Molecular studies on *Plocamium cartilagineum* complex (Plocamiales, Rhodophyta) suggest cryptic diversity in the Antarctic region

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Abstract

The red algae *Plocamium* (Plocamiales, Rhodopghyta) is a cosmopolitan genus, constituted by approximately 40 species (Wynne, 2002) that are widely distributed throughout the world's oceans. Morphological and anatomical traits, as well as biogeographical criteria have been traditionally employed to define most of the species. However, recent molecular systematic studies (e.g. Cremades et al. (2011); Saunders and Lehmkuhl (2005); Yano et al. (2006)) indicate that classical morphological-based delimitations have failed to accurately discern diversity and evolutionary relationship within this group of seaweeds. In this context, the type species *Plocamium cartilagineum* (Linnaeus) P.S. Dixon, with a worldwide distribution and showing considerably morphological variability, represents a particularly complex study case from a taxonomic and systematic point of view. Using a combination of morphological, anatomical and molecular information, Cremades et al. (2011) proposed a new taxonomic framework for North Atlantic *Plocamium* entities formerly considered *P. cartilagineum*. These and other authors also reported cryptic diversity in specimens originally described as P. cartilagineum from other distant areas of the world (e.g. Asia, Yano et al. (2006); or Oceania, Cooper and Wynn (2017)). However, other oceanic regions – such as Antarctic continent – have been relatively overlooked in previous works devoted to study the taxonomic and evolutionary diversity of *P. cartilagineum*. Here, our main aim is to study the diversity of *P. cartilagineum* from the Antarctic region using morphological and molecular information to compare with samples from other points of the world. In addition, we will investigate the variability of certain cytogenetic traits, as potential alternative markers for discriminating between taxonomic and evolutionary units within this species.

The Antarctic samplings were carried out in South Bay and False Bay of Livingston Island (South Shetland Islands), during the austral summer of 2019. The Mediterranean samples were collected at the north coast of Catalonia (Spain), at Ras Cape (Colera, Girona) and La Trona (Roses, Girona). Samples were divided in two types of conservation and pre-processing methods. For molecular analyses, fresh samples were immediately conserved in silica gel just after collection from the field. For nuclear DNA content and morpho-anatomic study, collected algae were frozen (-20°C) until their arrival to our laboratory at the University of Barcelona. Once there, the specimens were defrosted for their determination. Little fragments of the specimens were fixed with Carnoy solution (3:1 of 95% ethanol-glacial acetic acid) during 24 hours and stored in 70% ethanol at 4°C for subsequent nuclear DNA content analysis. Reference specimens were pressed and deposited in the BCN-Phyc Herbarium (Documentation Center of Plant Biodiversity, University of Barcelona, Spain).

We made molecular analysis of the samples by barcoding following Saunders and Lehmkuhl (2005) and Cremades et al. (2011) procedures. Specific primers for mitochondrial cox1 and the plastid rbcL markers were designed using reference genomic data of *P. cartilagineum* from Genbank (KJ398160; NC031179). The obtained sequence data of *P. cartilagineum* from Antarctic and Mediterranean regions were visualized and processed using Geneious Prime 2019 and aligned to other sequences of P. cartilagineum obtained from Genbank. Evolutionary groups were visualized with the construction of a neighbour-joining tree using PAUP as implemented in Geneious to compare with the phylogenetic representation of the genus obtained by Cremades et al. (2011). Nuclear DNA content estimates based on image analysis of DAPI-stained specimens followed a procedure using a Cooled CCD Miramax RTE 782-Y high performance digital camera placed on a Leica DMRB fluorescence microscope and subsequently analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). Nuclear DNA content was obtained by comparing the fluorescence intensity of sample's nuclei with those of *Gallus gallus* (Linnaeus) erythrocytes, since the later have a stable nuclear DNA content of 2.4 pg. For each species, histograms were constructed with the obtained nuclear DNA values to determine the C-value and assigning ploidy levels. To conduct the morphological study we measured diagnostic parameters based on previous descriptions (Cremades et al., 2011; Saunders and Lehmkuhl, 2005) for the comparison of the samples of the two localities. Our molecular phylogenetic analyses revealed that samples of *P. cartilagineum* from Antarctic region constitute an evolutionary distinct group. These results suggest at least one cryptic entity, not associated with *P. cartilagineum* sensu stricto, exists in Antarctic region. From the morphological point of view, the Antarctic specimens show subtle differences that could represent useful traits for potential taxonomic discrimination. Interestingly, our nuclear DNA content study of samples from the Mediterranean and the Antarctic region support the hypothesis of Antarctic *P. cartilagineum* being a separated evolutionary entity. Even though ploidy levels measured in the samples of both geographical areas are similar (with ploidy ranging from 2C = 0.05 to 32C = 0.8 picograms), the nuclear patterning observed are different between them 1, reinforcing the idea of divergent groups. In the case of Antarctic samples, we have observed a nuclear patterning consisting of cells with a big polyploid nucleus and several smaller nuclei. In the other hand, the nuclear pattern observed in the Mediterranean samples consists only in cells with a big polyploid nuclei, being dissimilar to the previous description of nuclear patterning given for *P. cartilagineum*.

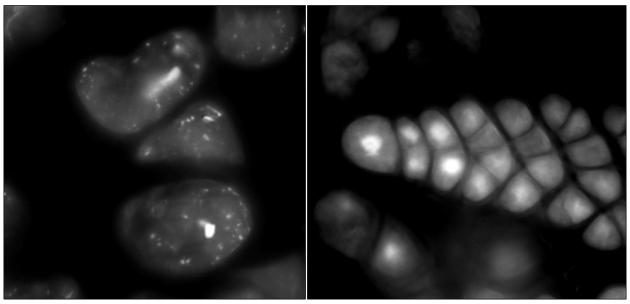

Figure 1: Samples of *P. cartilagineum*.

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