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PLASTOME REPORT

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Complete chloroplast genome sequence and phylogenetic analysis of *Gloiopeltis furcata* (Gigartinales, Florideophyceae)

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ABSTRACT

Gloiopeltis furcata is an annual red alga of Endocladiaceae, Florideophyceae, showing high economic and medical values. The chloroplast genome is instrumental for evolutionary studies, yet that of *G. furcata* remains unreported. Here, the chloroplast genome of *G. furcata* was assembled and analyzed. The genome size is 177,606 bp, which contains 184 genes (152 protein-coding genes, 29 tRNAs, and 3 rRNAs). Four simple sequence repeat (SSR) types were detected. The AGA-encoded arginine had the highest relative synonymous codon usage (RSCU) value, while CGG-encoded arginine had the lowest. Phylogenetic analysis indicated that *G. furcata* is a sister to *Chondrus crispus*.

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Gloiopeltis furcata; chloroplast genome; phylogenetic; simple sequence repeat, Codon usage

Introduction

Gloiopeltis furcata (Postels et Ruprecht) J. Agardh 1851 (Gigartinales, Florideophyceae) is an annual red alga of Endocladiaceae, Florideophyceae, and is commonly grown in the intertidal rocks throughout Pacific regions (Yu et al. 2010). *Gloiopeltis* possesses significant economic value, primarily as a carbohydrate source, and contains polysaccharides with antibacterial, anti-inflammatory, and antitumor properties (Saeki et al. 1996; Park et al. 2005). However, the *Gloiopeltis* genus is now facing population decline due to environmental pollution and excessive development. A study of the red algal genus *Gloiopeltis* identified five species (*G. furcata*, *G. dura*, *G. tenax*, *G. complanata*, and *G. frutex*) that formed nine highly supported clades (Hanyuda et al. 2020). Among these, six clades corresponded morphologically to *G. furcata*. The identification of the *G. furcata* clade is typically based on morphological characteristics, though this approach is often inconsistent or insufficient for precise delineation. The chloroplast genome structure and sequence are valuable in revealing the origin and evolution of different species. However, the complete chloroplast genome of many algae, including *G. furcata*, has not been recovered. Herein, we assembled and annotated a complete chloroplast genome of *G. furcata* for the first time. To investigate its evolution in the context of previously annotated cp genomes from other Florideophyceae species, we conducted a phylogenetic analysis. Moreover, we identified the simple sequence repeat (SSR) and Codon Usage in *G. furcata*.

Materials and methods

Samples of *G. furcata* were deposited at Qingdao Academy of Agricultural Sciences (http://nw.qingdao.gov.cn/; Wenjun Li, wenjunlinky@163.com) under the voucher number: QDSNKY-S109. *G. furcata* was initially collected from Yantai City in Shandong province, China (120°39′21.31″ E, 37°58′44.25″ N) (Figure 1). No specific permissions were required for *G. furcata* because they are not privately owned. Moreover, *G. furcata* was not endangered or protected.

Fresh young thalli were collected, immediately immersed in liquid nitrogen, and stored at −80 °C. A plant chloroplast DNA column extraction kit (BNT120406, Baiao Laibo Technology Co., Ltd., Beijing, China) was used for DNA extraction according to the manufacturer's protocol. Qualified DNA was sequenced by the Illumina NovaSeq 6000 platform (Illumina, Inc., USA).

The complete chloroplast genome of *G. furcata* was assembled by clean reads using NOVOPlasty v4.2. The average depth of coverage is 6872× (Figure S1). Gene prediction analysis was performed using GeSeq. Finally, we have generated circular genetic maps of *G. furcata* using the Organellar Genome DRAW tool (Marc et al. 2013). SSRs in the chloroplast genome of *Gloiopeltis* species were identified using the

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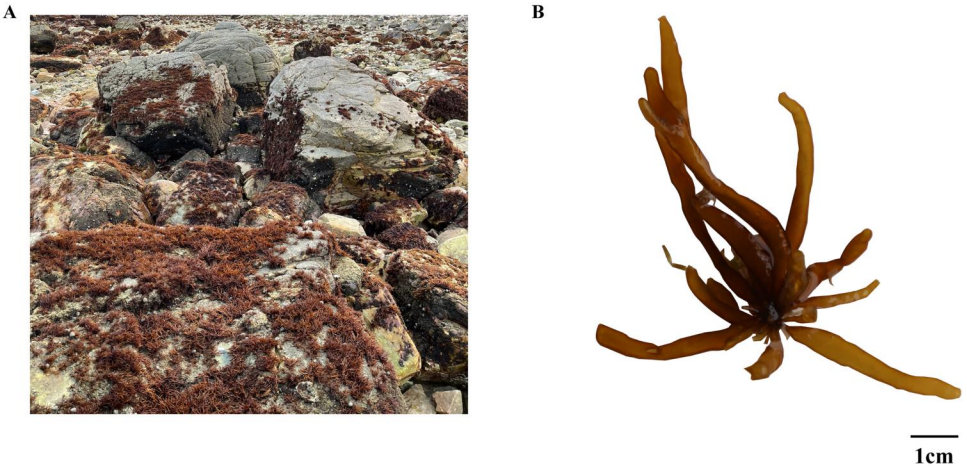

Figure 1. Growth environments and morphological characteristics of *G. furcata*. (A) The *G. furcata* inhabits the upper intertidal zone. (B) Morphological characteristics of *G. furcata*: the thallus appears brown or brownish-yellow, growing in clusters, with a disk-shaped base, and irregular fork-like branching; the main branches are cylindrical, gradually tapering to a point or becoming rounded at the top, and the mature thallus has a hollow interior. The photos were taken by Wenjun Li in Yantai City, Shandong province, China, and are free of copyright issues.

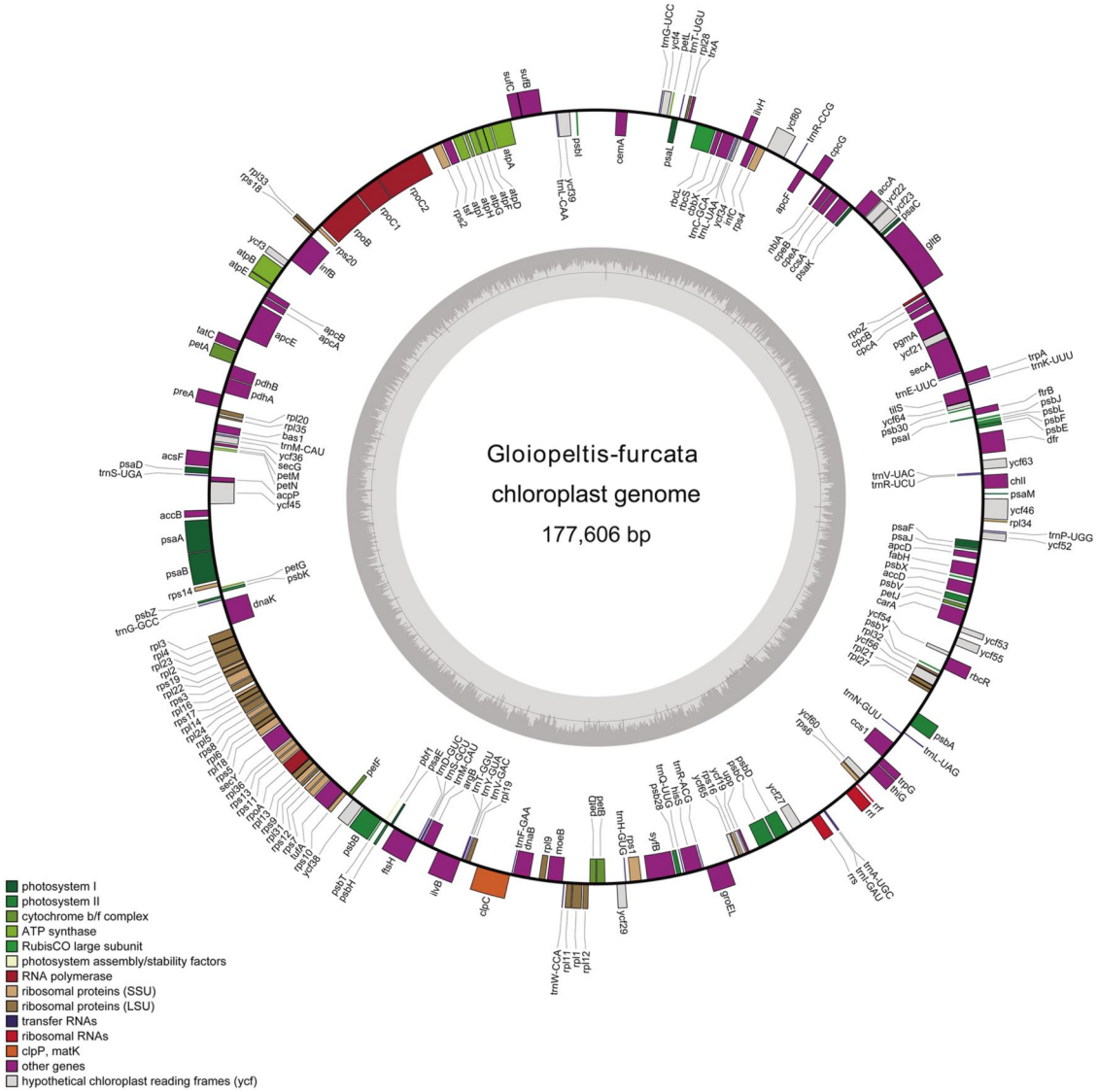

Figure 2. The circular map of the assembled *G. furcata* chloroplast genome consists of 152 protein-coding, 29 transfer RNA, and three ribosomal RNA genes. Genes drawn inside the circle are transcribed clockwise, and those outside are transcribed counterclockwise. Genes belonging to different functional groups are color-coded. The darker gray in the inner circle corresponds to DNA G/C content, while the lighter gray corresponds to A/T content. The map was drawn using the OGDRAW web server.

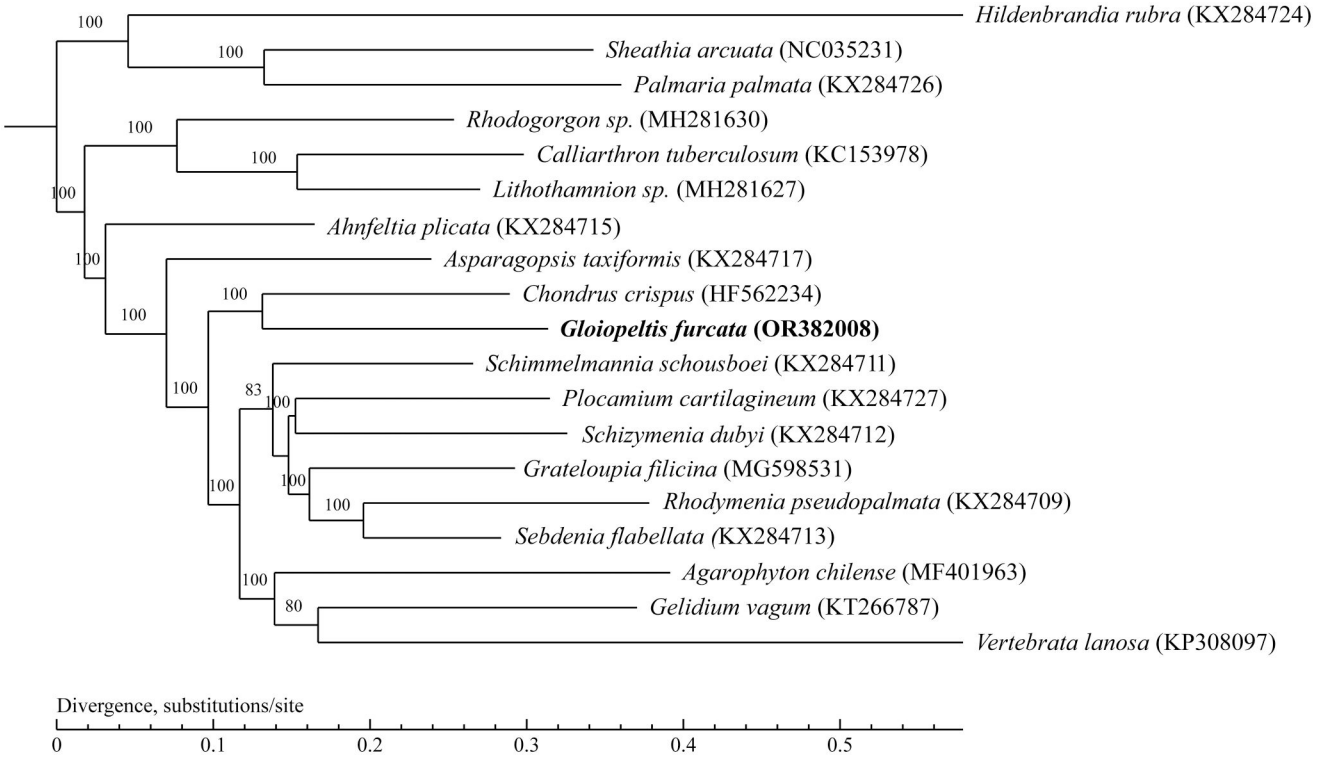

Figure 3. The maximum-likelihood (ML) phylogenetic tree of *G. furcata* was constructed from 151 common single-copy homologous genes across 19 species. The following sequences were used: *Rhodymenia pseudopalmata* (KX284709), *Sebdenia flabellata* (KX284713), *Plocamium cartilagineum* (KX284727), *Schizymenia dubyi* (KX284712), *Schimmelmannia schousboei* (KX284711), *Asparagopsis taxiformis* (KX284717), *Ahnfeltia plicata* (KX284715), *Grateloupia filicina* (MG598531) (Zhang et al. 2018), *Gelidium vagum* (KT266787) (Lee et al. 2016), *Chondrus crispus* (HF562234), *Calliarthron tuberculosum* (KC153978) (Janouškovec et al. 2013), *Lithothamnion sp.* (MH281627), *Rhodogorgon sp.* (MH281630) (Lee et al. 2018), *Sheathia arcuata* (NC035231) (Nan et al. 2017), *Palmaria palmata* (KX284726) (Cho et al. 2018), *Vertebrata lanosa* (KP308097), and *Agarophyton chilense* (MF401963). *G. furcata* (OR382008) is the target chloroplast sequence of this study, highlighted with color. *Hildenbrandia rubra* (KX284724) (Lee et al. 2016) were served as the outgroup. The phylogenomic tree was constructed using the GTR + G + I model of the maximum-likelihood method with 1000 bootstrap replications.

Microsatellite identification tool (Beier et al. 2017). We estimated the ratio of specific codon frequency to the expected frequency by RSCU using cusp (EMBOSS v6.6) with default parameters (Liu et al. 2019). The phylogenetic tree was constructed using the general time-reversible (GTR) + G model, selected with jModelTest v2.1.7 (Darriba et al. 2012).

To explore the phylogenetic position of *G. furcata*, the molecular phylogeny of the class Florideophyceae was investigated. A total of 151 common single copies of homologous genes (Supplementary File 1) from 19 species were used for phylogenetic analysis in MEGA 11.0 (Tamura et al. 2021). The phylogenomic tree was constructed by the maximum-likelihood (ML) method based on the substitution model GTR + G + I in PhyML 3.0 (Larkin et al. 2007; Guindon et al. 2010) with 1,000 bootstrap replicates (Letunic and Bork 2016).

Results and discussion

Among chloroplast diversity, the chloroplast genomes of red algae are considered ancestral and evolutionarily stable in terms of gene content and genome organization (Sergio et al. 2017). Costa et al. (2016) revealed the phylogenomic relationships of the Nemaliales (Rhodophyta) using chloroplast genomes from 22 species of this order, which were well supported by phylogenetic analyses. To better characterize the diversity of chloroplast genomes in red algae, we sequenced the chloroplast genome of *G. furcata*. A total of 4913.8 Mb raw data was generated, with 4864 Mb clean data remaining. The newly assembled chloroplast genome of *G. furcata* is 177,606 bp in length and has a GC content of 33.58% (Figure 2). Compared with the previously published chloroplast genome (GenBank: OP612670.1), the chloroplast genomes assembled in this study contained an additional 460 bp and were more complete. A total of 184 genes, including 152 protein-coding genes (mRNAs), 29 tRNAs, and three rRNAs, were annotated in this chloroplast. The characteristics of the chloroplast genome of *G. furcata* are consistent with those of other red algae: relatively large, gene-dense, and with highly conserved gene sequences. Similar to other algae (Turmel et al. 1999; Janouškovec et al. 2013), *G. furcata* showed no LSC, small single copy (SSC), and IR structure (Supplementary File 2).

SSRs have also been employed as significant markers in species variation, plant population genetics, and evolution research, owing to their high repeatability, high variability, and codominant inheritance (Huang et al. 2014; Zhang et al. 2016). In our present study, four SSR types were detected, including mononucleotide, dinucleotide, trinucleotide, and tetranucleotide repeats. The most abundant SSR was mononucleotide repeats (84) (Figure S2A). Most of the SSR repeats were composed of A/T, which may contribute to *G. furcata*'s low GC content.

We estimated the Codon Usage frequency across all coding genes. The higher-frequency codons were AGA (RSCU = 3.21), TTA (RSCU = 2.60), and TCT (RSCU = 1.88; Supplementary File 3). The codons were used mainly by arginine, leucine, and serine (Figure S2B). Codon Usage bias is characteristic of all biological systems, since the frequencies of synonymous codon use for each amino acid are unequal (Ikemura 1981). The synonymous Codon Usage frequencies of arginine appear to be significantly different, with the AGA-encoded arginine having the highest RSCU value, and the CGG-encoded arginine having the lowest (average RSCU = 0.15).

To gain insight into the position of *G. furcata* within the Florideophyceae, a phylogenetic analysis was performed using chloroplast genome sequences of *G. furcata* and other species. The phylogenetic tree showed that 16 *Gloiopeltis* species were grouped into two major branches (Figure 3). The results strongly support the positioning of the *G. furcata* within Florideophyceae and the close relationship of *G. furcata* to *Chondrus crispus* (HF562234), similar to a previous mitochondrial phylogenetic tree (Watanabe et al. 2019).

Conclusion

Our present study revealed the complete chloroplast genome of *G. furcata* using Illumina sequencing for the first time, providing significant information for phylogeny and plant molecular identification of the genus *Gloiopeltis*. Moreover, chloroplast phylogenomics is an attractive approach for phylogenetic studies in red algae. The genomic, SSR, and Codon Usage analyses may provide useful biomarkers for plant identification, phylogenetic analysis, and population genetics.

Author contributions

CRediT: **Xiaoxue Liu**: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing; **Chunchen Liu**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Visualization, Writing – original draft; **Wenjun Li**: Investigation, Methodology, Resources, Writing – original draft; **Kaida Xu**: Conceptualization, Funding acquisition, Writing – review & editing.

Disclosure of interest

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. OR382008. The associated BioProject, BioSample, and SRA accession numbers are PRJNA1000415, SAMN36767670, and SRX21194139, respectively.

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