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The complete chloroplast genome of the green algae *Hariotina reticulata* (Scenedesmaceae, Sphaeropleales, Chlorophyta)

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Abstract

In this study, the chloroplast genome of *Hariotina reticulata* was fully sequenced and compared to other Sphaeropleales chloroplast genomes. It is 210,757 bp larger than most Sphaeropleales cpDNAs. It presents a traditional chloroplast structure, and contains 103 genes, including 68 protein-coding genes, six rRNA genes and 29 tRNA genes. The coding region constitutes of 43% of the whole cpDNA. Eighteen introns are found in 11 genes and six introns are unique for *Hariotina*. 11 open reading frames are identified among these introns. The synteny between *Hariotina* and *Acutodesmus* cpDNAs is in general identical, while within Sphaeropleales order, high variability in cpDNA architecture is indicated by general high DCJ distances. *Ankyra judayi* exhibits the greatest dissimilarity in gene synteny to the others and share some unique gene clusters with *Treubaria triappendiculata*. The phylogenomic analyses show that *A. judayi* is clustered with Treubariaceae species and sister to Chlorophyceae incertae sedis and other Sphaeropleales species. The monophyly of Sphaeropleales is rejected.

Keywords *Hariotina* · Sphaeropleales · Chloroplast genome · Synteny · Phylogenomic analyses

Introduction

Scenedesmaceae is the largest family in alga order Sphaeropleales with over three hundred species. This group exhibits high adaption to freshwater and terrestrial habitat and high level of phenotypic homoplasy which leads to taxonomic confusion and covers species diversity within this family.

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This family has undergone a rather complicated taxonomic history with several revisions over the years from sole genus to present 49 genera classification (Chodat 1926; Trainor et al. 1976; Hegewald 1978, 2000; Hegewald and Silva 1988; Kessler et al. 1997; An et al. 1999; Van Hannen et al. 2002; Hegewald et al. 2010; Krienitz and Bock 2012; Guiry and Guiry 2016). Krienitz and Bock (2012) listed that only 13 genera were well phylogenetically and morphologically defined. Other phylogenetically accepted genera need further test. Besides, certain relationships still remain poorly resolved (Vanormelingen et al. 2007; Eliáš et al. 2010; Hegewald et al. 2010).

Plastid genome has been proved to be a useful tool for phylogenetic studies for resolving complex evolutionary relationships. So far, *Acutodesmus obliquus* is the only taxon with complete chloroplast genome and mitochondrial genome within Scenedesmaceae (de Cambiaire et al. 2006). More taxon sampling is needed to gain further insight into the family evolutionary trend. Previously, we reported the mitochondrial genome of *Hariotina reticulata* (MMOGRB0030F) which exhibits the similar genome organization to *A. obliquus* except a unique CUU anticodon for tRNA-Lys (He et al. 2016). *Hariotina* is a typical well supported small genus including only two recognized species in subfamily Coelastroidea. In this study, we sequenced its

chloroplast genome and compared to *A. obliquus* and other 11 cpDNAs in Sphaeropleales in the aspects of genome features, gene content and genome structure. The introduction of chloroplast genome of *H. reticulata* will provide material for deeper understanding of phylogeny and the genome evolution in Sphaeropleales as well as in Scenedesmaceae.

Materials and methods

Strain isolation, cultivation and microscopy

The strain was isolated from Furong Lake in Xiamen, Fujian, China and deposited in Marine Medicinal Organism Germplasm Resources Bank of Third Institute of Oceanography (MMOGRB 0030F). It is preserved under a 12:12 light:dark cycle and 40 μmol photons $m^{-2} s^{-1}$ from cool-white fluorescent tubes. Cells were observed under light microscope with Zeiss Scope A1.

Genome sequencing, assembly and annotation

Approximately 1 g of fresh microalgae was collected by centrifugation to extract the total genome using DNA extraction kit (DP305, Tiangen Biotech Co., LTD). Total DNA was used to generate the 500 bp paired-end library, following the Illumina Hiseq2500 standard protocol (Illumina Inc., San Diego, IL). About 3.7 G raw data was generated with reads length 250 bp, and the chloroplast genome sequencing depth is nearly to 1259.9×.

Reads were assembled with SOAPdenovo2-r240 (http://soap.genomics.org.cn). Since the assembled contigs contain a mixture of sequences from both organellar and nuclear genomes, the methods were used to isolate the chloroplast sequences based on the high correlation between contig read depth and the number of copies in the genome. Firstly, we sorted the assembled contigs by contig-read depth analysis of assemblies, that is, the raw reads sequence were mapped to the assembled contigs and the read depth of each contig was calculated through reads mapping. Taking the advantage of the difference of read depths among contigs, we could isolate the chloroplast contigs with high-coverage (more than 500×) from the nuclear contigs. Secondly, we also used all published chloroplast genome sequence to capture reads with BWA, then assembly those reads to chloroplast contigs. Finally, combined all isolated chloroplast contigs and recaptured reads again to isolate more completely chloroplast DNA reads, reassembly and contigs extended to get completely chloroplast genome sequencing.

Gene annotation was carried out with Organellar GenoMe Annotator (Wyman et al. 2004) and CPGAVAS (Cheng et al. 2013) with plastid/bacterial genetic code and default conditions. The cpDNA of *A. obliquus* chloroplast genome was

applied as the referee. To verify the exact gene and exon boundaries, putative gene sequences and protein sequences were BLAST searched in Nt and Nr database. All tRNA genes were further confirmed through online tRNAscan-SE and tRNADB-CE search sever (Griffiths-Jones et al. 2003; Schattner et al. 2005; Abe et al. 2011). The graphical map of the circular plastome was drawn with Organellar Genome DRAW v1.2 (Lohse et al. 2007). UniMoG (Hilker et al. 2012) was applied to run the minimal histories of rearrangements among the pairwise aligned genomes under a double cut and join (DCJ) model.

Phylogenomic analyses

Phylogenomic analyses were based on amino acid sequences of 48 shared protein-coding genes of chloroplast genomes from 33 members in Chlorophyceae. Alignments were performed by MUSCLE v3.8 (Edgar 2004) with gaps or missing data eliminated. Amino acids substitution was estimated using ProtTest v3.4 (Darriba et al. 2011). Maximum likelihood (ML) analyses were conducted by RAxML v.8 (Stamatakis 2014) with LG + G + I + F model. Branch support was evaluated by 1000 replications of bootstrap. Bayesian analyses were applied with MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012) running one million generations with four independent runs under CpREV+I+G+F model. The first 25% of samples were discarded as burn-in.

Results and discussion

Genome features

The complete Hariotina cpDNA (KX131180) assembles as a double-stranded circular with 210,757 bp larger than *A. obliquus* and other 12 cpDNAs in Sphaeropleales except *Bracteacoccus giganteus* and *Pseudomuriella schumacherensis*. It displays a typical quardripartite structure as most Chlorophyta species including a large single copy (LSC, 104,555 bp), a small single copy (SSC, 74,102 bp) and two 16,050 bp inverted repeats (IRA and IRB). 103 genes were identified, including 68 protein coding genes (PCGs), 29 tRNA genes and six rRNA genes (Table 1; Fig. 1). Of the 103 genes, we identified that one PCG, six rRNA genes and six tRNA genes are located within IRs. The LSC region contains 34 PCGs and eight tRNA genes while the SSC region has 33 PCGs and 15 tRNA genes. The psbC is the unique gene located in the boundary region between IRB and LSC. The total coding region accounts for 43.6% of the whole cp genome and the overall GC content was about 29.2% which is in the range of Sphaeropleales cpDNAs (Table 1).

Table 1 Summary of the Hariotina cpDNA and comparison with other Sphaeropleales cpDNAs

| Taxon	Accession no.	cpDNA size (bp)	GC content (%)	Gene number (ORF excluded)	Protein coding genes	rRNA genes	tRNA genes	% Coding	Intron (%)	Intron number	IR size (bp)	IR genes
Hariotina reticulata	KX131180	210,757	29.2	103	68/68	6	29	43.6	7.69	18	16,050	7
Acutodesmus obliquus	DQ396875	161,452	26.9	106	68/70	6	30	67.2	7.89	10	12,022	6
Ankyra judayi	KT199255	157,224	28.3	109	68/70	6	33	57.0	1.95	2	8247	6
Bracteacoccus aerius	KT199254	165,732	31.7	103	67/69	6	28	54.9	0.35	2	7271	5
Bracteacoccus giganteus	NC028586	242,897	33.3	107	69/73	6	28	40.0	10.76	15	14,766	6
Bracteacoccus minor	KT199253	192,761	31.9	104	68/70	6	28	48.4	1.53	3	9577	6
Chlorotetraedron incus	KT199252	193,197	27.1	106	68/70	6	30	46.7	4.62	10	13,490	8
Chromochloris zofingiensis	KT199251	188,937	30.9	106	67/69	6	31	47.2	0.25	2	6375	6
Kirchneriella aperta	KT199250	207,516	34.1	106	68/70	6	30	42.3	20.94	27	35,503	7
Mychonastes homosphaera	KT199249	102,718	39.8	105	68/70	6	29	80.1	0.14	1	6472	6
Mychonastes jurisii	NC_028579	103,047	39.6	107	70/72	6	29	79.8	0.54	2/6	6718	7
Neochloris aquatica	KT199248	166,767	30.3	102	65/67	6	29	50.9	16.62	32	18,217	8
Pseudomuriella schumacherensis	KT199256	220,357	31.2	109	69/71	6	32	41.6	3.49	8	22,004	7
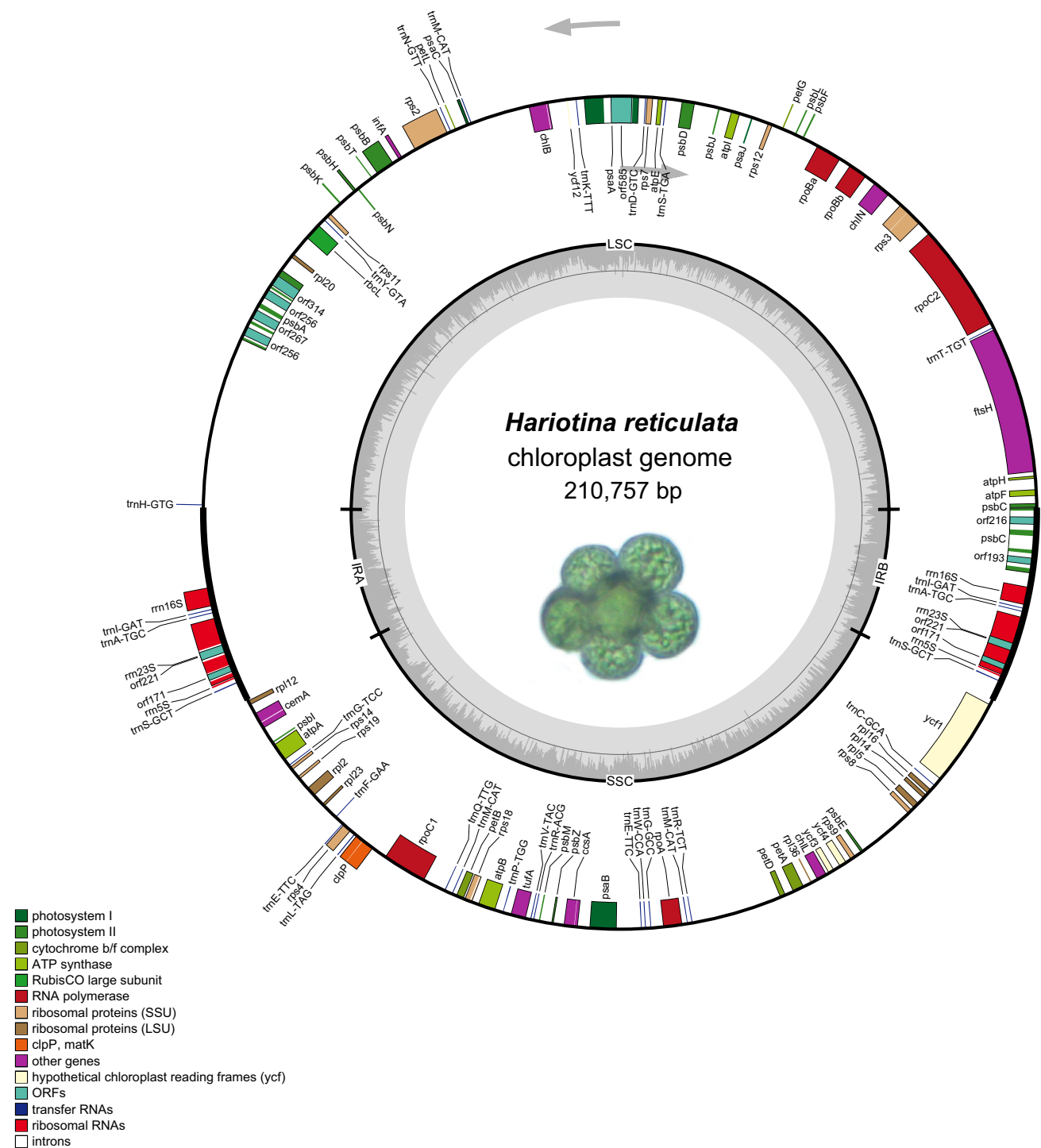
Fig. 1 Gene map of the chloroplast genome of *Hariotina reticulata*. Genes shown on the outside of the circle are transcribed counterclockwise. Annotated genes are colored according to the functional categories shown in the legend bottom left. (Color figure online)

The cpDNA of *Hariotina* strain exhibits great similarity with that of *A. obliquus* in the gene content with few genes absent e.g. *rps2a* and a copy of *trnL*. The differentiation of *psaA* gene structure is also observed between two cpDNAs. In *A. obliquus*, this gene is separated to two parts by five genes. The separation of *psaA* gene into two to four parts is common among Sphaeropleales cpDNAs. However, in *Hariotina*, it keeps intact with one intron and an ORF inserted which encodes putative maturase and HNH homing endonuclease.

Introns

Among the known Sphaeropleales cpDNAs, *Kirchneriella aperta* has the highest proportion of introns up to 20.94% of the whole plastid genome while *Mychonastes homosphaera* the least with only 0.14% (Table 1). In *Hariotina* chloroplast genome, eighteen introns are identified in 11 genes (Table 2), comprising 7.69% (including intronic ORFs) of the total cpDNA. 11 introns were identified as group I type, and seven as group II. Except introns found in *psaA, psbA* and *rrn23S*, other introns are rarely found or have not yet been observed in previously reported Sphaeropleales plastids (Supplementary Table S1). 11 open reading frames (ORFs) are distinguished within these introns (Table 2, Supplementary Table S2). Only one ORF encoding HNH is present in group II introns and ten ORFs in group I introns.

In Sphaeropleales, the introns in six genes including *rps3, chlB, cemA, clpP, rps18* and *ccsA* are only present in *Hariotina* cpDNA. All are identified as group II type and no ORF is recognized. The other group II intron in *psaA* is consistent in Sphaeropleales with the exception of *M. jurisii*.

The *psbA* gene in Sphaeropleales chloroplast genomes comprises a large number of introns. For examples, nine introns are present in *Neochloris aquatica* cpDNA, eight in *K. aperta* and seven in *B. giganteus*. In six reported cpDNAs, e.g. *Chromochloris zofingiensis, B. minor, B. aerius*, the *psbA* introns are missing. Four group IA introns are identified in *Hariotina psbA* gene containing four ORFs: three encoding HNH homing endonuclease and one encoding GIY-YIG endonuclease. The majority of *psbA* introns in Sphaeropleales are group I introns with the exception of four group II introns in *K. aperta*.

The introns in *rrn23S* gene are only found in *P. schumacherensis* and *C. zofingiensis* clade (Fig. 3) with both IA and IB types identified. Only IA type is present in *Hariotina* and two introns exhibits 74% similarity to that of *A. obliquus*. The intron in *psbC* is rarely observed in Sphareopleales plastid genome. Besides *Hariotina*, it is only observed in *N. aquatica*. All are identified as group IA type.

The group-I intron in tRNA (Leu) gene was reported to be conservative through Rhodophyta to high plants. This intron was thought to have been inherited by vertical transmission from the common ancestor of all chloroplasts (Besendahl et al. 2000). In Sphaeropleales order, it is present in most species as IB type except the IC3 type in *A. obliquus* cpDNA, while in *Hariotina*, this intron is lost as well as in *A. judayi* and two *Mychonastes* species.

Synteny and gene clusters

High variability in cpDNA architecture is widespread in Chlorophyceae (de Cambiaire et al. 2006; Turmel et al. 2015) as well as in Sphaeropleals as indicated by high DCJ

Table 2 Distribution and characteristics of introns and ORFs in *Hariotina* cpDNA

Designation	psbA						psbC			rrn23S*		ccsA	cemA	chlB	clpP	psaA	rps3	rps18
Intron size (bp)	1151	1569	1359	1378	1342	1251	1722	1039ᵃ/1111ᵇ	791ᵃ/719ᵇ	30	66	51	30	2484	27	90
Intron type	IA	IA	IA	IA	IA	IA	IA	IA	IA	II	II	II	II	II	II	II
AT content	70.98	68.77	72.11	71.04	70.49	69.22	66.43	68.23	70.10	63.33	75.76	70.59	70.00	61.39	66.67	63.33
ORF size (bp)	314	256	267	256	193	–	216	221	171	–	–	–	–	585	–	–
ORF type	HNH	GIY-YIG	HNH	HNH	GIY-YIG	–	GIY-YIG	LAGLIDADG	LAGLIDADG	–	–	–	–	HNH	–	–

Asterisks indicate a copy of the gene

ᵃ, ᵇ indicates rrn23S in IRA and IRB respectively

Table 3 DCJ distance comparisons of *Hariotina* and other Sphaeropleales cpDNAs

	Hariotina reticulata	Acutodesmus obliquus	Chlorotetraedron incus	Neochloris aquatica	Kirchneriella aperta	Chromochloris zofingiensis	Pseudomuriella schumacherensis	Bracteacoccus minor	Bracteacoccus aerius	Bracteacoccus giganteus	Mychonastes homosphaera	Mychonastes jurisii	Ankyra judayi
Hariotina reticulata	0	5	20	32	25	23	27	37	33	37	28	28	47
Acutodesmus obliquus	3	0	15	27	20	18	26	33	35	36	23	27	45
Chlorotetraedron incus	14	11	0	14	21	18	29	35	35	36	22	26	45
Neochloris aquatica	20	17	8	0	32	29	34	39	40	40	28	28	48
Kirchneriella aperta	16	14	17	21	0	23	31	38	38	36	26	30	47
Chromochloris zofingiensis	19	16	13	17	18	0	19	35	33	33	19	24	47
Pseudomuriella schumacherensis	20	21	22	23	23	15	0	35	38	32	20	19	45
Bracteacoccus minor	23	21	20	21	25	21	24	0	14	10	29	33	44
Bracteacoccus aerius	21	20	20	20	25	20	23	5	0	16	36	38	41
Bracteacoccus giganteus	21	22	23	22	25	21	22	7	6	0	29	28	42
Mychonastes homosphaera	16	13	13	15	17	10	15	21	20	23	0	7	47
Mychonastes jurisii	14	15	15	14	17	12	13	22	20	19	4	0	46
Ankyra judayi	31	30	29	32	32	33	32	28	26	28	34	34	0

Values on the upper right represent DCJ distance when all genes are considered. Values on the lower left indicate DCJ distance when all the tRNA genes are removed
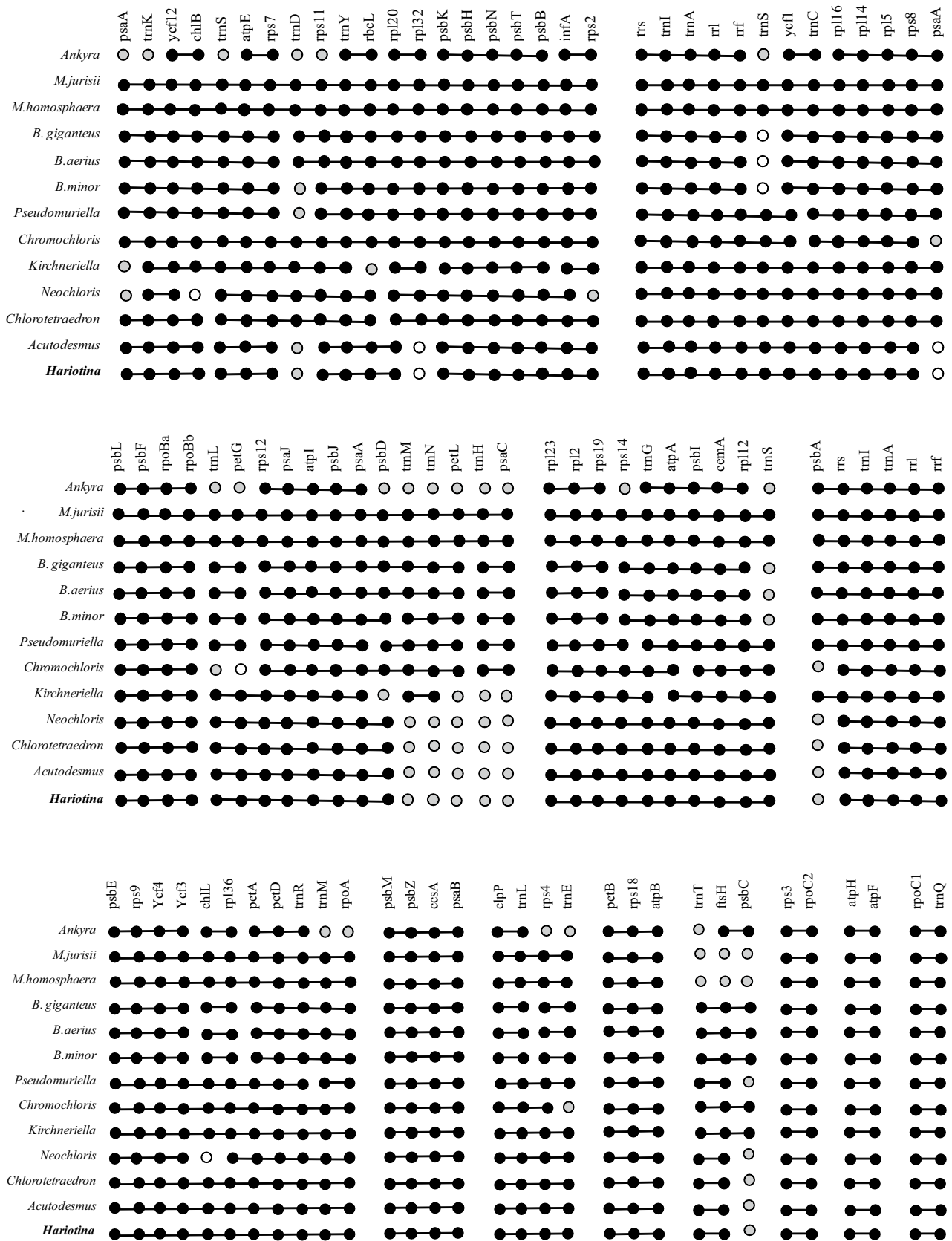
Fig. 2 Comparison of conserved genes clusters in Sphaeropleales. Black connected circles represent gene clusters. Grey circles indicate that genes are not adjacent, and white circles indicate genes that are absent from the cpDNA

Fig. 3 Bayesian phylogenetic tree of *Hariotina reticulata* and other Chlorophyceae species based on amino acids of 48 chloroplast protein-coding genes. Values above branches indicate Bayesian posterior probabilities and maximum likelihood bootstrap values. Asterisks indicate full support in each analysis. Values below 70 and 0.60, respectively, are not shown
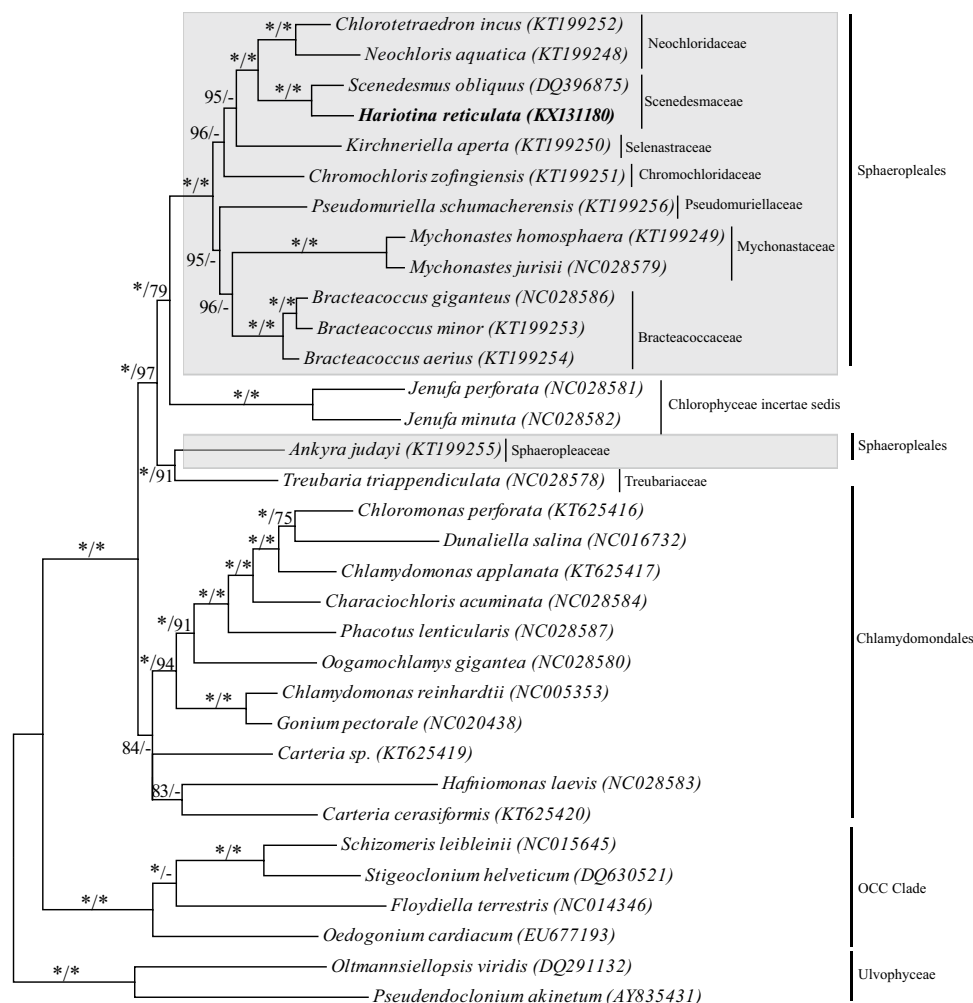
distances (Table 3). Except *A. judayi*, the average DCJ distance among the Sphaeropleales cpDNAs is 28. When exluding all the tRNA genes from the analysis, the average DCJ distance reduces to 18. The cpDNA of *A. judayi* exhibits the highest variability in gene synteny to the others with the DCJ distance of 46 on average (31 excluding all tRNA genes).

The gene order of *Hariotina* cpDNA is identical to that of *Acutodesmus* with the DCJ distance of 5 (3 excluding all tRNA genes), indicating that the genome organization of cpDNAs is conservative at the level of the family Scenedesmaceae. The DCJ distance among the three *Bracteacoccus* taxon is higher than that between *Hariotina* and *Acutodesmus*. The average distance value of the three *Bracteacoccus* species reduces from 13 to 6 after all tRNA genes reduced. It might indicate a higher level of gene rearrangement in the family Bracteacoccaceae than in Scenedesmaceae.

Six gene clusters conserved throughout Chlorophyta cpDNAs (de Cambiaire et al. 2006) are also conserved in Sphaeropleales, e.g. *psbH–psbN–psbT–psbB* cluster,

rrs–trnI–trnA–rrl–rrf cluster. Besides the above ones, ten unique gene clusters are found to be conservative throughout Sphaeropleales order, e.g. *psbM–psbZ–ccsA–psaB, psbE–rps9–ycf4–ycf3*, and *rps12–psaJ–atpI–psbJ–psaA* (Fig. 2). The plastid genome of *A. judayi* generates the most dissimilarity to other Sphaeropleales cpDNAs in gene clusters and exhibits similarity to *Treubaria triappendiculata* instead. Some unique gene clusters are observed between the two plastid genomes, e.g. *psaA–psbJ–atpI–psaJ–rpl36–rps12–chlL–rps2* cluster.

Phylogenomic analyses

The Bayesian topology is almost identical to that of maximum likelihood analyses and is presented in Fig. 3. The topology of the trees obtained in this study was similar to that from the previous studies based on few markers or phylogenomic analyses (Wolf et al. 2002; Keller et al. 2008; Fučíková et al. 2016). The Ulvophyceae is sister to Chlorophyceae which includes fiver orders, e.g. OCC clade, Chlamydomonadales and Sphaeropleales. *Hariotina reticulata*

was tightly linked with *A. obliquus*, forming the deepest branch with Neochloridaceae subclade (Fig. 3) and Neochloridaceae species (Fučíková et al. 2016) within Sphaeropleales clade.

To our surprise, the monophyly of Sphaeropleales is rejected. *A. judayi*, which had been strongly assigned to Sphaeropleales with other 'Sphaeroplea' species in several studies (Wolf et al. 2002; Keller et al. 2008; Buchheim et al. 2012; Caisová et al. 2013; Fučíková et al. 2016), is not clustered with Sphaeropleales members. Instead, it is shown to be closely related to Treubariaceae member, *T. triappendiculata* and sister to Chlorophyceae incertae sedis and Sphaeropleales. Besides, the high DCJ distance between *A. judayi* and other Sphaeropleales members (Table 3) also suggests *A. judayi* exhibits the greatest dissimilarity in cpDNA architecture. It also shares a few unique gene clusters with *T. triappendiculata*, e.g. *psaA–psbJ–atpI–psaJ–rpl36–rps12–chlL–rps2* cluster. These evidences bring about questions to reconsider the Sphaeropleales definition.

Conclusion

H. reticulata is the second Scenedesmaceae species to have its complete chloroplast and mitochondrial genomes sequenced. It provides important information to explore plastid evolution and phylogeny in Sphaeropleales as well as in Scenedesmaceae. Our most surprising advance is the sister relationship between *Treubaria* and *Ankyra*. We suggest a broader sampling of the *Golenkinia*, Chlorophyceae incertae sedis and Sphaeropleales and a probable reconsideration of the definition of Sphaeropleales. Additional chloroplast genome sequencing of Scenedesmaceae species is also necessary in order to explore a robust intrafamilial phylogenetic topology.

Compliance with ethical standards

Conflict of interest Lijuan He declares that she does not have conflict of interest. Zhaokai Wang declares that he does not have conflict of interest. Sulin Lou declares that she does not have conflict of interest. Xiangzhi Lin declares that he does not have conflict of interest. Fan Hu declares that he does not have conflict of interest.

Research involving with human and animal participants This article does not contain any animal or human subjects. The research material is not in the endangered species list.

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