Building-up knowledge on green marine macroalgae diversity in the Western Antarctic Peninsula: data from two molecular markers reveals numerous species with amphipolar distribution

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Acrosiphonia arcta kindly illustrated by Enzo Mardones (www.behance.net/enzomardones); illustration was based on photography available at www.algaebase.com.
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INTRODUCTION

In green algae, the so-called “core” Chlorophyta includes the three major classes Chlorophyceae, Ulvophyceae, Trebouxiophyceae and a few smaller classes (Fang et al. 2018; Del Cortona et al. 2020). They have diverged early from the Prasinophyceae during the Paleozoic era (Leliaert et al. 2012; Fučíková et al. 2014; Del Cortona et al. 2020), and some 6500 different species are described nowadays (Guiry & Guiry 2019). These species are ecologically and morphologically very diverse and are found in a wide variety of terrestrial, marine and freshwater environments. In the cold waters surrounding Antarctica, 15 to 17 species belonging to Ulvophyceae, Trebouxiophyceae and Chlorophyceae have historically been reported (Gallardo et al. 1999; Ramirez 2010; Wiencze & Clayton 2002; Wiencze et al. 2014). However, the recent study of Pellizzari et al. (2017) updated this number to 24 along the coasts of the South Shetland Islands (SShs), with five new records for the area (Chaetomorpha irregularis (Zaneveld) M.Cormaci, G.Furnari & G.Alongi, Rhizoclonium ambiguum (J.D.Hooker & Harvey) Kützing, Monostroma grevillei (Thuret) Wittrock, Spongocarpa arctica (Dillwyn) Kützing and Ulvella

TABLE

<table>
<thead>
<tr>
<th>Species</th>
<th>Phyllum</th>
<th>High latitudes</th>
<th>Low latitudes</th>
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<tr>
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<td>No</td>
</tr>
<tr>
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<td>Ulvophyceae</td>
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ABSTRACT

Low levels of diversity and endemism, when compared to red or brown algae, have been reported for Antarctic green marine macroalgae (Chlorophyta). However, recent studies including the use of molecular markers have allowed us to revisit the taxonomical status of species thought to be well known, underlying the existence of unexpected Antarctic flora diversity at local and regional scale. In the present study, samples of green macroalgae along the Western Antarctic Peninsula (from the 62°S down to the 66°S) were sequenced for two genetic markers regularly used for species determination and barcoding in Chlorophyta (i.e., the plastid genes tufA and rbcL). From the 122 specimens of Chlorophyta sampled, 85 were sequenced for the gene tufA and 16 for the gene rbcL. Using the NCBI Nucleotide Blast Tool to compare our sequences to the ones available in public data repositories allowed the identification of 11 species. Three new species were reported for the area: Rosenvingiella radicans (Kütz.) Rindi, L.McVor & Guiry, Uropsora wormskioldii (Mertens) Rosenvinge and Ulvella islandica R.Nielsen & K.Gunnarsson. Furthermore, molecular identification revealed strong match (> 95%) between our Antarctic sequences and the ones obtained for samples from the northern hemisphere for Acrosiphonia arctica (Dillwyn) Gain, Prasiola crispa (Lightfoot) Kützing, Prasiola antarctica Kützing 1849, R. radicans, Ulva sp. A-GW, U. islandica, Urospora penicilliformis (Roth) Areschoug and U. wormskioldii confirming the amphipolar distribution of various taxa of Antarctic Trebouxiophyceae and Ulvophyceae. Amphipolar distribution seems more common in green than red or brown Antarctic seaweeds, so here we hypothesize that recurrent occurrence of long dispersal events could explain the low level of endemism observed for this phylum along the Antarctic coasts.

RÉSUMÉ

Un patron de distribution amphipolaire chez plusieurs algues vertes Antarctique détecté à l’aide d’outils moléculaires.

Comparer aux algues marines rouges ou brunes, des niveaux faibles de diversité et d’endémisme ont historiquement été reportés pour les macroalgues vertes de l’Antarctique (Chlorophyta). Cependant, des études récentes incluant l’utilisation de marqueurs moléculaires ont permis de revoir le statut taxonomique d’espèces que l’on croyait bien connues, révélant une diversité inattendue de la flore antarctique à l’échelle locale et régionale. Dans cette étude, des échantillons de macroalgues vertes prélevés le long de la péninsule Antarctique occidentale (de 62°S à 66°S) ont été séquencés pour deux marqueurs génétiques régulièrement utilisés pour l’identification des espèces de Chlorophytes (i.e. gènes plastidiques tufA et rbcL). Sur les 122 spécimens de Chlorophyta échantillonnés, 85 ont été séquencés pour le gène tufA et 16 pour le gène rbcL. Grâce à l’outil Nucleotide Blast de la plateforme NCBI utilisé pour comparer nos séquences à celles disponibles dans la base de données publique, nous avons identifié 11 espèces dont trois reportées pour la première fois dans la région : Rosenvingiella radicans (Kütz.) Rindi, L.McVor & Guiry, Uropsora wormskioldii (Mertens) Rosenvinge et Ulvella islandica R.Nielsen & K.Gunnarsson. De plus, l’identification moléculaire a révélé une forte correspondance (> 95%) entre nos séquences antarctiques et celles obtenues pour des espèces de l’hémisphère nord, incluant Acrosiphonia arctica (Dillwyn) Gain, Prasiola crispa (Lightfoot) Kützing, Prasiola antarctica Kützing 1849, R. radicans, Ulva sp. A-GW, U. islandica, Urospora penicilliformis (Roth) Areschoug et U. wormskioldii confirmant la distribution amphipolaire de divers taxons de Trebouxiophyceées et Ulvophyceées antarctiques. La distribution amphipolaire semble plus fréquente chez les algues vertes antarctiques que chez les algues rouges ou brunes. Nous émettons l’hypothèse que des épisodes récurrents de dispersion à longue distance pourrait expliquer le faible niveau d’endémisme observé pour ce phylum le long des côtes antarctiques.

KEY WORDS

Antarctic, Chlorophyta, barcoding ADN, tufA et rbcL, amphipolar distribution, endemism.

MOTS CLÉS

Antarctique, Chlorophyte, barcoding ADN, tufA et rbcL, distribution amphipolaire, endémisme.
viridis (Reinke) R.Nielsen, C.J.O’Kelly & B.Wysor), and one putative new species (i.e., Prasiola sp. distinct from Prasiola crispa (Lightfoot) Kützing already mentioned for the area). Some of these new records are supported by results obtained with molecular markers (i.e., cytochrome c oxidase - COI-5P; UPA genes and Internal Transcribed Spacer - ITS - region for M. grevillei, Protomonostroma sp. and Prasiola sp.).

Recent studies including molecular tools have allowed to revisit the taxonomical status of species thought to be well known, improving knowledge on diversity and level of endemism characterizing the Antarctic flora (red algae: Hommersand et al. 2009; Pellizzari et al. 2017; Dubrasquet et al. 2018; Guillemin et al. 2018; Ocaranza-Barrera et al. 2019; green algae: De Wever et al. 2009; Moniz et al. 2012; Garrido-Benavent et al. 2017; Pellizzari et al. 2017; brown algae: Peters et al. 1997, 2000). In Chlorophyta, studies using plastid sequences have underlined unexpected diversity at local and regional scale along the Antarctic coasts for Chlorophyceae and Trebouxiophyceae (De Wever et al. 2009; Moniz et al. 2012) and highlighted that the supposedly well-known Antarctic green macroalgae diversity, with very few species reported in comparison with other marine realms (Griffiths 2010), could be underestimated (De Wever et al. 2009; Moniz et al. 2012; Mystikou et al. 2014). However, few molecular data are available in public data repositories for Antarctic green algae (i.e., 70 sequences of macroalgae obtained as result for a search for “Antarctic marine Chlorophyta” in GenBank database considering all available molecular markers, author’s pers. obs.).

Accurate and exhaustive understanding of the native algal flora biodiversity and distribution is a key factor for monitoring Antarctic seaweeds (Wiencke et al 2014). In the Western Antarctic Peninsula (WAP), recent transformations of the physical environment linked to global climate change (e.g. increasing sea temperatures and sea ice melting; Etourneau et al. 2019; Holland et al. 2019; Meehl et al. 2019; Valdivia et al. 2020) may for example favor the arrival and settlement of non-native species, affecting the functions of whole benthic communities (Wiencke et al. 2014; Hughes & Ashton 2016; McCarthy et al. 2019; Hughes et al. 2020). The development of molecular tools associated with comprehensive sampling have allowed for rapid and efficient detection of marine non-native species (Bott et al. 2010). In Antarctica, the Patagonian mussel Mytilus cf. platensis (Cárdenas et al. 2020) and the bryozoan Membranipora membraanae (Avila et al. 2020) were reported for the first time in the WAP in 2020. Both species have been categorized as “invasive non-native species likely to threaten biodiversity and ecosystems” in Antarctica (Hughes et al. 2020). Regarding green algae, some species of Chlorophyta are recorded among the most invasive marine organisms (Williams & Smith 2007) and have demonstrated drastic effects on coastal ecosystem functions (e.g. Caulerpa taxifolia introduction in Mediterranean Sea; Bellan-Santini et al. 1996; Jousson et al. 1998). The only non-native photosynthetic marine organism reported to be recently established in Antarctica is the green alga Ulva intestinalis (Clayton et al. 1997). The species was observed in highly touristic sites and close to human settlements (e.g. scientific bases) around the SSJs and the WAP, and its arrival was related to maritime transport (i.e., specimens found as biofouling on ship hull; Clayton et al. 1997; Chown et al. 2012; Chown et al. 2015; Hughes & Ashton 2016). As increasing shipping traffic augments propagule pressure of potential new colonizer (Lee & Chown 2009; Hughes & Ashton 2016; Cárdenas et al. 2020; Hughes et al. 2020), being able to detect early arrival of non-native species and to monitor their possible settlement and distribution range extension will rely on a comprehensive sampling design associated with long-term monitoring and available molecular data (Wiencke et al. 2014; McCarthy et al. 2019). However, apart from the efforts of Wiencke et al. (2014) to resume the current state of knowledge about Antarctic seaweed diversity and distribution, long-term data monitoring is still lacking for these taxa at regional scale (Grant & Linse 2009; De Broyer & Danis 2011).

MATERIAL AND METHODS

SAMPLING

Sampling was realized during austral summers between 2011 and 2014 within the framework of four campaigns organized by the Chilean Antarctic Institute (INACH). Five areas were sampled (Fig. 1), two located in the SSJs (near the Chilean Capitán Arturo Prat base in Greenwich Island and at Bahia Fildes in King George Island, hereafter referred as PRAT and KGI, respectively) and three areas along the Northern and Central part of the WAP (near the Chilean O’Higgins Antarctic base, noted OHI; in Paradise Bay, near the Chilean Presidente Gabriel González Videla Antarctic base, noted GGV and in Marguerite Bay, noted MAR). In all areas, intertidal samplings were conducted during diurnal low tide hours while subtidal samples were collected by SCUBA diving. Specimens showing different morphotypes (e.g. presenting noticeable variations in thallus shape, color or thickness and elasticity) were collected. All specimens were pressed as vouchers after removing a small portion of the thallus that was stored in silica gel for subsequent DNA analysis. Voucher specimens are housed in the herbarium of the Universidad Austral de Chile and available on request. All voucher specimens were identified, to the lowest possible taxonomic level, on the basis of morphological criteria using floristic keys and species lists available for the region (Wiencke & Clayton 2002; Ramirez 2010; Pellizzari et al. 2017).
DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

For each specimen stored in the herbarium, a fraction corresponding to some 30 mm² of dried tissue was milled in a Mini-BeadBeater 24 (BioSpec Products, Inc. Bortlesville, United States) and DNA extraction was performed with an E.Z.N.A tissue DNA kit (Omega Bio-tek, Inc. Georgia, United States) following the manufacturer instructions.

A fragment of the plastid gene *tufA*, encoding for protein synthesis elongation factor Tu (EF-Tu), was amplified for all samples. This gene, well conserved in a wide variety of
photosynthetic species, allows for reliable plant and green algae species determination (Fama et al. 2002; Saunders & Kucera 2010) and has been largely used to infer green macroalgae phylogeny (Leliaert et al. 2012). Amplification of tuFA was realized using the primers (TuFAg4: 5'TGAAACA-GAAAMAWAWGTCAATTATGC-3' and TuFAr: 5'CCTTTC-NCGAATMGCCRAAWCGC-3') developed by Fama et al. (2002) following the published protocol.

For a subsampling of green algae specimens (i.e., one or a few specimens per distinct genetic entities detected with the gene tuFA), the plastid gene rbcL coding for the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase was amplified. The primers GrbCLnF (5' GCTGGWGTAAAGAT-TAYCG 3') and GrbcLR (5'TCACGCCCAACGCATRAASGG 3') developed by Saunders & Kucera (2010) were used and the PCR reaction mix and program followed the protocol of Pirian et al. (2016).

All PCR reactions were performed in a Perkin Elmer Gene Amp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, United States). PCR products were purified using the commercial kit UltraCleanTM (MO BIO Laboratories, Carlsbad, USA). Quality and concentration of purified PCR products were verified by electrophoresis on 2% agarose gel dyed with GelRed™ (Biotium Inc, Hayward, United States). Sequencing was performed in AUSTRAL-omics Core-Facility (Universidad Austral de Chile, Chile) using an ABI PRISM®310 Genetic Analyzer (Applied Biosystems, Foster City, United States).

DATA ANALYSES
Sequences were edited using Chromas v.2.33 (McCarty 1997), and aligned using MEGA v.5 (Tamura et al. 2011). Molecular species identification was performed using the basic local alignment search tool (BLAST) from NCBI (Altschul et al. 1990) and comparing the sequences obtained in this study with those available in GenBank. Only the highest score and percentage identity values were kept for recording a match (see Appendices 1 & 2).

RESULTS
A total of 122 specimens of Chlorophyta were sampled between the high intertidal down to a depth of 30 m in the sampling area: 34 in PRAT, 22 in KGI, 15 in OHL, 34 in GGV and 17 in MAR. Because of low quality and/or quantity of DNA extracted for some specimens, tuFA sequences were obtained only for 92 specimens, representing 75% of the samples. Among these 92 sequences, four were contaminated by the bacterium Granulosioccus antarcticus (Lee et al. 2008) and three by the diatom Seminavis robusta D.B.Daniellidis & D.G.Mann and removed from the dataset. The remaining 85 sequences obtained for green macroalgae belonged to Ulvophyceae (69 specimens of Ulotrichales and 11 specimens of Ulvales) and Trebouxiiophyceae (five specimens belonging to Prasiolales). Eleven distinct putative species were detected in the present study using tuFA molecular dataset: eight are part of the Ulvophyceae class and three of the Trebouxiiophyceae class (Appendix 1). In order to confirm the species assignation based on the tuFA gene, the rbcL gene was amplified in a sub-sample of randomly selected specimens (N = 31 in total) belonging to each putative species. The rbcL sequences were obtained for only half of tested specimen (N = 15) belonging to seven putative species out of the twelve detected with the tuFA gene (Appendix 2). No rbcL. PCR products were obtained for Monostroma hariotii, even after testing amplification using the DNA of the 37 specimens available. Among the 15 rbcL obtained sequences, 11 were congruent with tuFA sequences identification (Appendix 1). The remaining four sequences were identified as Ulva sp. A-GW and congruent with morphological identification but tuFA gene sequences were lacking for these specimens. All sequences were deposited in the public depository (GenBank NCBI Public Database; see Appendices 1 and 2). Following the classification of Guiry & Guiry (2019), taxonomic status regarding species-specific results provided by tuFA and rbcL datasets are registered below. Reported distribution also follows Guiry & Guiry (2019).

Acrosiphonia arcta (Dillwyn) Gain
(SShs: PRAT, WAP, GGV)

REPORTED DISTRIBUTION. — Arctic, North Europe (Sweden, Denmark, Britain, Faroe Islands), North America (Alaska, Oregon, Canada, British Columbia), South America (Chile, Argentina, Falkland Islands), Asia (East Russia and Kamchatka, Bering Sea), Antarctic and subAntarctic islands (South Georgia, SSs, Kerguelen Islands, Auckland Islands, Campbell Island), New Caledonia.

COMMENT
Closest match (98,31%, GenBank Access Number HQ610211, Appendix 1) with Antarctic tuFA sequences was a Canadian specimen of Acrosiphonia arcta from British Columbia. No rbcL sequences were obtained for this species. Acrosiphonia arcta has previously been reported in Antarctic waters (Ramirez, 2010), mainly under the name Spongomorpha arcta (Pappenfuss 1964; Pellizari et al. 2017) considered as synonymous for this species (Guiry & Guiry 2019). We provide here the first genetic data for A. arcta in the southern part of its area of distribution (i.e., GGV in the WAP), and confirm its amphipolar distribution (Van Oppen et al. 1993; Saunders & Kucera 2010, Fig. 2).

Capsisiphon sp. Gobi
(WAP: GGV)

REPORTED DISTRIBUTION. — Capsisiphon groelandicus has been reported in Arctic (Svalbard), North Asia (China, Japan, East Russia, Kamchatka, Commander Islands), Antarctic and sub-
Antarctic islands (Adelaide Island). *Capsosiphon fulvescens* has been reported in Europe (United Kingdom, Belgium, Crimea, Denmark, Faroe Islands, Greenland, Iceland, France, Germany, Ireland, Italy, Netherlands, Norway, Sweden, Spain, Ukraine), United States (Alaska, California, Connecticut, Maine, New Hampshire, New Jersey), Canada (British Columbia, New Brunswick), North Asia (China, Japan, Korea), Argentina, Sub-Antarctic Islands (Saint Paul).

**Comment**
The closest match for the *tufA* and *rbcL* sequences were obtained with the *Capsosiphon fulvescens* plastid complete genome (93.26% and 98.53%, respectively, GenBank Access Number NC_039920, Appendices 1 and 2). The lower percentage of identity between our sequences and the *Capsosiphon fulvescens* plastid genome for the *tufA* than the *rbcL* molecular marker could be explained by a slightly higher mutation rate of the *tufA* gene in Antarctic green algae. Indeed, in their previous work, Saunders & Kucera (2010) reported both within and between species sequences divergence slightly higher for the *tufA* than the *rbcL*-5P (see in particular the results in *Ulva*, the only genus for which a high number of sequences were obtained). *Capsosiphon fulvescens* has never been reported in the Antarctic. However, using morphological identification combined with the information from various nuclear markers (18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 50-28S rRNA gene), Mystikou et al. (2014) reported the presence of *Capsosiphon groenlandicus* in the WAP (i.e., Adelaide Island in Marguerite Bay). Since species identification could not be clarified with the help of our two combined molecular markers, we choose to keep the name *Capsosiphon* sp. for the specimens sampled in the present study until further taxonomic work.

**Protomonostroma sp. A-GW**
(SSHs: PRAT)

**Reported distribution.** — *P. undulatum* has been reported in Europe (United Kingdom, Norway, Germany, Faroe Islands, Iceland, Greenland, Denmark), United States (Maine, Alaska), Canada (British Columbia, New Brunswick), North Asia (Japan, China, Korea, Kamchatka, East Russia), Argentina. *P. roslatum* Vinogradova, has only been reported in the South Shetland Islands.

**Comment**
The closest match for the *tufA* gene (99.87%, GenBank Access Number MG646367, Appendix 1) was found with *Protomonostroma* sp. A-GW from King George Island. Exact match with *rbcL* sequences of *Protomonostroma* sp. A-GW from King George Island was observed (100%, GenBank Access Number MG711514, Appendix 2). Based on previous reports (Medeiros 2013) and complementing morphological characters with molecular markers, Pellizzari et al. (2017) reported the presence of *Protomonostroma roslatum* in the SSHs instead of *P. undulatum*, as described in the early study of Vinogradova (1984). However, since closest matches obtained for both genes for our sequences were with *Protomonostroma* sp. A-GW we decided to use this last name for specimens sampled in the present study until further taxonomic work.

**Genus Urospora Areschoug**

**Urospora sp. 1 penicilliformis**
(SSHs: PRAT, WAP; GGV, MAR)

**Reported distribution of Urospora penicilliformis.** — Worldwide except tropical waters.

**Comment**
Exact match was found with *Urospora* sp. 1 *penicilliformis* *tufA* sequence from Nome, Alaska (GenBank Access Number MH571163, Appendix 1). Another close match was found (99.48%, GenBank Access Number HQ610440, Appendix 1) with *Urospora* sp. 1 *penicilliformis* *tufA* sequence from Canada. Exact match was found for the *rbcL* gene with *Urospora* sp. 1 *penicilliformis* sequence from United States, Maine (GenBank Access Number HQ603674, Appendix 2). Type locality for *Urospora penicilliformis* is located in the northern hemisphere, probably in Germany (Guiry & Guiry 2019), but the species has previously been reported (based on morphological character) in the southern hemisphere along the Chilean and Argentinean coasts (Ramirez & Santelices 1991; Boraso de Zaixso 2004, 2013), Antarctica and Sub-Antarctic Islands (Papenfuss 1964; Wiencke & Clayton 2002; Mystikou et al. 2014), Australia and New Zealand (Womersley 1984; Broady et al. 2012). Amphipolar distribution of *Urospora* sp. 1 *penicilliformis* is supported by molecular data (Alaska: Bringloe & Saunders 2019; British Columbia: Saunders & Kucera 2010; Antarctic and sub-Antarctic Islands: the present study, Fig. 2).

**Urospora wormskioldii** (Mertens) Rosenvinge
(WAP; OHI)

**Reported distribution.** — Arctic (Canada, Svalbard, Greenland, Iceland & Faroe Islands), North Europe (Germany, Denmark, Britain, Baltic Sea, Norway, Spitzberg), North America (both Pacific and Atlantic coasts down to Mexico), North Asia (China, East Russia, Kamchatka).

**Comment**
The closest matches for three specimens formerly identified as *Urospora penicilliformis* based on morphological characters were observed with sequences of *U. wormskioldii* from British Columbia, Canada for both *tufA* and *rbcL* genes (99.87%, GenBank Access Number HQ610441 and HQ603676 for *tufA* and *rbcL*, respectively, Appendices 1 and 2). The present molecular data represent the first report of a second *Urospora* species in Antarctic waters, underlying the unknown amphipolar distribution pattern of *U. wormskioldii* (Lindstrom & Hanic 2005, Fig. 2).
Fig. 2. — Amphipolar distribution of four species of Chlorophyta detected in the SSHs and/or WAP during our study. General distributions (shown as colored lines) follow information given in AlgaeBase repository (Guiry & Guiry 2019). Genetic data (i.e., tufA) available for the species are indicated with stars: red stars, present study; black stars, already published in GenBank/NCBI Database. All illustrations were kindly realized by Enzo Mardones, based on photographs available in www.algaebase.com (for Acrosiphonia arcta), www.seaweedsofalaska.com (for Urospora wormskioldii, Urospora sp.1 penicilliformis, Ulva sp A-GW). Cell illustrations for Urospora wormskioldii and Urospora sp.1 penicilliformis were based on www.algaebase.com photographs available for Urospora penicilliformis and Urospora wormskioldii. Ulva sp. A-GW illustration was based on Ulva linza photography available at www.seaweedsofalaska.com/
Family MONOSTROMATEAE Kunieda
Genus Monostroma Thur.

Monostroma hariotii Gain
(SShs: PRAT, KGI; WAP: OHI, GGV and MAR)

Reported distribution. — Antarctic and SubAntarctic Islands (Kerguelen Islands, Macquarie Island, South Georgia, South Orkney Islands, SSnts, Antarctic Peninsula, Wilkes Land), South America (Argentina, Falkland Islands).

Comment
Exact match for tufA gene was observed with sequence of a specimen named Monostroma angicava from King Georges Island (100%, GenBank Access Number MG646366, Appendix 1). Comparison with other sequences of specimens of Monostroma available in public repositories showed a lower percentage of similarity (e.g. 92.60% of similarity with Monostroma grevillei sp. 1 from Canada, GenBank Access Number HQ610257). The species Monostroma grevillei sp. 1 has, however, been reported, identified by molecular approaches, in the South Shetland Islands (Peulvast et al. 2017). No rbcL sequences were obtained for this species in the present study. Since our samples were first determined as M. hariotii based on morphological characters and due to the fact that M. hariotii has been reported as an emblematic specie of the Antarctic and SubAntarctic waters (Wiencke & Clayton 2002) while M. angicava has only been reported in the northern hemisphere, we decided to retain the name M. hariotii for specimens sequenced in the present study. Monostroma hariotii has been reported as common in the Falklands, Kerguelen and Macquarie Islands (Wiencke & Clayton 2002), South Shetland Islands including King George Island (Wiencke & Clayton 2002; Quartino et al. 2005; Al-Handal & Wulff 2008; Peulvast et al. 2017), Wilkes Land (Runcie & Riddle 2006) and the Antarctic Peninsula (Lamb & Zimmermann 1977; Amsler et al. 2005; Mystikou et al. 2014).

Ulva islandica R.Nielsen & K.Gunnarson
(SShs: PRAT)

Reported distribution. — North Europe (Iceland).

Comment
After sequencing one specimen formerly identified as Monostroma hariotii using morphological characters, molecular data provided an unexpected close match with the Ulvella islandica tufA sequence from Iceland (98.12%, GenBank Access Number KF444924, Appendix 1). It is probable that the sequence obtained in the present study corresponds to an epiphytic Ulvella stage living on M. hariotii thallus. No rbcL sequence was obtained for this specimen. Ulvella islandica has been recently described in Icelandic waters (Nielsen et al. 2014) but has never been reported in Antarctic waters. Moreover, Ulvella species sequences reported in Antarctica (Mystikou et al. 2014; Peulvast et al. 2017) and deposited in GenBank were less related to our tufA sequence (Ulvella reticulata, 96.54%, GenBank Access Number JQ930309; Ulvella viridis, 95.61%, GenBank Access Number EF595286; Ulvella leptochaeta, 95.07%, GenBank Access Number JQ930313). This could represent the first evidence of an amphipolar distribution for Ulvella islandica, but caution should be taken as our study relies upon one single specimen sampled in PRAT.

Class TREBOUXIOPHYCEAE Friedl
Order PRASIOLALES Schaffner
Family PRASIOLACEAE F.F.Blackman & A.G.Tansley
Genus Prasiola (C.Agardh) Menegh.

Prasiola crispa (Lightfoot) Kützing
(WAP: OHI, GGV)

Reported distribution. — Worldwide.

Comment
Two of the three specimens sequenced for tufA showed exact matches with GenBank sequence of Prasiola crispa strain n°43 from King George Island (100%, GenBank Access Number KF993450, Appendix 1) while the other one exactly matched a sequence of P. crispa from Svalbard (100%, GenBank Access Number LN877821, Appendix 1). rbcL gene confirms Prasiola
crispa identification and an exact match was encountered with a specimen from Antarctica (100%, GenBank Access Number KR017748, Appendix 2). Our findings are congruent with previous works reporting the presence of the species in Antarctica and its amphipolar distribution pattern (Moniz et al. 2012; Garrido-Benavent et al. 2017).

**Prasiola crispa** subsp. antarctica (Kützing) Knebel (WAP: GGV).

**REPORTED DISTRIBUTION.** — Antarctic and the subAntarctic islands (Macquarie Island, South Georgia, SShs, Antarctic Peninsula), South America (Chile, Argentina).

**COMMENT**
A close match was found for a single specimen formerly identified as *Prasiola* sp. with *P. antarctica* strain P31 from the SShs for the *tufA* gene (99.31%, GenBank Access Number KF993447, Appendix 1). Exact match for *rbcL* sequence was found with the same specimen of *P. antarctica* strain P31 from the SShs (100%, GenBank Access Number JQ669712, Appendix 2). Moniz et al. (2012) proposed the resurrection of *P. antarctica* as a true species. However, this decision has not yet been approved and *P. antarctica* is still considered as a synonym of *Prasiola crispa* in AlgaeBase. Our sequence was thus named *Prasiola crispa* subsp. antarctica, after AlgaeBase nomenclature. Our work expands the distribution of *Prasiola crispa* subsp. antarctica from the SShs, 62°S (Moniz et al. 2012) down to the Gerlache Strait, 64°S.

Genus *Rosenvingiella* P.C. Silva

*Rosenvingiella radicans* (Kützing) Rindi, L.McVor & Guiry (SShs: PRAT)

**REPORTED DISTRIBUTION.** — North Europe (Britain, Ireland, Baltic Sea, France, Faroe Island, Spain), North America (California, Washington), Arctic (White Sea), Australia & New Zealand, Argentina.

**COMMENT**
One specimen, formerly identified as *Blidingia minima* using morphological characters, showed close match with *Rosenvingiella radicans* from Norway for the *tufA* gene (98.02%, GenBank Access Number LN877834, Appendix 1). *Rosenvingiella radicans* has only been described in the Northern hemisphere. Another species of *Rosenvingiella*, *R. simplex*, has been described along the coasts of King George Island (Vinogradova 1984). To the best of our knowledge, no *tufA* sequence representing this species has been deposited in public repositories, while 8 *rbcL* sequences are available from Norway (GenBank Access Number LN877833 - NY694199: NY694204, Heesch et al. 2016). Unfortunately, *rbcL* gene failed to amplify in the present study limiting further identification.

**DISCUSSION AND CONCLUSION**

**NEW RECORDS OF CHLOROPHYTA IN ANTARCTIC WATERS**

Molecular data obtained for 85 specimens of our 122 Chlorophyta samples allowed the detection of eleven species including three new reports (*Rosenvingiella radicans*, *Urospora wormskioldii* and *Ulvella islandica*) in the SShs and WAP area. *Urospora wormskioldii* was previously reported in the northern hemisphere close to the polar circle, along the coasts of Greenland, Canada, Europe and East Russia (Guiry & Guiry 2019; Fig. 2), *Ulvella islandica* in Iceland and *Rosenvingiella radicans* at mid-high latitudes in both hemispheres (Guiry & Guiry 2019). A wide distribution (except in the tropics, Guiry & Guiry 2019) has been reported for *Urospora penicilliformis*, a species considered as common in the intertidal zone and reported in the SShs and WAP since first being registered in the middle of the 20th century (Papenfuss 1964; Lamb & Zimmermann 1977; Roleda et al. 2009; Wiencke & Clayton 2002; Mystikou et al. 2014). We reported here, for the first time, the presence of a second specie of *Urospora*, *U. wormskioldii*, in Antarctica.

The present work improves the Chlorophyta genetic database in a region within which only a few green macroalgae have been sequenced (*Ulvoxiphycaceae: Monostroma gresilleii*, Pellizzari et al. 2017; *Ulvota* sp.: Khan 2017 GenBank direct submission; Trebouxiphycaceae: *Prasiola crispa* and *Prasiola antarctica*: Garrido-Benavent et al. 2017; Moniz et al. 2012). Lack of molecular data, especially of sequences available in public repositories for comparison in barcoding studies, has been identified as a clear limitation for studies focused on Antarctic algae (Dubrasquet et al. 2018). Even if only a few species of green algae are reported in Antarctica, the use of morphological characters without confirmation by molecular data could lead to confusion and inaccuracy in assessing marine flora diversity. In the present study, even if only a few sequences of Antarctic green algae were available in public repositories, identification matching with GenBank reference sequences were obtained with at least one of the two genetic markers (i.e., *tufA* and *rbcL* genes) for each putative species, most of them with sequences from specimens sampled in the northern hemisphere. These new *tufA* and *rbcL* data, including sequences of common intertidal species such as *Acrospiophyta arctica*, *Monostroma harioiti*, *Ulvota* sp. A-GW and *Urospora penicilliformis* could help in Antarctic algae diversity long-term monitoring. However, as in other polar areas (i.e., Alaska, Bringleee & Saunders 2019), assessing the current state of marine flora diversity in Antarctica will require sustained sampling effort in order to include specimens from other non-glaciated coasts, such as East Antarctic coasts located between 45°E and 160°E (Wiencke et al. 2014), to complete the information already obtained for the SShs and the WAP (Papenfuss 1964; Wiencke & Clayton 2002; Ramirez 2010; Wiencke et al. 2014; present study). Our sampling strategy was limited by logistics of the Antarctic campaigns and sampling restrictions: sampling effort happened only during summer season, with one sampling event in each region and scuba diving down to only 30 m (e.g. the emblematic species *Lambia*...
**antarctica**, that generally live at greater depth as reported by Wiencke et al. 2014, was not sampled in any of our five sites). However, our effort still allowed us to sample and sequence almost half of the reported species in the SSs and WAP (11 over 24, Pellizzari et al. 2017).

**VARIOUS ANTARCTIC GREEN ALGAE ARE AMPHIPOLAR SPECIES**

The use of molecular data allows a better understanding of marine flora diversity but also to better defines species biogeographic limits in order to study their evolutionary history. Recent studies have focused on current diversity and distribution pattern of red (Billard et al. 2015; Dubrasquet et al. 2018; Guillemin et al. 2018; Ocaranza-Barrera et al. 2019) and brown (Peters et al. 1997, 2000) Antarctic algae. Cryptic species have been found in several well-known and widely distributed red algae (Billard et al. 2015; Dubrasquet et al. 2018; Guillemin et al. 2018) and in terrestrial green algae (De Wéver et al. 2009), underlying the limitation of taxonomic knowledge for these seaweeds.

For Antarctic species, as for the canopy forming brown algae *Desmarestia* spp. and the common red algae *Gigartina skottsbergii*, divergence from species living outside of the Antarctic waters has been estimated to date back some 10 Million years (Mya) (Peters et al. 1997; Billard et al. 2015). The deep divergence between specimens previously named as *Gigartina skottsbergii* have been recently recognized at the taxonomical level and two species are now acknowledged in the region: *Sarcoleptis skottsbergii* in South America and *S. antarctica* in Antarctica (Hughes et al. 2020). As a result of long-time isolation from the rest of the marine realms, red and brown Antarctic algae display a high percentage of endemic species nowadays (36% and 44% respectively) and clear adaptations to Antarctic marine environment. However, some cold-water species of *Desmarestia* (i.e., *D. aculeata*, *D. viridis/confervoides*, Peters et al. 1997; *D. viridis/willi*, Van Oppen et al. 1993) have been reported in both cold Arctic and Antarctic waters (note that *D. confervoides* is considered synonymous with *D. willi*, Guiry & Guiry 2019). For Antarctic green algae, a much lower percentage of endemic species has been recorded (18%, Wiencke & Clayton 2002) and amphipolar distribution has been observed for the common intertidal species *Acrosiphonia arcta* (Van Oppen et al. 1993). For both *D. viridis/willi* and *A. arcta*, an amphipolar distribution has been related to recurrent equator-barrier crossing during the cooling temperatures events of the Pleistocene (Van Oppen et al. 1993). The ability of early life stages to survive extreme temperatures is crucial when considering a possible connection from pole to pole and phytogeographical patterns and endemism levels are shaped by this physiological requirement (Bartsch et al. 2012 and references therein). Gametophyte stages of *D. viridis/willi* and *A. arcta* present great tolerance to warm temperatures (i.e., survival up to 26–27°C for *D. viridis/willi* and at least up to 25°C for *A. arcta*; Peters & Breeman 1992; Van Oppen et al. 1993). These two species could have survived the passage of the tropics through deep-water dispersion of gametophyte stages. Early life stages of several Antarctic marine green algae as *A. arcta*, *Ulva* sp. and *U. penicilliformis* have been shown to present better tolerance to high temperatures (upper survival temperature above 20°C) than endemic red or brown algae (upper survival temperature between 11°C and 19°C, Wiencke & Dieck 1990). In general, green algae propagules have been shown to support long dark periods (e.g. *Ulva flexuosa*, Imchen 2012) and to be able to travel over very long distances on oceanic currents (more than hundreds of kilometers; Watanabe et al. 2009) attached to rafting algae (Saunders 2014; Arroyo & Bondauff 2016; Macaya et al. 2016). This could explain in part their success as invasive species (e.g. *Caulerpa taxifolia*: Bellan Santini et al. 1996; Smith & Walters 1999; Fama et al. 2002; Arnaud-Haond et al. 2017; *Codium fragile* sp. fragile; Watanabe et al. 2009) or the high number of species with a reported amphipolar distribution. A recent study on evolutionary history of the lichen-associated green algae *Prasiola crispa* species complex proposed a combined theory of vicariance events associated with long distance deep-water dispersal across the tropics during the Pleistocene in order to explain their disjunct distribution in both polar areas (Garrido-Benavent et al. 2017). Our findings confirm the existence of an amphipolar distribution for *A. arcta*, *P. crispa*, *U. penicilliformis*, *U. wormskiolii*, *Ulva* sp. A-GW and *Ulva islandica* and show that amphipolar distribution seems to be much more common in Antarctic green than red or brown algae. It could explain in part the low level of endemism found in Antarctica for these studied taxa (Wiencke & Clayton 2002).

**CONCLUSION**

Studies of species diversity including genetic data provide key information for correct assessment of flora and fauna diversity in Antarctica, an area still difficult to access (Grant & Linse 2009; De Broyer & Danis 2011; Leliært et al. 2014; Dubrasquet et al. 2018). Fast environmental changes have been reported in Antarctica, especially in the SSs and the WAP, leading to increasing pressures and threats over the Antarctic biota (Chown et al. 2015). Among them, introduction of non – native species associated to human activities such as scientific research and tourism have been reported as an important threat (Broady & Smith 1994; Olech 1996; Radulovic et al. 2010; McCarthy et al. 2019; Cardenas et al. 2020). However, basic information is still lacking that could allow to properly monitor the timing and magnitude of these arrivals. Quick detection of alien species settlement in Antarctic waters could help building environmental recommendation for shipping, including tourism and fishing activities. As these organisms present great dispersal potential and include several potential invaders, the availability of genetic sequences in public depository for species commonly found in the Western Antarctic Peninsula and the South Shetland Islands will help to monitor the state of green Antarctic flora.

**Acknowledgements**

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REFERENCES


Hughes J. R., Leister G. L., Gabrielson P. W. & Hommersand M. H. 2020. — Sareprendisella gen. nov. (Gigartinaceae, Rhodophyta), with S. skottsbergii comb. nov. from southern South America and S. antarctica sp. nov. from the Antarctic Peninsula. Phytotaxa 468: 75-88. https://doi.org/10.11646/phytotaxa.468.1.4


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### APPENDICES

**APPENDIX 1.** — Genbank (GB) Access Number for the tufA gene for green macroalgae specimens from the South Shetlands Islands and Western Antarctic Peninsula. Specie names are given following molecular assignation. Closest match with existing tufA sequence in Genbank data repository are given (percentage of similarity). References are given for closest match sequences. Specimens ID marked with * or ** correspond to subtidal samples (*sampling depth between 0-15m and ** sampling depth between 15-30m). Specimens ID written in **bold** correspond to samples for which rbcL sequences are available.

<table>
<thead>
<tr>
<th>Specie name (AlgaeBase Current Accepted name)</th>
<th>Specimen ID</th>
<th>Sampling Area - Sampling Site</th>
<th>GB Access Number for tufA gene</th>
<th>Closest match (Percentage Identity) for tufA in GB repository</th>
<th>BLAST Highest score (bits)</th>
<th>GenBank Access Number for closest match</th>
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<td>MLG-0680*</td>
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<td>Specie name (AlgaeBase Current Accepted name)</td>
<td>Specimen ID</td>
<td>Sampling Area - Sampling Site</td>
<td>GB Access Number for tufA gene</td>
<td>Closest match (Percentage Identity) for tufA in GB repository</td>
<td>BLAST Highest score (bits)</td>
<td>GenBank Access Number for closest match</td>
<td>References</td>
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<td>Protomonostroma sp. A-GW</td>
<td>MLG-0236A</td>
<td>South Shetland Islands - Greenwich Island</td>
<td>MN145890</td>
<td>99.87% with Protomonostroma sp. A-GW from King George Island, South Shetland Islands.</td>
<td>1421</td>
<td>MG646387</td>
<td>Khan et al. Unpublished</td>
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<td>Ulva sp. A-GW</td>
<td>MLG-0524</td>
<td>Antarctic Peninsula - Paradise Bay</td>
<td>MN145923</td>
<td>100% with Ulva sp. A-GW from King George Island, South Shetland Islands.</td>
<td>1415</td>
<td>MG646388</td>
<td>Khan et al. Unpublished</td>
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<td>Ulvella islandica</td>
<td>MLG-0249</td>
<td>South Shetland Islands - Greenwich Island</td>
<td>MN145931</td>
<td>98.12% with Ulvella islandica from Iceland</td>
<td>1299</td>
<td>KF444924</td>
<td>Nielsen et al. 2014</td>
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<tr>
<td>Urospora sp. 1 penicilliformis</td>
<td>MLG-0226</td>
<td>South Shetland Islands - Greenwich Island</td>
<td>MN145895</td>
<td>100% with Urospora sp. 1 penicilliformis from Nome, Alaska</td>
<td>1426</td>
<td>MH571163</td>
<td>Bringloe &amp; Saunders 2019</td>
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<td>Urospora wormskioldii (Mertens)</td>
<td>MLG-0145</td>
<td>Antarctic Peninsula - O’Higgins</td>
<td>MN145892</td>
<td>99.87% with U. wormskioldii from British Columbia, Canada.</td>
<td>1421</td>
<td>HQ610441</td>
<td>Saunders &amp; Kucera 2010</td>
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<td>Rosenvingiella radicans (Kützing)</td>
<td>MLG-0150</td>
<td>Antarctic Peninsula - O’Higgins</td>
<td>MN145932</td>
<td>100% with Prasiola crispa strain P43 from King George Island, South Shetland Islands.</td>
<td>1293</td>
<td>KF993450</td>
<td>Moniz et al. 2012</td>
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<tr>
<td>Prasiola crispa (Lightfoot) Kützing</td>
<td>MLG-0199</td>
<td>Antarctic Peninsula - O’Higgins</td>
<td>MN145933</td>
<td>99.31% with Prasiola antarctica strain P91 from King George Island, South Shetland Islands.</td>
<td>1336</td>
<td>LN877821</td>
<td>Heesh et al. 2016</td>
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<tr>
<td>Prasiola crispa subsp. antarctica (Kützing) Knebel</td>
<td>MLG-0576</td>
<td>Antarctic Peninsula - Paradise Bay</td>
<td>MN145935</td>
<td>98.02% with Rosenvingiella radicans from Nordland, Norway.</td>
<td>1303</td>
<td>LN877834</td>
<td>Heesh et al. 2016</td>
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APPENDIX 2. — Genbank (GB) Access Number for the rbcL gene for green macroalgae specimens from the South Shetlands Islands and Western Antarctic Peninsula. Specie names are given following molecular assignation. All specimens were collected in the intertidal zone. Only the closest match (percentage identity) with existing rbcL sequence in Genbank data repository are given. References are given for closest match sequences deposited in Genbank. Specimens ID written in **bold** correspond to samples for which tufA sequences are available.

<table>
<thead>
<tr>
<th>Specie Name</th>
<th>Specimen ID</th>
<th>Sampling Area - Sampling Site</th>
<th>GB Access Number for rbcL gene</th>
<th>Closest match (Percentage Identity) with rbcL in GB repository</th>
<th>BLAST Highest score (bits)</th>
<th>GenBank Access Number for closest match</th>
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<td>Ulvophyceae</td>
<td><strong>Capsosiphon</strong> sp. <strong>MLG-0523</strong></td>
<td>Antarctic Peninsula - Paradise Bay</td>
<td>MN164670</td>
<td>98.53% with <em>Capsosiphon fulvescens</em> plastid complete genome from South Korea.</td>
<td>1205</td>
<td><strong>NC_039920</strong></td>
<td>Kim et al. 2019</td>
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<td><strong>Protomonostroma</strong> sp. A-GW <strong>MLG-0236A</strong></td>
<td>South Shetland Islands - Greenwich Island</td>
<td>MN164665</td>
<td>100% with <em>Protomonostroma</em> sp. A-GW from King George Island, South Shetland Islands.</td>
<td>1260</td>
<td><strong>MG711514</strong></td>
<td>Khan et al. Unpublished</td>
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<td>MN164676</td>
<td>100% with <em>Ulva</em> sp. A-GW from King George Island.</td>
<td>1260</td>
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<td><strong>MLG-0413A</strong></td>
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<td>Ulva sp. A-GW</td>
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<td>Antarctic Peninsula - Marguerite Bay</td>
<td>MN164678</td>
<td>South Shetland Islands.</td>
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<td><strong>MG711515</strong></td>
<td>Khan et al. Unpublished</td>
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<td><strong>MLG-0608A</strong></td>
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<td>Uropsora sp.1 penicilliformis</td>
<td><strong>MLG-0226</strong></td>
<td>South Shetland Islands - Greenwich Island</td>
<td>MN164666</td>
<td>100% with <em>Uropsora</em> sp.1 <em>penicilliformis</em> from USA, Maine.</td>
<td>1242</td>
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<td><strong>MLG-0677</strong></td>
<td>Island Antarctic Peninsula - Marguerite Bay</td>
<td>MN164667</td>
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<td>Uropsora wormskioldii (Mertens) Rosenvinge</td>
<td><strong>MLG-0148</strong></td>
<td>Antarctic Peninsula - O’Higgins Base</td>
<td>MN164668</td>
<td>99.85% with <em>Uropsora</em> <em>wormskioldii</em> from British Columbia, Canada.</td>
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<td>Antarctic Peninsula - O’Higgins Base</td>
<td>MN164669</td>
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<td>Trebouxioiphyceae</td>
<td><strong>Prasiola crispa</strong> (Lightfoot) Kützing</td>
<td><strong>MLG-0189</strong></td>
<td>Antarctic Peninsula - O’Higgins</td>
<td>99.71% with <em>Prasiola crispa</em> from Antarctica (unknown location).</td>
<td>1245</td>
<td><strong>KR017748</strong></td>
<td>Carvalho et al. 2015</td>
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<td><strong>MLG-0148</strong></td>
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