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Couverture / Cover:
Inaccessible Island, Tristan da Cunha, 25th November 2007. Steep bedrock at *c.* 6 m depth. Red algae include *Sarcothalia* (synonym of *Iridaea*) sp. (large flat dark red); *Pseudophycodrys pulcherrima* (pale pink), *Streblocladia atrata* (filamentous, upper centre), *Rhodymenia* (synonym of *Epymenia*) sp. (photo Sue Scott)

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Preliminary DNA barcode report on the marine red algae (Rhodophyta) from the British Overseas Territory of Tristan da Cunha

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ABSTRACT

The current manuscript is the first in a series intended to publish accumulating DNA barcode data to make them accessible to the scientific community. Focused on 135 specimens of red algae from the remote islands of Tristan da Cunha, part of the British Overseas Territory of Saint Helena, Ascension and Tristan da Cunha, the 47 (possibly 48; see notes with *Lophurella* sp. 1Tris) genetic groups uncovered during this project are compared to the only detailed floristic work for this region completed by Baardseth in 1941. A number of taxonomic anomalies are reported with indications for eventual solutions that await study of the type material of the associated morphospecies. Species previously assigned by Baardseth to the genus *Epymenia* Kützing are formally transferred to *Rhodymenia* Greville as *R. elongata* (Baardseth), comb. nov., (including *E. marginifera* Baardseth) and *R. flabellata* (Baardseth), comb. nov. A number of range extensions are reported including species such as *Ceramium secundatum* (Lyngbye) C.Agardh, *Colaconema caespitosum* (J.Agardh) Jackelman, Stegenga & J.J.Bolton, *Helminthocladia calvadosii* (J.V.Lamour. ex Duby) Setch. and *Porphyra mumfordii* S.C.Lindstrom & K.M.Cole, which have likely been distributed by human activities. We also note that the sporophyte of the supposedly narrowly distributed *Schimmelmannia elegans* has been collected from both British Columbia, Canada, and Queensland, Australia, consistent with other observations that sporophytes of red algal species with alternations of heteromorphic generations are commonly more broadly distributed than the gametophytic stage. This species, although originally described by Baardseth from these mid Atlantic islands, may also be introduced.

KEY WORDS
Biogeography,
COI-5P,
DNA barcode,
*rbc*L-3P,
Rhodophyta,
new combinations.

RÉSUMÉ

Rapport préliminaire de codes à barres ADN sur les algues rouges marines (rhodophytes) du territoire d'outre-mer britannique de Tristan da Cunha.

Le présent manuscrit est le premier d'une série destinée à publier des données de codes barres ADN afin de les rendre accessibles à la communauté scientifique. Centrés sur 135 spécimens d'algues rouges provenant des îles éloignées de Tristan da Cunha, qui font partie du territoire britannique d'outre-mer de Sainte-Hélène, Ascension et Tristan da Cunha, les 47 (peut-être 48 ; voir notes avec *Lophurella* sp. 1Tris) groupes génétiques découverts pendant ce projet sont comparés au seul travail floristique détaillé pour cette région réalisé par Baardseth en 1941. Un certain nombre d'anomalies taxonomiques sont signalées avec des indications pour d'éventuelles solutions qui nécessitent l'étude du matériel type des morphospèces associées. Les espèces précédemment assignées par Baardseth au genre *Epymenia* Kützing sont officiellement transférées à *Rhodymenia* Greville en tant que *R. elongata* (Baardseth), comb. nov., (y compris *E. marginifera* Baardseth) et *R. flabellata* (Baardseth), comb. nov. Un certain nombre d'extensions de l'aire de répartition sont signalées, y compris des espèces telles que *Ceramium secundatum* (Lyngbye) C.Agardh, *Colaconema caespitosum* (J.Agardh) Jackelman, Stegenga & J.J.Bolton, *Helminthocladia calvadosii* (J.V.Lamour. ex Duby) Setch. et *Porphyra mumfordii* S.C.Lindstrom & K.M.Cole, qui ont probablement été introduites par des activités humaines. Nous notons également que le sporophyte du *Schimmelmannia elegans*, dont la répartition est censée être étroite, a été prélevé en Colombie-Britannique (Canada) et au Queensland (Australie), conformément à d'autres observations selon lesquelles les sporophytes des espèces d'algues rouges ayant un cycle de vie avec une alternance de générations hétéromorphes sont généralement plus largement répartis que le stade gamétophytique. Cette espèce, bien que décrite à l'origine par Baardseth de ces îles du centre de l'Atlantique, pourrait également être introduite.

MOTS CLÉS
Biogéographie,
COI-5P,
code à barres ADN,
*rbc*L-3P,
Rhodophytes,
combinaisons nouvelles.

INTRODUCTION

Baardseth (1941) published the first and only detailed monograph of the marine macroalgal flora of Tristan da Cunha, part of the remote British Overseas Territory of Saint Helena, Ascension and Tristan da Cunha. Tristan is located in the South Atlantic, midway between South Africa and South America, at 37.078°S, 12.304°W. Inaccessible Island lies 30 km to the southwest, and Nightingale Island a similar distance to the southeast of Tristan (these three collectively referred to as the top islands). A fourth island, Gough, also belongs to the Tristan archipelago but is located around 200 km to the southeast, is floristically different and not included in this study. Ryan (2007) provided a brief overview of the islands, as well as a guide to terrestrial and marine life. The islands are extremely isolated, more than 2400 km from the nearest human settlement at St. Helena (another remote island to the north), and accessible only by sea. Tristan is the largest and only inhabited island, of classic volcano shape, roughly circular, 12 km across and 2060 m high. All the islands are volcanic and rugged, and extremely exposed with frequent gales.

Scott (2017) provided a more detailed biophysical profile of the Tristan islands, including marine life and habitats. Marine erosion has resulted in rocky coasts of mainly cliffs and boulders, with very few sandy beaches. In the subtidal an erosion platform of variable width but usually less than 2 km, eventually plunges to depths of over 3000 m. The climate is temperate maritime, with seawater temperatures ranging from 12-16°C in winter and 15-20°C in summer. The main residual surface currents are from the west, from South America, but also reach Tristan from the African coast via the South Atlantic gyre, a much longer route. Tidal range is small, only around a metre on spring tides. Conditions are ideal for seaweed growth, with clear waters allowing good light penetration, constant water movement replenishing nutrients, and few grazers on the shore and in shallow water. Smaller seaweeds are abundant on the shores and shallow subtidal rocks, with forests of the kelps *Macrocystis pyrifera* (L.) C.Agardh and *Laminaria pallida* Greville growing in water to at least 40 m deep (Scott & Tyler 2008; Scott 2010a, b, 2017). Urchins are abundant deeper than 12-15 m, so deeper rocks are heavily grazed, with few smaller seaweeds. Generally, the shallow water marine fauna and flora of the islands is extremely impoverished in terms of numbers of species, due to extreme isolation and young geological age, and the lack of any 'stepping-stone' islands to aid colonisation. Macroalgae are one of the more diverse groups, with around 125 species currently known, although further taxonomic work will undoubtedly reveal more. Baardseth (1941) noted that the level of endemism (40%) for the marine macroalgae was quite high, which are similar to levels for some terrestrial plant groups, including native ferns (40% endemic) and flowering plants (54% endemic) (Ryan 2007).

Baardseth (1941) acquired his collections during his doctoral years, which offered him a rare opportunity to participate in the Norwegian Scientific Expedition to Tristan da Cunha 1937-1938. He collected at all the northern islands (Tristan, Nightingale and Inaccessible), but was unable to visit Gough. As SCUBA was not available at the time, all Baardseth's subtidal specimens were obtained by dredging, or were drift material, with the obvious limitations this imposes on collecting from certain habitats such as vertical or overhanging rock. Fixing specimens in formalin and seawater, Baardseth completed his anatomical investigations at Botaniska Laboratoriet, Lund (Jan. 1939 to Aug. 1940) and at the Botanisk Museum, Oslo (Dec. 1940 to Apr. 1941) (Baardseth 1941). In his work Baardseth reported 76 species of red algae, but 67% of these were newly described or considered of uncertain species or genus assignment.

Efforts to document diversity for macroalgae have been plagued by morphological simplicity, exacerbated by convergent evolution, retention of ancestral features, and phenotypic plasticity (Saunders 2005). Molecular markers are an effective means of initial identification and establishment of genetically-based groups or species, but had suffered from the lack of a standardization marker (Saunders 2005). The DNA barcode, a portion of the mitochondrial cytochrome-c oxidase subunit I (COI) gene, had been championed as a standard for molecular species distinctions throughout eukaryotic life (Hebert *et al.* 2003). Although the initial hope that this marker would have broad taxonomic utility fell short (Chase & Fay 2009), it has been successfully used to discriminate among cryptic species of red algae (e.g., Saunders 2005, 2008). Genetic groups identified with this marker can then be subjected to morphological and anatomical study to supplement the molecular results in a process termed molecular-assisted alpha taxonomy (Saunders [2005], abbreviated MAAT [Cianciola *et al.* 2010]) and aid researchers in recognizing and correctly labeling species (Saunders 2008). With time, incomplete lineage sorting and introgression (e.g., Saunders *et al.* 2015), as well as unsuccessful attempts to amplify the COI-5P gene in some specimens (e.g., Kucera & Saunders 2012) have prompted the use of multiple markers emphasizing the nuclear internal transcribed spacer (ITS) and *rbc*L-3P (see Saunders & Moore 2013).

Over the years since Saunders (2005) first established the efficacy of COI-5P for species diagnosis in red algae, considerable barcode data have been generated (Saunders & Le Gall 2010) and, if our laboratory is any indication, much more have been generated than have been published. The issue is that generating sequence data is fast and easy to do, especially with robotics (e.g., Saunders & McDevit 2012a), but the taxonomic aspects of MAAT are time consuming requiring the examination of type material, which may or may not be available for study (a problem in the current case) and may or may not yield useable DNA for comparative purposes (e.g., Saunders & McDevit 2012b; Lindstrom *et al.* 2015). We propose that one solution to this backlog of unpublished barcode data is through the publication of short barcode accounts like that presented here and recently by Manghisi *et al.* (2019). More than simply releasing the sequences into GenBank, the purpose is to provide information on the sequences generated relative to the floristic region in the hopes that taxonomic research in other laboratories will be facilitated. Here we compile a total of 171 sequences representing 135 specimens and 47 (48?) genetic groups from Tristan da Cunha with notes directed at advancing taxonomic research on this little explored flora (Baardseth 1941).

METHODS

Specimens were collected during diving and shore surveys of Tristan, Nightingale and Inaccessible between 2004 and 2010 (Scott & Tyler 2008; Scott 2010a, b), mostly as part of Darwin Initiative projects (http://www.darwininitiative.org.uk). In each case a voucher was pressed with a subsample placed in silica gel for subsequent molecular work. Vouchers are stored with Sue Scott (address above), and will eventually be lodged with the Natural History Museum, London (NHM), where there is a small collection of Baardseth's Tristan specimens. The DNA was extracted following Saunders & McDevit (2012a) and the COI-5P and *rbc*L-3P (or extended fragment) amplified and sequenced as outlined in Saunders & Moore (2013). Meta data are recorded along with the sequences on the Barcode of Life Database (BOLD) website (http://www.boldsystems.org/) in the public dataset DS-SHTDC1 with the sequence data also loaded in GenBank (Appendix 1). Sequences generated here were subjected to blast searches in BOLD and GenBank to identify the closest matches. Typically 1% and 0.15% divergence for COI-5P (Saunders 2008) and *rbc*L (Saunders *et al.* 2015), respectively, were used as thresholds to define species with exceptions discussed below (Appendix 1).

Preparations for anatomical studies were made by rehydrating material in 4-5% formalin/seawater. Material was viewed as whole-mount squashes when feasible or following hand-sectioning or thin-sectioning in a cryostat (CM1850, Leica). Sections were typically stained with acidified 1% aniline blue before mounting in 40-50% corn syrup (Evans & Saunders 2017).

RESULTS AND DISCUSSION

From the 135 specimens subjected to molecular identification here, 171 sequences (COI-5P, n = 85; *rbc*L (including *rbc*L-3P), n = 86) were generated and assigned to 47 (possibly 48; see notes with *Lophurella* sp.1Tris) genetic groups (Appendix 1). Of those genetic groups, 33 were assigned to named species with 27 of these recorded in the detailed flora of Baardseth (1941; although many require taxonomic work at the genus level) and the other six newly reported here, i.e., they represent range extensions (Appendix 1). Five species are possibly introduced: *Ceramium secundatum* (Lyngbye) C.Agardh; *Colaconema caespitosum* (J.Agardh) Jackelman, Stegenga & J.J.Bolton; *Helminthocladia calvadosii* (J.V.Lamour. ex Duby) Setch.; *Porphyra mumfordii* S.C.Lindstrom & K.M.Cole(possibly a later synonym of *P. tristanensis* Baardseth); and *Schimmelmannia elegans* Baardseth, despite being described from this location. The remaining 14 genetic groups may or may not correspond to species listed in Baardseth (1941) and further taxonomic work is necessary (detailed in Appendix 1). Regrettably this taxonomic research will be hampered by the uncertainty as to the location of the type material for Baardseth's many new species (Dr. K-H. Larsson personal communication). Two new combinations are presented to resolve synonymy of the genus *Epymenia* Kützing with *Rhodymenia* Greville.

Interestingly, three sibling pairs with molecular differences at the threshold for recognition as distinct genetic species were uncovered, but which nonetheless were clearly distinct morphospecies: *Plocamium delicatulum* and *P. fuscorubrum*; *Rhodymenia elongata* and *R. flabellata*; and, *Schizoseris dichotoma* and *S. multifoliata*. This is in stark contrast to the usual case in which closely related genetic groups typically represent cryptic species (see Saunders 2008).

Baardseth (1941: 147-148) reported high levels of endemism (40% for the flora overall), which he logically associated with the isolation and geological age of TdC. This endemism was largely attributable to the red algae (45 of the 49 species reported as endemic; Baardseth 1941). Despite some observations that will erode Baardseth's estimates, including the recognition of putative introduced species (Appendix 1), our results will likely increase the overall percentage of endemism for TdC. Contributing to this are our records of cryptic species for which Baardseth (1941) had mis-assigned the name of a widely distributed species [e.g., *Centroceras* sp.1Tris versus *C. clavulatum* (C.Agardh) Montagne], as well as completely novel genetic species (lack matches in BOLD and GenBank) for which there were no records in Baardseth (1941) (e.g., *Wetherbeella* sp.1Tris; Appendix 1). Barcode surveys of the likely source floras, for which Baardseth (1941) emphasized South Africa and South America, which is consistent with our data (Appendix 1), are necessary to fully understand levels of endemism at TdC.

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APPENDIX

Appendix 1. — Searches of COI-5P (plain text accession numbers) and *rbc*L (including *rbc*L-3P) (***bold italicized*** accession numbers) data were carried out in BOLD and GenBank with the closest matches recorded where a meaningful relationship could be inferred. Abbreviations: **TdC**, Tristan da Cunha islands. Vouchers numbers correspond to TdC locations as follows: **IN**, Inaccessible Island; **NI** and Nightingale, Nightingale Island; **TR** and Tristan, Tristan da Cunha Island.

Taxonomy	Voucher	COI-5P *rbc*L	Comments
Bangiophyceae, Bangiales, Bangiaceae			
Porphyra mumfordii S.C.Lindstrom & K.M.Cole	NI05.R06	MK202379	COI-5P differs by only 1-3 bp (0.46%) from data in BOLD for our collections of *P. mumfordii* from British Columbia, Canada, and Chile [also reported in Muñoz-Muga *et al.* (2018)]. Given its distribution, this genetic group is likely widely introduced. A range extension for this species, but synonymy with *P. tristanensis* Baardseth needs consideration, which has priority.
	TR05 R20	MK202426	
Pyropia sp. 1TDC1732	IN07.R017	MK202381 ***MK185778***	COI-5P lacks a close match in BOLD and GenBank. Partial *rbc*L is close (0.2% divergent) to GenBank data for another specimen from TdC (GU046429; Broom *et al.* 2010).
Florideophyceae, Nemaliophycidae, Colaconematales, Colaconemataceae			
Colaconema caespitosum (J.Agardh) Jackelman, Stegenga & J.J.Bolton	Tristan 2010 084	***MK185765***	For the 1032 bp of *rbc*L available for comparison there is only 1 bp (0.1%) different from an Australian specimen of *C. caespitosum* (GenBank KC134354), while data for a specimen from near the type locality in France are a 100% match (Ireland: GWSC050; GenBank MK185384). This represents a significant range extension for this species, which is likely widely introduced. Not listed in Baardseth (1941), confusion with *Rhodothamniella,* which he lists, is a possibility (see Womersley 1994).
Florideophyceae, Nemaliophycidae, Nemaliales, Liagoraceae			
Helminthocladia calvadosii (J.V.Lamouroux ex Duby) Setchell	Tristan 2010 001	MK202404	COI-5P is similar (4 bp different over 664; 0.6% divergent) to data for this species from near the type locality in France (GenBank HQ603218). Not reported by Baardseth (1941) despite being a large, distinctive species growing in easily accessible rock pools and shallow subtidal habitat near the harbour on Tristan, this species may represent a subsequent introduction.
	Tristan 2010 017	MK202382	
Florideophyceae, Nemaliophycidae, Nemaliales, Scinaiaceae			
Nothogenia sp. 1Tris	IN07.R015	***MK185783***	*rbc*L similar to data for other species assigned to this genus in BOLD and GenBank, but there are no close matches. This species likely accounts for Baardseth's records of *N. ovalis* (Suhr) P.G. Parkinson [as *Chaetangium ovale* (Suhr) Papenfuss], type locality South Africa, but data for this species from near the type locality (Lindstrom *et al.* 2015) are not close to this genetic group. As such, this entity likely represents a new species of *Nothogenia*.
	Tristan 2010 026	***MK185821***	
Scinaia capensis (Setchell) Huisman	Nightingale 2010 048	***MK185759***	COI-5P differs by only 2 bp (0.3%) from data for an unpublished specimen from South Africa (type locality) in BOLD, which we assigned to *S. capensis*. The *rbc*L data lack a close match in BOLD and GenBank. This species may account for records of *Scinaia furcellata* (Turner) J.Agardh in Baardseth (1941), which has its type locality in England (Guiry & Guiry 2019).
	Tristan 2010 038	MK202414 ***MK185812***	
Florideophyceae, Rhodymeniophycidae, Acrosymphytales, Schimmelmanniaceae			
Schimmelmannia elegans Baardseth	Nightingale 2010 063	***MK185785***	Described from TdC by Baardseth (1941), this species lacked species-level matches for COI-5P and *rbc*L in GenBank. In BOLD the COI-5P is similar (1 bp different out of 660; 0.15% divergent) to crustose collections from British Columbia, Canada and Queensland, Australia. Presumably these represent the sporophyte stage in the life history, which for introduced species can typically be more widely distributed than the gametophyte (e.g. Saunders *et al.* 2015). This species is also likely introduced accounting for its presence at TdC. This species was considered an introduction to Cape Town, South Africa from TdC (De Clerck *et al.* 2002).
	Tristan 2010 019	MK202385 ***MK185780***	
Florideophyceae, Rhodymeniophycidae, Ceramiales, Callithamniaceae			
Aristothamnion ramelliferum Baardseth	Tristan 2010 065	MK202392	Weak match (*c.* 8% divergent) to various *Callithamnion* spp. in BOLD and GenBank. Morphologically and anatomically (including the presence of polysporangia) it best matches this species described in Baardseth (1941).
Florideophyceae, Rhodymeniophycidae, Ceramiales, Ceramiaceae			
Centroceras sp. 1Tris	Nightingale 2010 024	MK202400 ***MK185797***	*rbc*L is close to data for *Centroceras tetrachotomum* Won, T.O.Cho & Fredericq from South Africa in GenBank (DQ374323). There are, however, eight substitutions (over 1358 bp; 0.6% divergent), which is indicative of separate species. This species may account incorrectly for records of *C. clavulatum* (C.Agardh) Montagne in TdC (Baardseth 1941).
	Tristan 2010 069	MK202411	

Appendix 1. – Continuation.

Ceramium secundatum Lyngbye	IN07.R036	***MK185826***	Both COI-5P and *rbc*L indicate an alliance to the widely introduced *Ceramium secundatum* (Bruce & Saunders 2015). However, the COI-5P (*c.* 2% divergent from, e.g. KJ960521) and *rbc*L (*c.* 0.2% divergent from, e.g. KT250273) are slightly divergent from records in GenBank and the possibility of sibling species warrants investigation.
	Nightingale 2010 056	MK202407	
	Tristan 2010 068	MK202359	
	Tristan 2010 073	MK202399	
Microcladia alternata Baardseth	IN07.R008	***MK185800***	COI-5P lacked a strong match, while *rbc*L is 6% and 5% divergent from *Ceramium kondoi* Yendo and *C. sungminbooi* J.R.Hughey & G.H.Boo in BOLD and GenBank, respectively. This is a good morphological match to *M. alternata* in Baardseth (1941), although taxonomic work on the correct genus placement is required.
	Nightingale 2010 039	***MK185805***	
	Nightingale 2010 040	MK202416 ***MK185813***	
Florideophyceae, Rhodymeniophycidae, Ceramiales, Dasyaceae			
Heterosiphonia obscura (Dickie) Baardseth	Nightingale 2010 020	MK202417	*Heterosiphonia obscura* was originally described from TdC. COI-5P is only 2% divergent from a record in BOLD for a *Heterosiphonia* sp. from Chile collected in 2014 at Curiñanco (GWS038116), while there are no close matches in GenBank. The *rbc*L did not find a close match in BOLD or GenBank, which questions inclusion in this genus.
	Nightingale 2010 026	***MK185769***	
	Nightingale 2010 029	MK202396	
	Nightingale 2010 057	MK202397	
	Nightingale 2010 058	***MK185816***	
	Tristan 2010 066	***MK185792***	
Florideophyceae, Rhodymeniophycidae, Ceramiales, Delesseriaceae			
Myriogramme(?) sp. 1Tris	Nightingale 2010 034	***MK185762***	COI-5P and *rbc*L lack a close match in BOLD and GenBank. Preliminary phylogenetic analyses weakly ally this genetic group to *Myriogramme manginii* (Gain) Skottsberg, these two a distance sister to a variety of *Myriogramme* spp. including the type species *M. livida* (J.D.Hooker & Harvey) Kylin, as well as *Schizoseris hymenena* (Zanardini) Womersley. This species is assignable to the tribe Myriogrammeae but not *Myriogramme* sensu stricto and may represent a new species and genus.
	Nightingale 2010 036	MK202387 ***MK185781***	
	Tristan 2010 040	MK202405 ***MK185802***	
	Tristan 2010 041	MK202366 ***MK185757***	
Neuroglossum multilobum Baardseth(?)	IN07.R012	MK202386	COI-5P is distant from all records in BOLD and GenBank. Although our specimen is not a perfect match to Baardseth's *N. multilobum* in morphology, being larger in stature, the vegetative anatomy with the emphasis on the consistent size of cells throughout the costa and the growth from numerous marginal initials does match. Although fusion cells in the developing carposporophyte similar to those depicted for this species in Baardseth (1941: figure 60L) were observed, these same structures in some sections (not easily obtained from the dried specimens) appeared more similar to the *Neoharaldiophyllum* type as outlined in Kang *et al.* (2017, figure 56). Preliminary phylogenetic analyses place it in the tribe Myriogrammeae, which renders assignment of this specimen to *Neuroglossum* in the distant sister tribe Neuroglosseae untenable. Confirmation of our tentative identification is necessary, but this genetic group likely requires a new genus (and species?) in the Myriogrammeae as foreshadowed in Baardseth (1941).
Paraglossum minus (Baardseth) S.-M.Lin & Hommersand	TR05.R64	***MK185793***	*rbc*L data are only 7 bp different (0.5%) from a GenBank entry for *Paraglossum epiglossum* (J.Agardh) J.Agardh from the Falkland Islands (AF257410). This level of divergence is consistent with distinct species.
Pseudophycodrys pulcherrima Baardseth	IN07.R025	***MK185760***	COI-5P data lack a close match. The *rbc*L is only 3 bp different (0.2%) from two sequences assigned to *Pseudophycodrys phyllophora* (J.Agardh) Skottsberg in GenBank (AF257440, AF257441). AF257441 (and AF257440?) is from the Falkland Islands – the type locality of *P. phyllophora*. The level of divergence in *rbc*L between these two entities is at the edge of species distinction and possible synonymy needs study. Our collections are also 5.2% divergent in *rbc*L from a specimen assigned to *P. pulcherrima* (AF257442) collected from the Antarctic Peninsula. Our collections are from the type region and AF257442 is possibly mis-assigned to this species (see Lin *et al.* 2001).
	Tristan 2010 044	MK202373 ***MK185768***	
	Nightingale 2010 032	***MK185753***	

APPENDIX 1. – Continuation.

Schizoseris dichotoma (J.D.Hooker & Harvey) Kylin	Tristan 2010 056	MK202380	COI-5P data resolved closest (3.9% divergent) to a BOLD record for *S. griffithsia* (Suhr) M.J.Wynne from Chile; while GenBank indicates 5% divergence from a specimen assigned to *Phycodrys antarctica* (Skottsberg) Skottsberg (LN828741). Baardseth (1941) described two species of *Schizoseris* from the islands with our collections here assigned to *S. multifoliata* (below) only 0.76% divergent from the other species, *S. papenfussii* Baardseth [considered a synonym of *S. dichotoma* (J.D.Hooker & Harvey) Kylin, although molecular data are needed to assess this taxonomic proposal]. Nonetheless, the two genetic groups are clearly morphologically distinct based on the collections we have in hand and we continue to recognize both pending further study with nuclear markers.
Schizoseris multifoliata Baardseth	IN07.R022 Nightingale 2010 025 Nightingale 2010 035 Nightingale 2010 064	MK202390 MK202413 MK202372 MK202356	Comments above with *S. dichotoma*. A confounding factor is the degree of morphological variation among the four specimens included in this genetic group – more work is needed to confirm that they all are bona fide members of a single genetic group.
Florideophyceae, Rhodymeniophycidae, Ceramiales, Rhodomelaceae			
Bostrychia sp.	Nightingale 2010 068	MK202398 ***MK185794***	COI-5P and *rbc*L are not close to data in BOLD, but the former matches data for *B. intricata* (Bory) Montagne from Tristan da Cunha (identical; JN881516) and Chile (0.3% divergent; KM502835) in GenBank. However, the morphospecies *B. intricata* is assigned to specimens of considerable genetic variation in GenBank. Baardseth (1941) reported only *B. mixta* J.D.Hooker & Harvey, which is currently lumped into the broad concept of *B. intricata* – taxonomic work is needed.
Lophurella sp. 1Tris	NI07.R06 Tristan 2010 036	MK202421 MK202425 ***MK185824***	There are 8 bp divergence in COI-5P for the two TC specimens, which may indicate sibling species (indeed the overall size and richness of branching for the determinate branches differs between the two specimens). COI-5P data lack a close match in BOLD and GenBank being 5.7% divergent from *Leptosiphonia fibrillosa* (Dillwyn) Savoie & G.W.Saunders. The *rbc*L is 2% divergent from records for this same species in GenBank (AF342912). This species (complex?) may correspond to *Lophurella christophersenii* Baardseth from Tristan da Cunha, which would necessitate transfer to *Leptosiphonia*. Our specimens are larger than indicated in the type description (NI07.R06 being 23 cm tall) and richly branched along the entire length of the axes more similar to *L. comosa* (J.D.Hooker & Harvey) Falkenberg than *L. christophersenii*, but certainly with the thick axes and abundant trichoblasts of the latter (Baardseth 1941).
Pterosiphonia concinna Baardseth	Tristan 2010 051	***MK185801***	*rbc*L data are 5% divergent from a variety of *Pterosiphonia* spp. in GenBank and this entity is assignable to Baardseth's *P. concinna*, which is widely distributed throughout TdC.
Streblocladia atrata Baardseth	Nightingale 2010 017 Nightingale 2010 047 Tristan 2010 022	MK202391 MK202377 MK202406 ***MK185804***	This species is 3.4% and 1.3% divergent in COI-5P and *rbc*L, respectively, from data in BOLD for *S. camptoclada* (Montagne) Falkenberg from South Africa. These two species, however, are distant from *rbc*L data in GenBank reportedly from the type species of *Streblocladia* [KX499569; see Savoie & Saunders (2016)] refuting inclusion in this genus.
Florideophyceae, Rhodymeniophycidae, Gelidiales, Gelidiaceae			
Gelidium concinnum Baardseth	Nightingale 2010 008 Nightingale 2010 023 Tristan 2010 049	MK202363 ***MK185755*** MK202369 ***MK185763*** MK202424	COI-5P data lack a close match in BOLD or GenBank while *rbc*L data are only 2-3% divergent to collections of *G. isabelae* W.R.Taylor and *G. vittatum* (Linnaeus) Kützing. Baardseth (1941) described three new species of *Gelidium* and our larger specimens have vegetative and tetrasporangial (the actual nature of the sporangia needs further study) habit consistent with *G. concinnum*. A smaller specimen (Night 2010 023) is more consistent with Baardseth's *G. inflexum* and synonymy may be required.
Florideophyceae, Rhodymeniophycidae, Gelidiales, Gelidiellaceae			
Gelidiella feldmannii Baardseth	NI05.R13	***MK185782***	*rbc*L data are *c.* 6% divergent from *Pterocladiella caloglossoides* (M.Howe) Santelices and *P. luxurians* (Collins) G.H.Boo & K.A.Mill in BOLD and GenBank (KX423475), respectively. This specimen has vegetative (although we noted a few rhizoids in the medulla) and tetrasporangial details consistent with *G. feldmannii* in Baardseth (1941), however, with the closest matches being *Pterocladiella* spp. genus-level changes are necessary.

Appendix 1. – Continuation.

Florideophyceae, Rhodymeniophycidae, Gigartinales, Furcellariaceae			
Halarachnion (?) *pusillum* Baardseth	IN07.R023 Nightingale 2010 002 TR05.R38	MK202415 MK202430 MK202357	In describing this species Baardseth (1941) included it only tentatively in *Halarachnion*. This uncertainty is apparent in the COI-5P matches, which indicate *c.* 9.5% divergence from *H. ligulatum* (Woodward) Kützing in BOLD. This level of divergence is approaching saturation for COI-5P and a new genus assignable to the Furcellariaceae appears warranted.
Florideophyceae, Rhodymeniophycidae, Gigartinales, Gigartinaceae			
Iridaea ciliata Kützing	Nightingale 2010 011 Nightingale 2010 043 TR04.R12 TR06.R03 Tristan 2010 004	***MK185773*** ***MK185820*** ***MK185809*** ***MK185803*** ***MK185754***	This genetic group is only *c.* 0.5% divergent in *rbc*L from *Iridaea* sp. 1ciliata (below); *c.* 3.6% divergent from specimens assigned to *Sarcothalia stiriata* (Turner) Leister in BOLD, and only 0.8% divergent from a Chilean specimen assigned to *Sarcothalia crispata* (Bory) Leister in GenBank (U03085). Assuming that our identification is correct, this species (complex, see below) requires transfer to *Sarcothalia*.
Iridaea sp. 1ciliata	TR04.R02 TR04.R11	MK202423 ***MK185818*** MK202368	A second genetic group in the *I. ciliata* complex (see above), COI-5P data are *c.* 2.3% divergent from data for an unknown “*Iridaea*” specimen from Chile in BOLD. Nuclear data are necessary to determine if two separate TdC species should be recognized.
Mazzaella membranacea (J.Agardh) Fredericq	TR05.R55 TR05.R68 TR07.R001 Tristan 2010 018 Tristan 2010 063 Tristan 2010 077	***MK185777*** ***MK185828*** MK202375 ***MK185771*** MK202427 ***MK185790*** MK202374	COI-5P data are *c.* 7% divergent to a variety of species assigned to *Chondrus* and *Mazzaella* in BOLD and GenBank [not unexpected; see Saunders & Millar (2014)], while *rbc*L data are only 3 bp different from a record in GenBank assigned to this species from Chile (AF146214). More work is needed to determine if separate species are warranted between the two floras, but this genetic group corresponds to Baardseth’s records for *Iridaea membranacea* J.Agardh (= *M. membranacea*).
Rhodoglossum revolutum Baardseth	Nightingale 2010 022 Tristan 2010 028 Tristan 2010 030	MK202389 ***MK185788*** ***MK185784*** MK202362	COI-5P lack a close match to data in BOLD and GenBank, the closest being 6.8% divergent from *Mazzaella californica* (J.Agardh) G.De Toni (KF839772). Similarly, *rbc*L data are closest to *Sarcothalia decipiens* (J.D.Hooker & Harvey) Hommersand in BOLD and notably (only 0.6% divergent) to a specimen assigned to *Iridaea tuberculosa* (J.D.Hooker & Harvey) Leister from Chile in GenBank (AF146208). The generitype *R. gigartinoides* (Sonder) Edyvane & Womersley is a distant 9.2% in BOLD – transfer from *Rhodoglossum* is necessary.
Sarcothalia stiriata (Turner) Leister	Nightingale 2010 015 Nightingale 2010 052 Tristan 2010 024	***MK185823*** MK202408 ***MK185806*** MK202402	COI-5P is 5% divergent from *S. crassifolia* (C.Agardh) Edyvane & Womersley in BOLD, while *rbc*L are only 4 bp different (out of 1316 bp) from a South African specimen also assigned to *S. stiriata* (U03089). The possibility of sibling species requires further taxonomic study. This genetic group corresponds to Baardseth’s records for *Gigartina stiriata* (Turner) J.Agardh (synonym of *Sarcothalia stiriata*).
Florideophyceae, Rhodymeniophycidae, Gigartinales, Kallymeniaceae			
Callophyllis corollata Baardseth	IN07.R034 IN07.R038 Nightingale 2010 033 Night 2010 037 Night 2010 065 TR04.R06 TR05.R37 Tristan 2010 045	KX808067 KX808011 KX808032 ***KX783067*** KX808063 KX808061 KX808018 KX808069 KX808088	COI-5P is 4.6% divergent from *C. macrostiolata* Arakaki, Alveal & Ramírez from Chile in BOLD and GenBank, while *rbc*L is 1% divergent from Chilean specimens assigned to *C. atrosanguinea* (J.D.Hooker & Harvey) Hariot again in both databases. However, Saunders *et al.* (2017) reported that these three species form a genus-level lineage only distantly related to *Callophyllis* sensu stricto. Consequently, taxonomic work is needed at the genus level. At the species level, the cystocarps are clearly coronate and this genetic group is best assigned to this morphospecies.
Callophyllis (?) sp. 1Tris	NI07.R04	***MK185772***	This *rbc*L sequence is *c.* 5% divergent from data in BOLD for a wide variety of species assigned to *Callophyllis* sensu stricto, *Judithia*, and *Wendya* (see Saunders *et al.* 2017), while the closest match (4% divergent) in GenBank is the New Zealand taxon *Zuccarelloa ceramoides* D’Archino & W.A.Nelson despite the bladed habit of our specimen. This genetic group likely represents a new kallymeniacean genus.

Appendix 1. – Continuation.

Leniea kylinii (Baardseth) G.W.Saunders	IN07.R013	KX808090	This species, assigned to *Pugetia* by Baardseth (1941), was studied in Saunders *et al.* (2017) in which its affinities to the Antarctic genus *Leniea* were tentatively resolved.
	NI07.R01	***MK185810***	
	Tristan 2010 047	KX808051 KX783077	
Florideophyceae, Rhodymeniophycidae, Gigartinales, Phyllophoraceae			
Asterfilopsis sp. 1piurana	Tristan 2010 007	MK202422 ***MK185817***	COI-5P data are close (only 1.4% divergent) to a private record in BOLD for a specimen from Chile assigned to *Asterfilopsis disciplinalis* (Bory) M.S.Calderon & S.M.Boo, while GenBank (KU640310) reveals only 4 bp divergence from a specimen assigned to *Asterfilopsis piurana* M.S.Calderon & S.M.Boo from Peru. The *rbc*L data are relatively more divergent (0.5%) with the closest match being a specimen assigned to *A. disciplinalis* in GenBank (KU640230) rather than the expected *A. piurana* (1.6% divergent; KU640245). This genetic group likely accounts for records of *Ahnfeltiopsis polyclada* (Kützing) P.C.Silva & DeCew in Baardseth [1941; as *Gymnogongrus polycladus* (Kützing) J.Agardh]. Taxonomic work remains in assigning this genetic group to a morphospecies.
	Tristan 2010 050	MK202384 ***MK185779***	
	Tristan 2010 078	MK185764	
Gymnogongrus gregarius Baardseth	NI05.R09	MK202371 ***MK185767***	Our COI-5P sequences variously match phyllophoracean spp. in BOLD and GenBank notably an orphaned lineage at the genus level reported in Calderon & Boo (2016), which is confirmed by the *rbc*L data being 1.2% divergent from an unidentified specimen in GenBank (KU640229). This genetic group is a morphological match to *G. gregarius* described from Tristan da Cunha, but is incorrectly placed in *Gymnogongrus*.
	Nightingale 2010 054	MK202429 ***MK185832***	
	TR05 R48	MK185787	
Florideophyceae, Rhodymeniophycidae, Halymeniales, Tsengiaceae			
Tsengia pulchra (Baardseth) Masuda & Guiry	IN07.R011	MK202388	The COI-5P data for specimens of this genetic group are distant to a variety of *Tsengia* spp. in BOLD, while *rbc*L is closest to collections of *Tsengia laingii* (Kylin) Womersley & Kraft from Tasmania also in BOLD. Whereas Baardseth (1941) described one species of *Tsengia* (as *Nemastoma pulchra* Baardseth), we uncovered two divergent genetic groups.
	IN07.R027	MK202365	
	Nightingale 2010 001	MK202370 ***MK185766***	
	Nightingale 2010 005	MK202361	
	TR05.R59	***MK185829***	
	TR06.R02	***MK185798***	
	Tristan 2010 039	MK202393	
	Tristan 2010 059	MK202395	
	Tristan 2010 061	MK202401	
	Tristan 2010 076	MK202419	
Tsengia sp. 2Tris	IN07.R009	***MK185775***	This novel genetic group also had its best matches in BOLD being 6.6% and 3.7% divergent from southeastern Australian specimens of *T. feredayae* (Harvey) Womersley & Kraft for COI-5P and *rbc*L, respectively.
	IN07.R033	MK202394	
	NI07.R05	***MK185811***	
	Nightingale 2010 009	MK202383	
	Nightingale 2010 038	MK202360 ***MK185752***	
	Nightingale 2010 045	MK202412	
	TR05.R62	***MK185822***	
	Tristan 2010 037	***MK185770***	

Appendix 1. – Continuation.

Florideophyceae, Rhodymeniophycidae, Nemastomatales, Schizymeniaceae			
Platoma (?) sp. 1Tris	IN07.R032	***MK185830***	The *rbc*L generated for this specimen is distantly related to *Platoma gelatinosum* (M.Howe) C.W.Schneider, McDevit, G.W.Saunders & C.E.Lane in GenBank (FJ878849; 9.4% divergent). It is a close match to partial *rbc*L data for a second species from TdC (*Platoma* sp. 2Tris; below) in BOLD (1.5% divergent). There is no obvious match to species listed in the TdC flora by Baardseth (1941), although he did note that his collections of *Schizymenia obovata* J.Agardh were unusual in lacking gland cells. Whether his collections represent this species and or its sibling *Platoma* sp. 2Tris and or our report of *Schizymenia apoda* (J.Agardh) J.Agardh (both below) in part or in full is uncertain. The two species here assigned to *Platoma* (?) likely require assignment to a new genus in the Schizymeniaceae.
Platoma (?) sp. 2Tris	IN07.R010	***MK185791***	See comments above for *Platoma* sp. 1Tris.
Schizymenia apoda (J.Agardh) J.Agardh	Nightingale 2010 049	MK202367 ***MK185761***	COI-5P data are only 1% divergent from Australian collections assigned to *S. apoda* in BOLD and GenBank (e.g. KP725089), with *rbc*L also (only 2 bp divergent) similar to this species in both databases. Although a cryptic sibling species remains possible, this genetic group is best assigned this name joining the "pure apoda" group of Saunders *et al.* (2015). The last mentioned potentially occurs in the Azores and Namibia, but those records are based on *rbc*L data only leaving their assignment to a genetic group in the apoda/dubyi complex uncertain (see Saunders *et al.* 2015). In addition to the other species of this family reported here, this genetic group likely accounts for some of the records of *S. obovata* in Baardseth (1941).
Wetherbeella sp. 1Tris	Nightingale 2010 061 Tristan 2010 060	MK202358 ***MK185751*** ***MK185835***	COI-5P and *rbc*L are closest to *W. australica* (Womersley & Kraft) G.W.Saunders & Kraft, but 3.5% and 1.7% divergent, respectively (in both BOLD and GenBank). This genetic group thus represents a third species in this genus, which was previously confined to Australia (Saunders & Kraft 2002). As species of this genus lack gland cells (Saunders & Kraft 2002), collections of *Schizymenia* reported in Baardseth (1941) as lacking gland cells may have also included this species.
Florideophyceae, Rhodymeniophycidae, Plocamiales, Plocamiaceae			
Plocamium delicatulum Baardseth	Tristan 2010 058	MK202428 ***MK185831***	The three COI-5P sequences generated for specimens of *Plocamium* are 2-7 bp divergent and were 5.4% divergent from an unidentified species from Chile in BOLD (GWS038155) and 6.2% divergent from the Tasmania and New Zealand *Plocamium* sp. 1cirrhosum in GenBank (HQ919531). The *rbc*L sequences are identical for the three specimens and closest to a record in GenBank (0.02% divergent; U26817) incorrectly identified as *P. cartilagineum* (Linnaeus) P.S.Dixon from Chile (see Saunders & Lehmkuhl 2005; Cremades *et al.* 2011). Baardseth (1941) described two species from TdC, *P. delicatulum* and *P. fuscorubrum* Baardseth. Most of our collections are morphologically consistent with the latter including tetrasporangial details in Night 2010 050 (Baardseth 1941). Specimen Tristan 2010 058 is 5-7 bp divergent from the other two in COI-5P and is morphologically consistent with the morphospecies *P. delicatulum*. Despite the genetic similarities between this species and *P. fuscorubrum*, they are distinct morphospecies that we continue to recognize as discrete. Nuclear data are necessary to assess this taxonomic issue further.
Plocamium fuscorubrum Baardseth	Nightingale 2010 030	***MK185786***	See comments above under *P. delicatulum*.
	Nightingale 2010 050	MK202376 ***MK185774***	
	Tristan 2010 075	MK202420	

APPENDIX 1. – Continuation.

Florideophyceae, Rhodymeniophycidae, Plocamiales, Sarcodiaceae			
Trematocarpus sp. 1Tris	Tristan 2010 021	MK202403 ***MK185799***	The COI-5P is 7.3% divergent from a specimen (GWS038113) in BOLD assigned to *Trematocarpus dichotomus* Kützing from Chile. The *rbc*L is similarly closest to a specimen assigned to this species on GenBank (6.5% divergent; U26814), but found a closer match to a specimen of *T. affinis* (J.Agardh) De Toni from Tasmania in BOLD (GWS015207; 2.7% divergent). Baardseth (1941) reported an unknown *Dicurella* sp., which he considered similar to *D. scutellata* (Hering) Papenfuss. The last mentioned is included in the genus *Sarcothalia*, which is not remotely related to the family Sarcodiaceae to which our specimen is clearly assigned. However, some records of *D. scutellata* are considered misidentifications of *T. affinis* (see Womersley 1994). This genetic group is likely a new species assignable to *Trematocarpus* and accounts for the unknown *Dicurella* sp. in Baardseth (1941).
Florideophyceae, Rhodymeniophycidae, Rhodymeniales, Faucheaceae			
Fauchea (?) *furcellata* Baardseth	Nightingale 2010 003	MK202378 ***MK185776***	COI-5P data are distinct (saturated), while *rbc*L sequences were 7-8% divergent from various *Leptofauchea* and *Webervanbossea* spp. in BOLD and GenBank. This collection likely represents a new genus in the Faucheaceae and is a morphological match to Baardseth's *Fauchea* (?) *furcellata* for which only tetrasporangial material is known (Baardseth 1941).
Florideophyceae, Rhodymeniophycidae, Rhodymeniales, Lomentariaceae			
Lomentaria sp. 1Tris	Tristan 2010 053	MK202409 ***MK185807***	COI-5P is distant to various *Lomentaria* spp. in BOLD and GenBank, while *rbc*L is 3.9% divergent from *L. clavellosa* (Turner) Gaillon (KU726726). Nothing in Baardseth (1941) can be readily associated with this genetic group, however, *L. clavellosa* is one of the few species correctly assigned to *Lomentaria* (Filloramo & Saunders 2016a) – this TdC species is likely correctly allied to this genus as well. In our experience this species is common on subtidal stones, which Baardseth should have easily dredged – this entity may also be a recent introduction.
Florideophyceae, Rhodymeniophycidae, Rhodymeniales, Rhodymeniaceae			
Rhodymenia elongata (Baardseth) G.W.Saunders, C.M.Brooks & Su.Scott, comb. nov.	NI05 R11 NI05.R16 Nightingale 2010 006 Nightingale 2010 007 Tristan 2010 002 Tristan 2010 012	***MK185796*** ***MK185758*** MK202410 ***MK185808*** ***MK185819*** ***MK185827*** ***MK185795***	COI-5P sequences are only *c.* 1.5% divergent from *R. flabellata* (Baardseth) G.W.Saunders, C.M.Brooks & Su.Scott, comb. nov. (below) and an unpublished sequence in BOLD for a specimen we collected from Chile (GWS038160), while GenBank indicated *c.* 6% divergence to *R. obtusa* (Greville) Womersley (HM033136) from South Africa and *R. wilsonis* (Sonder) G.W.Saunders (KT781959) from Australia. The *rbc*L is *c.* 4% divergent from *R. capensis* J.Agardh (AF385646) and *R. obtusa* (AF385647) both from South Africa in GenBank, while the closest match in BOLD is *R. flabellata* (below) at only 0.4% divergence. Although closely related to *R. flabellata* (below), the morphological differences between these genetic groups necessitates recognition of separate species pending study with a nuclear marker. Further, this genetic group corresponds to two of Baardseth's three morphospecies assigned to *Epymenia* (Baardseth 1941), viz., *E. elongata* Baardseth and *E. marginifera* Baardseth. Morphological variation within and among species of the Rhodymeniaceae can be extreme, frustrating the task of matching genetic groups to morphospecies (e.g. Saunders & McDonald 2010; Filloramo & Saunders 2016b), but it is telling that while some of our TdC specimens have the tetrasporangial leaflets only on the blade (as in *E. elongata*; Tristan 002-11) or from the margins (as in *E. marginifera*; Night 2010 007) NI105.R11 has them abundantly from both locations questioning the utility of this character to recognize distinct species. As both names have equal priority, we choose *E. elongata* as the name for this genetic group. Assigned to *Epymenia*, which is now a synonym of *Rhodymenia* (Womersley 1996), a transfer is necessary. Regrettably, Baardseth's type collections cannot be located to facilitate this transfer. An interim solution is to lectotypify this species on an isotype in the Natural History Museum (BM; BM000610805, Egil Baardseth, #120), which we here implement. *Rhodymenia elongata* (Baardseth) G.W.Saunders, Su.Scott & C.M.Brooks, comb. nov. Basionym: *Epymenia elongata* Baardseth, *Results of the Norwegian Scientific Expedition to Tristan da Cunha, 1937-38.* No 9. p. 81-83, fig. 43A (1941). Synonymy of *Epymenia marginifera* with this species is proposed based on the variability of tetrasporangial features for specimens that are assigned to this genetic group.

APPENDIX 1. – Continuation.

Rhodymenia flabellata (Baardseth) G.W.Saunders, C.M.Brooks & Su.Scott, comb. nov.	IN07.R024 NI05.R15 Nightingale 2010 013 Tristan 2010 016 Tristan 2010 046 Tristan 2010 055	***MK185789*** ***MK185815*** MK202364 ***MK185756*** ***MK185825*** MK202418 ***MK185814*** ***MK185833***	See comments above regarding the low divergence between this genetic group and *R. elongata*. There is little doubt that this genetic group matches Baardseth's *Epymenia flabellata*, but that genus has been subsumed into *Rhodymenia*. As discussed above we lectotypify this species on an isotype in the Natural History Museum (BM; BM000610806, Egil Baardseth, #87). *Rhodymenia flabellata* (Baardseth) G.W.Saunders, Su.Scott & C.M.Brooks, comb. nov. Basionym: *Epymenia flabellata* Baardseth, *Results of the Norwegian Scientific Expedition to Tristan da Cunha, 1937-38.* No 9. p. 83-84, fig. 44 (1941).