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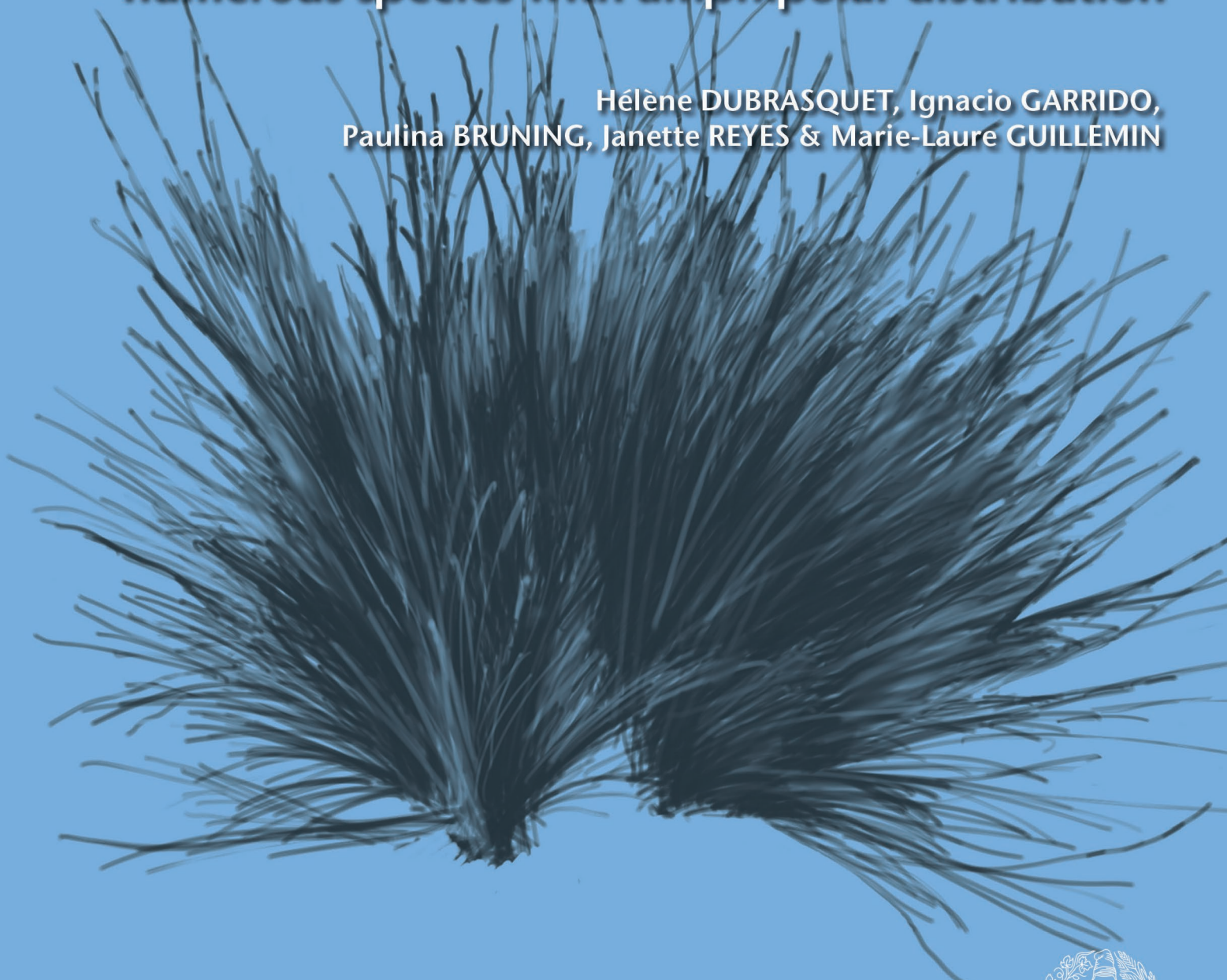

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Building-up knowledge on green marine macroalgae diversity in the Western Antarctic Peninsula: data from two molecular markers reveals numerous species with amphipolar distribution

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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 *tuf*A and *rbc*L). From the 122 specimens of Chlorophyta sampled, 85 were sequenced for the gene *tuf*A and 16 for the gene *rbc*L. Using the NCBI Nucleotide Blast Tool to compare our sequences to the ones available in public data depositories allowed the identification of 11 species. Three new species were reported for the area: *Rosenvingiella radicans* (Kütz.) Rindi, L.McIvor & Guiry, *Urospora 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 arcta* (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.

KEY WORDS
Antarctic, Chlorophyta, barcoding, *tuf*A and *rbc*L, amphipolar distribution, endemism.

RÉSUMÉ
Un patron de distribution amphipolaire chez plusieurs algues vertes Antarctique détecté à l'aide d'outils moléculaires.
Comparés 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 *tuf*A et *rbc*L). Sur les 122 spécimens de Chlorophyta échantillonnés, 85 ont été séquencés pour le gène *tufA* et 16 pour le gène *rbc*L. 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.McIvor & Guiry, *Urospora 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 arcta* (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* et confirmant la distribution amphipolaire de divers taxons de Trébouxiophycées et Ulvophycées 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.

MOTS CLÉS
Antarctique, Chlorophyte, barcoding ADN, *tufA* et *rbc*L, distribution amphipolaire, endémisme.

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; Ramírez 2010; Wiencke & Clayton 2002; Wiencke *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*, Spongomorpha arcta* (Dillwyn) Kützing and *Ulvella*

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 membranacea* (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 SShs 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 augment 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).

We propose revisiting marine Antarctic green macroalgae diversity using two molecular markers regularly used for species determination and barcoding in Chlorophyta (i.e., the plastid genes *tuf*A and *rbc*L; Lewis & Lewis 2005; Pröschold & Leliaert 2007; Leliaert *et al.* 2012). Our survey encompasses a wide area (i.e., more than 450 km) from the SShs to the center part of the WAP. The present work is part of an ongoing effort to monitor the benthic Antarctic flora, a group of taxa under increasing threat in the region due to the acceleration of climate change and intensification of anthropogenic activities.

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 SShs (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; Pellizari *et al.* 2017).

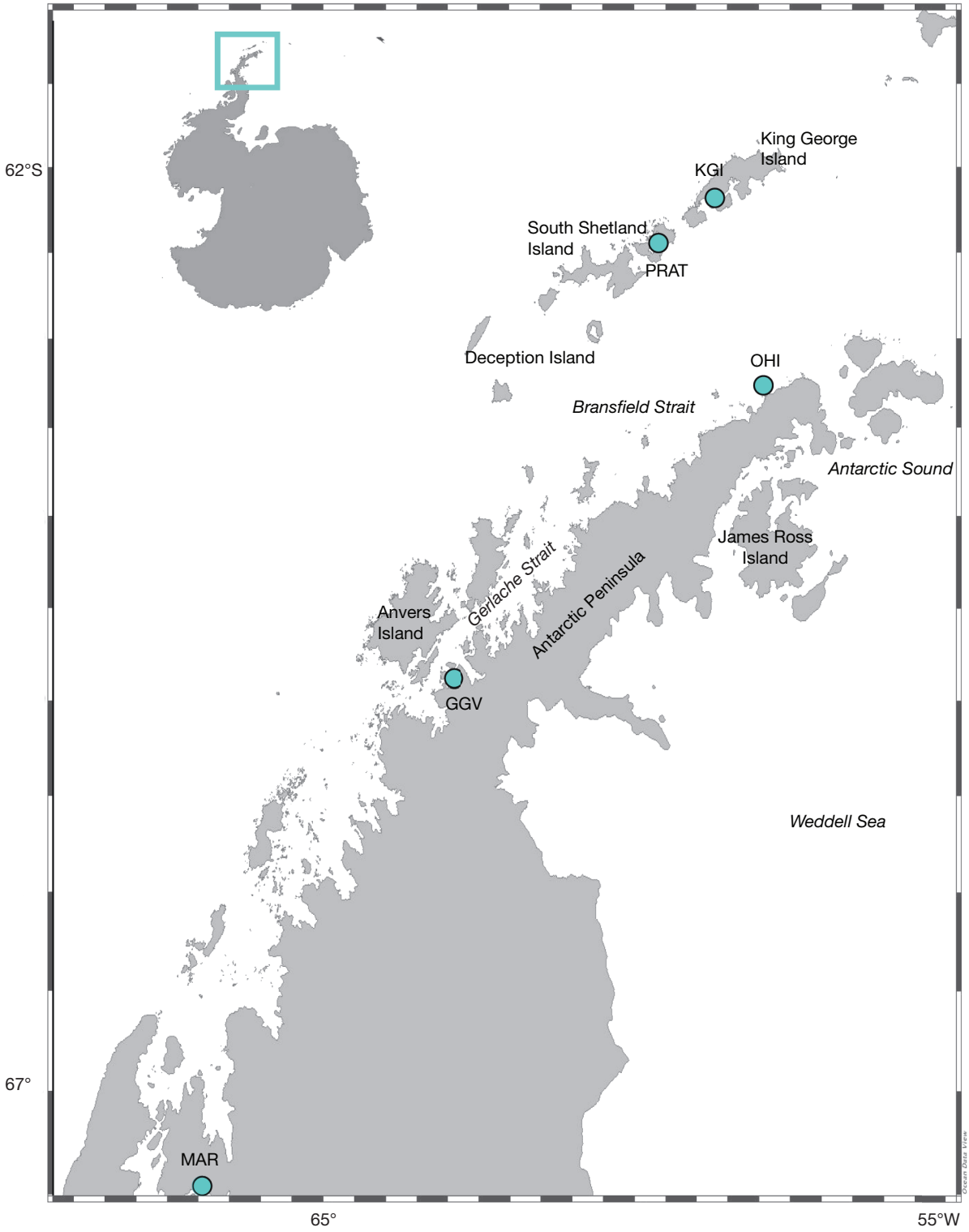

Fig. 1. — Map of the study area. Sampling Sites are shown as **blue circles**. Two sites are located in the South Shetland Islands (KGI, King George Island; PRAT, Greenwich Island) and three sites along the Western Antarctic Peninsula (OHI, Base O'Higgins; GGV, Paradise Bay; MAR, Margarita Bay).

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 *tuf*A, 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 *tuf*A was realized using the primers (TufAgf4: 5TGAAACAGAAMAWAWCGTCATTATGC-3 and TufAR: 5CCTTCNCGAATMGCRAAWCGC-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 *tuf*A), the plastid gene *rbc*L coding for the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase was amplified. The primers GrbcLnF (5' GCTGGWGTAAAAGATTAYCG 3') and GrbcLR (5' TCACGCCAACGCATRAASGG 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 (McCarthy 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 OHI, 34 in GGV and 17 in MAR. Because of low quality and/or quantity of DNA extracted for some specimens, *tuf*A sequences were obtained only for 92 specimens, representing 75% of the samples. Among these 92 sequences, four were contaminated by the bacteria *Granulosicoccus antarcticus* (Lee *et al.* 2008) and three by the diatom *Seminavis robusta* D.B.Danielidis & 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 Trebouxiophyceae (five specimens belonging to Prasiolales). Eleven distinct putative species were detected in the present study using *tuf*A molecular dataset: eight are part of the Ulvophyceae class and three of the Trebouxiophyceae class (Appendix 1). In order to confirm the species assignation based on the *tufA* gene, the *rbc*L gene was amplified in a sub-sample of randomly selected specimens (N = 31 in total) belonging to each putative species. The *rbc*L 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 *rbc*L PCR products were obtained for *Monostroma hariotii*, even after testing amplification using the DNA of the 37 specimens available. Among the 15 *rbc*L obtained sequences, 11 were congruent with *tuf*A sequences identification (Appendix 1). The remaining four sequences were identified as *Ulva* sp. A-GW and congruent with morphological identification but *tuf*A 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 *tuf*A and *rbc*L datasets are registered below. Reported distribution also follows Guiry & Guiry (2019).

Class ULVOPHYCEAE K.R.Mattox & K.D.Stewart
Order ULOTRICHALES Borzì
Family Ulotrichaceae Kützing
Genus *Acrosiphonia* J.Agardh

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, SShs, Kerguelen Islands, Auckland Islands, Campbell Island), New Caledonia.

Comment

Closest match (98.31%, GenBank Access Number HQ610211, Appendix 1) with Antarctic *tuf*A sequences was a Canadian specimen of *Acrosiphonia arcta* from British Columbia. No *rbc*L sequences were obtained for this species. *Acrosiphonia arcta* has previously been reported in Antarctic waters (Ramirez, 2010), mainly under the name *Spongomorpha arcta* (Papenfuss 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).

Capsosiphon sp. Gobi
(WAP: GGV)

Reported distribution. — *Capsosiphon groelandicus* has been reported in Arctic (Svalbard), North Asia (China, Japan, East Russia, Kamchatka, Commander Islands), Antarctic and sub-

Antartic 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, SubAntarctic Islands (Saint Paul).

Comment

The closest match for the *tuf*A and *rbc*L 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 *tuf*A than the *rbc*L molecular marker could be explained by a slightly higher mutation rate of the *tuf*A 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 *tuf*A than the *rbc*L-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. rosulatum* 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 *rbc*L 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 rosulatum* 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 tuf*A 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 tuf*A sequence from Canada. Exact match was found for the *rbc*L 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, Brittany, 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 *rbc*L genes (99.87%, GenBank Access Number HQ610441 and HQ603676 for *tufA* and *rbc*L, 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).

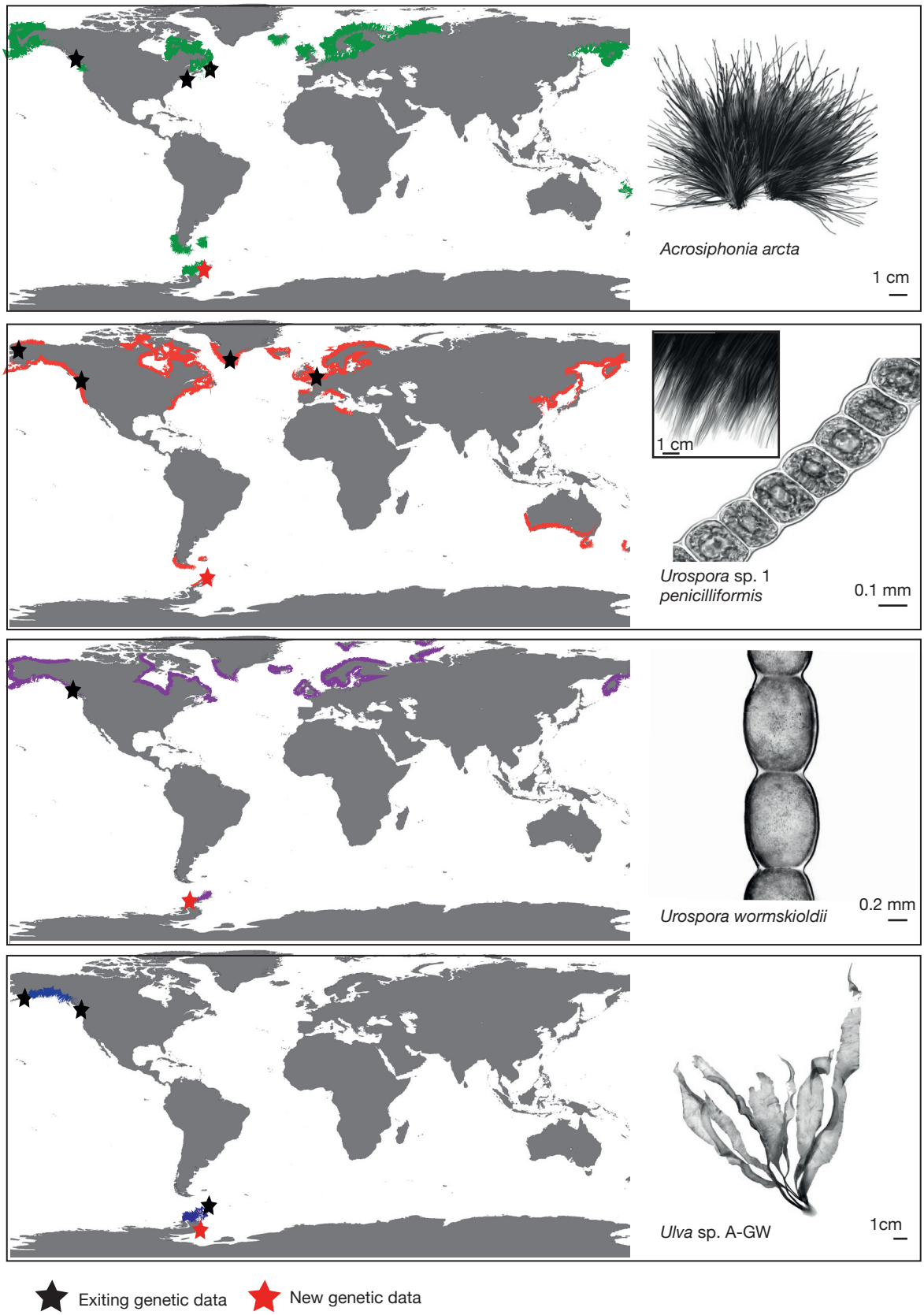

FIG. 2. — Amphipolar distribution of four species of Chlorophyta detected in the SShs and/or WAP during our study. General distributions (shown as colored lines) follow information given in AlgaeBase repository (Guiry & Guiry 2019). Genetic data (i.e., *tuf*A) available for the species are indicated with stars: red stars, present study; black stars, already published in GenBankNCBI 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 Monostromataceae 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, SShs, Antarctic Peninsula, Wilkes Land), South America (Argentina, Falkland Islands).

Comment
Exact match for *tuf*A 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 (Pellizari *et al.* 2017). No *rbc*L 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 SubAntartic 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 Orkney Islands (Wiencke & Clayton 2002), South Shetlands Islands including King George Island (Wiencke & Clayton 2002; Quartino *et al.* 2005; Al-Handal & Wulff 2008; Pellizzari *et al.* 2017), Wilkes Land (Runcie & Riddle 2006) and the Antarctic Peninsula (Lamb & Zimmermann 1977; Amsler *et al.* 2005; Peters *et al.* 2005; Mystikou *et al.* 2014).

Class ULVOPHYCEAE K.R.Mattox & K.D.Stewart
Order ULVALES Blackman & Tansley
Family Ulvaceae J.V.Lamouroux ex Dumortier
Genus *Ulva* L.

Ulva sp. A-GW
(WAP: GGV, MAR)

Reported distribution. — Unknown.

Comment
Both genes *tuf*A and *rbc*L confirmed the assignation of our samples to the species *Ulva* sp. A-GW with exact matches with sequences from King George Island, SShs (100%, GenBank Access Number MG646368 and MG711515 for *tuf*A and *rbc*L genes, respectively, Appendices 1 and 2). Close matches were also observed with *tuf*A sequences from British Columbia, Canada (98.83%, GenBank Access Number KM254999) and Nome, Alaska (98.61%; GenBank Access Number MF124264) suggesting that *Ulva* sp. A-GW displays an amphipolar distribution (Fig. 2). Our specimens were sampled in the southern part of the WAP (e.g. Gerlache Strait and Marguerite Bay), about 250 km from King George Island, suggesting an extensive distribution of *Ulva* sp. A-GW, at least in the Western Antarctic.

Family Ulvellaceae Schmidle
Genus *Ulvella* P. Crouan & H. Crouan

Ulvella islandica R.Nielsen & K.Gunnarsson
(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 tuf*A 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 *rbc*L 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; Pellizzari *et al.* 2017) and deposited in GenBank were less related to our *tuf*A sequence (*Ulvella reticulata*, 96.54%, GenBank Access Number JQ303009; *Ulvella viridis,* 95.61%, GenBank Access Number EF595286; *Ulvella leptochaete*, 95,07%, GenBank Access Number JQ303013). 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.

Classe 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 *tuf*A 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). *rbc*L 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 *tuf*A gene (99.31%, GenBank Access Number KF993447, Appendix 1). Exact match for *rbc*L 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.McIvor & 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 *tuf*A 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 *tuf*A sequence representing this species has been deposited in public repositories, while 8 *rbc*L sequences are available from Norway (GenBank Access Number LN877833 - AY694199: AY694204, Heesch *et al.* 2016). Unfortunately, *rbc*L 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 (Ulvophyceae: *Monostroma grevillei*, Pellizzari *et al.* 2017; *Ulva* sp.: Khan 2017 GenBank direct submission; Trebouxiophyceae: *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., *tuf*A and *rbc*L genes) for each putative species, most of them with sequences from specimens sampled in the northern hemisphere. These new *tuf*A and *rbc*L data, including sequences of common intertidal species such as *Acrosiphonia arcta, Monostroma hariotii, Ulva* sp. A-GW and *Urospora penicilliformis* could help in Antarctic algae diversity long-term monitoring. However, as in other polar areas (i.e., Alaska, Bringloe & 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 SShs 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 Wever *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: *Sarcopeltis skottsbergii* in South America and *S. antarctica* in Antarctica (Hughey *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., *Desmarestia aculeata*, *D. viridis/confervoides,* Peters *et al.* 1997; *D. viridis/willii,* Van Oppen *et al.* 1993) have been reported in both cold Arctic and Antarctic waters (note that *D. confervoides* is considered synonymous with *D. willii*, 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/willii* 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/willii* and *A. arcta* present great tolerance to warm temperatures (i.e., survival up to 26-27°C for *D. viridis/willii* 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 & Bonsdorff 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 disjoint 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. wormskioldii, Ulva* sp. A-GW and *Ulvella 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; Leliaert *et al.* 2014; Dubrasquet *et al.* 2018). Fast environmental changes have been reported in Antarctica, especially in the SShs 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; Radulovici *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.

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APPENDICES

Appendix 1. — Genbank (GB) Access Number for the *tuf*A 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 *tuf*A 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 *rbc*L sequences are available.

Specie name (AlgaeBase Current Accepted name)	Specimen ID	Sampling Area - Sampling Site	GB Access Number for tufA gene	Closest match (Percentage Identity) for tufA in GB repository	BLAST Highest score (bits)	GenBank Access Number for closest match	References
Ulvophyceae	–	–	–	–	–	–	–
Acrosiphonia arcta (Dillwyn) Gain"	MLG-0234B	South Shetland Islands - Greenwich Island	MN145911	98.31% with *Acrosiphonia arcta* from British Columbia, Canada	1351	HQ610211	Saunders & Kucera 2010
	MLG-0234C	South Shetland Islands - Greenwich Island	MN145912				
	MLG-0582A	Antarctic Peninsula - Paradise Bay	MN145913				
	MLG-0582B		MN145914				
	MLG-0582C		MN145915				
	MLG-0610A		MN145916				
	MLG-0610B		MN145917				
	MLG-0610C		MN145918				
Capsosiphon sp.	MLG-0585	Antarctic Peninsula - Paradise Bay	MN145920	93.26% with complete plastid genome of *Capsosiphon fulvescens*	1138	NC_039920	Kim *et al.* 2019
	MLG-0609		MN145921				
	MLG-0612		MN145922				
	MLG-0523		MN145919				
Monostroma hariotii Gain	ANT-2004-2	South Shetland Islands - King George Island	MK507414	100% with *Monostroma angicava* from King George Island, South Shetland Islands.	1426	MG646366	Khan *et al.* Unpublished
	ANT-2004-3		MK507415				
	ANT-2005-1		MK507416				
	ANT-2005-2		MK507417				
	ANT-2005-3		MK507418				
	ANT-2005-4		MK507419				
	ANT-2009-2		MK507420				
	ANT-2134-1*		MK507421				
	ANT-2134-3*		MK507422				
	ANT-2134-4*		MK507423				
	ANT-2164*		MK507424				
	MLG-0032*	Antarctic Peninsula - O'Higgins	MK507425				
	MLG-0109*		MK507426				
	MLG-0124**		MK507427				
	MLG-0142		MK507428				
	MLG-0153		MK507429				
	MLG-0185		MK507430				
	MLG-0223*	Antarctic Peninsula - Paradise Bay	MK507431				
	MLG-0233		MK507432				
	MLG-0241		MK507433				
	MLG-0259*		MK507434				
	MLG-0329B		MK507435				
	MLG-0506A*		MK507443				
	MLG-0506B*		MK507444				
	MLG-0526C		MK507445				
	MLG-0530*		MK507436				
	MLG-0541		MK507446				
	MLG-0550**		MK507447				
	MLG-0564*		MK507437				
	MLG-0575		MK507438				
	MLG-0607		MK507439				
	MLG-0617*		MK507440				
	MLG-0645	Antarctic Peninsula - Marguerite Bay	MK507441				
	MLG-655**		MK507442				
	MLG-0666A*		MK507448				
	MLG-0666B*		MK507449				
	MLG-0680*		MK507450				

APPENDIX 1. —Continuation

Specie name (AlgaeBase Current Accepted name)	Specimen ID	Sampling Area - Sampling Site	GB Access Number for tufA gene	Closest match (Percentage Identity) for tufA in GB repository	BLAST Highest score (bits)	GenBank Access Number for closest match	References
Protomonostroma sp. A-GW	**MLG-0236A**	South Shetland Islands - Greenwich Island	MN145890	99.87% with *Protomonostroma* sp. A-GW from King George Island, South Shetland Islands.	1421	MG646367	Khan *et al*. Unpublished
	MLG-0236B	South Shetland Islands - Greenwich Island	MN145891				
	MLG-0234A	South Shetland Islands - Greenwich Island	MN145889				
Ulva sp. A-GW	MLG-0524	Antarctic Peninsula - Paradise Bay	MN145923	100% with *Ulva* sp. A-GW from King George Island, South Shetlands Islands.	1415	MG646368	Khan *et al*. Unpublished
	MLG-0543A		MN145924				
	MLG-0543B		MN145925				
	MLG-0581B		MN145926				
	MLG-0583B		MN145901				
	MLG-0608B		MN145927				
	MLG-0608C		MN145928				
	MLG-0729A	Antarctic Peninsula - Marguerite Bay	MN145930				
	MLG-0647		MN145929				
Ulvella islandica R.Nielsen & K.Gunnarsson 2014.	MLG-0249	South Shetland Islands - Greenwich Island	MN145931	98.12% with *Ulvella islandica* from Iceland	1299	KF444924	Nielsen *et al*. 2014
Urospora sp. 1 *penicilliformis*	**MLG-0226**	South Shetland Islands - Greenwich Island	MN145895	100% with *Urospora* sp. 1 *penincilliformis* from Nome, Alaska	1426	MH571163	Bringloe & Saunder 2019
	MLG-0238		MN145896				
	MLG-0291		MN145897				
	MLG-0314		MN145898				
	MLG-0316		MN145899				
	MLG-0412		MN145900				
	MLG-0584	Antarctic Peninsula - Paradise Bay	MN145902				
	MLG-0644	Antarctic Peninsula - Marguerite Bay	MN145903				
	MLG-0676		MN145904				
	MLG-0677		MN145905				
	MLG-0709		MN145906				
	MLG-0724		MN145907				
	MLG-0726		MN145908				
	MLG-0746		MN145909				
Urospora wormskioldii (Mertens) Rosenvinge"	MLG-0145	Antarctic Peninsula - O'Higgins	MN145892	99.87% with *U. wormskioldii* from British Colombia, Canada.	1421	HQ610441	Saunders & Kucera 2010
	MLG-0148		MN145893				
	MLG-0181		MN145894				
Trebouxiophyceae	–	–	–	–	–	–	–
Prasiola crispa (Lightfoot) Kützing	MLG-0150	Antarctic Peninsula - O'Higgins	MN145932	100% with *Prasiola crispa* strain P43 from King George Island, South Shetland Islands.	1293	KF993450	Moniz *et al*. 2012
	MLG-0538	Antarctic Peninsula - Paradise Bay	MN145934				
	MLG-0189	Antarctic Peninsula - O'Higgins	MN145933	100% with *Prasiola crispa* from Svalbard.	1336	LN877821	Heesh *et al*. 2016
Prasiola crispa subsp. *antarctica* (Kützing) Knebel	**MLG-0576**	Antarctic Peninsula - Paradise Bay	MN145935	99,31% with *Prasiola antarctica* strain P31 from King George Island, South Shetland Islands.	1317	KF993447	Moniz *et al*. 2012
Rosenvingiella radicans (Kützing) Rindi, L.McIvor & Guiry	MLG-0313	South Shetland Islands - Greenwich Island	MN145936	98.02% with *Rosenvingiella radicans* from Nordland, Norway.	1303	LN877834	Heesh *et al*. 2016

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.

Specie Name	Specimen ID	Sampling Area - Sampling Site	GB Access Number for rbcL gene	Closest match (Percentage Identity) with rbcL in GB repository	BLAST Highest score (bits)	GenBank Access Number for closest match	References
Ulvophyceae	–	–	–	–	–	–	–
Capsosiphon sp.	**MLG-0523**	Antarctic Peninsula - Paradise Bay	MN164670	98.53% with *Capsosiphon fulvescens* plastid complete genome from South Korea.	1205	NC_039920	Kim *et al*. 2019
	MLG-0609		MN164671				
	MLG-0612		MN164672				
Protomonostroma sp. A-GW	**MLG-0236A**	South Shetland Islands - Greenwich Island	MN164665	100% with *Protomonostroma* sp. A-GW from King George Island, South Shetland Islands.	1260	MG711514	Khan *et al*. Unpublished
Ulva sp. A-GW	MLG-0317	South Shetland Islands - Greenwich Island	MN164676	100% with *Ulva* sp. A-GW from King George Island,	1260	MG711515	Khan *et al*. Unpublished
	MLG-0390D		MN164674				
	MLG-0413A		MN164677				
Ulva sp. A-GW	**MLG-0729A**	Antarctic Peninsula - Marguerite Bay	MN164678	South Shetland Islands.	1260	MG711515	Khan *et al*. Unpublished
	MLG-0608A	Antarctic Peninsula - Paradise Bay	MN164675				
Urospora sp.1 *penicilliformis*	**MLG-0226**	South Shetland Islands - Greenwich	MN164666	100% with *Urospora* sp.1 *penicilliformis* from USA, Maine.	1242	HQ603674	Saunders & Kucera 2010
	MLG-0677	Island Antarctic Peninsula - Marguerite Bay	MN164667				
Urospora wormskioldii (Mertens) Rosenvinge	**MLG-0148**	Antarctic Peninsula - O'Higgins Base	MN164668	99.85% with *Urospora wormskioldii* from British Columbia, Canada.	1254	HQ603676	Saunders & Kucera 2010
	MLG-0181	Antarctic Peninsula - O'Higgins Base	MN164669				
Trebouxiophyceae	–	–	–	–	–	–	–
Prasiola crispa (Lightfoot) Kützing	**MLG-0189**	Antarctic Peninsula - O'Higgins	MN164679	99.71% with *Prasiola crispa* from Antarctica (unknown location).	1245	KR017748	Carvalho *et al*. 2015