and their respective orders were examined through Mauve (Darling *et al.* 2004) genome alignments. Representatives of each genus present in the Halymeniales and Gracilariales phylogenies were selected for alignment, along with the sequences from this study. Alignments were performed by progressive

Mauve using the default parameters and HOXD scoring. Start positions for each alignment were standardized to the most common position in the taxon sampled. The Gracilariales alignment begins at rns while the Halymeniales alignment begins at *rbc*L. Possible plasmid-derived sequences (PDS) were identified by similarity to a custom BLASTn database of Rhodophyta plasmid sequences.

RESULTS

The assembled *M. abscissa* plastid genome has a length of 209,722 base pairs, and GC-content of 32.2% (Fig. 1). The average depth of coverage for the assembly is 55. A total of 190 protein-coding genes were identified along with 29 tRNA sequences and three rRNA genes, and 15 ORFs. Identified genes with overlap include *lys*R with *ycf*54, *suf*B with *suf*C, *atp*D with *atp*F, *rpl*33 with *rps*18, *ycf*59 with *leu*D, *rpl*4 with *rpl*23, *rpl*14 with *rpl*24, and *psb*C with *psb*D. Including ORFs identified, protein-coding regions account for 68.4% of the plastome.

Two simple sequence repeats (SSR) were found in the plastome of *M. abscissa*. A trinucleotide repeat of AGC was found at positions 11,417 to 11,431 within the *psb*A gene, and a dinucleotide repeat of AT was found at positions 174,075 to 174,088 within the *syf*B gene. No inverted repeat was found. Mauve alignments of the entire plastomes show locally collinear block (LCB) rearrangement is observed in the alignment between *Melanthalia abscissa* and *Melanthalia obtusata*. The plastid genome of *M. abscissa* is published on GenBank under the accession number PP328475.

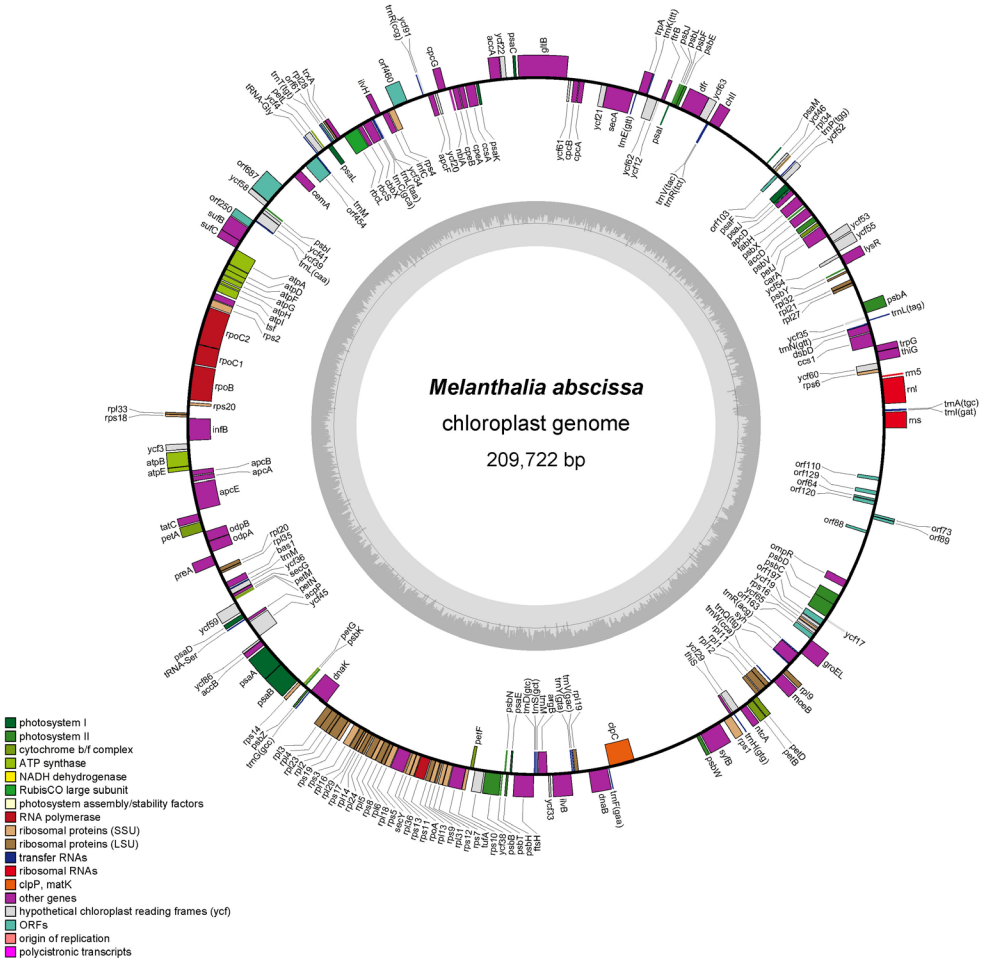

Figure 1: Map of the plastid genome of *Melanthalia abscissa*. Positioning on the ring indicates transcription direction. Genes on the outside of the ring are transcribed in the forward direction while genes inside are transcribed in the reverse direction. The inner ring shows GC-content.

A mitochondrial genome 26,057 base pairs long with a GC content of 30.1% was assembled (Fig. 2). The average depth of coverage for the assembly is 75. The mitochondrial genome contains 24 protein-coding genes, one 537 base- pair ORF, and 20 tRNAs. No SSRs were found by MISA in the *M. abscissa* mitochondrial genome. Starting from the *trn*A gene, all genes occur on the forward strand until the *trn*L gene around the midpoint of the genome. All genes between *cob* and *trn*N occur on the reverse strand. The mitochondrial genome of *M. abscissa* is published on GenBank under the accession number PP335805.

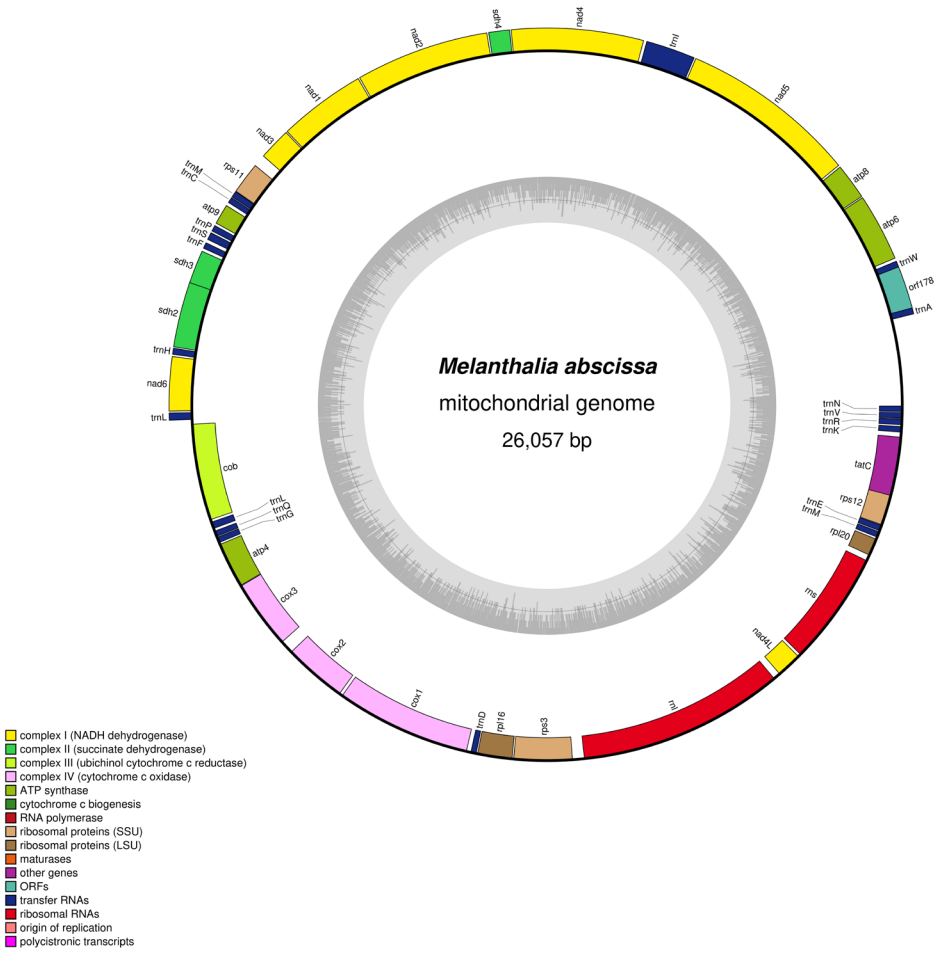

Figure 2: Map of the mitochondrial genome of *Melanthalia abscissa*. Positioning on the ring indicates transcription direction. Genes on the outside of the ring are transcribed in the forward direction while genes inside are transcribed in the reverse direction. The inner ring shows GC-content.

The *P. polyideoides* plastome comprises 201,550 base pairs, 192 protein coding genes, and 29, tRNA sequences, three rRNA sequences, and 17 ORFs (Fig. 3). The average depth of coverage for the assembly is 230. Like *M. abscissa*, a few identified genes have overlapping regions, including *rpl*24 with *rpl*14, *rpl*23 with *rpl*4, *trp*G with *ccs*1, and *psb*D with *psb*C. Protein coding regions account for 74.2% of the plastome.

Five SSRs were located in the plastid genome of *Polyopes polyideoides*. A monomeric repeat of A was found within the *rps*1 gene at positions 179,843 to 179,852. Monomeric repeats of T were found at positions 146,373 to 146,382 within the *rpl*21 gene as well as 195,608 to 195,617 at the end of *ycf*33. A dinucleotide repeat of AT is present at positions 185,213 to 185,224, and a dinucleotide repeat of TA from 108,513 to 108,524.

No large inverted repeats were found. However, ORFs 122 and 121 at the beginning of the plastome are identical for the first 313 base pairs. The ORFs are 377 and 366 base pairs in length respectively. Sparse rearrangements between *P. polyideoides* and the rest of Halymeniales are seen in the Mauve alignment. The plastid genome of *P. polyideoides* is published on GenBank under the accession number PP338773.

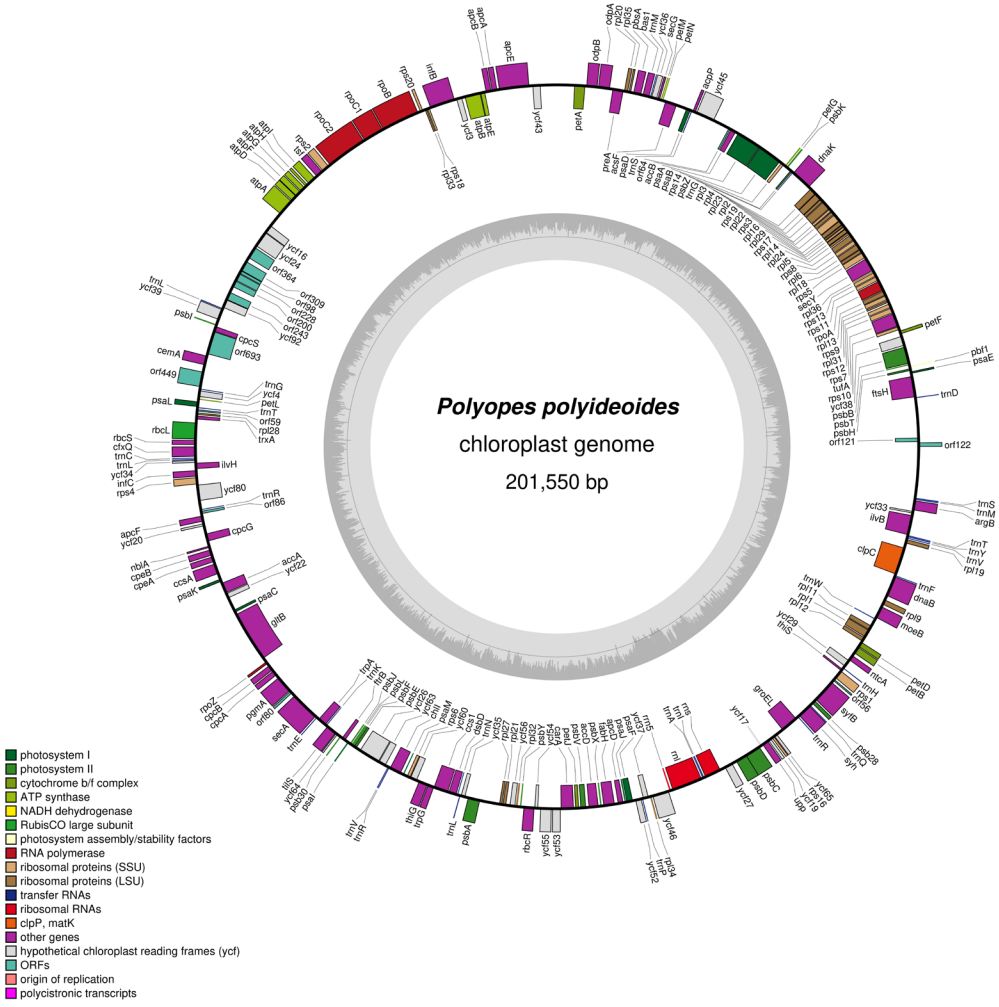

Figure 3: Map of the plastid genome of *Polyopes polyideoides*. Positioning on the ring indicates transcription direction. Genes on the outside of the ring are transcribed in the forward direction while genes inside are transcribed in the reverse direction. The inner ring shows GC-content.

The *P. polyideoides* mitochondrial genome is 26,499 base pairs long and has a GC-content of 30.7% (Fig. 4). The average depth of coverage for the assembly is 174. Three SSRs were identified in the mitochondrial genome of *P. polyideoides*. None of them were in coding regions. A trinucleotide of AGT is present from positions 26,071 to 26,085. Dinucleotide repeats of TA and AT can be found at positions 26,162 to 26,175 and 26,221 and 26,238, respectively.

Like the *M. abscissa* mitochondrial genome, 24 protein-coding genes and one ORF are present. However, four additional tRNAs were found, for a total of 24. The gene order and direction are the same as seen in the mitochondrial genome of *M. abscissa*. The mitochondrial genome of *P. polyideoides* is published on GenBank under the accession number PP338774.

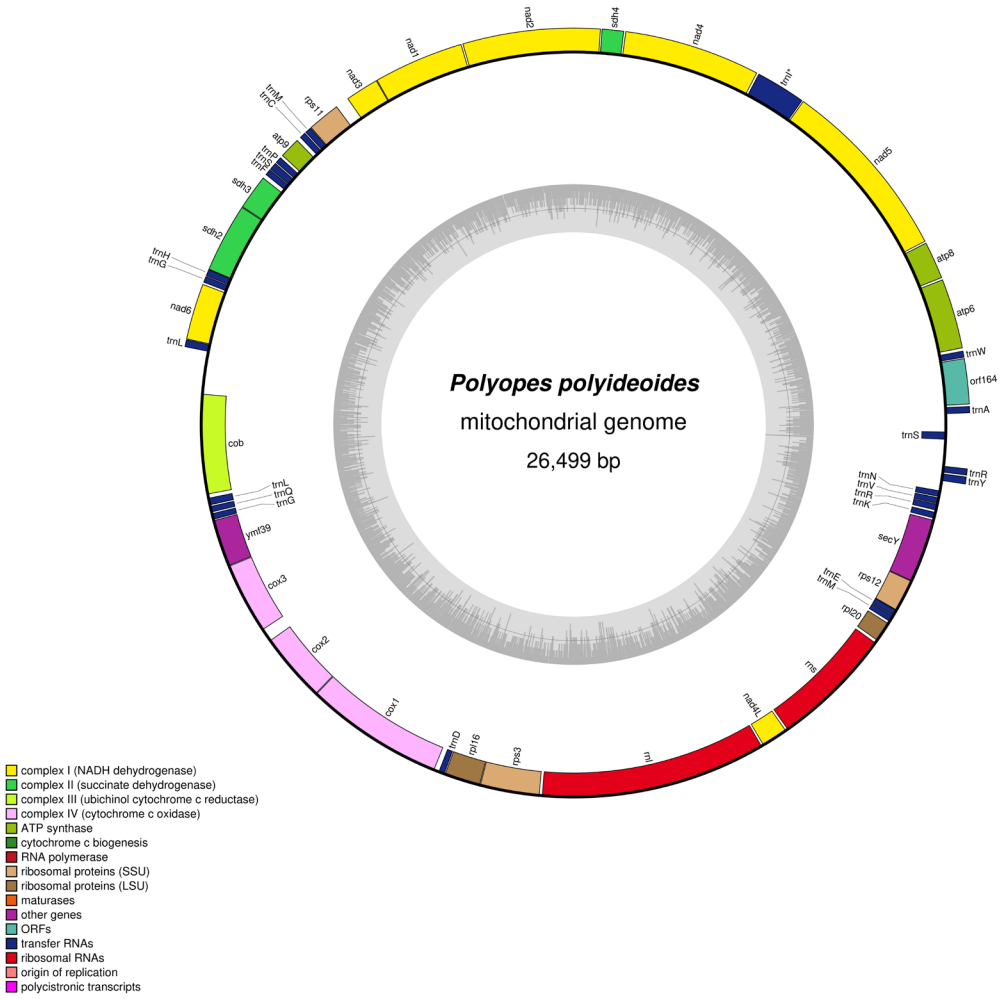

Figure 4: Map of the mitochondrial genome of *Polyopes polyideoides*. Positioning on the ring indicates transcription direction. Genes on the outside of the ring are transcribed in the forward direction while genes inside are transcribed in the reverse direction. The inner ring shows GC-content.

The phylogenetic analysis placed the novel *M. abscissa* plastome within *Melanthalia* alongside the sister species *M. obtusata* with a bootstrap value of 100 (Fig. 5). All genera sampled from Gracilariales formed monophyletic clades. However, there are varying degrees of support for these nodes.

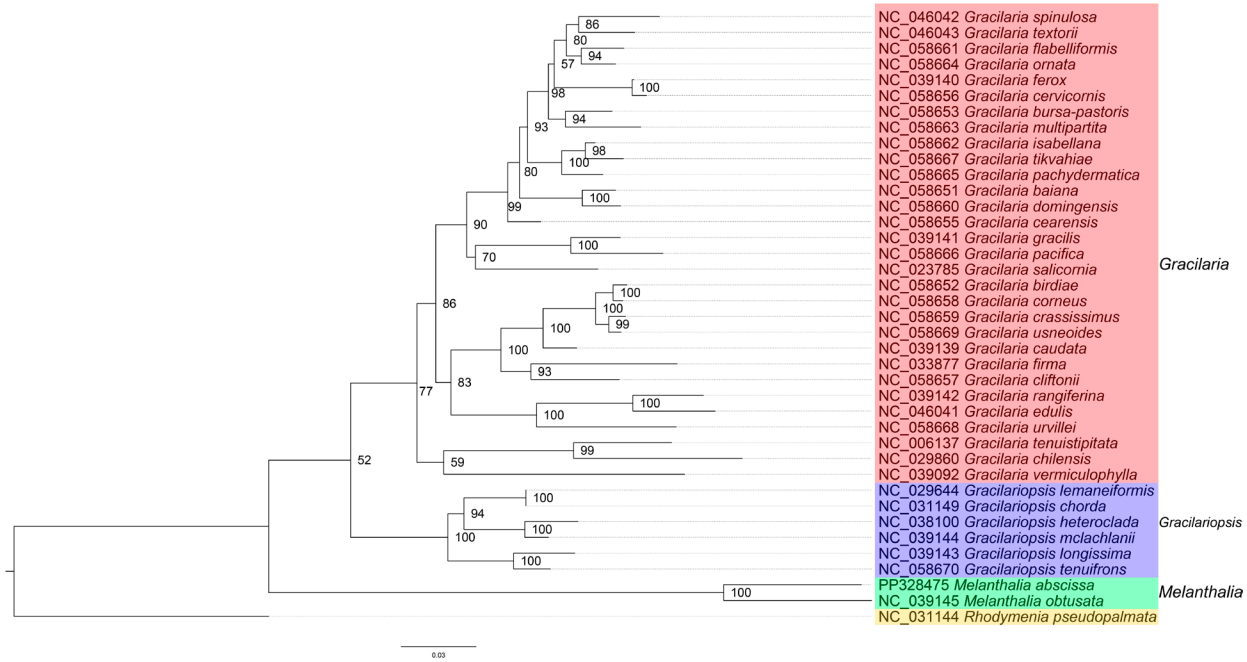

Figure 5: Maximum likelihood *rbc*L phylogeny of Gracilariales. Node values are based on 10,000 bootstrap replicates. Colors on the right are based on current genus classification.

Due to the sparse number of complete plastid genome assemblies from Halymeniales on RefSeq, full-length *rbc*L sequences could not be used to construct a useful phylogeny in the same manner as Gracilariales. A larger phylogeny with 31 taxa and an outgroup, was able to be constructed, but with a widely varying support (Fig. 6). *Halymenia floridana* was placed in a well-supported clade with members of the genus Cryptonemia rather than with the other member of *Halymenia* in the phylogeny, *H. maculata*.

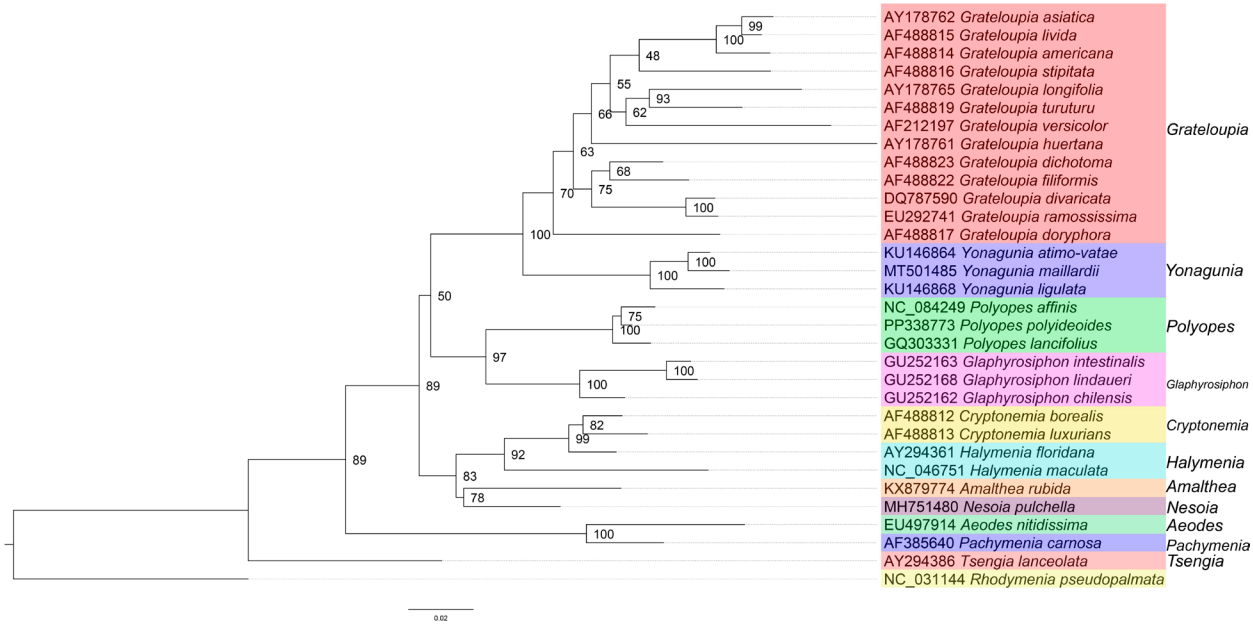

Figure 6: Maximum likelihood *rbc*L phylogeny of Halymeniales. Node values are based on 10,000 bootstrap replicates. Colors on the right are based on current genus classification.

Large scale rearrangements were examined using Mauve alignments of complete plastid genomes from representatives of each genus from Gracilariales and Halymeniales with plastomes available on RefSeq. Halymeniales plastid genomes are strongly conserved in order. Most of each plastome is contained within one locally collinear block (LCB), with no further rearrangements (Fig. 7). Smaller LCBs are present at the ends of the genomes. This contrasts the rearrangement observed in the Gracilariales alignment, particularly in the genus *Melanthalia* (Fig. 8). White space within the LCBs signifies regions unique to that genome compared to the others in the alignment.

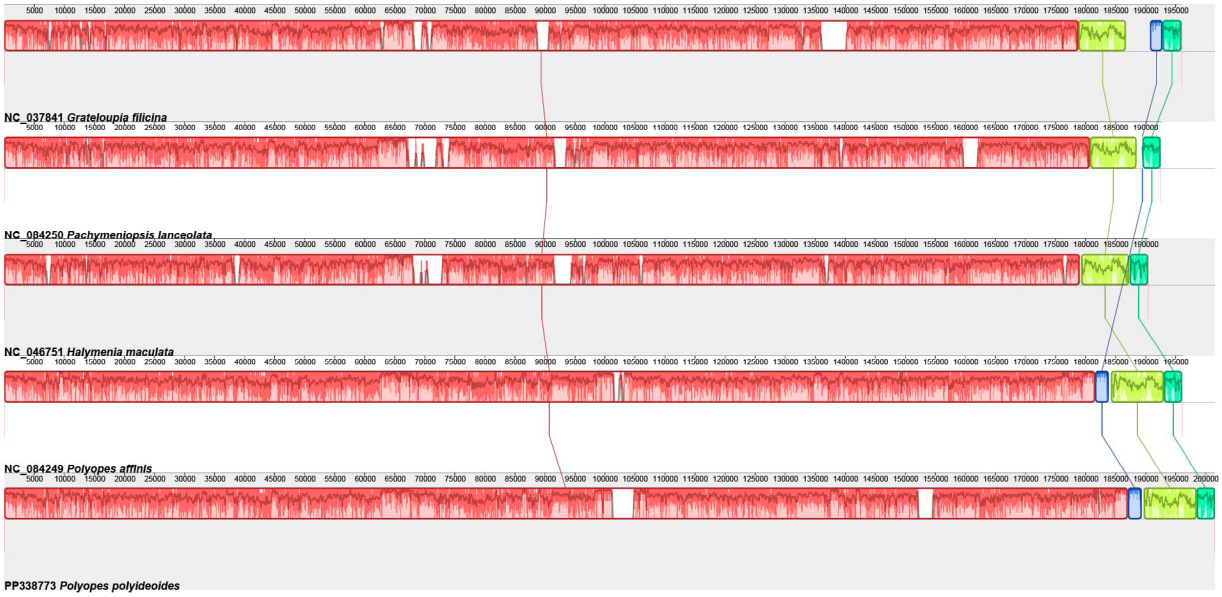

Figure 7: Progressive Mauve alignment of select representatives of Halymeniales. Colored blocks represent homologous, collinear blocks. White regions represent unique sequences.

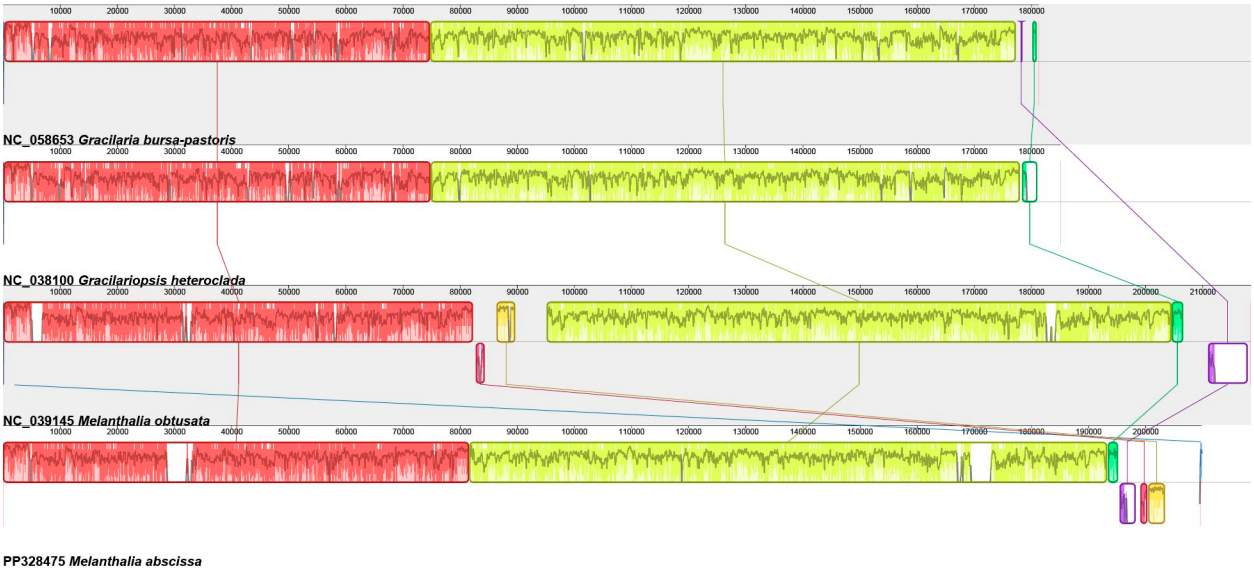

Figure 8: Progressive Mauve alignment of select representatives of Gracilariales. Colored blocks represent homologous, collinear blocks. White regions represent unique sequences.

BLASTn searches show the plasmid GL3.5 from *Gracilaria lemaneiformis* (Goff & Coleman 1990), matching short sequences of the *M. abscissa* plastome in three separate locations, within these unique regions (positions 30,463 to 31,076, 199,377 to 199,608, and 202,884 to 203,362). Two of these locations contain ORFs (orf88 and orf129). Similarity to the plasmid Gve4548 from *Gracilaria vermiculophylla* was also found in one sequence (positions 205,209 to 205,381).

The ORFs *orf*88, *orf*89, *orf*73, *orf*120, *orf*64, and *orf*129 return a similarity to 'plasmid- derived' ORFs in *Melanthalia obtusata* in BLASTp searches of the RefSeq database, but do not match any plasmid genes directly (Table 1). No plasmid matches were found in *P. polyideoides*.

Query Name	Subject Name	Subject Accession	Subject Length	Query Cover	E-Value	Percent Identity
orf88	*orf1202*	YP_009511647.1	174 aa	79%	2.00E-29	74.29%
orf89	*orf212*	YP_009511649.1	212 aa	97%	6.00E-36	77.01%
orf120	*orf328*	YP_009511648.1	328 aa	76%	5.00E-42	80.43%
orf64	*orf328*	YP_009511648.1	328 aa	93%	3.00E-27	91.67%
orf129	*orf159*	YP_009511650.1	159 aa	82%	3.00E-55	78.30%

Table 1: Top BLASTp matches of potentially plasmid-derived ORFs in *Melanthalia abscissa*. The standard non-redundant protein sequences database was used. All subjects are from the *Melanthalia obtusata* plastid genome.

DISCUSSION

The 209,722 base pair length of the *M. abscissa* plastid genome makes it the second longest within the Florideophyceae sequenced to date, just behind *M. obtusata*. Their plastomes are also the most GC-rich plastomes of the Gracilariales. *M. abscissa* has the highest GC-content of the order at 32.2%. With a length of 201,550, *P. polyideoides* is the third longest within the class, and 5,377 base-pairs longer than the other published member of its genus *P. affinis*. Neither plastome was found to possess inverted repeats. This is consistent with other published members of *Melanthalia* and *Polyopes*.

Microsatellites, or simple sequence repeats (SSRs) in the chloroplasts of red algae have been used to distinguish between individuals from different geographical locations (Song *et al.* 2014). SSRs are also useful for diversity, gene flow, and evolutionary studies. (Vieira *et al.* 2016). Both of the SSRs identified in the *M. abscissa* plastid genome are located within the coding regions of genes. The trinucleotide repeat found in the *psb*A gene of *M. abscissa* results in a repeat of alanine in the translation. This repeat is also found in the same gene of the *M. obtusata* plastid genome. The monomeric repeat within the *rpl*21 gene of *P. polyideoides* can also be found within the same gene in the plastid genome of *P. affinis*. The retention of these repeats across different species may be functionally significant, as mutation rates of tandem repeats is typically much higher than the rest of the genome (Gemayel *et al.* 2012).

Phylogenetic analysis confirms the positions of our samples of *M. abscissa* and *P. polyideoides* within the genera *Melanthalia* and *Polyopes*, respectively. All genera represented in the Gracilariales phylogeny are monophyletic (Fig. 5). However, not all families of Halymeniales represented were recovered as monophyletic (Fig. 6). Halymeniaceae excludes *Yonagunia* S. Kawaguchi & M. Masuda and *Grateloupia* C. Agardh, which belong to Grateloupiaceae though they share a common ancestor with *Polyopes* and *Glaphyrosiphon* Hommersand &

Leister, members of Halymeniaceae. Both *Yonagunia* and *Grateloupia* were previously considered members of Halymeniaceae until the resurrection of the family Grateloupiaceae was supported by an extensive, multigene phylogeny from 47 taxa (Kim *et al.* 2021). The resurrection of Grateloupiaceae is controversial and disputed on a morphological basis (Nguyen *et al.* 2023). Further studies are needed to clarify their systematic position.

Halymenia C. Agardh was the only genus found as non-monophyletic in our phylogeny. *Halymenia floridana* J. Agardh was placed with strong bootstrap support within *Cryptonemia* J. Agardh rather than with the other member of *Halymenia, H. maculata* J. Agardh. This is not surprising since extensive *rbc*L phylogenies have shown both *Cryptonemia* and *Halymenia* to be polyphyletic (Rodríguez-Prieto *et al.* 2018). Specimens of *H. floridana* from Brazil do not exhibit the summer seasonality or subtidal habitat of other species of *Halymenia*, but rather the all-season presence and intertidal habitat of *Cryptonemia* (Azvedo *et al.* 2016). Furthermore, other *rbc*L phylogenies have placed it within *Cryptonemia* (Azvedo *et al.* 2018, Rodríguez-Prieto *et al.* 2018). Despite the support for the transfer of *H. floridana* to *Cryptonemia*, it remains a member of *Halymenia* due to a lack of analysis of specimens from Florida, the type locality (Schneider *et al.* 2018). The specimen included in our phylogeny presented here is from Brazil, which may not be the same species as the true *H. floridana*.

Prior to this study, only one complete plastid genome from *Polyopes* was available on GenBank. The represented members of the genus in the phylogeny presented here form a monophyletic genus. The *rbc*L sequence from our sample of *P. polyideoides* was placed as a sister clade to *P. affinis*, with *P. lancifolius* diverging earlier. The intra-ordinal relationships of Halymeniales remain uncertain, with several genera such as *Halymenia* and *Grateloupia* not appearing as monophyletic in *rbc*L or multigene phylogenies (Kim *et al.* 2021).

No LCB rearrangement occurred between the plastomes of the two members of *Polyopes* (Fig. 8). This was not the case in *Melanthalia*; where two LCBs are relocated, one of which is reversed. *M. abscissa* and *M. obtusata* also exhibit more regions unique to their plastome compared to the other members of Gracilariles (Fig. 7). Unique regions appear more commonly in the plastomes of Halymeniales. In Halymeniales, many of these regions lacking similarity appear in the same approximate position in the plastome. These regions may be the result of plasmid insertions (Ng *et al.* 2017). Plasmids that originate and self-replicate within red algae have been observed in other Florideophycean plastid genomes (Iha *et al.* 2018, Ng *et al.* 2017). An early characterization of plasmids in the genome of *Gracilaria* did not find any exchange between plasmids and the nuclear or organellar genomes (Goff & Coleman 1990). However, more recent studies have found evidence for the movement of DNA from red algal plasmids to mitochondrial and plastid genomes (Lee *et al.* 2016).

Plasmid-derived sequences (PDS) appear to be present in the plastid genome of *M. abscissa*. Short, nucleotide sequences that matched circular plasmids from other members of Gracilariales were found in the regions of the genome that are unique according to the Mauve alignment. Some of these locations contain ORFs, and multiple ORFs were found to have similarities to ORFs designated as 'plasmid-derived' in the plastid genome of *Melanthalia obtusata*. These sites are potentially the result of horizontal gene transfer. However, no direct matches to plasmid ORFs or plasmid protein coding genes were found in the plastome. No sequences were found to match plasmids in the plastome of *P. polyideoides*. This may be due to genuine absence of PDS, or lack of reference data. No plasmids from Halymeniales have been published on GenBank to date, compared to the eight published from Gracilariales.

Gene order and direction are identical in the *Melanthalia abscissa* and *Polyopes polyideoides* mitochondrial genomes. The split directionality of these mitochondrial genomes is commonly seen in other mitogenomes of Florideophyceae (Iha *et al.* 2018). Possible reasons for this occurrence do not appear well documented and warrant further investigation.

CONCLUSIONS

The subfamily Melanthalioideae that contains *Melanthalia* was determined to be the sister group to the rest of Gracilariaceae based on *rbc*L phylogenies and morphological data (Gurgel *et al.* 2018). As found in this study, the genus *Melanthalia* has characteristically large and GC-rich plastid genomes compared to the rest of Gracilariales. Sequence data for the other genus belonging to Melanthalioideae, *Curdiea* is very limited. No complete plastid genomes have been published to date. Mauve alignments also show more rearrangement between *Melanthalia* plastid genomes and the other representatives of the order. The early divergence from the rest of Gracilariales, and their limited geographic range may contribute to these unique characteristics.

Limited plastid sequence data for Halymeniales makes drawing conclusions for *Polyopes* more

problematic. However, rearrangements between members of the order seem to occur less frequently than in Gracilariales. The contribution of more organellar genomes and plasmid sequences is needed for a more comprehensive analysis of this order. The data presented here adds to the limited knowledge base of both *Melanthalia* and *Polyopes*, as well as their respective ordinal status.

DATA AVAILABILITY

Organellar genomes are published on GenBank under the accession numbers, PP328475, PP335805, PP338773, and PP338774. The voucher specimen for *Melanthalia abscissa* is located at the Te Papa Tongarewa Museum of New Zealand Herbarium in Wellington, New Zealand with the voucher number WELT A024150. DNA from this specimen stored at The University of Alabama PhycoLab under the number UA 816. DNA for *P. polyideoides* is stored under the number UA 733.

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CRÉDITO DE FOTO DE LA PORTADA

Núcleos del hospedero (dinocarión, cromosomas condensados) y del endosimbionte de *Durinskia báltica* (Levander) Carty et Cox. Microscopía confocal con epifluorescencia teñida con DAPI. Lago de Xochimilco. Microvideo de Edgar Jiménez Díaz (UNICUA) Fac. Ciencias, UNAM. Foto fija publicada en Lira et al. 2023. Botanical Sciences 101 (4): 1102-1114. DOI: 10.17129/botsci.3259. Agradecemos a *Botanical Sciences* y a su editora, Dra. Teresa Terrazas, por el permiso para publicar esta versión en video.