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New records and observations of macroalgae and associated pathogens from the Falkland Islands, Patagonia and Tierra del Fuego

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Abstract: Subantarctic and Antarctic regions remain little explored with regards to their seaweed diversity. This study is based upon collections in the early 1970s and 2007–2013. It is supported by sequencing *COI* (mitochondrial cytochrome oxidase I) and reports new records for four species of brown algae (*Hincksia granulosa*, *Hincksia sandriana*, *Myriotrichia clavaeformis*, *Syringoderma australe*), four red algae (*Erythrotrichia carnea*, *Paraglossum*

salicifolium, *Phycodrys antarctica*, *Plumariopsis eatonii*), one green alga (*Chaetomorpha aerea*) and of the oomycete *Anisolpidium ectocarpii*. A further four brown algae are reported at genus level and discussed (*Cladostephus* sp., *Colpomenia* sp., *Dictyota* sp., *Punctaria* sp.). Observations of the biology of three brown algal taxa (*Cladothela decaisnei*, *Geminocarpus geminatus*, *Halopteris obovata*) from the region are also reported here.

Keywords: *Anisolpidium ectocarpii*; *COI*; Falkland Islands; Patagonia; Phaeophyceae; Rhodophyta; Subantarctic seaweeds; Tierra del Fuego; Ulvophyceae.

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Introduction

Major gaps remain in the knowledge of the seaweed diversity of the Falkland Islands, South Georgia and southernmost South America. The results of a 3 year survey of 50 brown algal species from the region of Puerto Deseado, Argentine Patagonia were reported in Asensi and Küpper (2012). This covered the systematics and ecology of brown algae in the region since 1968, while records from Chilean Patagonia were summarized in Ramírez and Santelices (1991). Further south, at the southern tip of Patagonia, in Tierra del Fuego and the Falkland Islands, explorations by Skottsberg provided a first major overview of the seaweeds in these regions (Skottsberg 1907, Skottsberg 1921, Skottsberg 1941). More recently, synopses of Antarctic seaweeds, which also covered several taxa from the Falklands, South Georgia and Tierra del Fuego, have been published (Wiencke and Clayton 2002, Wiencke et al. 2014), while Broom et al. (2010) surveyed the *Porphyra* flora of the Falklands. Despite such efforts, large areas remain poorly studied for their seaweed diversity, as highlighted by a recent survey around Rothera Research Station (Adelaide Island) off the south-western Antarctic Peninsula, which resulted in 18 new records for this region (Mystikou et al. 2014).

Inevitably, a number of questions still remain unanswered and more have arisen in recent years. Studying seaweeds in such remote areas faces logistical and safety challenges (as discussed recently for similar fieldwork in the Canadian Arctic; Sayer et al. 2013) and new records were anticipated by the investigators of this study because of the first-ever use of SCUBA diving in studying seaweeds in the areas surveyed here. All previous investigators relied on collections from the shore or occasionally by dredging and bottom grab, missing much of the deep-water flora. The use of molecular methods has improved the identification of seaweeds, jointly with algal culturing techniques. Incubation of natural substrata in culture media may reveal taxa otherwise invisible or inaccessible at the remote study sites visited, usually with limited time and laboratory facilities (Kornmann and Sahling 1980, Müller and Ramírez 1994, Tsiamis et al. 2014). Among several of the taxa described by Skottsberg (1907, 1921, 1941), especially the smaller and filamentous species need re-investigation because of the taxonomic revisions that have been made since then – additionally, some of the taxa reported by Skottsberg (e.g. *Cladochroa chnoosporiformis*) have not been reported since then.

The area covered by this study is the region north of the Antarctic Convergence in the Southern Ocean (chiefly the Atlantic, but also the Pacific coast of South America). The light conditions are strongly seasonal, with sea temperatures ranging from a minimum of around 2°C in winter in Tierra del Fuego and the Falklands to a maximum of around 14°C in summer around Puerto Montt. The region is generally cold-temperate and ice free.

Samples and specimens were collected during four expeditions to Patagonia, Tierra del Fuego and the Falkland Islands in 2007, 2010–2011 and 2013, respectively. We also describe previously unpublished records and materials collected by A.O. Asensi from Puerto Deseado and Tierra del Fuego from the early 1970s. Furthermore, a number of cultures of mostly filamentous macroalgae were isolated from substratum samples collected in sterile tubes during the aforementioned expeditions.

Materials and methods

Study sites

Surveys and collections were conducted at the sites mapped in Figure 1.

Collection of materials

Seaweed specimens were collected as entire thalli in the intertidal or by SCUBA diving. They were subsequently conserved as herbarium specimens on Bristol paper or on microscope slides using acetocarmine as fixative and dye and 50% Karo™ syrup as mounting medium (Müller and Ramírez 1994). Occasionally, algal specimens were investigated in the field with a small compound microscope with 25×, 100× and 750× magnification.

Diving also enabled the collection of sediment and benthic substratum samples in sterile 15- or 50-ml Falcon™ tubes. Uni-algal laboratory cultures from such samples were established following the modified technique described by Müller and Ramírez (1994) and Peters et al. (2015).

Seaweed tissue samples were conserved in parallel in Silica gel and cetyltrimethylammonium bromide (CTAB; e.g. Gachon et al. 2009) buffer for further molecular study. Representative herbarium specimens and permanent slides were deposited at the British Natural History Museum (BM) in London or the National Museum of Natural History (PC) in Paris.

DNA extraction, PCR amplification and sequencing

DNA extractions were carried out using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) on material from specimens preserved in silica gel which had been collected from Puerto Montt (Chile), Tierra del Fuego (Chile and Argentina) and the Falkland Islands. The partial mitochondrial cytochrome oxidase I (*COI*) was amplified using either the primer pair COI-789F & COI-1378R (Silberfeld et al. 2010) or *cox1-GazF1* and *cox1-GazR1* (Saunders 2005).

PCR amplification was performed in a total volume of 25 µl, containing 1.25 units µl⁻¹ (0.25 µl stock) of Taq DNA Polymerase (Promega), 5 µl GoTaq™ buffer, 5 mM MgCl₂, 1.25 mM dNTPs (desoxy nucleotides), 1.87 mM of each primer and 1 µl of template DNA (5–50 ng µl⁻¹). PCR amplification for the primer pair COI-789F/COI-1378R was carried out with an initial denaturation at 94°C for 5 min; followed by 40 cycles of amplification consisting of denaturation at 94°C for 30 s, annealing at 46°C for 30 s and elongation at 72°C for 40 s, followed by a final extension at 72°C for 5 min. For the *cox1-GazF1/cox1-GazR1* pair, PCR amplification was carried out with an initial denaturation at 94°C for 2 min; followed by 35 cycles of amplification

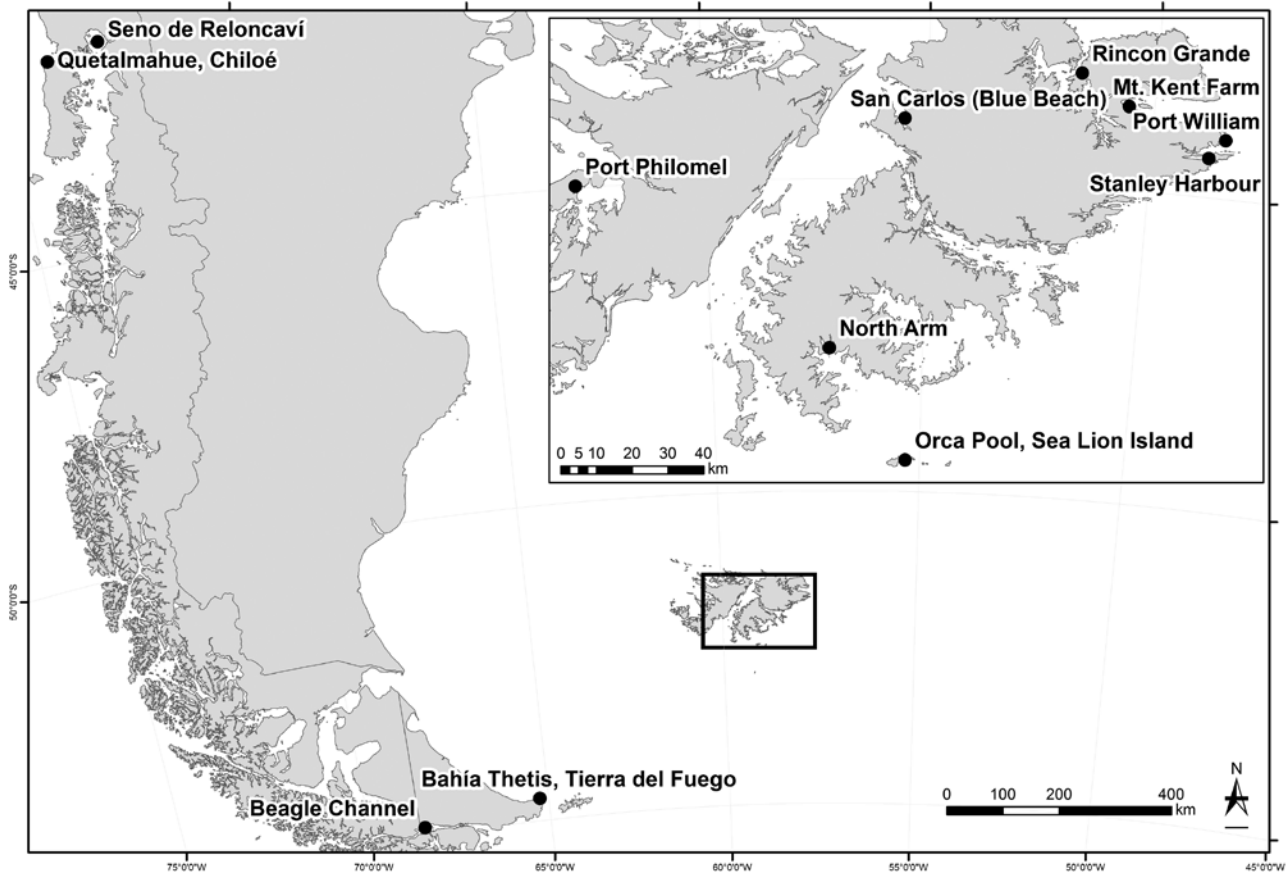


Figure 1: Sampling areas around southern South America surveyed by this study: Port Philomel, West Falkland (W 60° 22' 42.66" S 51° 42' 51.54"), San Carlos (Blue Beach), East Falkland (W 59° 2' 12.36" S 51° 34' 26.76"), Rincon Grande (W 58° 19' 4.86" S 51° 28' 15.66"), Mt. Kent Farm, East Falkland (W 58° 7' 50.88" S 51° 33' 24.84"), Port William (W 57° 44' 32.1" S 51° 38' 47.64"), Beatrice Cove/Port William (W 57° 45' 29.52" S 51° 38' 36.23"), Stanley Harbour (W 57° 48' 42.47" S 51° 41' 25.43"), Orca Pool at Sea Lion Island (W 59° 4' 33.42" S 52° 25' 55.8"), North Arm (W 59° 22' 17.4" S 52° 8' 40.14"), Bahía Thetis, Tierra del Fuego (W 65° 14' 31" S 54° 38' 35"), Seno de Reloncavi (W 72° 49' 59.88" S 41° 42' 0"), Quetalmahue, Chiloé (W 73° 57' 18" S 41° 51' 33"). The following three sites in Tierra del Fuego correspond to the point marked Beagle Channel: Playa Larga, Ushuaia (W 68° 11' 04.49" S 54° 49' 06.92"), Isla Redonda, Tierra del Fuego (W 68° 28' 36.98" S 54° 51' 43.64") and Bahía Ensenada (W 68° 28' 53.08" S 54° 50' 48.78").

consisting of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 1 min, followed by a final extension at 72°C for 5 min.

Sequences have been deposited in the EBI (European Bioinformatics Institute) database (accession numbers are provided in Table 1).

For the *Dictyota* specimens, DNA extractions were performed using CTAB buffer as described by De Clerck et al. (2006) and Silberfeld et al. (2010). The *psbA* sequences were generated using the primers *psbAF* and *psbAR* reported by Yoon et al. (2002). The *COI* sequences were generated using *Dictyota*-specific primers developed by Tronholm et al. (2010). This corresponds to the standard *COI* barcode but with primer sequences adapted to *Dictyota* based on the mitochondrial genome of *Dictyota dichotoma*, *cox1F* and *cox1R* (Secq et al. 2006). PCR

products were checked for amplification and correct length by electrophoresis on a 1.2% (w/v) agarose gel prepared with Tris/Borate/EDTA buffer (TBE; Brody and Kern 2004) with post-staining in GelRed™ (Biotium). For sequencing a single reaction product of approximately 50 ng DNA was purified using the QIAquick PCR Purification Kit (Qiagen) and sequencing of both strands was carried out by the Source Bioscience sequencing service using the same primers as employed for PCR. Returned chromatograms (in ABI/Applied Biosystems format) were imported into BioEdit (Hall 1999) for quality control and resulting consensus sequences were queried against the GenBank online database using BLASTn (Altschul et al. 1997). A phylogeny was generated from *psbA* sequences, edited and aligned in MEGA6 (Tamura et al. 2013) and added to a representative selection of 28 *Dictyota*

Table 1: Overview of sequenced taxa covered by this study, with corresponding specimen and EBI (European Bioinformatics Institute) sequence accession numbers. The *E-value* (expect value) is a parameter for the number of hits that can be expected to be seen by chance when searching a nucleotide database of a particular size. The lower the *E-value*, the more “significant” a match to a database sequence (i.e. there is a smaller probability of finding a match just by chance). The query coverage denotes the fraction (percent) of the query sequence that overlaps the subject sequence.

Specimen number	Herbarium sheets	Microscope slides	Taxa	% Identity to closest relative (in parentheses, with accession number) with publicly available sequences	Query cover (where >0)	E value (where >0)	EBI accession numbers for new sequences
041213-01	BM001180498	BM000701838	<i>Cladostephus</i> sp.	<i>COI</i> (5'-3') 94% (<i>Cladostephus spongiosus</i> , EU681396.1)	98%		LN828731
KUC4a		PC0167415	<i>Cladathele decaisneii</i> J. D. Hooker et Harvey	99% (Ectocarpoid, KM254290.1)	90%		LN828732
010213-20	BM001180499	BM000701835	<i>Dictyota</i> sp.	93% (<i>Hecatonema maculans</i> , LM995318.1)	96%		LN828733
KUA5a	PC0720276	PC0167416	<i>Dictyota</i> sp.	90% (Chordariaceae, LM995296.1)	98%		LN828734
PM 71.1	BM 000701834	PC0167413	<i>Geminocarpus geminatus</i> (Hooker et Harvey) Skottsberg	88% (Acinetosporaceae, LM995398.1)	94%		LN828735
PM 72.2		PC0167414	<i>Hincksia granulosa</i> (Smith) P. C. Silva	89% (Acinetosporaceae, LT546278)	98%		LN828736
Fl 7-53.1	BM 000701833	PC0167412	<i>Hincksia sandriana</i> (Zanardini) Silva	91% (<i>Hincksia hincksiae</i> , LM995181.1)	97%		LN828738
270113-3	BM 001063406		<i>Myriotrichia claviformis</i> Harvey	92% (<i>Myriotrichia claviformis</i> , LM994973.1)	95%		LN828739
020213-13			<i>Paraglossum salicifolium</i> (Reinsch) S.-M.Lin, Fredericq et Hommersand*	82% (<i>Callithamnion</i> sp., EU194967.1)	90%	2e-138	LN828740
			<i>Phycodryx antarctica</i> (Skottsberg) Skottsberg	91% (<i>Phycodryx isabelliae</i> , KM254542.1)	69%		LN828741
010213-20	BM001180499	BM000701835	<i>Dictyota</i> sp.	<i>COI</i> (3'-5') 90% (<i>Dictyota spiralis</i> , GQ425136.1)	100%		KU708623
	PC0720276		<i>Dictyota</i> sp.	86% (<i>Dictyota kunthii</i> , GU290240.1)	71%	8e-107	KU708624
010213-20	BM001180499	BM000701835	<i>Dictyota</i> sp.	psbA 95% (<i>Dictyota canariensis</i> , KF322229.1)	100%		KU708625
	PC0720276		<i>Dictyota</i> sp.	89% (<i>Dictyota</i> sp., KF270587.1)	100%		KU708626

Table 2: Taxon sampling for the *Dictyota* phylogeny.

Taxon	Voucher	Accession no.
<i>Canistrocarpus cervicornis</i>	MAD1676	LN831805
<i>Canistrocarpus crispatus</i>	DAP39	EU395605
<i>Dictyota acutiloba1</i>	ODC888	EU395602
<i>Dictyota adnata</i>	SD712204	GU265788
<i>Dictyota binghamiae</i>	HV1542	JQ061015
<i>Dictyota binghamiae</i>	HV1801	JQ061016
<i>Dictyota binghamiae</i>	HV1802	JQ061017
<i>Dictyota canaliculata1</i>	ODC1477	GQ425190
<i>Dictyota canariensis</i>	UAMIZ1600	KF322229
<i>Dictyota caribaea</i>	HV623	EU395608
<i>Dictyota ceylanica5</i>	MAD0751	LN831811
<i>Dictyota coriacea</i>	CSUF003	EU395619
<i>Dictyota coriacea</i>	JALee11	AY748323
<i>Dictyota crenulata</i>	HV1074	GU265782
<i>Dictyota cymatophila</i>	D406	GQ425201
<i>Dictyota dichotoma1</i>	FS0160	FJ869842
<i>Dictyota dichotoma2</i>	JALee02	AY748320
<i>Dictyota dichotoma3</i>	SALee5	AY528443
<i>Dictyota diemensis</i>	LT0108	LN871942
<i>Dictyota fasciola</i>	ODC1035	EU395616
<i>Dictyota friabilis1.d</i>	HV153	GU265786
<i>Dictyota implexa</i>	FS0264	GU265773
<i>Dictyota intermedia</i>	D173	EU395615
<i>Dictyota kunthii</i>	D102	EU395618
<i>Dictyota linearis</i>	FS0343	GU255863
<i>Dictyota mediterranea</i>	D653	GU265785
<i>Dictyota mertensii2</i>	DR31	GQ425215
<i>Dictyota naevosa</i>	KZN2241	EU395609
<i>Dictyota sandvicensis</i>	ODC889	GU265783
<i>Dictyota stolonifera</i>	MAD0573	LN831818
<i>Dilophus fastigiatus</i>	D96	EU395614

sequences (Table 2). Sequences of *Canistrocarpus cervicornis*, *Canistrocarpus crispatus* and *Dilophus fastigiatus* were added as outgroup taxa.

Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic species trees were generated using MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003) and RAxML v.8.1.21 (Stamatakis 2014), respectively. The Bayesian analyses, initiated with a random starting tree under a GTR+G model, four chains of MCMC iterations ran for 5 million generations, saving every 1000th tree. All other parameters were set as default. The first 1000 (20%) trees were discarded as burnin. Stationarity of lnL was assessed using Tracer version 1.6 (Rambaut et al. 2014). A consensus topology and posterior probability values were calculated from the remaining trees. The ML analyses used a GTR+CAT model. The robustness of the resulting phylogeny was tested using 1000 replicates of a rapid bootstrap heuristic (Stamatakis 2006).

Results and discussion

Phaeophyceae

Cladostephus sp.

Cladostephus sp. was collected from the intertidal zone in western Port Philomel, West Falkland, Falkland Islands on December 4, 2013 (specimen # 041213-01); by snorkeling at 7 m depth at Mt. Kent Farm, East Falkland (Figure 1) on February 2, 2013 (specimen # 020213-25) and at 5 m depth in San Carlos (Blue Beach), East Falkland on February 1, 2013 (specimen # 010213-16; Figure 2A).

A *COI* sequence was obtained of the specimen from Port Philomel (LN828731), supporting the identification of *Cladostephus* sp.

It should be noted that Skottsberg's (1907) identification as *Cladostephus spongiosus* f. *hedwigioides* (Bory de Saint-Vincent) Prud'homme van Reine from the Falklands will arguably have to be re-examined as the type specimen requires further examination and until then this taxon should be treated as *taxon inquirendum*.

Herbarium specimen – BM001180498, microscope slide – BM000701838.

Cladothele decaisnei J. D. Hooker et Harvey

Cladothele decaisnei J. D. Hooker et Harvey appeared in raw culture of a sediment/substratum sample collected at ca. 10 m depth by diving from Ensenada near Ushuaia, Tierra del Fuego (Figures 1, 3A,B). This taxon had been previously reported from southern South America by Papenfuss (1964), Asensi (1976) and Asensi and Küpper (2012), and from Macquarie Island by Ricker (1987); our collection confirms its presence in Tierra del Fuego. Here, thalli of up to 5 cm in size were observed, which were uniseriate in their upper parts, with multiple longitudinal cell walls in basal parts. Terminal parts of thalli were trichothallic, while prominent apical cells and phaeophyceean hairs were absent. Its appearance was bushy with rich branching and its plurilocular zoidangia were hemispherical, formed by transformation of peripheral thallus cells, singly or in groups. Plurizoids developed directly into new macrothalli.

This study reports the first *COI* sequence for the genus *Cladothele*, which showed that the specimen belongs in the family Chordariaceae. Together with *Hincksia granulosa* (below), this is a case where there are no publicly available DNA sequences for a given species, although the

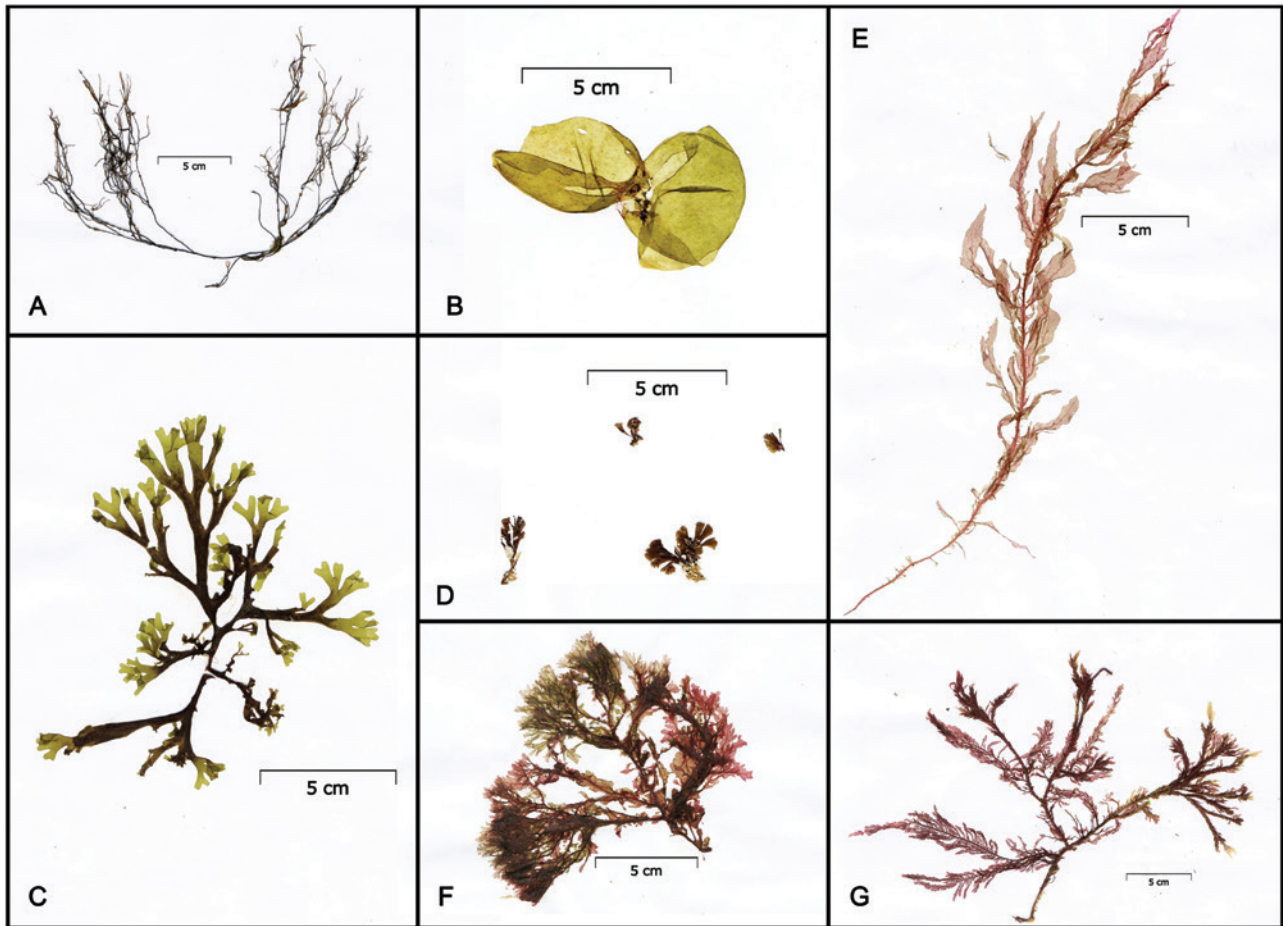


Figure 2: Herbarium specimens of the taxa from the Falkland Islands covered here. (A) *Cladostephus* sp. from Port Philomel, West Falkland, Falkland Islands (BM 001180498). (B) *Colpomenia* sp. from San Carlos (Blue Beach), East Falkland, Falkland Islands (BM 001180497). (C) *Dictyota* sp. from San Carlos (Blue Beach), East Falkland (BM 001180499). (D) *Syringoderma australe* herbarium specimen (BM 001063405) from Port William, East Falkland, Falkland Islands. (E) *Paraglossum salicifolium* herbarium specimen from Orca Pool, Sea Lion Island, Falkland Islands (BM 001063406). (F) *Phycodrys antarctica* herbarium specimen from North Arm, East Falkland, Falkland Islands (BM 001063408). (G) *Plumariopsis eatonii* herbarium specimen from Orca Pool, Sea Lion Island, Falkland Islands (BM 001063407).

DNA sequence agreed on a higher hierarchical level with the morphological identification (LN828732).

Microscope slide – PC0167415.

Colpomenia sp.

Colpomenia sp. (specimen # 010213-04) was collected from the intertidal at San Carlos (Blue Beach), East Falkland (Figure 1) on February 1, 2013 and constitutes the first report of the genus for the Falkland Islands (Figure 2B).

Herbarium specimen – BM001180497.

Dictyota sp.

Dictyota sp. was found by snorkeling in the shallow subtidal (1–2 m depth at low tide) at North Arm, East

Falkland (Figure 1) on January 30, 2013 (specimens # 300113-21, # 300113-34) constituting the first report of the genus for the Falkland Islands. *Dictyota* sp. (Figure 2C) was also found at San Carlos (Blue Beach; Figure 1), East Falkland on February 1, 2013 (specimen # 010213-20). *psbA* has been sequenced for the San Carlos specimen (LN828733).

Herbarium specimen – BM001180499, microscope slide – BM000701835.

A thallus corresponding to the genus *Dictyota* was found at Bahía Thetis in Tierra del Fuego, November 22, 1969 (Figure 4). Remarkably, this constitutes the only record of the genus for Tierra del Fuego. Thalli were sterile, but relatively well developed. The *psbA* sequence agreed with the morphological identification (LN828734) and furthermore suggested the specimen is conspecific with the *Dictyota* species collected in the Falklands (p distance <0.5%; Figure 5).

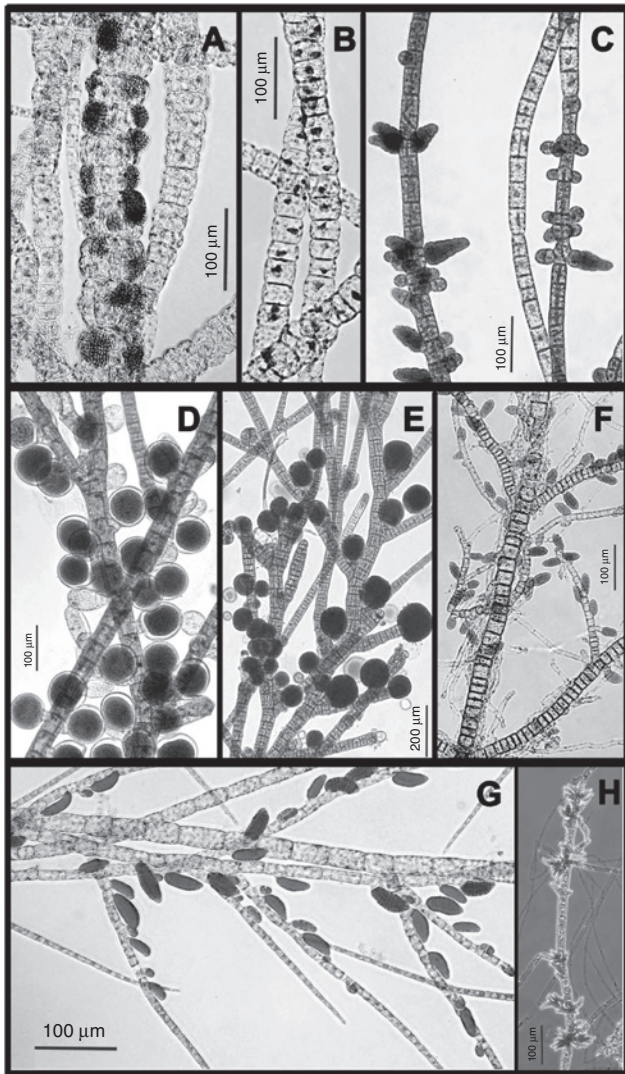


Figure 3: Micrographs of the brown algal taxa from Patagonia, Tierra del Fuego and the Falkland Islands covered here. (A and B) *Cladothelae decaisnei*: plurilocular zoidangia can be seen as dark, hemispherical structures (A); (C) *Geminocarpus geminatus* with cone-shaped plurilocular zoidangia; (D and E) *Halopteris obovata*: the micrographs were taken on clonal cultures: generation with unilocular sporangia (D) and succeeding generation with plurilocular zoidangia (E); (F) *Hincksia granulosa*; (G) *Hincksia sandriana*; (H) *Myriotrichia clavaeformis*: sessile plurilocular zoidangia can be seen lateral to the filaments – either singly, in pairs or in groups.

Herbarium specimen – PC 0720276.

These two records are significant since they may constitute the southernmost records of *Dictyota* sp. in the world. *Dictyota* is typically a tropical to warm temperate genus with distinctly fewer species in cold temperate regions. Species described from high latitudes are scarce. The only truly Subantarctic species described so far is *Dictyota decumbens* (Ricker) Hörnig, Schnetter et Prud'homme van Reine from Macquarie Island, 54° S



Figure 4: *Dictyota* sp. from Bahía Thetis, Tierra del Fuego, Argentina (PC 0720276).

(Ricker 1987 as *Dilophus decumbens*). The latter species is clearly distinct from the Falkland Islands and Tierra del Fuego specimens by its prostrate growth form. Our species is also very different from *Dictyota kunthii*, originally described from Peru but occurring along most of the Chilean coastline (Malbrán and Hoffmann 1990) and also being present in New Zealand (Nelson 2013). *Dictyota kunthii* is characterized by terete surface proliferations which formed the basis for its classification in the former genus *Glossophora* (De Clerck et al. 2006). Such proliferations are absent in the Falkland Islands and Tierra del Fuego specimens. Even though it seems likely that the specimens constitute a hitherto undescribed lineage (Figure 5), further research is needed to examine its relationships to, for example, *Dictyota phlyctaenodes* Montagne (Montagne 1852), described from Juan Fernandez Island and other southern hemisphere *Dictyota* occurring in New Zealand.

***Geminocarpus geminatus* (Hooker et Harvey) Skottsberg**

Geminocarpus geminatus (Hooker et Harvey) Skottsberg appeared in the raw culture of a sediment sample collected at 12 m depth at Isla Redonda (near Ushuaia);

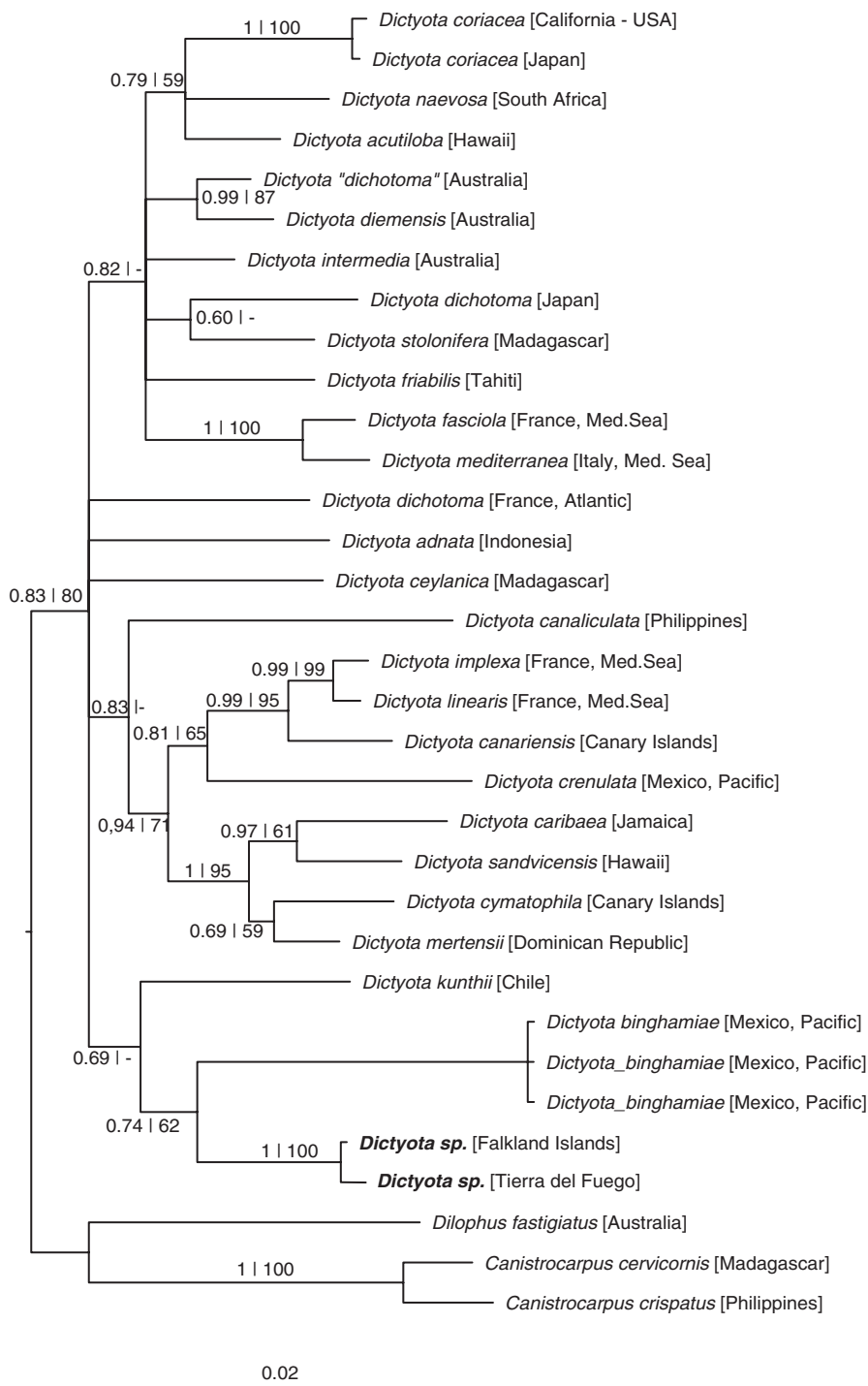


Figure 5: Bayesian tree, generated with MrBayes, based on *psbA* gene sequences (see Table 2 for taxon sampling). The values shown at each node represent posterior probability (left) and the maximum likelihood bootstrap values (right). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bold indicates samples from Falklands and Tierra del Fuego.

Figure 1), Tierra del Fuego, January 10, 2007 (Figure 3C). Thalli were up to 2 cm long, uniseriate, occasionally with longitudinal walls, rarely branched. They had plurilocular cone-shaped zoidangia, which occurred singly, paired or in local clusters. Direct reproduction

occurred with pluri-zoids; unilocular zoidangia were not seen.

This taxon had been previously reported for the region of southern South America (Papenfuss 1964), from Macquarie Island (Ricker 1987) and from Tierra del Fuego

by Asensi and Küpper (2012); our record confirms its presence in the area. This study reports the first *COI* sequence of the genus *Geminocarpus* (LN828735).

Microscope slide – PC0167416.

***Halopteris obovata* (J. D. Hooker et Harvey) Sauvageau**

Halopteris obovata (J. D. Hooker et Harvey) Sauvageau appeared in a sediment sample collected at ca. 12 m depth at Isla Redonda (in the Beagle Channel near Ushuaia), Tierra del Fuego (Figure 1) on January 10, 2007 (Figure 3D,E).

Two apical cells with 0.5 mm sub-apical somatic tissue were isolated. Both resulting unialgal clonal cultures gave identical results: The isolated apices continued to grow and produced typical *Halopteris* thalli of 1–2 cm in length. Dominant main axes formed lateral branches and occasionally new main axes. The regenerates of both initial fragments became fertile, forming rich crops of unilocular zoidangia, sessile or with one or a few stalk cells, randomly distributed on main axes or side branches.

Uni-zoids were released in great numbers. They germinated into new *Halopteris* thalli with the same morphology and size of their parent individual. The unispore-derived *Halopteris* individuals became fertile, exclusively forming plurilocular zoidangia, of similar size as the unilocular zoidangia in the preceding generation.

Loculi of plurilocular zoidangia were all of equal size, and released motile zoids. No interaction between pluri-zoids was seen, and upon settling on the bottom of culture dishes they immediately germinated. All progeny from pluri-zoids formed *Halopteris*-thalli bearing unilocular zoidangia. Careful screening confirmed repeatedly that the alternation between uni- and pluri-zoid forming generations is obligatory, and that no thalli were found with both types of zoidangia side by side.

The observations described here suggest that the *Halopteris* taxon from Beagle Channel exhibits what looks like a typical brown algal alternation of isomorphic generations: a presumed sporophyte with unilocular meio-sporangia alternates with a generation carrying plurilocular gametangia with iso-gametes. However, the absence of evidence for sexuality is puzzling, and suggests the following interpretation:

Our culture isolates may be a genuinely apomictic taxon descending from a predecessor with a complete sexual life cycle. This is supported by the fact that both our original isolates directly regenerated from field material. They undisputedly had sporophytic character, which is normally expected to produce both sexes in its unilocular meio-sporangia.

Similar cases of loss of sexuality through apomeiosis and apomixis, with unilocular zoidangia as former site of meiosis, and plurilocular “gametangia” functioning as mitosporengia are not uncommon in brown algae (Müller and Meel 1982, Müller 1986, Müller and Schmidt 1988, Müller and Stache 1989). Morphological characters as well as the geographical origin fit well with the description of *Halopteris obovata* (Hooker et Harvey) Sauvageau. In the literature available for this taxon (Sauvageau 1904, Wiencke and Clayton 2002), reproductive characters are poorly documented, and a final conclusion cannot be made.

This taxon had been previously reported from southern South America (Skottsberg 1907), from the Antarctic Peninsula and surrounding islands (Skottsberg 1907, Papenfuss 1964, Wiencke and Clayton 2002, Peters et al. 2005, Aumack et al. 2010), the South Shetland Islands (Wiencke and Clayton 2002, Quartino et al. 2005), and from Tierra del Fuego (John et al. 1994, Wiencke and Clayton 2002, Wells et al. 2011); our record confirms its presence in Tierra del Fuego.

Microscope slides – PC0167417, PC0167418.

***Hincksia granulosa* (Smith) P. C. Silva**

Hincksia granulosa (Smith) P. C. Silva (Figure 3F) was recorded and isolated from the Falkland Islands, Stanley Harbour, East Falkland (Figure 1) on January 18, 2007 for the first time.

Hincksia granulosa was also collected in a mariculture installation, in a loose brown algal tuft on soft bottom at 13 m depth, Seno de Reloncaví, Llanquihue, Chile (Figure 1), on February 2, 2007.

Unialgal cultures developed thalli up to 3 cm long, with richly branched main axes, and frequently opposite branches. Plurilocular zoidangia were numerous, ovoid, irregular on main and side branches, sessile or on few-celled stalks. Reproduction was observed to be direct by pluri-zoids. Unilocular zoidangia were not observed.

This taxon has been previously reported from Chile (Ramírez and Santelices 1991) and from Argentina by Asensi and Küpper (2012) and Boraso et al. (2011) and Boraso de Zaixso (2013); our specimen represents the first report for the Falkland Islands. The *COI* sequence obtained for the isolate from Chile (LN828736) showed only 85% similarity with the numerous sequences of European *H. granulosa* (e.g. EU681410). It was 89% similar with an unidentified species of Acinetosporaceae from Baffin Island (Küpper et al. 2016). The Chilean material, although morphologically similar to *H. granulosa*, apparently belongs to a different species.

Herbarium specimen – BM000701834, microscope slide – PC0167413.

Hincksia sandriana (Zanardini) Silva

Hincksia sandriana (Zanardini) Silva was collected in a mariculture installation, in a loose brown algal tuft on soft bottom at 13 m depth, Seno de Reloncaví, Llanquihue, Chile (Figure 1), February 2, 2007 (Figure 3G).

In unialgal culture, filaments up to 1 cm in length developed, with dominant main axes with rich branching. Plurilocular sessile zoidangia were observed. They were of elongated ovoid morphology in loose unilateral series on side branches. Unilocular zoidangia were absent. Direct reproduction by pluri-zoids was observed.

Previously discussed by Cardinal (1964) and Kornmann and Sahling (1977) for South America, and by Boraso et al. (2011), Boraso de Zaixso (2013) and Asensi and Küpper (2012) for Argentina, this is the first record for Chile and the first *COI* sequence (LN828738) reported for this taxon. The latter clearly confirms its affinity with the genus *Hincksia*.

Microscope slide – PC 0167414.

Myriotrichia clavaeformis Harvey

A specimen of this taxon was collected at Bahía Thetis, Tierra del Fuego (Figure 1), Argentina, November 22, 1969. This is the first record of any species of *Myriotrichia* from Tierra del Fuego. It was previously known from considerably further north, e.g. from the intertidal platforms of Piedras Coloradas near Las Grutas (Müller et al. 1996) and the Valdes Peninsula (Peters et al. 2004) – but it was not found at Puerto Deseado (Asensi and Küpper 2012).

Myriotrichia clavaeformis also appeared in a raw culture started with *Pylaiella* and *Ectocarpus* from the intertidal of Stanley Harbour, East Falkland (Figure 1), January 18, 2007 (Figure 3H). This establishes the first record of the genus for the Falkland Islands. Thalli were up to 5 mm in size and predominantly uniseriate. Terminal and lateral hairs were with basal meristem without sheath. Plurilocular zoidangia were lateral, sessile, singly, in pairs or groups. Pluri-zoids developed directly to new thalli with the same characteristics. Unilocular zoidangia were absent. A *COI* sequence has been obtained (LN828739) from this material, however it differed by 8% from a sequence of European *M. clavaeformis*.

Herbarium specimen – BM 000701833, microscope slide – PC 0167412.

Punctaria sp.

Punctaria sp. was found by snorkeling at 5 m depth at San Carlos (Blue Beach), East Falkland (Figure 1), February 1, 2013 (Specimen # 010213-17).

There were several parietal plastids per cell. Sporocysts were observed as single surface cells. *Punctaria* is a cosmopolitan genus that is often being transported by shipping activities. Both *P. latifolia* and *P. plantaginea* are reported from the adjacent coast of Atlantic Patagonia. However, based on morphology (2–4 cells in thickness, soft and flaccid; thallus ovate linear-lanceolate or oblong), the available specimen can likely be attributed to *P. latifolia* – even though a degree of uncertainty remains since it is a juvenile. This constitutes the first record of a *Punctaria* sp. from the Falklands.

Microscope slide – BM000701837.

Syringoderma australe Levring

A specimen of *Syringoderma australe* was found at ca. 12 m depth during a dive at Beatrice Cove, East Falkland (Figure 1), December 8, 2010 (specimen # 081210-7) and again close to this site in Port William, East Falkland (Figure 1) at ca. 20 m depth, February 10, 2013 (specimen # 100213-13; Figures 2D, 6). The thallus grew attached to rocks in the coralligenous zone underneath the kelp canopy (*Macrocystis pyrifera* and *Lessonia* sp.). This

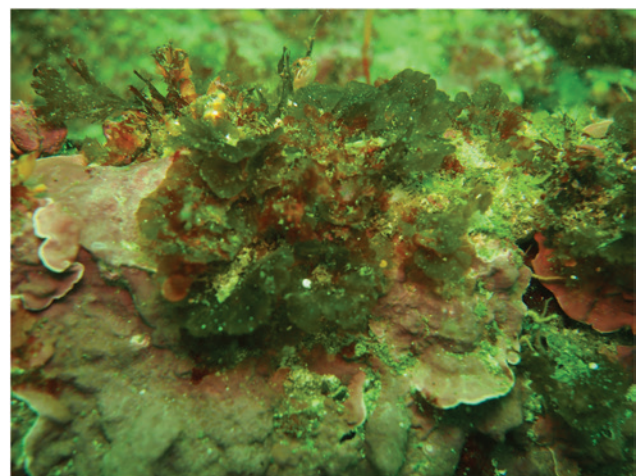


Figure 6: Close-up view of *Syringoderma australe* in its habitat at Port William, East Falkland, Falkland Islands.

constitutes the first record of this taxon north of the Antarctic Convergence and from the Falkland Islands.

Herbarium specimen – BM 001063405.

Rhodophyta

Erythrotrichia carnea (Dillwyn) J. Agardh

This taxon was found by snorkeling at about 5 m depth as a single epiphyte on the thallus of the brown alga *Dictyota* sp., at San Carlos (Blue Beach), East Falkland (Figure 1), February 1, 2013 (specimen # 010213-20). *Erythrotrichia carnea* was violaceous, filamentous, terete, erect, up to 240 µm long, attached to the substratum by a basal cell. It consisted of simple, uniseriate filaments, ca. 20–24 µm in diameter, tapering towards the base, composed of rectangular cells, 15 µm long and 12 µm broad. Reproductive structures were not observed.

This is a common species with cosmopolitan distribution (Guiry and Guiry 2015). *Erythrotrichia carnea* has been reported from Antarctica (Papenfuss 1964), Tierra del Fuego and Victoria Land (Wiencke and Clayton 2002). This constitutes the first record from the Falkland Islands.

Paraglossum salicifolium (Reinsch) S.-M.Lin, Fredericq et Hommersand

This species was found in the shallow sublittoral (ca. 0.5 m depth at low tide) in the Orca Pool at Sea Lion Island (Figure 1), Falkland Islands (specimen # 270113-3). This constitutes a new record for the Falkland Islands (Figure 2E), and this study reports the first *COI* sequence of this taxon (LN828740).

Herbarium specimen – BM 001063406.

Phycodrys antarctica (Skottsberg) Skottsberg

This taxon was collected at North Arm, East Falkland (Figure 1) at 3 m depth (specimen # 300113-13), January 30, 2013, and at Rincon Grande, East Falkland (Figure 1) at 5 m depth (specimen # 020213-13), February 2, 2013. A *COI* sequence was obtained for the material from Rincon Grande (LN828741).

Thalli were erect, up to 16 cm high, membranous, red to brownish in color, and attached to the substratum through a solid holdfast. They had ligulate blades, up to 2 cm wide, monostromatic, with margin irregularly serrate; conspicuous midrib present, alternately to

oppositely branched. New blades were found to arise from the midrib. Reproductive structures were not observed. All measurements were taken on dried specimens (Figure 2F).

Several thalli of *Phycodrys antarctica* were found on rocky sublittoral at 3 m (North Arm, Lafonia, East Falkland) and 5 m (Rincon Grande, East Falkland) depth.

This species is endemic to the Antarctic and Subantarctic regions (Papenfuss 1964, Wiencke and Clayton 2002). This study constitutes the first report from the Falkland Islands. In light of the records of *Phycodrys quercifolia* (Bory) Skottsberg from the Falklands (Kylin and Skottsberg 1919, Lin et al. 2001, Hommersand et al. 2009, Wynne 2014), the identity of the material discussed here was double-checked using the key of Wiencke and Clayton (2002). Future molecular studies may well reveal a taxonomic distinctness of this material from *P. antarctica* from the Antarctic.

Herbarium specimen – BM 001063408.

Plumariopsis eatonii (Dickie) De Toni

A single thallus (specimen # 270113-5) of this rare species was found as an epiphyte on *Ballia callitricha* (C.Agardh) Kützinger on a rocky coast at 0.5 m depth at low tide in the Orca Pool on Sea Lion Island (Figure 1), January 27, 2013.

Specimens were feathery, delicate, bright red in color, erect, up to 16 cm long, pinnately branched in one plane. Main axes were flattened, up to 500 µm wide, irregularly branched, composed of rectangular axial cells which tapered towards the upper parts, ending in apical cells of 20 µm in diameter, always overtopped by incurving lateral branches. A pair of two uniseriate simple uncorticated filaments, up to 500 µm long and 70 µm wide, were found to arise oppositely from each axial cell, which were determinate and composed of rectangular cells tapering towards the base and apex. Main axes were heavily corticated with the exception of the extreme upper part; cortication developing from one initial cell cut off from the base of each lateral branch, giving slightly elongated cells, measuring 25×10 µm, creating rhizoidal cortication. No fertile specimens were found. All measurements have been taken on dried specimens (Figure 2G).

Previously described from other areas in the Antarctic/Subantarctic region (Papenfuss 1964) including Heard Island (Wiencke and Clayton 2002), Kerguelen (Kylin and Skottsberg 1919, Wiencke and Clayton 2002), Macquarie Island (Moe and Silva 1983, Ricker 1987, Wiencke and Clayton 2002), South Georgia (Kylin and Skottsberg 1919, Wiencke and Clayton 2002), this constitutes a new record from the Falkland Islands.

Note: *Plumariopsis eatonii* shares similar morphology with *Plumariopsis peninsularis* R.L. Moe et P.C. Silva and *Georgiella confluens* (Reinsch) Kylin, but it can be distinguished by reproductive and vegetative characters (Moe and Silva 1983). Despite the lack of reproductive structures in our specimen, we attribute it to *P. eatonii* based on the morphology of the apical parts of the thallus, which is always overtopped by incurving lateral branches in *P. eatonii* but never overtopped in *P. peninsularis* and *G. confluens* (Moe and Silva 1983).

Herbarium specimen – BM 001063407.

Ulvophyceae

Chaetomorpha aerea (Dillwyn) Kützting

Chaetomorpha aerea was found as an epiphyte on *Cladostephus* sp. (specimen # 041213-01) in the intertidal of Port Philomel at West Falkland (Figure 1), and represents a new record for the Falkland Islands. Thalli were filamentous, wiry, green in color, up to 4 cm in length. Filaments were uniseriate, unbranched, terete, up to 200–250 µm in diameter, attached to the substratum by a basal elongated cell. Cell width was constant along filaments, but filaments tapered towards the base; cells large, rectangular, with a length/width ratio about 2–3; no noticeable constrictions at septa. Reproductive structures were not observed; all measurements have been taken on dried specimens.

This is a common species with worldwide distribution (Guiry and Guiry 2015). *C. aerea* is reported here for the first time from the Falklands Islands and also for the wider Antarctic and Subantarctic area.

It should be noted that *C. aerea* has been treated as a synonym of *Chaetomorpha linum* (O.F. Müller) Kützting by several authors (Burrows 1991, John et al. 2003), but their actual relationship remains uncertain. We note that the genus requires taxonomic re-investigation and we tentatively attribute our specimen to *C. aerea* until molecular investigations clarify its identity.

Herbarium specimen – BM 001063407.

Eukaryotic pathogens

During the expedition in January–February 2007 we encountered the oomycete *Eurychasma dicksonii* infecting *Pylaiella* and *Ectocarpus* in Stanley Harbour, Falkland Islands (Figure 1) and infecting *Ectocarpus* at Playa Larga (Figure 1 near Ushuaia, Tierra del Fuego. Laboratory studies



Figure 7: Several putative *Anisolpidium* thalli within *Ectocarpus* cells from Quetalmahue, Chile. *Anisolpidium* thalli (arrowed) can be seen within the cells of *Ectocarpus*, measuring between 19 and 30 µm in diameter. These thalli can be differentiated from those of *Eurychasma*, another common pathogen of brown algae, by the presence of a single elongated exit tube (not shown here).

on these two *Eurychasma* strains have been reported by Gachon et al. (2009). We here report new records of *Anisolpidium ectocarpii* (Figure 7) from Quetalmahue, Chiloé (Figure 1), January 31, 2007 and Seno de Reloncaví, Llanquihue (Figure 1) on Feb 2, 2007. At Quetalmahue, the algal host *Ectocarpus* was growing in dense tufts (together with *Pylaiella*) on the shaded, immersed parts of the hulls of small fishing boats, while at Seno de Reloncaví, *A. ectocarpii* was present in numerous infections in intermingled *Ectocarpus* and the two *Hincksia* species reported from the same occasion above. This constitutes the first record of *A. ectocarpii* for South America. It was last reported by Johnson (Johnson 1957) 50 years earlier. This pathogen had so far only been observed at its type locality in North Carolina (Karling 1943, Johnson 1957) and, 2 years after the records reported here, in Greece in 2009 (Strittmatter et al. 2013). *Eurychasma* is the most basal genus of the oomycete lineage (Küpper et al. 2006) while the phylogenetic affinity of *A. ectocarpii* remains unresolved. These findings clearly demonstrate that these pathogens, previously only known from temperate localities in the Northern Hemisphere, occur on both sides of the tropical belt. They infect *Ectocarpales* species, in which they can occur in epidemic outbreaks (Küpper and Müller 1999). They are unknown in the tropics – *Eurychasma* does not survive tropical sea water temperatures (Müller et al. 1999). This suggests that they may have crossed the equator either during glaciation periods or with globally colder climates than today. Alternatively, they may have crossed the equator via deep-water kelp habitats thriving at lower temperatures in the lower

part of the euphotic zone throughout the tropics (Graham et al. 2007) in the more recent past.

Another possibility may be that anthropogenic vectors (such as shipping, which can transport fouling algae or ballast water over very large distances) have led to the distribution on both sides of the equator. However, this appears rather unlikely in an area with low human impact such as Tierra del Fuego or the Falklands.

Microscope slides:

- *A. ectocarpii* (infecting *Ectocarpus* sp.) from Quetalmahue, Chiloé – BM000701842
- *A. ectocarpii* (infecting *Ectocarpus* sp.) from Seno de Reloncaví, Llanquihue – BM000701841
- *E. dicksonii* (infecting *Ectocarpus* sp.) from Playa Larga, Ushuaia, Tierra del Fuego – BM000701831
- *E. dicksonii* (infecting *Pylaiella* sp.) from Stanley Harbour Falklands – BM000701832.

Conclusions

The results of our expeditions to the Falkland Islands, Patagonia and Tierra del Fuego – the number of new records relative to sampling effort – suggest that this region remains substantially under-sampled with regards to seaweed diversity. Techniques such as the germling emergence method (Peters et al. 2015), combined with molecular identification, offer great potential for unravelling more of the algal diversity of little-studied regions such as these. Also, the lack of observations of reproductive structures in many field surveys demonstrates the importance of laboratory culture studies for the resolution of taxonomic and biogeographic problems.

In total, our activities reported here produced 11 new seaweed records for the Falkland Islands, many of which are supported by genetic data. In addition, two new records from Tierra del Fuego as well as one new record from southern Chile are described, complemented by sequencing data.

The marine flora of the Falkland Islands and southern South America had not been studied before by SCUBA diving, which explains that small thalli attached to rocks in the deeper sublittoral like *Syringoderma australe* remained inaccessible to previous investigators.

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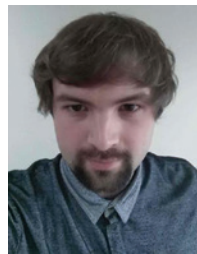
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