



Research Article

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Novel rearrangements in the mitochondrial genomes of the Ceramiales (Rhodophyta) and evolutionary implications

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The Ceramiales is the most diverse and species-rich group (2,669 spp.) of red algae, and it is widely distributed from tropical to polar oceans. Mitochondrial genomes (mitogenomes) and other genes have contributed to our knowledge regarding the classification and phylogeny of this diverse red algal group; however, the mitogenome architecture remains understudied. Here, we compared 42 mitogenomes, including 19 newly generated in this study, to expand our knowledge. The number of genes in mitogenome varied from 43 to 68 due to gene duplication. The mitogenome architecture was also variable, categorized into four types (A-D): type A = ancestral type with a basic composition; type B = those with inverse transpositions; type C = those with inverted duplications; and type D = those with both inversion and duplication. The palindromic and inverted repeats were consistently found in flanking regions of the rearrangement, especially near the *cob* and *nad6* genes. The three rearranged mitogenome architectures (types B, C, D) are the first report of these in red algae. Phylogenetic analyses of 23 protein-coding genes supported the current familial classification of the Ceramiales, implying that the diversity of mitogenome architecture preceded the phylogenetic relationships. Our study suggests that palindromic and inverted repeats may drive mitogenome architectural variation.

Keywords: genome rearrangement; mitogenome architecture; molecular evolution; palindromic repeat; red algae

INTRODUCTION

The order Ceramiales is the largest and most diverse group of red algae representing 2,669 species as listed in a global database (Guiry and Guiry 2023), and this makes the group interesting with regard to its evolutionary history and diversification. The Ceramiales is generally circumscribed by the uniaxial structure of thallus, the presence of periaxial cells, a non-motile male spermatium, a female reproductive structure with a post-fertilization process by the auxiliary cell formation directly from the

supporting cell and a triphasic life history (Kylin 1956, Hommersand 1963, Huisman 2018). Molecular studies have greatly expanded our knowledge of the diversity and phylogeny of the Ceramiales (Lin et al. 2001, Choi et al. 2002, 2008, Díaz-Tapia et al. 2017, Barros-Barreto et al. 2023). For many years, the Ceramiales employed a four-family scheme: Ceramiaceae, Dasyaceae, Delesseriaceae, and Rhodomelaceae (Hommersand 1963, Maggs and Hommersand 1993, Womersley 1998). Recently, using



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plastid genome data from 80 representative species, Díaz-Tapia et al. (2019) proposed the five-family system: Callithamniaceae, Ceramiaceae, Delesseriaceae (including Dasyaceae), Rhodomelaceae, and Wrangeliaceae (segregated from the early diverging Ceramiaceae).

The mitochondrial genome (hereafter mitogenome) of red algae includes fast evolving genes in a circular, maternally inherited molecule (Yang et al. 2015, Salomaki and Lane 2017). A previous study reported the rapid radiation and surprisingly high conservation of mitochondrial gene synteny among the morphologically divergent multicellular lineages of the Rhodymeniophycidae, a subclass of red algae including the Ceramiales (Yang et al. 2015). Mitogenomes and individual mitochondrial genes have greatly resolved phylogenetic relationships and provided insights into the diversification and its evolutionary histories of red algae (e.g., Hughey et al. 2014, Boo et al. 2016, 2020, Salomaki and Lane 2017, Iha et al. 2018).

Previous studies have investigated mitogenome architecture in other groups of cyanidiophycan and florideophycidean red algae (Lee et al. 2015, 2018, Yang et al. 2015, Cho et al. 2020). In this study, we generated 19 mitogenomes from representative taxa of the order Ceramiales. Specifically, we completed nine mitogenomes for the Ceramiaceae, two each in the Delesseriaceae and Wrangeliaceae, and six in the Rhodomelaceae; however, we were not able to add any taxa from the Callithamniaceae. We focused on the variation of mitogenome architecture from 42 representatives including 23 published mitogenome data in the Ceramiales. From this study, we found that there are four types of mitogenome structure that highlight our knowledge of evolutionary dynamics of the Ceramiales as well as red algae.

MATERIALS AND METHODS

Sample preparation and DNA extraction

Seventeen culture strains of the Ceramiales were obtained from the National Marine Biodiversity Institute of Korea (MABIK). The strains were maintained in the laboratory using L1 enriched seawater medium at 20°C with a 14 : 10 light / dark cycle. Culture tissue (~30 mg) was frozen in liquid nitrogen and ground using Automill TK-AM5 (Tokken Inc., Chiba, Japan). Total genomic DNA was extracted with a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). The pre-heated (65°C) CTAB mixture (CTAB 750 µL, 1% 2-mercaptoethanol 8 µL, and 20 mg mL⁻¹ proteinase K 50 µL) was

added to ground tissue and incubated for 10 min at 65°C, followed by a 5 min incubation at room temperature with RNase A (1% v/v). Phenol : chloroform : isoamyl alcohol (25 : 24 : 1) was added in volume equal to sample and centrifuged for 10 min at 14,000 rpm. The supernatant was transferred to a new tube, mixed with 600 µL of chloroform and centrifuged under the same condition. DNA precipitation was achieved by adding 500–600 µL of pre-cooled isopropanol and a volume of 3 M sodium acetate equal to 1/10 of the suspension, followed by incubation at –20°C for 30 min. The supernatant was decanted after centrifugation at 14,000 rpm for 20 min at 4°C. The pellet was washed twice with 1 mL of 70% ethanol, each time inverted and centrifuged at 14,000 rpm for 15 min at 4°C. The final pellet was air-dried and suspended in 50–100 µL of D.W. The quantity and quality of DNA were assessed using the NanoDrop (NABI micro digital, Seongnam, Korea) and Qubit 2.0 fluorometer (Thermo Scientific, Waltham, MA, USA). The concentration of DNA varied in the range of 8–180.8 ng µL⁻¹. The 260/280 absorbance ratios were within the optimal range of 1.9 to 2.1. However, the 260/230 absorbance ratios showed a wider range of 0.97–2.32.

Whole genome sequencing, assembly, and annotation

Whole genome sequencing was conducted using Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) by DNA-link Inc. (Seoul, Korea). The pair-end sequencing library was prepared using the TruSeq Nano DNA Prep Kit (Illumina), which generated 10 Gb for each taxon. We obtained additional previously generated Illumina read data for *Chondria armata* (Kützinger) Okamura (SRR15927350) and *Digenea simplex* (Wulfen) C. Agardh (SRR8325636), which were retrieved from the NCBI Sequence Read Archive (SRA). The adapter and low-quality reads were removed using Trimmomatic v0.39 (Bolger et al. 2014) with the following parameters: ILLUMINACLIP: TruSeq3-PE.fa: 2: 30: 10: 2: True LEADING: 3 TRAILING: 3 MINLEN: 36.

The mitogenome was assembled using GetOrganelle v1.7.7.0 (Jin et al. 2020) and NOVOPlasty v4.3.1 (Dierckxsens et al. 2017). Genome assemblies obtained with GetOrganelle were manually inspected using Bandage (Wick et al. 2015). The Illumina reads were mapped to the assembled mitogenomes using Bowtie2 (Langmead and Salzberg 2012) for validation and error correction. Primary annotation was performed automatically using MFannot (Beck and Lang 2010). Transfer RNA (tRNA)

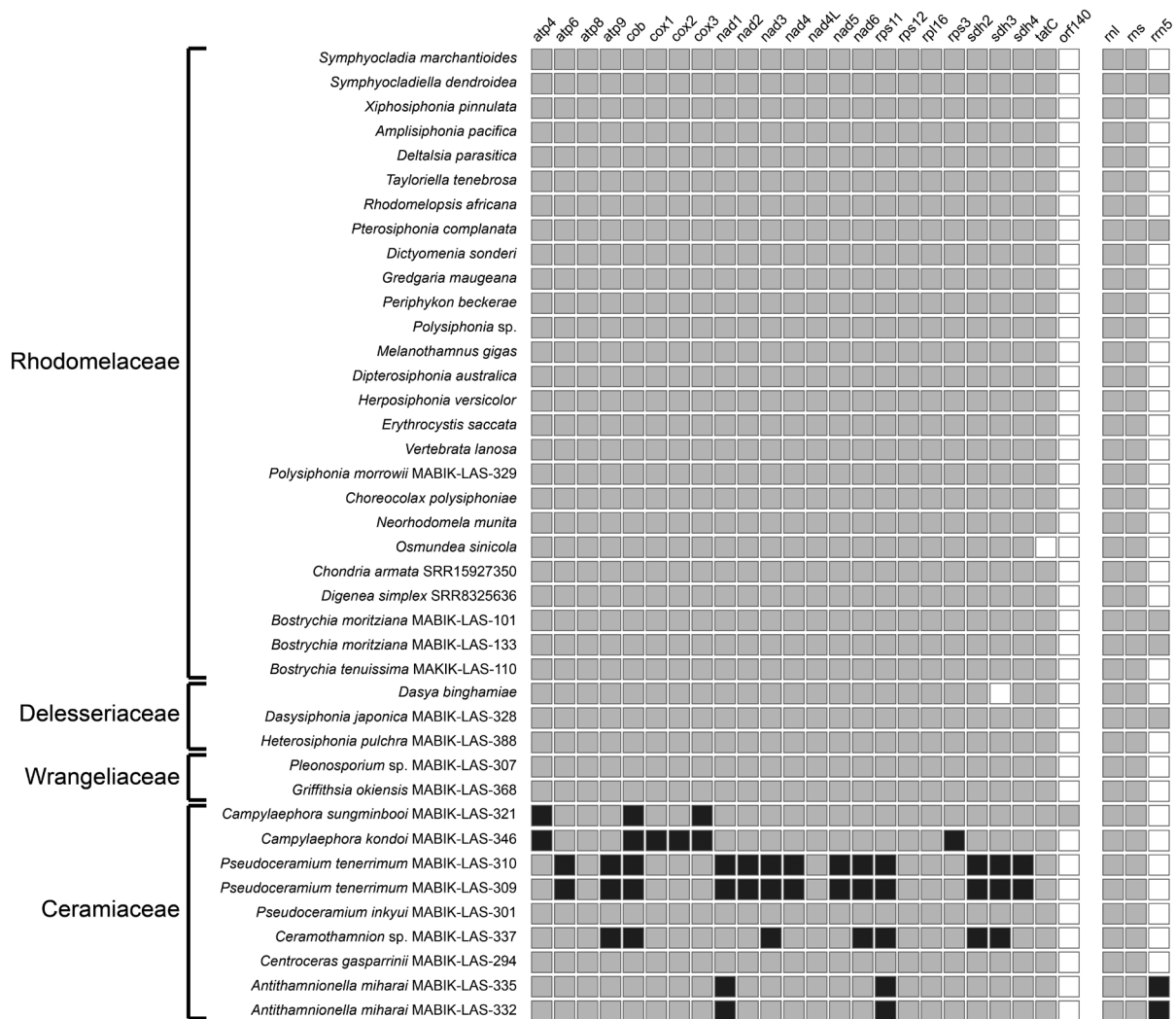


Fig. 1. Presence and absence of mitochondrial protein-coding genes and ribosomal RNAs in the Ceramiales. The order of species follows the phylogeny in Fig. 2. Grey: present, black: 2 copies, white: absent.

genes were verified using tRNAscan-SE v2.0 (Lowe and Chan 2016). Other possible open reading frames in the intergenic region were searched by using BLASTx. All the predicted genes were confirmed manually by using Geneious Prime 2020.2.4 (<https://www.geneious.com>) and BLASTp (e-value, $1e-05$). Inverted repeats were identified using Palindrome analyzer (Brázda et al. 2016). Palindrome analyzer describes the features of palindromic and inverted repeats through similarity, localization, and visualization. Long repeat sequences were analyzed using the Repeat Finder in Geneious Prime with minimum length of 20 bp and 5% of mismatch. The mitochondrial genome sequences have been deposited at GenBank under the accession numbers OR885323–OR885339, BK064963, and BK064964.

Phylogenetic analysis

We collected a total of 42 mitogenomes, including 19 newly generated in this study and 23 from previously published genomes (Table 1). *Rhodymenia pseudopal-mata* (J.V.Lamouroux) P.C.Silva (KC875852) and *Schimmelmannia schousboei* (J.Agardh) J.Agardh (KJ398162) were selected as outgroup. Nucleotide sequences of 23 protein-coding genes (PCGs) were aligned with MACSE v2 (Ranwez et al. 2018) and manually adjusted. The ambiguous region was trimmed with GBLOCKS v0.19b (Castresana 2000) under a 50% gap threshold. Maximum likelihood analysis was performed using IQ-Tree2 v2.0.6 (Minh et al. 2020) with 1,000 ultrafast bootstrap replications under the GTR + F + I + R5 model. Phylogenetic trees

Table 1. Sample list used in phylogenomic analyses including published mitochondrial genomes

Family	Tribe	Species	Culture code	Collection site; latitude, longitude	GenBank accession No.	Reference
Ceramiales	Anthamnieae	<i>Anthamnieella miharai</i>	MABIK-LAS-332	Daecheon beach, Boryeong, Korea; 36°17'57.69" N, 126°31'00.33" E	OR885323	In this study
		<i>Anthamnieella miharai</i>	MABIK-LAS-335	Daecheonhang, Boryeong, Korea; 36°19'30.46" N, 126°29'58.88" E	OR885324	In this study
	Ceramieae	<i>Campylaeophora kondoi</i>	MABIK-LAS-346	Euihang-Beach, Taean, Korea; 36°50'33.39" N, 126°10'19.72" E	OR885328	In this study
		<i>Campylaeophora sungminbooi</i>	MABIK-LAS-321	Jangpyeongri, Tongyeong, Korea; 34°52'20.14" N, 128°28'02.80" E	OR885336	In this study
		<i>Campylaeophora sungminbooi</i>	-	Egerslev Røn, Limfjorden, Denmark	KU145004	Hughey and Boo (2016)
		<i>Centroceras gasparrinii</i>	MABIK-LAS-294	Guryongpo Beach, Pohang, Korea; 35°59'42.21" N, 129°33'59.79" E	OR885329	In this study
		<i>Ceramothenion japonicum</i>	-	Gijang, Busan, Korea	KI398159	Yang et al. (2015)
		<i>Ceramothenion</i> sp.	MABIK-LAS-337	Dumunpo, Dolsando(east), Yeosu, Korea; 34°38'43.51" N, 127°47'50.55" E	OR885337	In this study
		<i>Pseudocerarium inkyui</i>	MABIK-LAS-301	Janggilri, Pohang, Korea; 35°56'59.02" N, 129°32'33.82" E	OR885335	In this study
		<i>Pseudocerarium tenerrimum</i>	MABIK-LAS-309	Myeongsasipri, Wando, Korea; 34°19'31.20" N, 126°48'15.60" E	OR885338	In this study
		<i>Pseudocerarium tenerrimum</i>	MABIK-LAS-310	Myeongsasipri, Wando, Korea; 34°19'31.20" N, 126°48'15.60" E	OR885339	In this study
		<i>Dasya binghamiae</i>	-	Haida Gwaii, British Columbia, Canada	KX247283	Tamayo and Hughey (2016)
Delesseriaceae	-	<i>Dasya binghamiae</i>	-	Haida Gwaii, British Columbia, Canada	KX247283	Tamayo and Hughey (2016)
Rhodomeleaceae	-	<i>Dasydiphonia japonica</i>	MABIK-LAS-328	Songjeong, Gijang, Busan, Korea; 35°10'32.30" N, 129°11'52.08" E	OR885330	In this study
		<i>Heterosiphonia pulchra</i>	MABIK-LAS-388	Woohak-ri, Geumdo, Yeosu, Korea; 34°30.43' N, 127°46.30' E	OR885332	In this study
	-	<i>Amplisiphonia pacifica</i>	-	Haida Gwaii, British Columbia, Canada	OP748284	Díaz-Tapia et al. (2023)
		<i>Deltalsia parasitica</i>	-	Skomer Island, Wales, UK	OP748283	Díaz-Tapia et al. (2023)
		<i>Rhodomeleopsis africana</i>	-	Shelley Beach, South Africa	OP748274	Díaz-Tapia et al. (2023)
		<i>Tayloriella tenebrosa</i>	-	Hernanaus, South Africa	OP748271	Díaz-Tapia et al. (2023)
	Alsidieae	<i>Digenea simplex</i>	SRR8325636	Onna, Okinawa Prefecture, Japan; 26°29'39.90" N, 127°50'22.90" E	BK064964	In this study
	Bostrychieae	<i>Bostrychia moritziana</i>	MABIK-LAS-101	Tooradin, Western Port Bay, VIC, Australia; 38°14'02.10" S, 145°24'43.30" E	OR885325	In this study
	Bostrychieae	<i>Bostrychia moritziana</i>	MABIK-LAS-133	Tempusak (near Kota Belud), Sabah, Malaysia; 6°23'12.35" N, 16°20'49.71" E	OR885326	In this study
	Bostrychieae	<i>Bostrychia tenuissima</i>	MAKIK-LAS-110	Broughton Ck., NSW, Australia; 34°46'30.12" S, 150°49'06.22" E	OR885327	In this study

Table 1. Continued

Family	Tribe	Species	Culture code	Collection site; latitude, longitude	GenBank accession No.	Reference
Rhodomelaceae	Chondrieae	<i>Chondria armata</i>	SRR15927350	Kyushu Island, Japan; 31°06'36.00" N, 130°18'04.09" E	BK064963	In this study
	Dipterosiphonieae	<i>Dipterosiphonia australica</i>	-	Killornei, Victoria, Australia	OP748281	Díaz-Tapia et al. (2023)
	Herposiphonieae	<i>Gredgarria maugiana</i>	-	The Rip, Victoria, Australia	OP748280	Díaz-Tapia et al. (2023)
	Herposiphonieae	<i>Herposiphonia versicolor</i>	-	Sant Leonards, Victoria, Australia	OP748279	Díaz-Tapia et al. (2023)
	Laurencieae	<i>Osmundea sinicola</i>	-	Eureka, near La Paz, Mexico	MH989940	Hughley and Miller (2021)
	Polysiphonieae	<i>Polysiphonia morrowii</i>	MABIK-LAS-329	Tappori, Geoje, Korea; 34°45'50.56" N, 128°35'59.69" E	OR885334	In this study
	Polysiphonieae	<i>Polysiphonia</i> sp.	-	Coral Bay, Western Australia, Australia	OP748276	Díaz-Tapia et al. (2023)
	Pterosiphonieae	<i>Dictyomenia sonderi</i>	-	Green Head, Western Australia, Australia	OP748282	Díaz-Tapia et al. (2023)
	Pterosiphonieae	<i>Periphykon beckeriae</i>	-	Barrow Island, Western Australia, Australia	OP748277	Díaz-Tapia et al. (2023)
	Pterosiphonieae	<i>Pterosiphonia complanata</i>	-	Ártabra, A Coruña, Spain	OP748275	Díaz-Tapia et al. (2023)
	Pterosiphonieae	<i>Symphycycladia marchantioides</i>	-	Praia do Populo, Azores, Portugal	OP748273	Díaz-Tapia et al. (2023)
	Pterosiphonieae	<i>Symphycycladia dendroidea</i>	-	-	OP748272	Díaz-Tapia et al. (2023)
	Pterosiphonieae	<i>Xiphosiphonia pinnulata</i>	-	Bastagueiro, A Coruña, Spain	OP748270	Díaz-Tapia et al. (2023)
	Rhodomeleae	<i>Choreocolax polysiphoniae</i>	-	Beavertail State Park, Jamestown, Rhode Island, USA	KX687877	Salomaki and Lane (2017)
	Rhodomeleae	<i>Neorhodomela munita</i>	-	Badaguan Area, Qingdao, Shandong Province, China	MW750196	Jiang et al. (2021)
	Strebloladiaceae	<i>Erythrocytis saccata</i>	-	Pebble Beach, California, USA	MW810348	Amos et al. (2021)
	Strebloladiaceae	<i>Melanothamnus gigas</i>	-	Barrow Island, Western Australia, Australia	OP748278	Díaz-Tapia et al. (2023)
	Strebloladiaceae	<i>Vertebrata lanosa</i>	-	Beavertail State Park, Jamestown, Rhode Island, USA	KX687880	Salomaki and Lane (2017)
Wrangeliaceae	Griffithsieae	<i>Griffithsia okiensis</i>	MABIK-LAS-368	Namae Beach, Yangyang, Korea; 37°56'46.81" N, 128°47'12.73" E	OR885331	In this study
	Spongoclonieae	<i>Pleorosprium</i> sp.	MABIK-LAS-307	Myeongsasipri, Wando, Korea; 34°19'31.20" N, 126°48'15.60" E	OR885333	In this study

Dash (-) indicates not available. Bold letter indicates newly analyzed sequences in this study.

were edited and visualized using FigTree v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>).

RESULTS

General feature of mitochondrial genomes

Nineteen newly assembled mitogenomes had an average coverage between 500× and 1,000×. We observed a general decrease in coverage, often to 300×, in palindromic regions. However, the lowest coverage in these regions was >100×, maintaining the integrity and reliability of our data (Supplementary Figs S1 & S2). Genome sizes and gene contents varied at the family and species level, ranging from 24,508 bp in *Campylaephora sungminbooi* (J.R.Hughey & G.H.Boo) Barros-Barreto & Maggs (KU145004) to 36,710 bp in *Pseudoceramium tenerrimum* (G.Martens) Barros-Barreto & Maggs (MABIK-LAS-310);

the GC content was $25.0 \pm 2.4\%$ (Table 2). Overall, the mitogenomes contained 43–68 genes, consisting of 21–36 PCGs, 18–33 tRNAs, and 2–4 rRNA subunits (Table 2). A core set of 23 PCGs were shared in all families, however, up to 36 PCGs were found in the family Ceramiaceae (Table 2, Fig. 1). Most of the Ceramiales species contained two copies of the ribosomal RNA operon, while, depending on the number of copies of the 5S rRNA, three to four rRNAs were found in five species: *Antithamnionella miharai* (Tokida) Itono (4 rRNAs), *Dasysiphonia japonica* (Yendo) S.-H.Kim (3 rRNAs), *Bostrychia moritziana* (Sonder ex Kützinger) J.Agardh (3 rRNAs), *Pterosiphonia complanata* (Clemente) Falkenberg (3 rRNAs), and *Symphocodiella dendroidea* (Montagne) Bustamante, B.Y.Won, S.C.Lindstrom & T.O.Cho (3 rRNAs). All 19 newly analyzed mitogenomes had a group II intron between the *nad5* and *nad4* genes, and *Pseudoceramium tenerrimum* contain two introns (Table 2).

Table 2. General features of the mitochondrial genomes used in the present study

Family	Species	Size (bp)	No. of total genes	Protein-coding gene	rRNAs	tRNAs	Group II intron	G + C (%)
Ceramiaceae	<i>Antithamnionella miharai</i> MABIK-LAS-332	26,796	55	25	4	26	1	28.3
	<i>Antithamnionella miharai</i> MABIK-LAS-335	27,190	58	25	4	29	1	28.3
	<i>Campylaephora kondoi</i> MABIK-LAS-346	31,430	57	29	2	26	1	30.2
	<i>Campylaephora sungminbooi</i> MABIK-LAS-321	27,664	55	27	2	26	1	29.3
	<i>Centroceras gasparrinii</i> MABIK-LAS-294	24,915	48	23	2	23	1	27.6
	<i>Ceramothamnion</i> sp. MABIK-LAS-337	29,876	65	30	2	33	1	26.9
	<i>Pseudoceramium inkyui</i> MABIK-LAS-301	25,516	48	23	2	23	1	28.6
	<i>Pseudoceramium tenerrimum</i> MABIK-LAS-309	36,678	68	36	2	30	2	28.5
	<i>Pseudoceramium tenerrimum</i> MABIK-LAS-310	36,710	68	36	2	30	2	28.5
	<i>Dasysiphonia japonica</i> MABIK-LAS-328	25,918	49	23	3	23	1	23
	<i>Heterosiphonia pulchra</i> MABIK-LAS-388	25,591	47	23	2	22	1	22
	<i>Bostrychia moritziana</i> MABIK-LAS-101	25,508	45	23	3	19	1	25.6
	<i>Bostrychia moritziana</i> MABIK-LAS-133	25,401	45	23	3	19	1	25.6
Rhodomelaceae	<i>Bostrychia tenuissima</i> MAKIK-LAS-110	25,423	45	23	2	20	1	27.9
	<i>Chondria armata</i> SRR15927350	25,853	49	23	2	24	1	22.6
	<i>Digenea simplex</i> SRR8325636	25,141	48	23	2	23	1	24
	<i>Polysiphonia morrowii</i> MABIK-LAS-329	25,235	47	23	2	22	1	23.9
	<i>Griffithsia okiensis</i> MABIK-LAS-368	24,865	46	23	2	21	1	22.1
Wrangeliaceae	<i>Pleonosporium</i> sp. MABIK-LAS-307	24,963	48	23	2	23	1	25.8

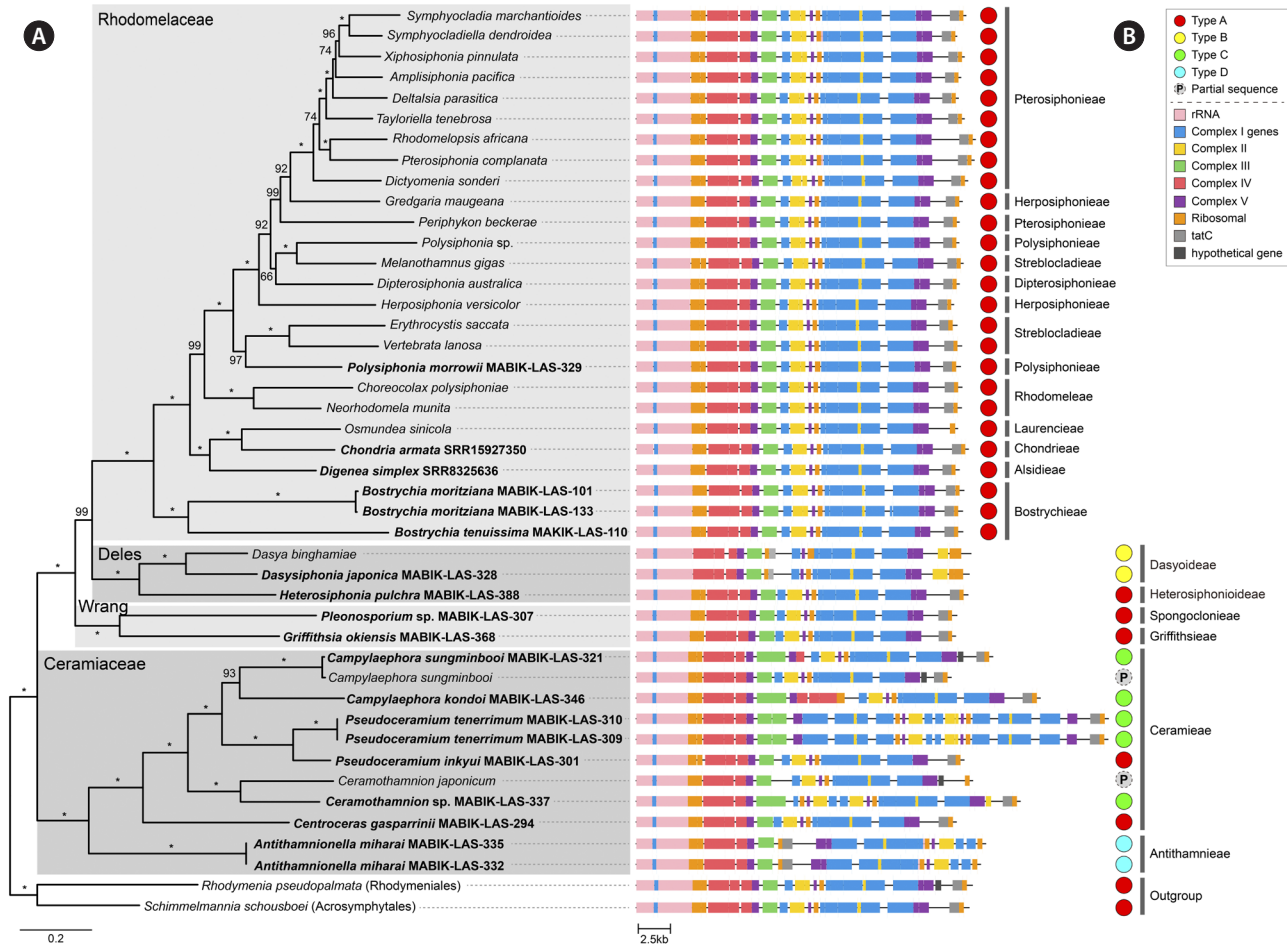


Fig. 2. (A) Maximum likelihood phylogeny of the Ceramiales based on nucleotide sequences of 23 protein-coding genes. Asterisk (*) indicates fully supported bootstrap value. Bold letter indicates newly generated sequences in this study. Deles, Delesseriaceae; Wrang, Wrangeliaceae. (B) Synteny of mitochondrial genome. Four types of mitochondrial structure and gene classification are colored as shown in the key.

Mitogenome phylogeny

Phylogenomic analysis resolved the relationships of families in the Ceramiales using a concatenated dataset of 23 PCGs (16,263 bp in alignment), excluding duplicated PCGs (Fig. 2A). The Ceramiales was monophyletic, including four families, which were fully supported. The Rhodomelaceae and Delesseriaceae formed a monophyletic clade, which was a sister to the Wrangeliaceae. The Ceramiaceae diverged first within the Celamiales, which was far related from the remaining families.

Genome rearrangement and expansion in the Ceramiales

We categorized four types of mitogenome architecture in the Ceramiales (Figs 2B & 3). Type A consisted of 23

PCGs, with 10 PCGs in the forward direction and 13 PCGs of the *atp6-nad6* region in reverse direction between the *cob* and *tatC* genes. Type A was found throughout the four families and the outgroup species, and here we refer to this as the putative ancestral type.

Type B mitogenomes were characterized by having inverse transpositions (Fig. 3A), as found in *Dasya binghamiae* A.J.K.Millar and *Dasysiphonia japonica*. The inverse transpositions occurred in three sets of paired-genes: *rps3-rpl16*, *sdh2-sdh3*, *tatC-rps12*. A deletion of *sdh3* gene was found in *Dasya binghamiae* only.

Type C mitogenomes had inverted duplications by repeats, where each pair of duplicated segments revealed a palindromic structure (Fig. 3B). The duplication was centered on either the 3' end of the *cob* or *nad6* genes, and the number of duplicated genes varied among species. Type C occurred in four species of the tribe Ceramiaceae:

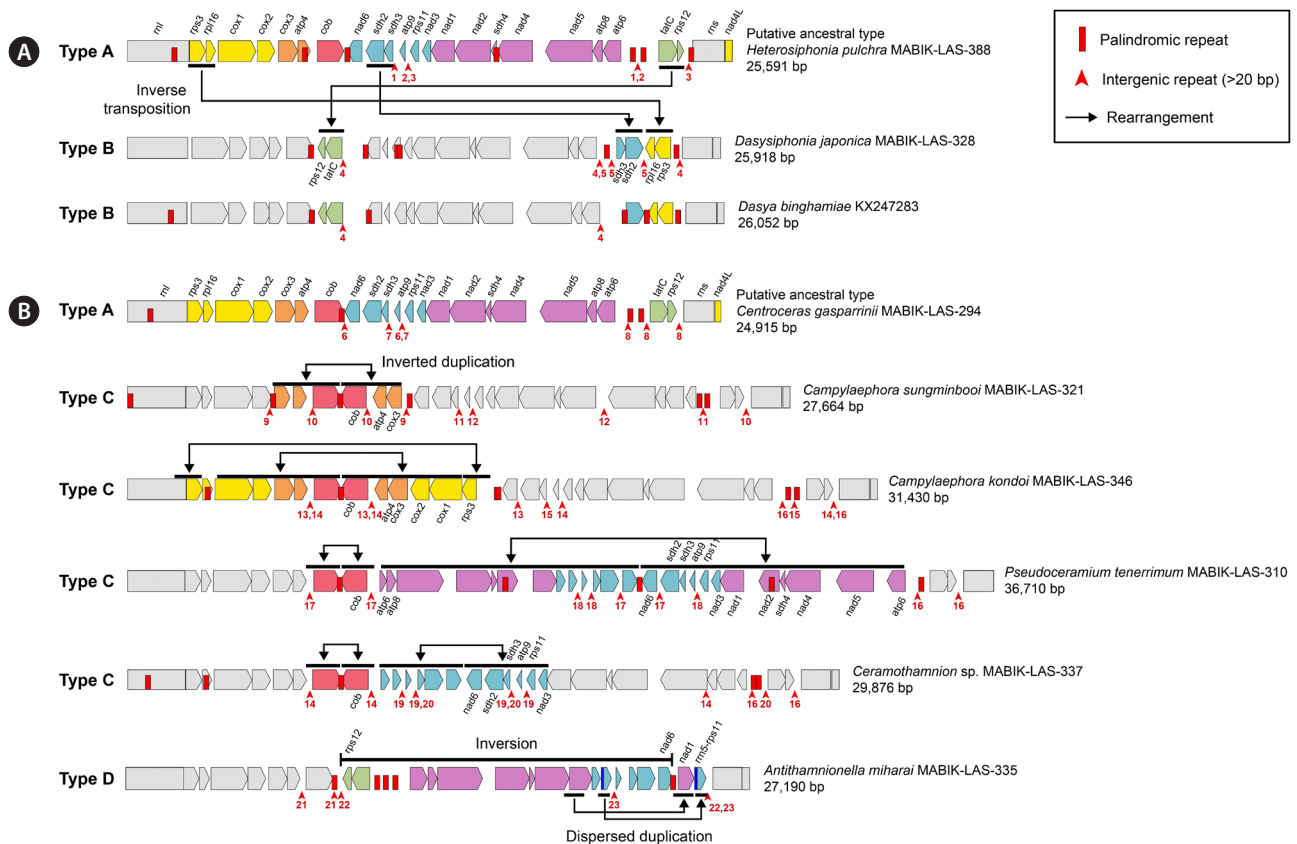


Fig. 3. Syntenic differences among mitochondrial genome in the order Ceramiales. (A) Type B (inverse transpositions) was found in the family Delesseriaceae. (B) Type C (inverted duplications) and type D (inversion and duplications) were found in the family Ceramiaceae. Type A is a putative ancestral type.

Campylaephora kondoi (Yendo) Barros-Barreto & Maggs, *Campylaephora sungminbooi*, *Ceramothamnion* sp. MABIK-LAS-337, and *Pseudoceramium tenerrimum*. The duplication began with the *cob* gene in *Ca. kondoi* (*cob-cox3*) and *Ca. sungminbooi* (*cob-rps3*). *Pseudoceramium tenerrimum* and *Ceramothamnion* sp. MABIK-LAS-337 displayed inverted duplications that started from *cob* and *nad6* genes, respectively. The *nad6-nad3* region was duplicated in *Ceramothamnion* sp. A similar but longer duplication observed in *Pseudoceramium tenerrimum*, which spanned from *nad6* to *atp6*.

Type D mitogenome had both an inversion and a duplication (Fig. 3B), and this type was found in the tribe Antithamnieae (*Antithamnionella miharai*). Specifically, there was an inversion of the genomic segment from *nad6* to *rps12* genes and duplications occurred for the *rrn5-rps11* and *nad1* genes.

DISCUSSION

Our mitogenome phylogeny of the Ceramiales is congruent with previous plastid genome-based as well as multigene-based phylogenetic relationships (Díaz-Tapia et al. 2019, Barros-Barreto et al. 2023). Within the Rhodomelaceae, which is the most species-rich family in the Ceramiales (about 1,100 spp.) (Guiry and Guiry 2023), the present study confirmed the relationships of 10 tribes. The topology was similar with those of plastid genomes (Díaz-Tapia et al. 2017), except three were non-monophyletic. For example, *Melanothamnus gigas* Huisman did not cluster with the other species of the Strebloladiaceae, *Erythrocytis saccata* (J. Agardh) P.C. Silva and *Vertebrata lanosa* (Linnaeus) T.A. Christensen. *Gredgaria maugeana* Womersley was distantly related to *Herposiphonia versicolor* (Hooker & Harvey) Reinbold (the tribe Herposiphonieae), but nested in the tribe Pterosiphonieae, making the Pterosiphonieae paraphyletic. This is likely due to low taxon sampling or misidentification.

These tribes have been suggested for taxonomic revision in a recent study (Díaz-Tapia et al. 2023).

In the Ceramiaceae, the topology of the tribe Ceramieae is consistent with a recent study based on three-gene phylogeny and morphological characters (Barros-Barreto et al. 2023). Three new genera, *Pseudoceramium* Barros-Barreto & Maggs, *Yoneshiguea* Barros-Barreto, Maggs & M.A.Jaramillo, and *Stirkia* Barros-Barreto & Maggs were established, and previously synonymized genera, *Celceras* Kützing and *Reinboldiella* De Toni, were reinstated (Barros-Barreto et al. 2023). However, due to the nomenclatural priority, the genus *Ceramothamnion* H.Richards was reinstated from the superfluous *Stirkia* (Wynne and Schneider 2023).

Our comparative analyses of mitogenomes in the Ceramiales demonstrate conserved gene content and synteny, with the exception of the Ceramiaceae. A core set of 23 PCGs was shared in all ceramialean species, with additional PCGs (2–13 genes) resulted from gene duplication in the Ceramiaceae. Two previously published mitogenomes in the Ceramiaceae (*Campylaeophora sungminbooi* KU145004 and *Ceramothamnion japonicum* KJ398159) were excluded from the comparative analysis because they were only partially sequenced (Yang et al. 2015, Hughey and Boo 2016). Hughey and Boo (2016) found a long inverted repeat (about 700 bp) in *Ca. sungminbooi*, which might disrupt the complete mitogenome assembly of *Ca. sungminbooi*.

The main finding of this study is the identification of four syntenic types of mitogenome in the Ceramiales, of which three of them are newly reported structures in florideophycan red algae. Ancestral type (type A) was widely distributed, including in all four families of the order and outgroup species, Acrosymphytales and Rhodymeniales. The overall gene content and gene synteny of this ancestral type are highly conserved in other reported florideophycidean red algal mitogenomes (Yang et al. 2015, Lee et al. 2018). However, *rpl20* gene has been lost in the Ceramiales as well as in other red algal species, but this lineage-specific gene loss does not correspond to phylogenetic relationships (Yang et al. 2015).

Because three new structures were located within the ancestral type A at different phylogenetic positions (i.e., type B in the Delesseriaceae, type C and type D in the Ceramiaceae), it is highly likely that these three new structures originated independently. It is interesting that these mitogenomic rearrangement typically occurs near the *cob* and *nad6* genes, where palindromic and inverted repeats were found in flanking regions of the rearrangement. The consistent presence of palindromic and in-

verted repeats in the rearrangement regions suggests that they may initiate or promote these genomic rearrangements. This hypothesis is supported by the observation that palindromic repeats, known to form stable hairpin structures, may cause replication errors and genomic instability (Cunningham et al. 2003, Miklenić and Svetec 2021). Additionally, the presence of repeated sequences can facilitate genomic rearrangements through unequal crossing over or replication slippage (Witte et al. 2001, Achaz et al. 2003, Carvalho et al. 2011, Reams and Roth 2015). Therefore, palindromic and inverted repeats of the Ceramiales may initiate the rearrangement of mitogenome structure. However, a question arises: why do other lineages (e.g., the Rhodomelaceae and Wrangelia-ceae) not show these syntenic rearrangement, given that ancestral type A still contains palindromic and inverted repeats? We are unable to figure out this mystery; however, a possible scenario is proposed that DNA replication, repair, and recombination-related genes (e.g., *RAD52*, *MSH1*) could be involved in unique mitogenome reshaping (e.g., minicircular chromosomes) (Lee et al. 2023). Nuclear genome analysis would provide some insight into this abnormal phenomenon in future studies.

In conclusion, our taxon-wide comparative study of the Ceramiales mitogenomes provides valuable insights into genome architecture with phylogenetic relationships, revealing significant genomic rearrangements and expansions. The palindromic and inverted repeats likely play an important role in genomic rearrangements. The various mitogenome structures of the Ceramiales are first reported in red algae, enhancing our understanding of the diversity and complexity of red algal mitogenome evolution. Our study encourages reexamination of mitogenome architecture in florideophycidean red algae as well as the Callithamniaceae, not covered in the present study, to understand its mechanism and how many variant types occur during the evolutionary process of red algae.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Fig. S1. Mapping coverage of established Ceramiales mitogenomes. (A) *Antithamnionella miharai* (MABIK-LAS-332). (B) *Antithamnionella miharai* (MABIK-LAS-335). (C) *Bostrychia moritziana* (MABIK-LAS-101). (D) *Bostrychia moritziana* (MABIK-LAS-133). (E) *Bostrychia tenuissima* (MABIK-LAS-110). (F) *Campylaeophora kondoi* (MABIK-LAS-346). (G) *Campylaeophora sungminbooi* (MABIK-LAS-321). (H) *Centroceras gasparinii* (MABIK-LAS-294). (I) *Ceramothamnion* sp. (MABIK-LAS-337). (J) *Chondria armata* (SRR15927350) (<https://www.e-algae.org>).

Supplementary Fig. S2. Mapping coverage of established Ceramiales mitogenomes. (A) *Dasysiphonia japonica* (MABIK-LAS-328). (B) *Digenea simplex* (SRR8325636). (C) *Griffithsia okiensis* (MABIK-LAS-368). (D) *Heterosiphonia pulchra* (MABIK-LAS-388). (E) *Pleonosporium* sp. (MABIK-LAS-307). (F) *Polysiphonia morrowii* (MABIK-LAS-329). (G) *Pseudoceramium inkyui* (MABIK-LAS-301). (H) *Pseudoceramium tenerrimum* (MABIK-LAS-309). (I) *Pseudoceramium tenerrimum* (MABIK-LAS-310) (<https://www.e-algae.org>).

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