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Research Note

Unique repeat and plasmid sequences in the mitochondrial genome of *Gracilaria chilensis* (Gracilariales, Rhodophyta)

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Abstract: We described the complete mitochondrial genome of *Gracilaria chilensis* (Gracilariales), an economically important agarophyte alga. The genome was a 26,898 bp circular DNA with 27.6% guanine-cytosine (GC) content, and it encoded 25 protein-coding genes, two ribosomal RNAs (rRNAs), 26 transfer RNAs (tRNAs), and an intron (499 bp) in *trn*I. The gene composition was similar to that of the published mitogenome of *G. salicornia*; however, one inverted and several direct repeat sequences occurred between *sec*Y and *orf*148 genes, resulting in duplicated *trn*R and *trn*S tRNA sequences. The mitochondrial genome also contained three integrated partial plasmid sequences, which were reported for *G. robusta* (Gro4059).

Key Words: Complete mitochondrial genome, Florideophyceae, *Gracilaria chilensis*, Gracilariales, Repeat sequences, Rhodophyta

Gracilaria chilensis C. J. Bird, McLachlan & E. C. Oliveira is the most economically important agar-producing red alga in South America, especially in Chile (Hemmingson *et al*. 1996; Alveal *et al*. 1997; Troell *et al*. 1997). Mature thalli reach 5 m in length, allowing agar-producing companies to cultivate this purple-red alga in both the intertidal and subtidal zones. *Gracilaria chilensis,* as with other Gracilariales species, plays an important role in the ecosystem, serving as a shelter for diverse marine organisms (Fredericq & Hommersand 1989; Yang *et al*. 2012). Here we describe the complete mitochondrial genome of *G. chilensis* and compare it to the published genome of *G. salicornia* (C. Agardh) Dawson (Campbell *et al*. 2014), as well as other four Gracilariales species (Hancock *et al*. 2010; Zhang *et al*. 2012; Yang *et al*. 2014).

A mixture of gametophytes and tetrasporophytes of *G. chilensis* were collected from Ancud, Chile on 1 February 2003, and they were desiccated using silica gel. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and used in next-generation sequencing with the Ion Torrent PGM platform (Life Technologies, San Francisco, California, USA). A sequencing library (400 bp size selection) was constructed using an Ion Xpress Plus gDNA Fragment Library Kit, and genome sequencing was conducted with the Ion PGM Template OT2 400 Kit and the Ion PGM Sequencing 400 Kit following manuals provided by Life Technologies. The mitochondrial genome was assembled using a CLC Genomics Workbench 5.5.1 (CLC bio, Aarhus, Denmark) and a MIRA assembler that were incorporated in Ion Server (Life Technologies). Gene annotation was conducted using programs of the Geneious 6.1.6 (http://www.geneious.com/), RNAmmer 1.2 Server (Lagesen *et al*. 2007) and tRNAscan-SE 1.21 (Schattner *et al*. 2005). Twenty five protein coding genes were aligned using MAFFT 7.110 (Katoh & Toh 2008) including all available mitochondrial genomes of the Gracilariales and *Rhodymenia pseudopalmata* (V.J. Lamouroux) P.C. Silva as an outgroup taxa (Kim *et al*. 2014). Phylogenetic relationships were inferred by the maximum likelihood (ML) method using RaxML 8.0.0 with a LG4MF model (Stamatakis 2014). The same evolutionary model was used for the ML bootstrap analysis with 1000 replications.

More than 6 Mbp of mitochondrial genome data comprising 27,806 total mitogenome reads was obtained from the genome sequencing data (506 Mbp). It provided 209× average coverage when mapped onto the mitochondrial genome. The total mitochondrial genome was 26,898 bp in length and had a 27.6% guanine-cytosine (GC) content. The *Gracilaria chilensis* mitochondrial genome contained 53 genes (Fig. 1), including 25 protein-coding genes, two ribosomal RNAs (rRNAs), and 26 transfer RNAs (tRNAs) with an intron (499 bp) in *trn*I. The gene synteny was basically identical with that of *G. salicornia* (Campbell *et al*. 2014; this study). Only two exceptions were found in a directional change in *trn*H, and a 1600 bp repeat region between *sec*Y and *orf*148 (Fig. 2). It included five additional tRNAs (1 *trn*Y, 2 *trn*R, and 2 *trn*S) and several copies of repeat sequences in opposite and parallel directions. Inverted repeats (IR in Fig. 2) comprised two identical sequences (268 bp) including two tRNAs of *trn*S and *trn*R. Two different types of direct repeats were present (DR-1 and DR-2 in Fig. 2). Direct repeat-1 included two highly similar DNA fragments of 302 bp and 321 bp (19 bp insertion and 1 bp

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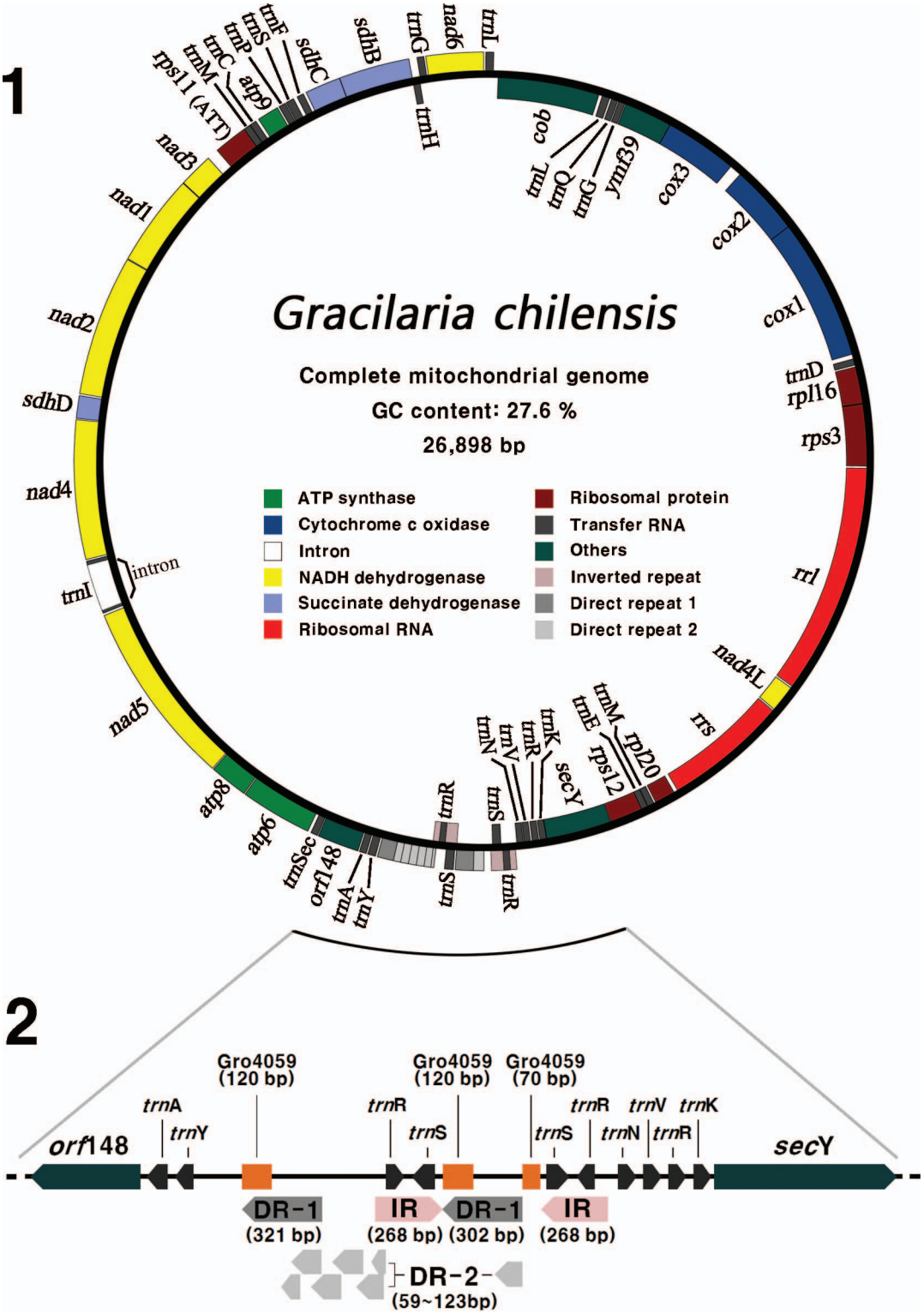

Fig 1. Mitochondrial genome map of *Gracilaria chilensis*. Functional gene groups are color-coded. Genes drawn inside of the circle indicate the transcriptionally clockwise strand, whereas genes on outside indicate the counter-clockwise strand. The *trn*I tRNA has an intron. The alternative start codon of the *rps*11 gene is marked in parentheses.

Fig 2. The linear gene synteny between *orf*148 and *sec*Y. Inverted repeat (IR) sequences and two types of direct repeat (DR-1 and DR-2) sequences are shown with their direction and length. Orange boxes indicate partial plasmid sequences reported from *Gracilaria robusta* (Gro4970).

Table 1. Comparison of mitochondrial genomes among species in the Gracilariales. Genes in boldface indicate unique feature in certain species. Plus or minus indicates gene direction; percentage in parentheses shows amino acid similarity compared to *Gracilaria chilensis*; blank indicates absence of gene; * and ** indicate un-annotated or mis-annotated genes, respectively, in previous studies.

Gene	*Gracilaria chilensis*	*Gracilaria salicornia*	*Gracilariopsis chorda*	*Gracilariopsis lemanaeformis*	*Gracilariopsis andersonii*	*Gracilariophila oryzoides*
	This study	Campbell *et al*. 2014	Yang *et al*. 2014	Zhang *et al*. 2012	Hancock *et al*. 2010	Hancock *et al*. 2010
rrs	−	−	−	−	−	−
*nad*4L	−	− (92)	− (90)	− (90)	− (90)	− (89)
rrl	−	−	−	−	−	−
*rps*3	−	− (68)	− (64)	− (65)	− (64)	− (63)
*rpl*16	−	− (77)	− (75)	− (75)	− (75)	− (72)
*trn*D	−	−	−	−	− *	− *
*cox*1	−	− (96)	− (97)	− (97)	− (97)	− (97)
*cox*2	−	− (88)	− (87)	− (87)	− (89)	− (87)
*cox*3	−	− (90)	− (85)	− (87)	− (88)	− (85)
*ymf*39	−	− (63)	− (56)	− (56)	− * (57)	− (53)
*trn*G	−	−	−	−	−	−
*trn*Q	−	−	−	−	−	−
*tnr*L	−	−	−	−	−	−
cob	−	− (93)	− (94)	− (94)	− (94) + ***orf*87** + ***orf*95** + ***orf*61**	− (93)
***trn*H**			−	−	− *	−
*trn*L	+	+	+	+	+	+
*nad*6	+	+ (79)	+ (74)	+ (75)	+ (74)	+ (73)
*trn*G	+	+	+	+	+	+
***trn*H**	−	+				
			− ***orf*60** **	− ***orf*60**		
*sdh*B	+	+ (85)	+ (84)	+ (84)	+ (83)	+ (83)
*sdh*C	+	+ (54)	+ (49)	+ (49)	+ (53)	**(pseudo)**
*trn*F	+	+	+	+	+	+
*trn*S	+	+	+	+	+	+
*trn*P	+	+	+	+	+	+
*atp*9	+	+ (100)	+ (100)	+ (100)	+ (100)	+ (100)
*trn*C	+	+	+	+	+	+
*trn*M	+	+	+	+	+	+
*rps*11	+	+ (71)	+ (58)	+ (57)	− **(43)**	+ (69)
*nad*3	+	+ (92)	+ (87)	+ (87)	+ (88)	+ (87)
*nad*1	+	+ (94)	+ (94)	+ (94)	+ (93)	+ (92)
*nad*2	+	+ (78)	+ (71)	+ (71)	+ (70)	+ (73)
*sdh*D	+	+ (59)	+ (61)	+ (61)	+ (62)	+ (65)
*nad*4	+	+ (90)	+ (87)	+ (87)	+ (86)	+ (85)
*trn*I	+ (intron)	+ * (intron*)	+ * (intron*)	+ * (intron*)	+ * (intron*)	+ * (intron*)
*nad*5	+	+ (84)	+ (84)	+ (84)	+ (83)	+ (82)
*atp*8	+	+ (71)	+ (61)	+ (62)	+ (60)	**(pseudo)**
*atp*6	+	+ (91)	+ (90)	+ (90)	+ (90)	+ (88)
*trn*Sec	+	+ **	+	+ **	+	+
*orf*148 or *orf*143	+	+ (39)	+ (37)	+ (37)	+ (31)	+ (37)
*trn*A	+	+	+	+	+	+
***trn*Y**	+					
	Gro4059 (120 bp)		**Gro4059 (111 bp)**	**Gro4059 (110 bp)**		
***trn*R**	−					
***trn*S**	+					
	Gro4059 (120 bp)					
	Gro4059 (70 bp)					
***trn*S**	−		−			
***trn*R**	+		+			
***trn*Y**			+			
			Gro4059 (110 bp)			
*trn*N	−	−	−	−	−	− **
*trn*V	−	−	−	−	−	−
*trn*R	−	−	−	−	−	− **
*trn*K	−	−	−	−	−	− **
*sec*Y	−	− (59)	− (56)	− (56)	− (56)	− (53)
*rps*12	−	− (80)	− (79)	− (79)	− (44)	− (77)
*trn*E	−	−	−	−	−	−
*trn*M	−	−	−	−	−	−
*rpl*20	−	− (58)	− (68)	− (68)	− (67)	− (66)

substitution), respectively. Within DR-1, a fragment of 120 bp was identified as a non-coding sequence of *G. robusta* Setchell plasmid (Gro4059, NCBI gi: 11466333) by BLASTn search (94–96% identities with e-value 1.0 e^{-20}–e^{-36}). Another repeat of plasmid Gro4059 (70 bp) was located between DR-1 and the IR (see orange boxes in Fig. 2). DR-2 comprised a group of seven short repeats that ranged from 59–123 bp and lacked coding genes. Short repeat sequences were overlapping, which then overlapped again with DR-1 and IR regions (Fig. 2). These features of the repeat and plasmid sequences make the *G. chilensis* mitogenome unique in comparison to other red algal species and distinct even from *G. salicornia*.

We extended comparison to the other Gracilariales species including three *Gracilariopsis* species (*Gp. chorda* (E.M. Holmes) Ohmi, *Gp. lemanaeformis* (Bory de Saint-Vincent) Dawson, Acleto, & Foldvik, and *Gp. andersonii* (Grunow) Dawson) and *Gracilariophila oryzoides* Setchell & Wilson, a colorless parasite of *Gracilaria* and *Gracilariopsis* species (Hancock *et al*. 2010; Zhang *et al*. 2012; Yang *et al*. 2014). A multigene phylogeny showed that the monophyletic clade of two *Gracilaria* species was separated from a clade of other species [(*Gp. chorda Gp. lemanaeformis*), (*Gp. andersonii*, *Gracilariophila oryzoides*)] (data not shown). Similarities of the amino acid sequences to *G. chilensis* varied from 31% (*orf*148) to 100% (*atp*9). The gene synteny was basically similar to those of four Gracilariales species (Table 1). However, one translocation of *trn*H was found between two *Gracilaria* species and others. Independent gene gain (a fragment of *orf*87-*orf*95-*orf*61) was located between *cob* and *trn*H in *Gp. andersonii*, while one homologous *orf*60 was found in both *Gp. chorda* and *Gp. lemanaeformis*. Two pseudogenized genes (*sdh*C and *atp*8) were found in the parasite species of *Gracilariophila oryzoides* (see Table 1). It is interesting to see that *Gp. chorda* and *Gp. lemanaeformis* contained the *Gracilaria robusta* plasmid sequences (Gro4059) in same location of *G. chilensis*. In *Gp. chorda,* this plasmid fragment was duplicated (i.e. 111 bp and 110 bp), and three tRNAs (*trn*S, *trn*R, and *trn*Y) were identified similar to those of *G. chilensis*. Because it is unlikely to insert these DNA fragments independently in exactly the same position, these plasmid-related fragments with tRNAs were likely inserted in the common ancestor of the Gracilariales, then retained in *Gp. lemanaeformis,* but duplicated in *G. chilensis* and *Gp. chorda,* while other species lost this fragment. Therefore we postulate that an insertion of a foreign gene into the organellar genome is mediated by the plasmid sequence and a copy-and-paste transposition mechanism frequently found in transposable elements (e.g. McClintock 1950).

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