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Marine benthic algal flora of Ascension Island, South Atlantic

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This paper provides a comprehensive checklist of the marine benthic macroalgal flora of Ascension Island (tropical South Atlantic Ocean), based on both new collections and previous literature. 82 marine macroalgae were identified from our work, including 18 green algae (Ulvophyceae), 15 brown algae (Phaeophyceae) and 49 red algae (Rhodophyta). Among our collections, 38 species and infraspecific taxa are reported for the first time from Ascension Island, including seven green, three brown and 28 red macroalgae, raising the total number of seaweeds recorded in Ascension so far to 112 taxa in species and infraspecific level. No seagrasses have been recorded at Ascension Island.

Keywords: algae, seagrasses, checklist, seaweeds, marine vegetation

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INTRODUCTION

Ascension Island is one of the remotest volcanic islands worldwide, located 07°57′ south of the Equator, near the Mid-Atlantic Ridge, about 1600 km from West Africa and 2400 km from South America. St Helena Island is the nearest island to Ascension, about 1130 km to the south-east.

The coastline of Ascension varies, ranging from sea cliffs and steep slopes (mainly eastern coasts) to sandy beaches and smooth sublittoral areas and rocky beaches (mainly western coasts), thus creating several different types of coastal habitats for various seaweed communities (Price & John, 1980). However, the most important factor governing seaweed development in Ascension is the presence of the marauding blackfish (*Melichthys niger* Bloch, 1786), the most dominant fish species around the coast and seemingly mainly responsible for the paucity of the island's benthic algal flora (Price & John, 1979, 1980).

Only a few studies regarding the marine seaweed flora of Ascension Island have been carried out previously. Osbeck (1757, 1765, 1771) was the first to report marine algae from Ascension, based on drift specimens. In the 19th Century sparse records were provided by Bory (1829) and Askenasy (1888), while about a century later a single green seaweed (*Valonia* sp.) was reported from the inland saline pools of the island (Chace & Manning, 1972). Finally, a more detailed survey of Ascension's seaweeds was performed in October and November 1976 by Price & John (1979, 1980). Taking into account their original work, additional records from Ascension Island were included in the series of 'Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment' (Lawson & Price, 1969; Price *et al*., 1978, 1986, 1988, 1992; John *et al*., 1979, 1994; Lawson *et al*., 1995; Woelkerling *et al*., 1998) as well as in the updated catalogue of seaweeds of the western coast of Africa and adjacent islands (John *et al*., 2004)

After 36 years since the seaweed collections of Price & John (1980) at Ascension shores, we conducted an extensive seaweed survey in the island's coastal environment, aiming to update the knowledge about Ascension marine benthic macroalgal flora. Older records from the island are also covered, providing a comprehensive check list of the marine seaweeds of Ascension Island.

MATERIALS AND METHODS

The seaweed flora of Ascension Island was investigated during a one-week period in February 2011 and a two-and-a-half-week period in September 2012. Several rocky sites were chosen along the island's shores (Table 1), from the eulittoral zone down to 38 m depth. Similar to previous work by Price & John (1980) we sampled seaweeds in various types of coastal habitat: rock pools (Catherine Point, Collyer Point, Shelly Beach, Wideawake Plains); blow-hole areas (Catherine Point); turtle ponds (Georgetown); midlittoral rocks and beach rocks (Pillar Bay, Collyer Point, Clarkes Beach); the outfall channel from the island's main power station (Derby wreck); and sublittoral rocks (English Bay, Comfortless Cove, Sudan wreck site, Derby wreck site, Horseshoe Reef, Red Rock Cave, and a sublittoral reef west of Georgetown).

Seaweeds were collected by snorkelling and SCUBA-diving through the destructive method (scraping the macroalgae off the substratum). Underwater photographs were taken using

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Table 1. Seaweed taxa recorded in inshore waters of Ascension Island, based both on our collections and previous known literature. Taxa are listed alphabetically for each group (Ulvophyceae, Phaeophyceae and Rhodophyta), in order to make their detection easier. Synonyms used in previous publications are also given. Superscript numbers in taxa refer to notes. The exact location where each taxon was collected during our samplings as well as habitat details are provided (1, Catherine Point; 2, Clarkes Beach; 3, Collyer Point; 4, Comfortless Cove; 5, Derby wreck (in English Bay); 6, English Bay; 7, Georgetown; 8, Horseshoe Reef; 9, Pillar Bay; 10, Red Rock Cave; 11, Shelly Beach; 12, Sublittoral reef west of Georgetown; 13, Sudan wreck; 14, Wideawake Plains).

	Location found	Habitat	Previous records
ULVOPHYCEAE			
Bryopsis duplex De Notaris	4	Upper sublittoral rocks	
Bryopsis plumosa (Hudson) C. Agardh	5, 6	Upper sublittoral rocks	
Caulerpella ambigua (Okamura) Prud'homme van Reine & Lokhorst = *Caulerpa vickersiae* Børgesen	3	Beach rock	Price & John (1980)
Chaetomorpha aerea (Dillwyn) Kützing			John *et al.* (2004)
Chaetomorpha antennina (Bory de Saint-Vincent) Kützing	3, 7	Turtle Ponds, midlittoral rocks	Price & John (1980)
Cladophora coelothrix Kützing			Price & John (1980)
Cladophora corallicola Børgesen	13	Sublittoral rocks	
Cladophora hutchinsiae (Dillwyn) Kützing	7	Turtle ponds	
Cladophora laetevirens (Dillwyn) Kützing	5	Outfall channel	
Cladophora lehmanniana (Lindenberg) Kützing	4, 5	Upper sublittoral rocks	
Codium taylorii P.C. Silva	1, 3	Blow-hole areas, rock pools	Price & John (1980)
Dictyosphaeria versluysii Weber-van Bosse	1	Rock pools	Price & John (1980)
Neomeris annulata Dickie	4	Sublittoral rocks	Price & John (1980)
Phyllodictyon anastomosans (Harvey) Kraft & M.J. Wynne = *Struvea anastomosans* (Harvey) Piccone & Grunow *ex* Piccone	6, 12	Sublittoral rocks	Price & John (1980)
Rhizoclonium riparium (Roth) Harvey			John *et al.* (2004)
Ulva clathrata (Roth) C. Agardh	5	Outfall channel, substratum culture	John *et al.* (2004)
Ulva compressa Linnaeus			Lawson & Price (1969)
Ulva flexuosa Wulfen = *Enteromorpha flexuosa* (Wulfen) J. Agardh = *E. lingulata* J. Agardh	3	Rock pools	Price & John (1980)
Ulva intestinalis Linnaeus = *Enteromorpha intestinalis* (Linnaeus) Nees	5	Outfall channel	Price & John (1980)
Ulva lactuca Linnaeus			Price & John (1980)
Ulvella leptochaete (Huber) R. Nielsen, C.J.O' Kelly & B. Wysor	6	Appeared in raw culture of filamentous brown alga	
Valonia macrophysa Kützing	3, 6	Rock pools, sublittoral rocks	John *et al.* (2004)
Valonia ventricosa J. Agardh = *Ventricaria ventricosa* (J. Agardh) J.L. Olsen & J.A. West	3, 11	Rock pools	Price & John (1980)
PHAEOPHYCEAE			
Asteronema breviarticulatum (J. Agardh) Ouriques & Bouzon = *Ectocarpus breviarticulatus* J. Agardh	3, 9	Midlittoral rocks and beach rock, rock pools	Price & John (1980)
Bachelotia antillarum (Grunow) Gerloff	3, 7	Turtle ponds, midlittoral rocks	Price & John (1980)
Chnoospora minima (Hering) Papenfuss	1, 3, 14	Blow-hole areas, midlittoral rocks, rock pools	Price & John (1980)
Colpomenia sinuosa (Mertens) Derbès & Solier	1, 3	Blow-hole areas, rock pools	Price & John (1980)
Dictyota bartayresiana J.V. Lamouroux = *Dictyota bartayresii* J.V. Lamouroux	1, 4	Rock pools, sublittoral rocks	Price & John (1980)
Feldmannia irregularis (Kützing) Hamel	3	Rock pools	
Feldmannia paradoxa (Montagne) Hamel	3	Rock pools	
Feldmannia mitchelliae (Harvey) H.-S. Kim = *Giffordia mitchelliae* (Harvey) Hamel = *Hincksia mitchelliae* (Harvey) P.C. Silva	3, 7	Turtle ponds, rock pools	Price & John (1980); John *et al.* (2004)
Levringia brasiliensis (Montagne) A.B. Joly	1, 3, 14	Blow-hole areas, beach rock, rock pools	
Levringia sordida (Bory de Saint-Vincent) Kylin			Price & John (1980)
Lobophora variegata (J.V. Lamouroux) Womersley *ex* E.C. Oliveira	6	Sublittoral rocks	Price & John (1980)
Neoralfsia expansa (J. Agardh) Cormaci & G. Furnari = *Ralfsia expansa* (J. Agardh) J. Agardh	1, 3	Blow-hole areas, rock pools	Price & John (1980)
Padina gymnospora (Kützing) Sonder = *Padina vickersiae* Hoyt	1, 3	Blow-hole areas, rock pools	Price & John (1980)
Padina pavonica (Linnaeus) Thivy			John *et al.* (2004)
Sargassum lendigerum (Linnaeus) C. Agardh[a] = *Fucus lendigerum* Linnaeus			Linnaeus (1753)
Sargassum vulgare C. Agardh (nom. illeg.)	1, 3	Beach rock, rock pools	Price & John (1980)
Sphacelaria novae-hollandiae Sonder	1, 3, 9	Midlittoral rocks, rock pools	Price & John (1980)
Sphacelaria rigidula Kützing = *Sphacelaria furcigera* Kützing	6	Sublittoral rocks, substratum culture	Price & John (1980)
RHODOPHYTA			
Acanthophora muscoides (Linnaeus) Bory de Saint-Vincent[b]			John *et al.* (2004)
Acanthophora ramulosa Lindenberg *ex* Kützing	3	Beach rock	

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Table 1. Continued

	Location found	Habitat	Previous records
Acrochaetium microscopicum (Nägeli *ex* Kützing) Nägeli	3, 13	Epiphyte on various macroalgae	
Aglaothamnion sp.	4	Sublittoral rocks	
Ahnfeltia plicata (Hudson) E.M. Fries	3	Beach rock	John *et al.* (2004)
Amphiroa fragilissima (Linnaeus) J.V. Lamouroux			Price & John (1980)
Amphiroa rigida J.V. Lamouroux	1	Rock pools	Price & John (1979)
Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon[c]	3, 6, 13	Rock pools, sublittoral rocks	
Bostrychia intricata (Bory de Saint-Vincent) Montagne = *Bostrychia mixta* J.D. Hooker & Harvey			John *et al.* (2004)
Catenella caespitosa (Withering) L.M. Irvine			John *et al.* (2004)
Centroceras clavulatum (C. Agardh) Montagne[d]			Price & John (1980)
Centroceras gasparrinii (Meneghini) Kützing	3, 6	Rock pools, sublittoral rocks	
Ceramium cimbricum H.E. Petersen cf. *cimbricum* = *Ceramium fastigiatum* Harvey			Price & John (1980)
Ceramium cimbricum cf. *flaccidum* (H.E. Petersen) Furnari & Serio	5	Sublittoral rocks	
Ceramium diaphanum (Lightfoot) Roth = *Ceramium gracillimum* (Kützing) Zanardini = *Ceramium tenuissimum* (Roth) Areschoug nom. illeg.	6	Sublittoral rocks	Price & John (1980)
Ceratodictyon intricatum (C. Agardh) R.E. Norris = *Gelidiopsis intricata* (C. Agardh) Vickers	11	Rock pools	Price & John (1980)
Champia parvula (C. Agardh) Harvey			John *et al.* (2004)
Champia cf. *puertoricensis* Lozada-Troche & D.L. Ballantine	12	Sublittoral rocks	
Coelothrix irregularis (Harvey) Børgesen			Price & John (1980)
Corallophila sp.	8, 12	Sublittoral rocks	
Crouania attenuata (C. Agardh) J. Agardh	4, 10	Sublittoral rocks	
Dasya ocellata (Grateloup) Harvey	4	Sublittoral rocks	
Dasya rigidula (Kützing) Ardissone	4	Sublittoral rocks	
Digenea simplex (Wulfen) C. Agardh			Price & John (1979)
Diplothamnion tetrastichum A.B. Joly & Yamaguishi			Price & John (1980)
Erythrotrichia bertholdii Batters	3	Epiphyte on various macroalgae	
Erythrotrichia carnea (Dillwyn) J. Agardh	4, 6, 7	Epiphyte on various macroalgae	John *et al.* (2004)
Galaxaura rugosa (J.Ellis & Solander) J.V. Lamouroux			John *et al.* (2004)
Gayliella flaccida (Harvey *ex* Kützing) T.O. Cho & L.J. McIvor[e] = *Ceramium flaccidum* (Harvey *ex* Kützing) Ardissone			John *et al.* (2004)
Gayliella mazoyerae T.O. Cho, Fredericq & Hommersand = *Ceramium gracillimum* var. *byssoideum* (Harvey) Mazoyer			Price & John (1980)
Gayliella transversalis (F.S. Collins & Hervey) T.O. Cho & Fredericq	4, 6	Sublittoral rocks	
Gelidium pusillum (Stackhouse) Le Jolis	1, 3, 4	Beach rock, rock pools, sublittoral rocks	Price & John (1980)
Griffithsia schousboei Montagne	6	Sublittoral rocks	
Gymnothamnion elegans (Schousboe *ex* C. Agardh) J. Agardh			John *et al.* (2004)
Halydictyon mirabile Zanardini	12	Sublittoral rocks	
Herposiphonia brachyclados Pilger	4, 6	Sublittoral rocks	Price & John (1980)
Herposiphonia secunda (C. Agardh) Ambronn	6	Sublittoral rocks	John *et al.* (2004)
Herposiphonia tenella (C.Agardh) Ambronn	9	Rock pools	Price & John (1980)
Heterosiphonia crispella (C. Agardh) M.J. Wynne = *Heterosiphonia wurdemannii* (J. Bailey *ex* Harvey) Falkenberg			Price & John (1980)
Hildenbrandia rubra (Sommerfelt) Meneghini			Price & John (1980)
Hydrolithon cruciatum (Bressan) Y.M. Chamberlain	4	Epiphyte on macroalgae	
Hydrolithon farinosum (J.V. Lamouroux) D. Penrose & Y.M. Chamberlain	4, 6	Epiphyte on macroalgae	
Hypnea spinella (C. Agardh) Kützing	4, 12	Sublittoral rocks	Price & John (1980)
Hypoglossum anomalum M.J. Wynne & D.L. Ballantine	4	Sublittoral rocks	
Hypoglossum barbatum Okamura	6	Sublittoral rocks	
Jania capillacea Harvey			Price & John (1980)
Jania pumila J.V. Lamouroux	3	Rock pools	John *et al.* (2004)
Jania rubens (Linnaeus) J.V. Lamouroux			Price & John (1980)
Laurencia brachyclados Pilger	9	Midlittoral rocks, rock pools	Price & John (1980)
Laurencia caduciramulosa Masuda & Kawaguchi	2	Beach rock	
Leptosiphonia schousboei (Thuret) Kylin			John *et al.* (2004)
Liagora albicans J.V. Lamouroux = *Liagora decussata* Montagne			Price & John (1980)
Liagora ceranoides J.V. Lamouroux			John *et al.* (2004)
Lithophyllum sp.	8, 12, 13	Sublittoral rocks	
Lomentaria corallicola Børgesen	6	Sublittoral rocks	
Lophosiphonia adhaerens Pilger	8	Sublittoral rocks	

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Table 1. Continued

	Location found	Habitat	Previous records
Lophosiphonia cristata Falkenberg			John *et al.* (2004)
Lophosiphonia obscura (C. Agardh) Falkenberg	3	Rock pools	
Lophosiphonia reptabunda (Suhr) Kylin	7	Turtle ponds	John *et al.* (2004)
Monosporus indicus Børgesen	12	Sublittoral rocks	
Nitophyllum punctatum (Stackhouse) Greville	12	Sublittoral rocks	
Palisada perforata (Bory de Saint-Vincent) K.W. Nam = *Chondrophycus papillosus* (C. Agardh) D.J. Garbary & J.T. Harper = *Laurencia papillosa* (C. Agardh) Greville	1, 3, 13	Blow-hole areas, midlittoral rocks, beach rock, rock pools	Price & John (1980); John *et al.* (2004)
Parviphycus setaceus (Feldmann) J. Afonso-Carrillo, M. Sanson, C. Sangil & T. Diaz-Villa	3	Rock pools	
Phymatolithon calcareum (Pallas) W.H. Adey & D.L. McKibbin			John *et al.* (2004)
Plocamium cartilagineum (Linnaeus) P.S. Dixon			John *et al.* (2004)
Polysiphonia scopulorum Harvey	7	Turtle ponds	
Polysiphonia subtilissima Montagne	4, 6	Sublittoral rocks	Price & John (1980)
Rhodymenia holmesii Ardissone			John *et al.* (2004)
Rhodymenia sp.	10	Sublittoral rocks	
Sahlingia subintegra (Rosenvinge) Kornmann	4, 7	Epiphyte on *Cladophora hutchinsiae* and *Bryopsis* spp.	
Spyridia filamentosa (Wulfen) Harvey			John *et al.* (2004)
Stylonema alsidii (Zanardini) K.M. Drew = *Goniotrichum alsidii* (Zanardini) M.A. Howe	4	Sublittoral rocks, substratum culture	John *et al.* (2004)
Tiffaniella gorgonea (Montagne) Doty & Meñez	1	Epiphyte on *Codium taylorii*	
Vickersia baccata (J. Agardh) Karsakoff	6, 13	Sublittoral rocks	
Wrangelia argus (Montagne) Montagne	4, 14	Sublittoral rocks	Price & John (1980)

[a], originally described from Ascension Island as *Fucus lendigerum* Linnaeus (1753), based on drift material collected by Osbeck (1757, 1765, 1771). However, neither the following phycologists visiting the island nor we have managed to detect the species, and we cite this species as *taxon inquirendum*; [b], based on Perrone *et al.* (2006), this species should be treated as *taxon inquirendum*, since its neotype and other herbarium specimens studied probably correspond to different species; [c], only the tetrasporophytic stage found: *Falkenbergia hillebrandii* (Bornet) Falkenberg; [d], according to Won *et al.* (2009) specimens identified using the 'traditional' concept for *Centroceras clavulatum* usually fall into other species. The actual *C. clavulatum* is restricted to the Pacific Ocean and thus the Price & John (1980) record probably corresponds to another species of the genus, possibly *C. gasparrinii*; [e], according to the phylogenetic study by Cho *et al.* (2008) the '*Ceramium flaccidum*' complex accommodates several *Gayliella* species. Thus, the single record of *C. flaccidum* by John *et al.* (2004) requires further investigation.

an Olympus PTWC-01 camera with PTDP-EP05 housing. The material collected was preserved in 4% buffered formalin/seawater and/or mounted on Bristol paper, pressed, air dried and prepared as herbarium specimens and silica-gel samples.

Specimens were studied in the laboratory of the Ascension Conservation Centre, under dissecting or compound microscopes. When necessary, they were sectioned manually with a razor blade. Species were identified down to the lowest possible taxonomic level. In several cases permanent material was prepared as microscope slides using Karo™ corn syrup (ACH Food Companies, Memphis, TN, USA).

Herbarium specimens have been deposited in the herbarium at the University of Aberdeen (School of Biological Sciences). For present-day taxonomic and nomenclatural opinions the following on-line taxonomic databases were consulted: *Index Nominum Algarum* (Silva, 2014) and *AlgaeBase* (Guiry & Guiry, 2014).

Given the limited time and logistic constraints at this remote island, inevitably leading to a limited coverage of the smaller representatives of the flora, collections of seaweed specimens were supplemented by collections of substratum samples stored in sterile tubes during the survey dives. Following return to Europe and based upon a protocol developed for a similar study in the Juan Fernandez Islands (Müller & Ramirez, 1994), these samples were incubated in Provasoli-enriched seawater (Starr & Zeikus, 1993) under white fluorescent light (12 h day: 12 h night) and a temperature of 18°C, which is slightly below the lower limit of the sea surface temperature present around Ascension. Over the next roughly three months, they were monitored for algal outgrowth, from which uni-algal isolates were made. Isolates were characterized and identified morphologically using a Zeiss PrimoVert inverted microscope and a Zeiss Axio Imager.D2 compound microscope, and by DNA sequencing and comparison with published data. The isolates have been deposited in the Culture Collection of Algae and Protozoa (CCAP, Oban).

DNA extractions were performed on four of the cultured isolates using CTAB buffer as described previously (Gachon *et al.*, 2009), or by using the GeneJET plant DNA kit (ThermoScientific; Cat. No. K0791) and following the manufacturer's instructions. Polymerase chain reactions (PCRs) were performed using specific primers for the internal transcribed spacer (ITS) and the small subunit rDNA (SSU). For ITS amplification, the primer pairs PI (Tai *et al.*, 2001) and KIRI (Lane *et al.*, 2006), or ITS1 and ITS4 (White *et al.*, 1990) were used. SSU fragment amplification was achieved using the primer pair NS1 and NS4 (White *et al.*, 1990). Table 2 gives details of the primer sequences and some of the PCR conditions used. PCR 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,

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then annealing and extension steps as shown in Table 2. The 40 cycles were followed by a final extension at 72°C for 5 min. PCR amplification was performed using BIOTAQ™ DNA Polymerase (5 units μl^{-1}; Bioline) and following the manufacturer's instructions while incorporating 1 μl of template DNA. PCR products were electrophoresed on 1.2% (w/v) agarose gels which were stained in GelRed nucleic acid stain and resulting DNA bands were visualized using a gel imager. Products of interest were sequenced by sending samples to either MWG Eurofins or Source Bioscience.

The alignment of each DNA sequence returned was conducted using the BioEdit Sequence Alignment Editor™ (Hall, 1999). For identifying taxa, sequences were compared to published data by means of NCBI BLAST searches (Altschul *et al.*, 1997).

RESULTS

In total, 82 marine macroalgae in species and infraspecific level were identified from our samplings (Table 1): 18 green algae (Ulvophyceae); 15 brown algae (Phaeophyceae); and 49 red algae (Rhodophyta). In addition, four taxa belonging to the genera *Aglaothamnion*, *Corallophila*, *Lithophyllum* and *Rhodymenia* were given under their generic names as identification to species level would require collection of fertile material and none of the material collected contained reproductive structures.

Among our results, 38 species and infraspecific taxa are reported for the first time from Ascension Island: seven green, three brown and 28 red macroalgae, raising the total number of seaweeds recorded in Ascension so far to 112 taxa in species and infraspecific level (Table 1).

Culturing substratum samples and monitoring algal outgrowth resulted in the isolation of four algal species which were further characterized by sequencing their partial SSU–ITS genes (Table 3). Two of them were confirmed (by both morphological identification and sequence homologies) to be *Ulvella leptochaete* and *Stylonema alsidii* respectively, of which *U. leptochaete* was not detected in our field samplings. *Sphacelaria rigidula* was identified morphologically (based on the presence of propagules) with a closest SSU sequence homology with *Sphacelaria* sp. UTEX LB800. Finally, some ambiguity remains about the *Ulva* isolate (Table 3). Based on classical taxonomy it was close to *U. clathrata* but the SSU was 99% similar to *U. pertusa* and *U. lactuca*. For ITS, however, the best BLAST hits (90% identity) were *U. prolifera*, *U. erecta*, *U. taeniata*, *Ulva* sp., and the two public sequences of *U. clathrata* showed only 88 and 87% identity. The identity of our isolate will have to be resolved in the context of currently ongoing taxonomic revisions of this genus.

DISCUSSION

Floristics

Seventy-four species and infraspecific taxa had already been reported from Ascension Island in previous studies (Lawson & Price, 1969; Price *et al.*, 1978, 1986, 1988, 1992; John *et al.*, 1979, 1994, 2004; Price & John, 1979, 1980; Lawson *et al.*, 1995; Woelkerling *et al.*, 1998). Based on our collections, 38 species and infraspecific taxa are added to the Ascension seaweed flora, raising the total number of recorded seaweeds so far to 112 taxa. As a result, the benthic flora of Ascension Island is richer than that of neighbouring St Helena, where only 47 species are known (Bolton *et al.*, 2003). However, this difference may reflect the fewer studies conducted in St Helena regarding seaweeds.

Among our new records, rare species of interest include the green alga *Cladophora corallicola* and the red algae *Acanthophora ramulosa, Champia* cf. *puertoricensis, Lophosiphonia adhaerens* and *Laurencia caduciramulosa.* The latter might correspond to a recent introduction to Ascension Island, since it was reported only recently from the Atlantic Ocean: Brazil, Cuba and the Canary Islands (Cassano *et al.*, 2008; Senties *et al.*, 2010).

Our new records of *Erythrotrichia bertholdii*, *Hydrolithon cruciatum* and *Vickersia baccata* for Ascension, all known from the north-east and subtropical Atlantic Ocean and the Mediterranean Sea (Tsiamis *et al.*, 2011; Guiry & Guiry, 2014) extend their distribution range to the tropical Atlantic Ocean. However, the vast majority of the recorded Ascension seaweeds are common in the tropical Atlantic Ocean. The algal flora of Ascension Island has features in common with those of both the eastern and western parts of the tropical and subtropical Atlantic Ocean (John *et al.*, 2004; Wynne, 2011). Only a few species occurring in Ascension Island are absent from the western tropical and subtropical Atlantic Ocean, such as the red algae *Herposiphonia brachyclados, Laurencia brachyclados, Lophosiphonia adhaerens* and *Rhodymenia holmesii.* On the other hand, some red algae found in Ascension, such as *Champia* cf. *puertoricensis*, *Diplothamnion tetrastichum*, *Lomentaria corallicola* and *Monosporus indicus,* are lacking from the western coasts of Africa.

Most of the algal species were encountered on sublittoral rocks, holes and crevices or in rock pools. A number of 31 taxa (36% of our collections) were found exclusively in the

Table 2. Sequences of primers used in polymerase chain reaction (PCR) for the amplification of partial nuclear ribosomal internal transcribed spacer and small subunit rDNA genes of 4 algal isolates (Table 3) from Ascension Island. Also detailed are the annealing and extension steps, along with the expected PCR product size.

Primer sequences (5′ → 3′)	Annealing	Extension	Product size	Reference
ITSP1: GGAAGGAGAAGTCGTAACAAGG	45°C for 30 s	72°C for 1 min	~500 bp	Tai *et al.* (2001)
ITSK1R1: TTCAAAGTTTTGATGATT				Lane *et al.* (2006)
ITS1: TCCGTAGGTGAACCTGCGG	55°C for 30 s	72°C 1 min 30 s	Variable bp	White *et al.* (1990)
ITS4: TCCTCCGCTTATTGATATGC				White *et al.* (1990)
NS1: GTAGTCATATGCTTGTCTC	55°C for 30 s	72°C 1 min 30 s	~1250 bp	White *et al.* (1990)
NS4: CTTCCGTCAATTCCTTTAAG				White *et al.* (1990)

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Table 3. Live isolates of 4 algal taxa included in the present study.

CCAP/[isolate number/original sample number]	Species name	Date of collection	Locality	% identity to closest relative with publicly available sequences	Query cover (%)	*e* value	EBI accession numbers for new sequences
CCAP 6038/1 [11-2-2/090211-5]	*Ulva clathrata*	9 Feb. 2011	Tidal pool at brown noddy colony north-east of English Bay	99% to *Ulva pertusa* and *Ulva lactuca* 18S, respectively	100	0.0	LM653284 (ITS1), LM653279 (18S)
CCAP 6037/1 [11-1-2/080211-1]	*Ulvella leptochaete*	8 Feb. 2011	English Bay, ca. 3m depth	100% to *Ulvella leptochaete* 18S	100	0.0	LM653285 (ITS1), LM653280 (18S)
CCAP 1329/1 [11-11-1/090211-7]	*Sphacelaria rigidula*	9 Feb. 2011	Off Comfortless Cove, ca. 28m depth	99% to *Sphacelaria* sp. UTEX LB800 18S	95	0.0	LM653281 (18S)
CCAP 2383/1 [11-3-3/100211-6]	*Stylonema alsidii*	10 Feb. 2011	Epiphytes on discarded fishing line, north-east off Georgetown, 29m depth	100% to *Stylonema alsidii* 18S	99	0.0	LM653282 (18S)

sublittoral zone, pointing out the importance of carrying out SCUBA diving-based collections in poorly explored areas.

The present study attempted to complement the findings of field-collected samples by culturing portions of substratum collected during dives followed by identification of any isolates obtained by means of light microscopes and molecular analyses. Some success was achieved since one of the four taxa isolated (*Ulvella leptochaete*) was not discovered in field-collected samples and was identified based on morphological and molecular analysis (Table 3). This approach has proven particularly rewarding in remote locations where time in the field and local laboratory facilities may be limited, for example in the Juan Fernandez Islands (Müller & Ramirez, 1994). Besides the green algae *Ulva clathrata* and *Ulvella leptochaete*, the red alga *Stylonema alsidii* and the brown alga *Sphacelaria rigidula*, we have generated numerous isolates of small multicellular algae whose identification is underway.

Overall marine vegetation

The marine vegetation around Ascension Island seems unchanged since the Price & John (1979, 1980) visit in 1976. As pointed out by the latter authors, sublittoral marine vegetation seems absent at first sight. Seagrasses do not exist, probably due to strong wave exposure occurring on the sandy beaches. When it comes to the rocky bottom, the only evident dominant seaweeds covering the sublittoral seafloor are the non-geniculate coralline red algae, which seem to remain tolerant to heavy grazing by the blackfish that prevail around Ascension. These corallines cover large surfaces and constitute crucial structuring elements to the seabed communities and dominate the underwater seascape (see also Price & John, 1979, 1980). They either form tower-like, reef-forming structures or loose piles on the seabed (maerl–rhodoliths). Most of them correspond probably to *Lithophyllum* species (Athanasios Athanasiadis, personal communication) and further investigations are underway to identify the crustose coralline algae at species-level. Finally, conspicuous cyanobacteria can be found growing on coralline formations, which apparently are not grazed by the blackfish.

However, a closer look around Ascension's sublittoral shores reveals the presence of inconspicuous non-coralline seaweeds (see Table 1), with diminutive and repent habit creating turf-like mats, an adaptation to the strong grazing pressure by the blackfish, while in crevices, where access for the blackfish is limited, more flourishing algal communities can be encountered, such as *Padina gymnospora* and *Cladophora* spp.

Far more luxuriant and extensive seaweed populations can be found in shallower waters, where access for the blackfish is restricted. Midlittoral rocks and beach rocks, rock pools (including the turtle pond), blow-hole areas and the outfall channel host the most abundant and diverse seaweed clumps in the island, as noted also by Price & John (1979, 1980), including various seaweeds such as *Chaetomorpha antennina, Codium taylorii, Asteronema breviarticulatum, Chnoospora minima*, *Colpomenia sinuosa*, *Levringia brasiliensis*, *Palisada perforata* and many others (see Table 1).

Finally, marine vegetation surveys did not reveal any major differences between the two samplings seasons (September and February), possibly due to the small seasonal variation in sea temperature: 23–25°C water temperature during the cold season and up to 28°C during the warm season

(Shallow Marine Surveys Group, Stanley, Falkland Islands, unpublished personal communication).

CONCLUSION

Our knowledge about the marine algal flora of Ascension is increased by the addition of 38 species and infraspecific taxa as a result of the present study, and the number of taxa now recorded for the island is 112. Still, there is no doubt that this number is an underestimation, since most parts of the island, and also, in particular the deeper parts of the euphotic benthos, remain unexplored. Therefore, additional surveys are crucial for further exploration of the marine benthic flora of Ascension.

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