J. Phycol. **50,** 149–166 (2014)

DOI: 10.1111/jpy.12148

LIGULATE *DESMARESTIA* (DESMARESTIALES, PHAEOPHYCEAE) REVISITED: *D. JAPONICA* SP. NOV. AND *D. DUDRESNAYI* DIFFER FROM *D. LIGULATA*[1]

Eun Chan Yang

Culture Collection of Algae and Protozoa (CCAP), The Scottish Association for Marine Science Dunstaffnage Marine Laboratory, Oban, Argyll, Scotland PA37 1QA, UK

Marine Ecosystem Research Division, Korea Institute of Ocean Science & Technology, 787 Haeanro, Ansan 426-744, Korea

Akira F. Peters

Bezhin Rosko, 40 rue des pêcheurs, 29250 Santec, Brittany, France

Hiroshi Kawai

Kobe University Research Center for Inland Seas, Rokkodai, Nadaku, Kobe 657-8501, Japan

Rowena Stern

SAHFOS, The Laboratory, Citadel Hill, The Hoe, Plymouth PL1 2PB, UK

Takeaki Hanyuda

Kobe University Research Center for Inland Seas, Rokkodai, Nadaku, Kobe 657-8501, Japan

Ignacio Bárbara

Coastal Biology Research Group, Facultad de Ciencias, Universidad de A Coruña, Campus de la Zapateira, A Coruña 15071, Spain

Dieter Gerhard Müller

Fachbereich Biologie, Universität Konstanz, Konstanz D-78457, Germany

Martina Strittmatter[2]

Culture Collection of Algae and Protozoa (CCAP), The Scottish Association for Marine Science Dunstaffnage Marine Laboratory, Oban, Argyll, Scotland PA37 1QA, UK

Willem F. Prud'Homme van Reine

Netherlands Centre for Biodiversity Naturalis, Leiden University (section NHN), P.O. Box 9514, RA Leiden 2300, The Netherlands

and Frithjof C. Küpper[3]

Culture Collection of Algae and Protozoa (CCAP), The Scottish Association for Marine Science Dunstaffnage Marine Laboratory, Oban, Argyll, Scotland PA37 1QA, UK

Oceanlab, University of Aberdeen, Main Street, Newburgh, Scotland AB41 6AA, UK

The phylogeny of ligulate and sulfuric-acid containing species of *Desmarestia*, occurring worldwide from polar to temperate regions, was revised using a multigenic and polyphasic approach. Sequence data, gametophyte characteristics, and sporophyte morphology support reducing a total of 16 taxa to four different species. (1) *D. herbacea*, containing broad-bladed and highly branched forms, has dioecious gametophytes. The three other species have monoecious gametophytes: (2) *D. ligulata* which is profusely branched and, except for one subspecies, narrow-bladed, (3) Japanese ligulate *Desmarestia*, here described as *D. japonica* sp. nov., which is morphologically similar to *D. ligulata* but genetically distant from all other ligulate taxa. This species may have conserved the morphology of original ligulate *Desmarestia*. (4) *D. dudresnayi*, including unbranched or little branched broad-bladed taxa. A figure of the holotype of *D. dudresnayi*, which was lost for decades, was relocated. The taxonomy is complemented by a comparison of internal transcribed spacer and

[1]Received 1 August 2012. Accepted 11 September 2013.

[2] Present address: Station Biologique de Roscoff, UMR 7139 CNRS-Université Pierre et Marie Curie-Paris VI, BP 74, Roscoff, F-29682, France.

[3]Author for correspondence: e-mail fkuepper@abdn.ac.uk

This article is dedicated to Aldo O. Asensi on the occasion of his 80th birthday (September 28, 2012) for his contributions to brown algal taxonomy and biogeography – including *Desmarestia*.

Editorial Responsibility: H. Verbruggen (Associate Editor)

cytochrome c oxidase subunit I (*cox*1) as potential barcode loci, with *cox*1 offering good resolution, reflecting species delimitations within the genus *Desmarestia*.

Key index words: **Brown algae; *cox*1; *Desmarestia*; DNA barcoding; multigene phylogeny; Phaeophyceae; *psa*A; *rbc*L; SSU-ITS; sulfuric acid**

***Abbreviations*: *cox*1, cytochrome c oxidase subunit I; ITS, internal transcribed spacer; ML, maximum likelihood**

The Desmarestiales is an order of large subtidal marine brown algae with a heteromorphic life history resembling that of the Laminariales or kelps. The macroscopic sporophytes are pseudoparenchymatous, they may be bushy, feather-like, or consist of a single or several blades (Ramirez and Peters 1992). The thalli are annual or perennial, can measure up to 8 m in length, as observed in a Northeast Pacific individual (Pease 1920), and these macroscopic forms alternate with microscopic gametophytes that are either monoecious or dioecious (Peters et al. 1997). A conspicuous character of most annual taxa of *Desmarestia* is a high concentration of sulfuric acid in the vacuoles (Schiff 1962, McClintock et al. 1982, Sasaki et al. 2004), which possibly serves to deter herbivores (Anderson and Velimirov 1982, Pelletreau and Muller-Parker 2002). In molecular phylogenies, the Desmarestiales forms a well-supported clade within the brown algal crown radiation (Draisma et al. 2003, Kawai et al. 2007, Phillips et al. 2008, Silberfeld et al. 2010). With a distribution from polar to warm-temperate climates, Desmarestiales comprise dominant components of the phytobenthos where other bed-forming brown algal taxa (i.e., Fucales, Laminariales and Phyllariaceae) are lacking (e.g., recolonization of barren grounds). This pattern is especially observed in the Southern Hemishpere and, to a lesser extent, in the Northern Hemisphere (Peters et al. 1997). Desmarestiales are also present in the understory of kelp forests (e.g., Stegenga et al. 1997). Few records of Desmarestiales exist from tropical latitudes, however, this may be due to the little studied deep-water refugia (Taylor 1945, Graham et al. 2007).

The type genus *Desmarestia* J.V. Lamouroux contains 30 species currently recognized (of 61 species described in www.algaebase.org search on March 05, 2012; Guiry and Guiry 2012) that are distributed worldwide from warm-temperate to polar regions. The type species of the genus, *D. aculeata* (Linnaeus) J.V. Lamouroux, is a perennial species which was described from Europe and occurs in the Arctic and in cold-temperate regions of the Northern Hemisphere (Lamouroux 1824, Lüning 1990). Morphology and ontogeny of sporophytes (Chapman 1972a,b, Anderson 1985, Stolpe et al. 1991, Wiencke et al. 1995, 1996), sporangial type (Moe and Silva 1977, 1981, 1989, Anderson 1985), dioecism versus monoecism of gametophytes (Anderson 1982, Peters and Müller 1986, Ramirez et al. 1986, Ramirez and Peters 1992), temperature tolerance of gametophytes (Peters and Breeman 1992, 1993), and nuclear ribosomal ITS sequence data (van Oppen et al. 1993, Peters et al. 1997, 2000) have been utilized to study the taxonomy, phylogeny, and biogeography of *Desmarestia* and the related monotypic genera *Arthrocladia* Duby, *Himantothallus* Skottsberg, and *Phaeurus* Skottsberg. Peters et al. (1997) hypothesized that *Desmarestia* originated in the Southern Hemisphere, possibly in high latitudes, and subsequently migrated to the Northern Hemisphere. They suggested that the characteristic of strong acidity of the sporophytic cells evolved only once in the desmarestialean lineage.

The annual species of *Desmarestia* with acid-containing thalli, which are in the focus of the present work, belong to a lineage of world-wide distribution which is subdivided into a small clade of taxa with terete thalli (e.g., *D. viridis* (O. F. Müller) J.V. Lamouroux) and a larger clade of taxa with bladed thalli (e.g., *D. ligulata* (Lightfoot) J.V. Lamouroux). Although Peters et al. (1997) have shown the major evolutionary and biogeographic tendencies within the Desmarestiales, the systematic position, taxonomy, and nomenclature of several species, especially from the clade with bladed and acid-containing thalli, have yet to be clarified. Opinions vary on how to treat this complex, ranging from a single variable species (*D. ligulata*; Chapman 1972a) to a number of at least six genetically isolated taxa, potentially corresponding to species (Peters et al. 1997). The situation is complicated by the fact that cases of significant morphological differences among co-occurring genetically similar forms exist (e.g., *D. ligulata, D. gayana* Montagne, and *D. muelleri* M.E. Ramírez et Peters in Chile), as well as a case of similar morphologies in genetically and geographically separated populations: isolates of *D. ligulata* from Japan differed genetically from *D. ligulata* isolates from Europe, South America, New Zealand, or the northeast Pacific (Peters et al. 1997). In the present work, we have examined more specimens from Japan and more genetic markers to confirm the distinctness of the Japanese entity, which justifies its description as a different species.

Desmarestia dudresnayi J.V. Lamouroux ex Léman is a little-known ligulate taxon distributed in cold to warm-temperate regions of Europe, where it is rare and confined to deep water. It is broad-bladed (>25 mm width) and sparsely branched or unbranched (Léman 1819, Drew and Robertson 1974, Anderson 1985) and opinions diverge whether it should be regarded as an independent species, a subspecies or form of *D. ligulata* (Chapman 1972b), or as conspecific with South African *D. firma* (C. Agardh) Skottsberg (Peters and Breeman 1992). The type locality of *D. dudresnayi* is St. Pol de Léon,

near Roscoff in northern Brittany (Sauvageau 1925). So far there have been no culture or molecular studies of this entity, which is of nomenclatural importance because its description predates that of all other unbranched and of most branched species of ligulate *Desmarestia*.

DNA barcoding aims at providing a rapid and unambiguous identification of biological materials, based upon the rapid and cost-effective sequencing of a short strand of DNA typically of the five primer region of *cox*1 but now extends to other loci (Hebert et al. 2003). In Phaeophyceae, DNA barcoding has been successful in identifying new and cryptic species. Mitochondrial *cox*1 and the nuclear rRNA ITS have been successful in identifying many brown algal species belonging to the Laminariales (Lane et al. 2007, Macaya and Zuccarello 2010, McDevit and Saunders 2010) and Fucales (Kucera and Saunders 2008, McDevit and Saunders 2009). The *cox*1 locus reveals biogeographic patterns and cryptic diversity, but it is not uniformly useful in all Phaeophyceae, such as *Macrocystis* (Macaya and Zuccarello 2010). The ITS has more variable sites and has proved useful in some genera but there have been difficulties interpreting results due to the presence of indels and genetic introgression (Kucera and Saunders 2008, McDevit and Saunders 2009, 2010).

The primary objective of this study was a reassessment of ligulate, acid-producing *Desmarestia* phylogeny, based on the sequences of multiple and phylogenetically informative markers such as nuclear small subunit (SSU) rDNA and ITS, mitochondrial *cox*1, plastid *psa*A (photosystem I P700 apoprotein A1), and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L). Including *D. dudresnayi* was essential for the revision of this species complex. Our results propose a practical nomenclature following Linnean classification criteria. Finally, we compared the barcode loci of mitochondrial *cox*1 and the nuclear rRNA ITS, to evaluate their potential suitability as barcode markers for the genus *Desmarestia*.

MATERIALS AND METHODS

Taxon sampling. A total of 52 specimens and cultures were investigated for this study (including four outgroup taxa; Table 1). Most of the Desmarestiales cultures and DNA extracts used in the present study were the same as in previous studies (Peters and Breeman 1992, Ramirez and Peters 1992, Peters et al. 1997) and they were deposited in the Culture Collection of Algae and Protozoa (CCAP; www.ccap.ac.uk). A specimen of ligulate *Desmarestia* was collected as drift material from the shore of Muroran (Western Hokkaido) on July 14, 1989. A gametophyte isolate was made (CCAP 1306/7), and a herbarium specimen was prepared (SAP109522). More specimens were collected from Oshoro (Northwestern Hokkaido) and Akkeshi (Eastern Hokkaido, Pacific Ocean) in May 2009 and a part of their thalli were dried in silicagel for DNA extractions, while the remainder of the thalli were pressed for herbarium specimens (*Desmarestia japonica* (Akkeshi, Type): SAP109521; *D. japonica* (Muroran): SAP109522). They were transported back to the laboratory in sterilized seawater, cleaned, and sorted carefully under a dissecting microscope. Epiphyte-free parts of the thalli were rapidly frozen in −75°C and freeze-dried for subsequent DNA extraction.

As *Desmarestia dudresnayi* is a rare species only a small number of sporophytes were collected in situ at the type locality near St. Pol de Léon in Brittany (France; $n = 4$; L'Hardy-Halos 1972) and Galicia (Spain; $n = 2$; Bàrbara et al. 2004, 2005). Morphological characters utilized by Chapman (1972b) were measured. The specimens of *D. dudresnayi* used for biometry were deposited in the herbarium of the Museum of Natural History, Paris (PC; unnumbered). Further specimens from Galicia were deposited in the herbarium of the University of Santiago de Compostela (SANT), and an individual from Brittany was deposited in the herbarium of the University of California at Berkeley (UC; #UC 1746473). The number of lateral blades was counted in previously collected specimens of *D. dudresnayi* housed at PC (Table 2).

Isolation of gametophytes. Fragments a few mm^2 in size were cut out of fertile blades of freshly collected sporophytes of *D. dudresnayi* from Brittany and Galicia and of a sporophyte of *D. ligulata* from Galicia and were inoculated in autoclaved Provasoli-enriched seawater (Starr and Zeikus 1987) containing GeO_2 ($6\ mg \cdot L^{-1}$) to prevent diatom growth. They were cultivated at 10°C and 15°C in white light of 25–30 μmol photons $\cdot\ m^{-2} \cdot s^{-1}$ at a day length of 14:10 h LD. Clonal gametophye cultures were subisolated by pipetting single germlings. A gametophyte strain of *D. dudresnayi* from Brittany and gametophyte strains of *D. dudresnayi* and *D. ligulata* from Galicia were deposited in CCAP (Table 1).

Sequencing and phylogenetic analyses. Genomic DNA was extracted from unialgal cultures or freeze-dried field samples using the DNeasy Plant Mini Kit™ (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reactions (PCR) were performed using specific primers for each gene with a Taq PCR Master Mix Kit™ (Qiagen). The nuclear SSU and ITS regions were amplified using the primers EAF3 and ITS055R, and sequenced using additional internal primers, such as 528F, 920F, EBR, 920R, 536R (Marin et al. 2003), a and b from Coleman et al. (1994). Plastidal *psa*A was amplified and sequenced using the primers psaA130F and psaA970R (Yoon et al. 2002). The mitochondrial *cox*1 was amplified using the primer pair GazF2 and GazR2 (Saunders 2005). To amplify and sequence desmarestialean *rbc*L, we designed the specific primers rbcL77F (5′-TGG GNT AYT GGG ATG CTG A-3′) and rbcL1471R (5′-ATS AGG TGT ATC TGT TGA TGT-3′). PCR amplification was performed in a total volume of 50 μL, containing 0.5 units $\cdot\ \mu L^{-1}$of Taq DNA Polymerase, 1 × Qiagen PCR Buffer, 1.5 mM $MgCl_2$, and 200 μM of each dNTP, 1 μM of each primer (except for *cox*1, for which 300 nM of each primer were used), and 1–10 ng of template DNA. PCR of the SSU-ITS region was carried out with an initial denaturation at 95°C for 3 min, followed by 30 cycles of amplification (denaturation at 95°C for 1 min, annealing at 50°C for 2 min and extension at 68°C for 3 min) with a final extension step at 72°C for 5 min. PCR of cox1 was performed as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min with one final extension at 72°C for 5 min. Amplified DNA was purified with the QIAquick™ PCR Purification Kit (Qiagen) and sent to commercial sequencing at the NERC Biomolecular Analytics Facility in Edinburgh. Electropherogram outputs for each were edited using the Chromas v.1.45 (http://www.technelysium.com.au/chromas.html). Assembled sequences of nuclear SSU and ITS were aligned using ClustalW implemented in SeaView v.4.3.3 (Gouy et al. 2012; http://pbil.univ-lyon1.fr/software/seaview.

TABLE 1. List of taxa and GenBank accession numbers used in the present study.

Taxa	Strain	Locality	Nuclear		Mitochondria	Plastid	
			SSU	ITS	*cox*1	*psa*A	*rbc*L
Desmarestia							
D. aculeata (Linnaeus)	CCAP 1306/35	Helgoland, Germany	HE866893	HE866855	HE866759	HE866785	HE866814
J.V. Lamouroux	CCAP 1306/38	San Juan Island, Washington, USA	HE866894	HE866856	HE866760	–	HE866815
	–	–	–	–	EU681402	EU579882	AJ287847
D. anceps Montagne	CCAP 1306/39	King George Island, Antarctica	HE866895	HE866857	–	HE866786	HE866816
D. antarctica R.L. Moe et P.C. Silva	CCAP 1306/41	King George Island, Antarctica	HE866896	HE866858	–	–	HE866817
D. chordalis J.D.	–	Cape Horn, Chile	HE866897	HE866859	–	–	HE866818
Hooker & Harvey	–	Sea Lion Island, Falkland Islands	–	HE866860	–	–	HE866829
D. dudresnayi	CCAP 1306/1	Ria de Arousa, Galicia, Spain	HE866898	HE866861	HE866761	HE866787	HE866820
Lamouroux ex Léman	CCAP 1306/2	Roscoff/St. Pol de Léon, Brittany, France (type locality)	HE866899	HE866862	–	HE866788	HE866821
D. dudresnayi subsp. *patagonica* Asensi	CCAP 1306/12	San Carlos, Valdivia, Chile	HE866900	HE866863	HE866762	HE866789	HE866822
D. dudresnayi subsp. *tabacoides* Okamura	CCAP 1306/13	Montana de Oro State Park, California, USA	HE866901	HE866864	HE866763	HE866790	HE866823
	–	Sageunjin 1, Gangreung, Korea	HE866902	HE866865	–	HE866791	HE866824
D. herbacea (Turner) Lamouroux	–	San Francisco, California, USA	HE866903	HE866866	HE866764	HE866792	HE866825
	–	Santa Barbara, California, USA	HE866904	HE866867	HE866765	HE866793	HE866826
	CCAP 1306/19	Playa Mendieta, Paracas, Peru	HE866907	HE866870	HE866768	HE866796	HE866829
	–	Horcon, Chile	HE866910	HE866873	HE866771	HE866799	HE866832
(=*D. latissima* Setchell & Gardner)	CCAP 1306/27	San Juan Island, Washington, USA	HE866905	HE866868	HE866766	HE866794	HE866827
(=*D. munda* Setchell et Gardner)	CCAP 1306/29	Bamfield, British Columbia, Canada	HE866906	HE866869	HE866767	HE866795	HE866828
D. herbacea subsp.	CCAP 1306/23	South Africa 1	HE866908	HE866871	HE866769	HE866797	HE866830
firma Skottsberg	CCAP 1306/31	South Africa 10	HE866909	HE866872	HE866770	HE866798	HE866831
D. herbacea subsp. *peruviana* Montagne	CCAP 1306/21	San Juan de Marcona, Peru	HE866911	HE866874	HE866772	HE866800	HE866833
D. japonica sp. nov.	CCAP 1306/7	Muroran, Hokkaido, Japan	HE866912	HE866875	HE866773	HE866801	HE866834
	Kawai	Japan	HE866913	–	–	–	HE866835
	KU-d5481, KU-d5486	Oshoro, Hokkaido, Japan	HE866914	AB623009	–	HE866802	AB623010
	KU-d5897, KU-d5899	Akkeshi, Hokkaido, Japan	–	–	–	–	–
	–	Japan	HE866915	–	–	HE866803	HE866836
D. latifrons (Ruprecht) Kützing	CCAP 1306/33	Hearst Beach, CA, USA	HE866916	–	–	HE866804	HE866837
D. ligulata (Lightfoot) Lamouroux	CCAP 1306/3	Faro de Larina, Galicia, Spain	HE866917	HE866876	–	–	HE866838
	CCAP 1306/5	Bamfield, Vancouver Island, British Columbia, Canada	HE866918	HE866877	HE866774	–	HE866839
	CCAP 1306/6	Cabo Raso, Chubut, Argentina	HE866919	HE866878	HE866775	HE866805	HE866840
	CCAP 1306/9	Kaikoura, New Zealand	HE866920	HE866879	HE866776	HE866806	HE866841
	CCAP 1306/10	Roscoff, Brittany, France	HE866921	HE866880	–	HE866807	HE866842
	CCAP 1306/11	San Carlos, Valdivia, Chile	HE866922	HE866881	HE866777	HE866808	HE866843
	–	–	L43060	–	–	EU681610	AJ287848
D. ligulata f. *distans*	CCAP 1306/8	Ushuaia, Argentina	HE866923	HE866882	HE866778	–	HE866844

(continued)

TABLE 1. Continued

Taxa	Strain	Locality	Nuclear		Mitochondria	Plastid	
			SSU	ITS	*cox*1	*psa*A	*rbc*L
D. ligulata subsp. *gayana* Montagne	CCAP 1306/15	Estaquilla, Los Muermos, X Region, Chile	–	HE866883	HE866779	–	HE866845
D. ligulata subsp. *muelleri* Ramirez et Peters	CCAP 1306/17	La Boca, Navidad, Chile	HE866924	HE866884	HE866780	–	HE866846
	CCAP 1306/18	Isla Bridges, Ushuaia, Argentina	HE866925	HE866885	HE866781	HE866809	HE866847
D. menziesii J. Agardh	CCAP 1306/40	King George Island, Antarctica	HE866926	HE866886	HE866782	HE866810	HE866848
	–	Candlemas Island	HE866927	HE866887	–	–	HE866849
	–	–	–	–	GQ368260	GQ368333	GQ368318
D. viridis (O.F. Müller) J.V. Lamouroux	CCAP 1306/14	Kiel, Germany	HE866928	HE866888	HE866783	HE866811	HE866850
	–	–	AJ295828	–	AY500367	EU681611	AJ287849
Arthrocladia villosa (Hudson) Duby	CCAP 1301/1	Topsail Island, North Carolina, USA	HE866929	HE866889	–	–	HE866851
	–	Villefranche, France	HE866930	HE866890	–	–	HE866852
	–	–	–	–	–	EU681603	EU681589
Himantothallus grandifolius (A. Gepp et E.S. Gepp) Zinova	CCAP 1313/1	King George Island, Antarctica	HE866931	HE866891	HE866784	HE866812	HE866853
	–	–	AJ229110	–	GQ368262	GQ368335	GQ368320
Phaeurus antarcticus Skottsberg	–	King George Island, Antarctica	HE866932	HE866892	–	HE866813	HE866854
Outgroup							
Fucus vesiculosus L.	–	–	–	–	AY494079	AY372960	DQ307680
Laminaria digitata (Hudson) J.V. Lamouroux	–	–	–	–	AJ344328	AY372964	AY372984
Saccorhiza polyschides (Lightfoot) Batters	–	–	L43059	–	EU681422	AY372965	AB045255
Sporochnus pedunculatus (Hudson) C. Agardh	–	–	–	–	–	EU681621	EU579937

TABLE 2. Occurrence of laterals in specimens of *Desmarestia dudresnayi* at the herbarium of the Museum of Natural History, Paris (PC).

Herbarium	Number of thalli		Number of laterals in branched individuals
	Without laterals	With laterals	
Thuret & Bornet	3	9	1–8
Sauvageau	17	2	1
Herbarium de France	9	7	1–3
Sum	29	18	

html) then refined by eye with Se-Al™ v2.0a11 (Sequencing Alignment Editor Version 2.0 alpha 11; http://tree.bio.ed.ac.uk/software/seal/). The plastid and mitochondrial protein coding genes were aligned manually with Se-Al™ based on inferred amino acid sequences.

Two data sets were used for phylogenetic analyses. First, in the DNA data set (a total of 5,138 bp; c5dna data), we combined all DNA alignments of *psa*A (675 bp), *rbc*L (1,257 bp), *cox*1 (655 bp), SSU (1,720 bp), and ITS (831 bp). Second, in the protein + DNA mixed data set (3,413 characters; c5mix data), translated *psa*A (225 aa), *rbc*L (389 aa), and *cox*1 (218 aa) were combined with SSU and ITS DNA sequences to avoid possible artifacts of phylogenetic calculations such as homoplasy at the third codon position. We used an independent evolution model for each partition (five individual genes) to minimize the effect on phylogeny of heterogeneity among genes. The selected evolutionary models were general time reversible substitution with the gamma distributed rate heterogeneity (GTR + G) model for DNA parts, the LG substitution (Le et al. 2008) with empirical amino acid frequencies and rate heterogeneity (LG + F + G) model for protein parts.

ML analyses were performed using the RAxML v.7.2.8 (Stamatakis 2006). We used "-f a" option for rapid bootstrap analysis and the best likelihood tree searching using "-# 1000" with default "-i" (automatically optimized SPR rearrangement) and "-c" (25 distinct rate categories) options of the program. The independent evolution model for all partition were unlinked by using "-m GTRGAMMA" and "-q" options. Bootstrap values (MLBS) were calculated using 1,000 replications under the same evolution model used for the best tree search.

DNA barcoding analysis. For DNA barcoding analysis, *cox*1 and ITS sequences were aligned with related phaeophycean sequences using BioEdit™ and MAFFT™ (Katoh et al. 1995). Phylogenetic analyses were conducted in MEGA5 (Tamura et al. 2011). For pairwise distance calculations, both uncorrected p-distances and kimura 2- parameter (Kimura 1980) models were calculated by MEGA5 and were found to be almost identical. The number of base differences per site was calculated from averaging over all sequence pairs within each species group. For *cox*1 and ITS, 556 and 447 positions were analyzed, respectively, in the final data set. The analysis involved 324 and 253 sequences for *cox*1 and ITS respectively.

Codon positions included were 1st, 2nd, 3rd, and noncoding. All positions containing gaps and missing data were eliminated. Species were defined based on clades obtained from phylogenetic analyses using all molecular markers in combination with nonmolecular characters (see Results and Discussion). Within species and between species pairwise distances were categorized into discrete bins and measured against their frequency. The barcoding cut-off was determined as the smallest distance that encompassed all within-species distances. Minimum genus-level distances were defined as the smallest pairwise distance observed between two species. This distance was applied to species to categorize them into barcode groups. The barcode groups were cross-compared with the combined morphological and multigene phylogenies to determine species-and genus level boundaries for each barcode marker.

RESULTS

Field collections, morphological and culture studies. **Desmarestia japonica *sp. nov.*** Ligulate *Desmarestia* is fairly common in northern Japan and an ecologically important component of seaweed communities. It grows on rocks of more or less exposed coasts in the shallow subtidal to 5–6 m (Fig. 1) and is distributed around Hokkaido and along the Pacific coast of Northern Honshu. The sporophytic thalli are annual, growing from winter to late summer, becoming fertile in late spring. The holdfast is cushion-shaped, bearing one to a few erect thalli. The erect thalli are light olive brown to brown in color, 0.6–1 (-2) m in length, with a conspicuous main axis 2–6 (-20) mm in width, oppositely branched in 2–3 orders. The shape and width of the main axis as well as the branchlets are variable, from linear to lanceolate. The main axis is compressed cylindrical at the base, and flattened in other parts, with a conspicuous midrib. The branchlets are stipitate, narrower at the base, broadest at the middle portion, and becoming tapered at the distal end. Young thalli have deciduous, trichothallic filaments, and the thalli are pseudoparenchymatous. Cells of the sporophytes are strongly acidic, and turn bluish green when immersed or soaked in fresh water, similar to *D. ligulata, D. viridis,* etc. (Sasaki et al. 2004). The thallus is composed of a large central axial cell surrounded by inner rhizoidal filaments, large, colorless medullary cells, and 1–2 layers of small, peripheral cells containing many discoid chloroplasts without pyrenoids. Unilocular zoidangia are conical, up to ~20 μm in height, embedded in the peripheral layer of the entire thallus except for the basal part of the main axis and tips of the thalli. Unizoids are ~8 × 5 μm in size, containing a chloroplast with eyespot, and with longer anterior and shorter posterior flagella. Gametophytes are minute, uniseriate branched filaments, monoecious, and oogamous (Nakahara 1984).

Desmarestia dudresnayi: In Brittany, *D. dudresnayi* was found on rock in the shade beneath an underwater cliff (Le Paradis) and on a sublittoral reef (Ar Tourtu) at 20–25 m depth on three occasions in July and August 1999 and 2000. A total of four specimens were available for measurement. The holdfast was smooth and conical with a diameter of 1–3 mm, the stipe was terete, 1.5–3 cm in length, and the blades had smooth margins. The phylloid of the individual collected on July 18, 1999 (Fig. 2a) was 28 cm in length and 6 cm in width. The three other individuals had blades of 20 cm length (apex eroded) and 8 cm width (Fig. 2b), 38 cm length and 9 cm width, and 30 cm length and 10.5 cm width (not illustrated). The specimen with the eroded apex had a pair of eroded laterals, the others were unbranched. The less eroded of the laterals was 12 cm in length and 5 cm in width. The connections of the laterals to the main blade were not terete like the stipe but flat and 4–5 mm wide. The central vein was distinct in the main blades of all specimens, but lateral veins were obvious only in one individual (Fig. 2b). They branched off at an angle of less than 90° and were bifurcated toward the margin.

In Galicia, *D. dudresnayi* was growing on a substratum of maërl, pebbles, and broken shells, near the

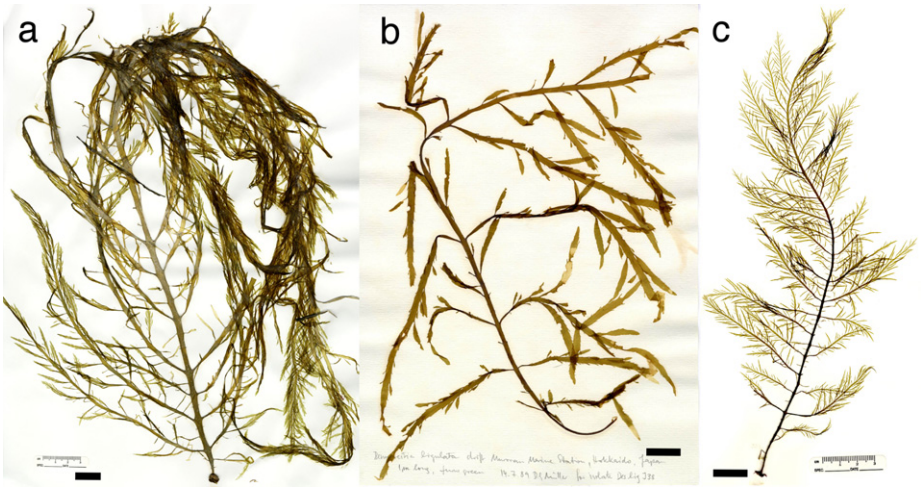

FIG. 1. *Desmarestia japonica* sp. nov. H. Kawai, T. Hanyuda, D.G. Müller, E.C. Yang, A.F. Peters et F.C. Küpper. Habit of grown-up specimens (a and b) and juvenile specimen. (a) Akkeshi, Eastern Hokkaido; (b) Muroran, South western Hokkaido; (c) Oshoro, North western Hokkaido; scale bars = 2 cm.

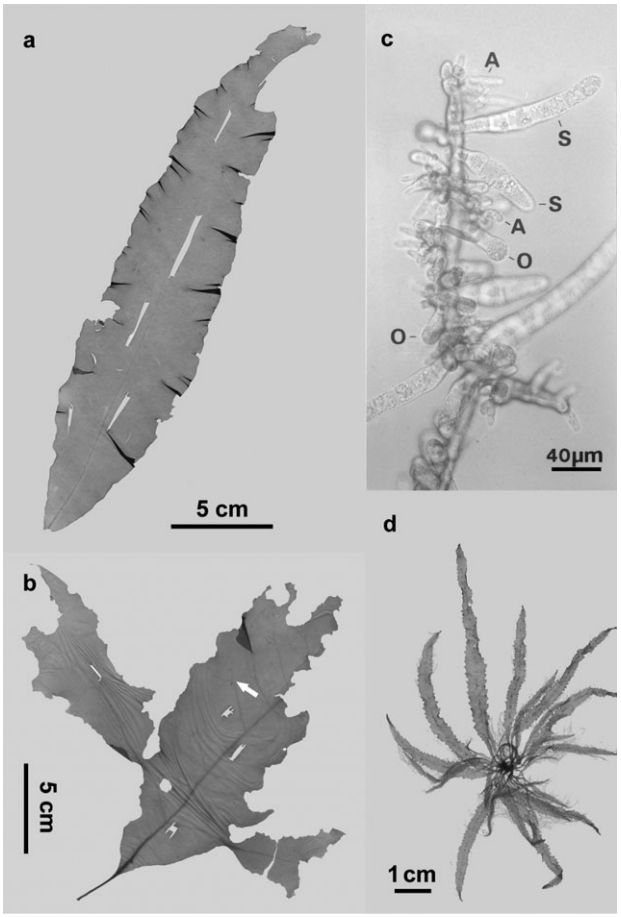

FIG. 2. *Desmarestia dudresnayi*. (a and b) Branched and unbranched individuals collected at the type locality, St. Pol de Léon (Brittany). Herbarium specimens, scanned. In the lower specimen (b), bifurcation of lateral veins (white arrow) is visible. (c) Fertile monoecious gametophyte, laboratory culture, isolate from Brittany. Branched laterals with antheridia (A), oogonia (O), and young sporophytes (S) which have developed in situ from fertilized oogonia. (d) Juvenile sporophytes from laboratory culture, after 2 months at 10°C.

central channel of the Ría de Arousa (Bàrbara et al. 2004). Collections for the present work were made at 13–15 m depth in September 1997, with two specimens measured. They had narrow terete stipes of 1.5 cm length, and in one a conical holdfast of 4 mm diameter was present. The blade of the first specimen was distally eroded, unbranched, 44 cm in length and 17 cm in width, the blade of the second individual was 61 cm in length and 23 cm in width. It had a single lateral of 9.5 cm length and 5 cm width whose connection to the main blade was 7 mm broad. The main axis and lateral veins were clearly visible. The lateral veins branched off at an angle of less than 90° and most of them were bifurcated. Like the individuals from Brittany, blades were devoid of marginal teeth or spines.

In Scotland, *D. dudresnayi* was found in the narrow sea straits between Dunstaffnage and Eilean Mhor (near Oban) on August 20, 2010. The habitat (pebbles and small rocks on a mostly sandy seabed) was different from the localities off Roscoff (underwater cliff faces), but more resembling that where *D. dudresnayi* was encountered in Galicia – a seabed consisting mostly of gravel at ~15 m below low tide level, with *D. dudresnayi* thalli growing attached to small pebbles and sea shells. Despite two searches using SCUBA, no *D. dudresnayi* was found at the same locality in the summer of 2011.

Gametophytes of *D. dudresnayi* from Brittany developed only in the culture from the unbranched individual collected on July 18, 1999 (Fig. 2c). From Galicia, we obtained gametophytes both from the unbranched and the branched individual. All three cultures gave rise to monoecious gametophytes, indistinguishable from each other. They consisted of branched creeping filaments 10–15 µm in diameter. Germlings became reproductive in 10°C and 15°C and bore antheridia and oogonia on the same thallus. Sporophytes developed from oogonia, without release of eggs (Fig. 2c). Sporophytes of *D. dudresnayi* grown in our cultures to a length of several centimeters remained unbranched (Fig. 2d).

Desmarestia ligulata: The specimen of *D. ligulata* collected in Galicia was profusely branched. The maximum width of the main axis was 6 mm (Fig. 3). Gametophytes of *D. ligulata* from Galicia were monoecious (not shown as they were similar to previously studied isolates, e.g., Peters and Müller 1986, Ramirez and Peters 1992). The time required for gametogenesis in *D. dudresnayi* and *D. ligulata* was compared. Vegetative gametophytes of both species from Galicia were simultaneously inoculated

FIG. 3. *Desmarestia ligulata* from Rio de Arousa, Galicia.

at 10°C and the appearance of first young sporophytes (like those illustrated in Fig. 2c) was recorded. *D. dudresnayi* gametogenesis took 14 d, whereas in *D. ligulata*, it required only 10 d.

Molecular phylogeny. Sequence statistics obtained for the alignments of the five markers used in the present study are summarized in Table 3. Nuclear SSU rDNA and ITS required gaps for multiple sequence alignment, however, no insertion and deletion were found in the three protein markers. SSU was the most conserved gene (98.2% constant positions) and mitochondrial *cox*1 was the most variable gene (27.2% variable positions) among the five genes used in the present study. The highest P-distance was found in *cox*1 (0.072 ± 0.01), followed by *psa*A (0.047 ± 0.005), ITS (0.026 ± 0.004; ligulate *Desmarestia* species only), *rbc*L (0.02 ± 0.002), and SSU (0.003). In each alignment, most variable positions were identified as phylogenetically informative sites. For example, mitochondrial *cox*1 showed the highest variable position content among genes (27.2%), and most variable positions (94%; 168/178 positions) were informative. However, in the ITS alignment, more than half of the variable positions were noninformative for phylogenetic analysis (52%; 57/110 positions). The three protein-coding organelle genes (*cox*1, *psa*A, and *rbc*L) had similar patterns in variation, proportion of informative site, base composition, and Ti/Tv ratio. The majority of substitutions occurred in the third codon position (e.g., 150 of 278 in *cox*1); AT bias was relatively stronger (i.e., higher than 0.6); and transition (Ti) was two times more abundant than transversion (i.e., Ti/Tv ratio higher than 2). To check for potentially misleading phylogenetic signals of the third codon position, we performed the saturation test for each gene. Uncorrected P distance and corrected distance with the Kimura 2-parameter evolution model were used for determining the coefficient of correlation. There were no significant saturation signals found in all tests (coefficients of correlation were higher than 0.91, $r^2 = 0.999$) except one; the third codon positions of *psa*A showed the lowest coefficient of correlation 0.797 ($r^2 = 0.999$). Rate heterogeneity of each gene was evaluated by shape parameter (alpha) estimation. ITS and *cox*1 showed relatively higher alpha values (≥0.2) and SSU showed the lowest heterogeneity (0.02) among the five markers.

A total of 5,138 positions of five concatenated DNA sequences (c5dna; SSU rDNA + ITS + *cox*1 + *psa*A + *rbc*L) and 3,413 positions of mixed DNA/protein sequences (c5mix; 862 aa from *cox*1, *psa*A and *rbc*L + 2,551 bp from SSU rDNA and ITS) were used for phylogenetic analyses, respectively. ML trees of c5dna and c5mix were highly congruent except for one different relationship. In the c5dna tree, *Phaeurus antarcticus* Skottsberg was grouped within a *Desmarestia-Himantothallus* (DH) clade; in the c5mix tree, *P. antarcticus* was a sister of the DH clade (indicated by dotted arrow line in Fig. 4). However, neither relationship had high statistical support. Since no saturation signals were found in the saturation test, we used the c5dna phylogeny as the best hypothesis. The type genus of the order *Desmarestia* was paraphyletic; i.e., *D. anceps* Montagne and *D. antarctica* R.L.Moe & P.C.Silva grouped with *Himantothallus* (MLBS 100% from c5dna and 91% from c5mix).

The sulfuric acid-containing *Desmarestia* species were monophyletic with high bootstrap supports (MLBS, 100% from c5dna and 89% from c5mix). A clade containing *D. aculeata* formed the sister group of the sulfuric acid-containing taxa (96% from c5dna and 77% from c5mix). The sulfuric acid-containing taxa were subdivided in five well-supported clades: (1) *D. viridis* branched first, as the sister species to all ligulate taxa which form a monophyletic, well supported group; (2) A Japanese species which will be described here as *D. japonica* sp. nov.; (3)

TABLE 3. Sequence statistics of Desmarestiales used in this study.

	Nuclear		Mitochondrial	Plastidial	
	SSU rDNA[a]	ITS[b]	*cox*1	*psa*A	*rbc*L
Number of sequences	41	26	30	34	47
Aligned positions (bp)	1720	730	655	675	1257
Indel positions	3	69	0	0	0
Variable positions, total	31 (1.8%)	110 (15.1%)	178 (27.2%)	135 (20%)	144 (11.5%)
[1st/2nd/3rd codon]	–	–	[23/5/150]	[20/2/113]	[28/7/109]
Informative positions, total	24 (1.4%)	53 (7.3%)	168 (25.6%)	100 (14.8%)	110 (8.8%)
[1st/2nd/3rd codon]	–	–	[20/3/145]	[11/0/89]	[21/6/83]
P-distance: Mean ± SD	0.003 ± 0.0004	0.026 ± 0.004	0.072 ± 0.010	0.047 ± 0.005	0.020 ± 0.002
Base frequencies (A/C/G)	0.24/0.21/0.27	0.20/0.29/0.28	0.21/0.19/0.21	0.29/0.15/0.20	0.28/0.16/0.23
Ti/Tv ratio	1.77	2.10	2.43	3.76	2.29
Saturation[c] ($r^2 > 0.99$)	0.994	0.942	0.952	0.797	0.915
Rate heterogeneity (alpha)	0.02	0.2	0.21	0.1	0.06

[a] *Himantothallus grandifolius* (GenBank AJ229110) was excluded because of missing data (only 503 bp in length).
[b] Sulfuric acidic *Desmarestia* taxa only.
[c] Coefficient of correlation between uncorrected P distance and corrected distance with Kimura 2-parameter model. All positions were used for distance calculation in SSU rDNA and ITS; the third codon position result shown for *cox*1, *psa*A, and *rbc*L.

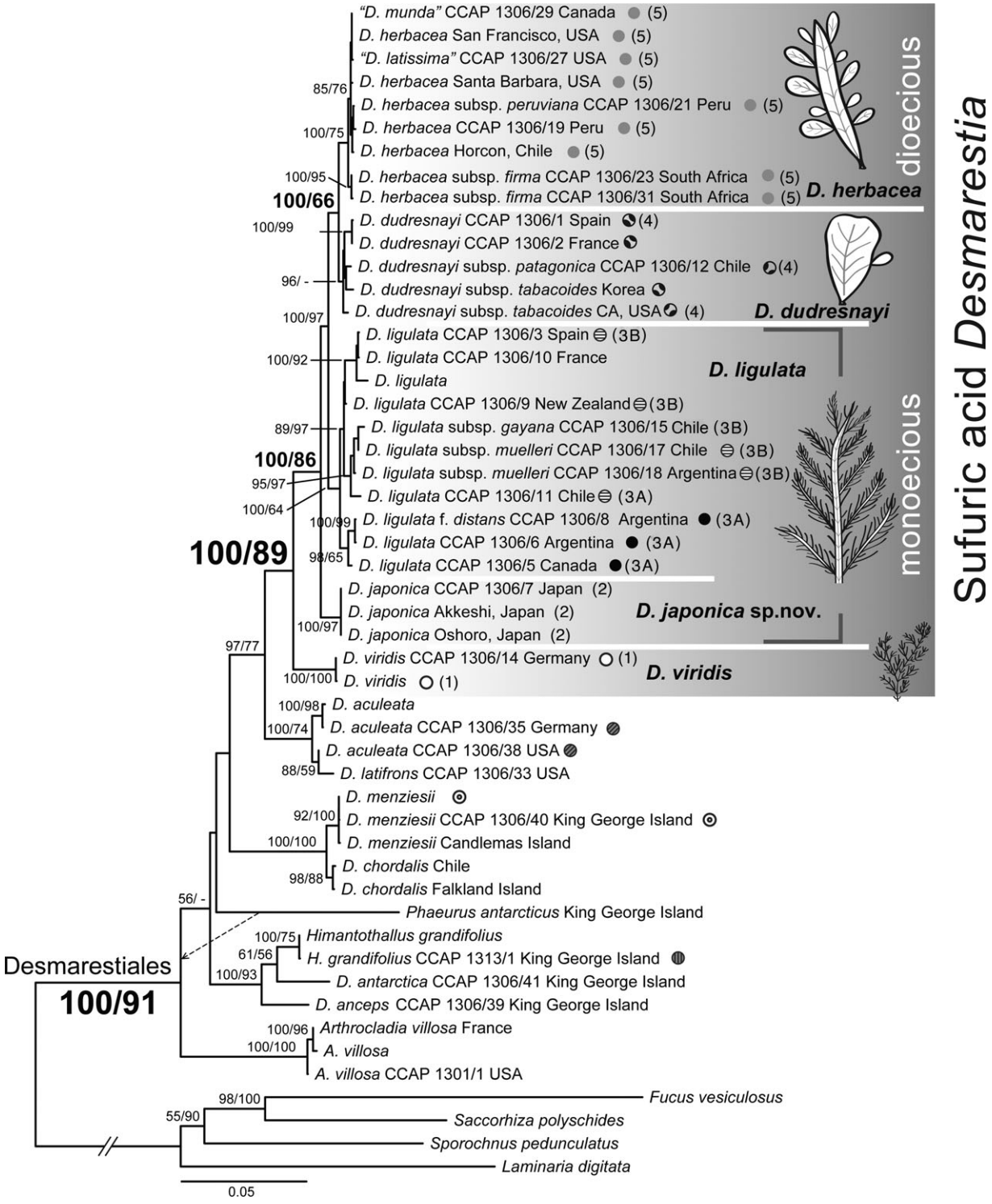

FIG. 4. Maximum likelihood phylogeny of the Desmarestiales based on combined small subunit + internal transcribed spacer (ITS) + cytochrome c oxidase subunit I (*cox*1) + *psa*A + *rbc*L sequences (5,138 bp; c5dna data, 5 DNA sequences concatenated) under the independent GTR + G model. The dotted arrow line indicates an alternative topology based on c5mix (3,413 characters; 3 protein sequences [862 aa; exclude the last stop codon of *rbc*L] + 2 DNA sequences [2,551 bp] concatenated). The numbers near branches refer to the maximum likelihood bootstrap values derived from 1,000 bootstrap analyses based on the c5dna and c5mix data sets. Strain ID and locality are followed by ITS and *cox*1 barcode labels (i.e. circle and number in parentheses, respectively). Numbers in parentheses (1–5) represent distinct *cox*1 barcode species groups with ≤1.2% pairwise distance (PWD). Different patterned circles show distinct ITS barcode groups with ≤1% PWD. For example, gray colored circles and number (5) in *Desmarestia herbacea* indicate that all samples grouped under the same species with ≤1.2% of *cox*1 and ≤1% of ITS PWD, respectively. In *D. ligulata*, two *cox*1 and ITS barcode groups were identified suggesting ongoing speciation.

D. ligulata isolates from Europe, Argentina, Chile, New Zealand, Canada, including strains originally identified as *D. distans*, *D. gayana* and *D. muelleri*; (4) *D. dudresnayi* (from France and Spain), *D. patagonica* (Chile), and *D. tabacoides* (from Korea and USA); (5) *D. herbacea* from the Pacific Coast of North and South America, *D. latissima* (USA) and *D. munda* (Bristish Columbia), *D. herbacea* ssp. *firma* (South Africa) and *D. herbacea* ssp. *peruviana* (Peru).

DNA barcoding. We compared the DNA barcoding utility of nuclear ITS and mitochondrial *cox*1. ITS and *cox*1 showed larger rate heterogeneity values (≥0.2) than the other genes (Table 3). Cytochrome c oxidase subunit I (*cox*1) sequence data were obtained from 30 Desmarestiales and three other phaeophycean specimens (*Fucus vesiculosus* Linnaeus, *Laminaria digitata* (Hudson) J.V. Lamouroux and *Saccorhiza polyschides* (Lightfoot) Batters). To determine the utility of *cox1* in delineating *Desmarestia* species, a comparison was made between genetic distances of *Desmarestia* compared to those of six Phaeophyceae genera (Fig. 5A). Specimen identifications of *Desmarestia* were based on the newly delimitated four species. Intraspecific PWDs were ≤1.2% in 98% of cases of Phaeophyceae. Interspecies distances started at 2.4%. For barcode assignments, identification of *Desmarestia* specimens were based on the newly delimitated four species. A cut-off value of 1.2% was used to define a species-barcode group.

*Desmarestia cox*1 species-level barcode groups conformed to their respective phylogenetic clades, only *D. ligulata* contained two groups (3A,B). *D. ligulata* (Spain) showed only partial identity to *D. ligulata* subspp. *gayana* and *muelleri* (Fig. 4). *D. ligulata* and *D. dudresnayi* barcode groups showed more variation in genetic distance compared with *D. herbacea*. Within the newly defined *D. herbacea* and *D. dudresnayi* groups all members formed a species group below the species-level cutoff of 1.2%. *D. viridis* formed its own separate species group that was at least 8.6% different to the ligulate specimens. Within ligulate *Desmarestia*, *D. japonica* sp. nov. (Japan; barcode group 2, Fig. 4) was clearly distinct and showed the greatest distance to other *Desmarestia* species, its nearest neighbor being *D. ligulata* (New Zealand) at 3.0% PWD.

Evaluation of the ITS barcode locus was performed with 36 sequences of *Desmarestia*, one sequence each from *Himantothallus grandifolius*, *Phaeurus antarcticus*, and *Arthrocladia villosa*, plus 214 phaeophycean sequences from six genera (five being common with *cox1* barcode analysis) available publically (Fig. 5B). Again, genetic distances were compared with the newly delimited species definitions here. In our data set 18/23 species comparisons showed equal or lower than 1.0% similarity (see Fig. 5B, dashed line), although the frequency of species between 1% and 1.14% is high because of greater representation from more divergent specimens. Genera- and species-level differences overlapped considerably, mostly due to *Alaria* spp. and only a modest genetic distance was found between species and genera. However, significantly, the ITS-barcodes did maintain the same groups as the multigene phylogeny. Using 1.0% as a species-level cut-off (see Fig. 5B, dashed line), ITS-barcode groups fell into two species-level groups and two single isolate groups in the sulfuric acid-containing species. *D. viridis* was clearly confirmed as a separate species to other *Desmarestia* (2.8%–3.4%). *D. japonica* sp. nov. (Japan) was at the species boundary to its nearest neighbors, the *D. dudresnayi* specimen group (0.8%–1.4%). The ITS sequences from the newly defined *D. ligulata* formed two major, closely related and partially overlapping groups that showed 1%–2.4% PWD difference to each other. *D. ligulata* (Spain) was distinct from both these groups. All members of the newly defined *D. dudresnayi* group and a publicly available sequence, AJ439832, were related at species-level. The *D. herbacea* group (*D. herbacea*, *D. herbacea* subsp. *firma*, and *D. herbacea* subsp. *peruviana*) were all related at species-level.

In summary *cox*1 shows better resolution with a distinct separation between species and genera compared to ITS. *cox*1 results confirm species limited by taxonomic and phylogenetic analysis.

DISCUSSION

Molecular phylogeny. Our new analyses employing nuclear, plastidial, and mitochondrial markers and four outgroup taxa have confirmed the previous phylogenetic tree of the Desmarestiales based on ITS sequences (Peters et al. 1997). As in the previous analysis, Antarctic and sub-Antarctic *Desmarestia* and *Himantothallus* as well as the monotypic genera *Arthrocladia* and *Phaeurus* formed the early branches, although their hierarchy remained ambiguous. Overall, our results confirm the monophyly of the sulfuric acid-producing *Desmarestia* clade. It is the sister group to the clade of the type species (Fig. 4). Furthermore, we confirmed that the sulfuric-acid clade is separated into *D. viridis,* branching off first, and all ligulate forms, in which we distinguish four major groups (Fig. 4): (1) Japanese *D. japonica*, (2) *D. ligulata* sensu stricto (including forma *distans*, subsp. *muelleri* and subsp. *gayana*), (3) *D. dudresnayi* (including subsp. *tabacoides* and subsp. *patagonica*, tentatively also subsp. *sivertsenii* [Tristan da Cunha] and subsp. *foliacea* [NE Pacific]) and (4) *D. herbacea* (incl. subsp. *peruviana*, subsp. *firma*, and the synonyms *D. latissima*, *D. munda,* and *D. mexicana*). Our classification recognizes four instead of 16 species of acid-containing ligulate *Desmarestia* (Table 4). The criteria for recognizing subspecies are the following: (i) Genetic distance, but insufficient for declaring different species; (ii) Geographically disjunct populations of the same species; (iii) Clear morphological differences.

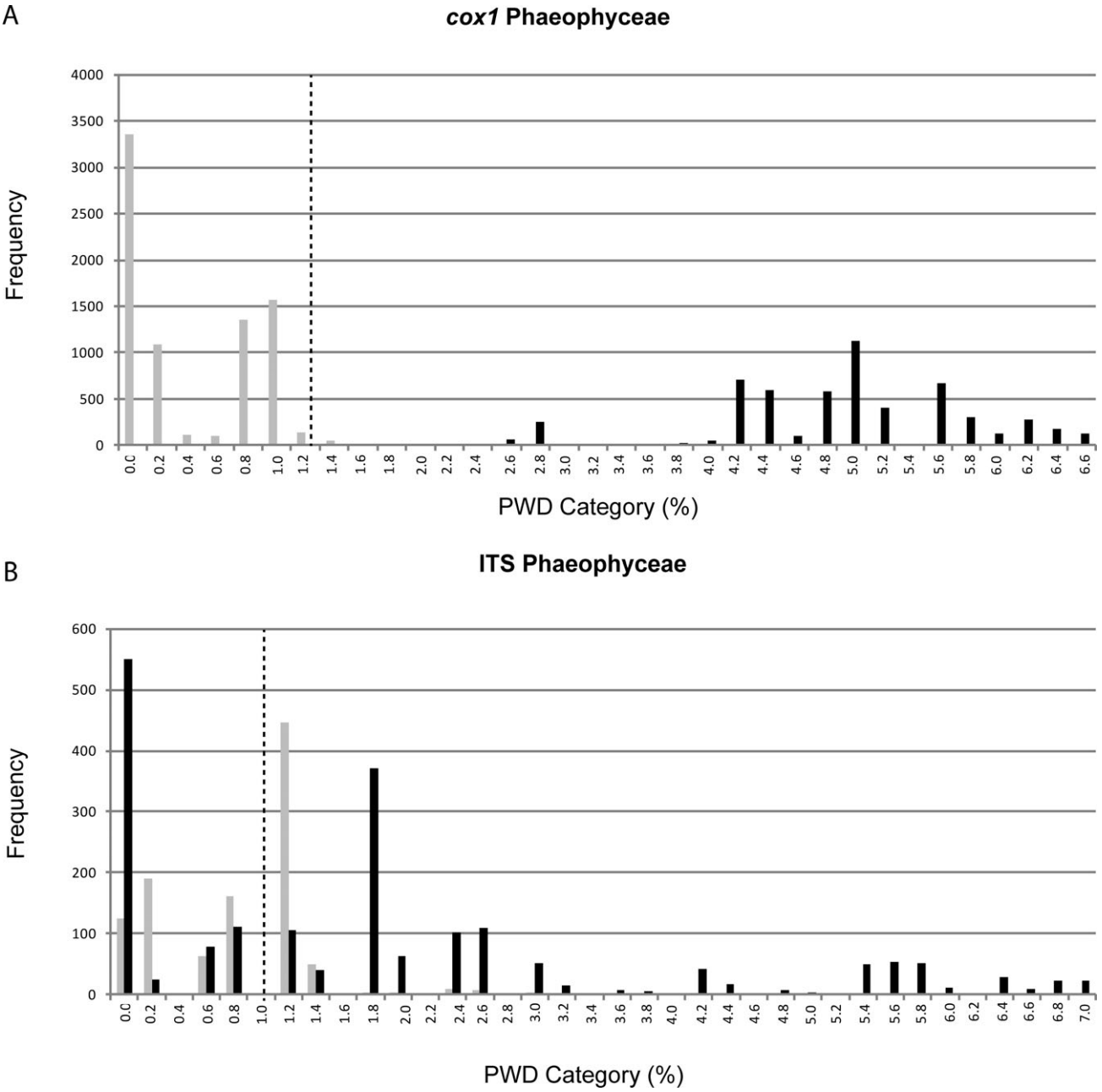

FIG. 5. Pairwise distance (PWD) distribution of Phaeophyceae for (A) *cox1* and (B) internal transcribed spacer (ITS) barcode markers. Grey and black bars indicate intra- and interspecies distances, respectively, and the dashed line indicates the species-level cut-off applied to Desmarestiales in each case.

Desmarestia ligulata: Our isolates of *D. ligulata* from Brittany (France) and from Galicia (Spain) clustered together showing that European samples of this species are slightly genetically different from individuals from Argentina, Chile, New Zealand, and western Canada. Nevertheless, all together they form a highly supported clade, which represents *D. ligulata* sensu stricto. However, samples belonging to three other taxa fell within the same clade: *D. distans* (C. Agardh) J. Agardh, *D. muelleri* M.E. Ramírez et A.F. Peters and *D. gayana* Montagne. The latter was not formally included in our analysis because of incomplete data; *rbc*L sequences place it close to *D. muelleri*, as did the previous analysis of ITS data (Peters et al. 1997). To accommodate these three taxa we propose to regard *D. distans*, which showed no genetic difference from a *D. ligulata* sample from Argentina, as a narrow growth form of *D. ligulata*. This classification would agree with the original treatment of this form (*Sporochnus ligulatus* var. *distans*; Agardh 1824). For *D. muelleri* and *D. gayana,* which show more significant mor-

TABLE 4. Recognized species of ligulate Desmarestiales with synonyms.

Species	Infraspecific taxa	Synonyms
D. ligulata (Stackhouse) J.V. Lamouroux (see AlgaeBase why Stackhouse and not Lightfoot)	subsp. *ligulata*	
	[subsp. *ligulata*] f. *distans* (C. Agardh) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. distans* (C. Agardh) J. Agardh
	subsp. *muelleri* (M.E.Ramírez et A.F.Peters) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. muelleri* M.E. Ramírez et A.F. Peters
	subsp. *gayana* (Montagne) comb. nov. A.F. Peters, E.C. Yang, & F.C. Küpper	*D. gayana* Montagne
D. dudresnayi J.V. Lamouroux ex Léman	subsp. *patagonica* (Asensi) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. patagonica* Asensi
	subsp. *tabacoides* (Okamura) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. tabacoides* Okamura
	subsp. *foliacea* (V.A. Pease) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. foliacea* V.A. Pease
	subsp. *sivertsenii* (Baardseth) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. sivertsenii* Baardseth
D. herbacea (Turner) J.V. Lamouroux	subsp. *firma* (C. Agardh) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. firma* (C. Agardh) Skottsberg
	subsp. *peruviana* (Montagne) comb. nov. A.F. Peters, E.C. Yang, F.C. Küpper & Prud'Homme van Reine	*D. peruviana* Montagne
	synonyms in subsp. *herbacea*: *D. latissima* Setchell & Gardner	
	D. munda Setchell et N.L. Gardner	
	D. mexicana E.Y. Dawson	
D. japonica H. Kawai, T. Hanyuda, D.G. Müller, E.C. Yang, A.F. Peters & F.C. Küpper	–	*D. ligulata* (Lightfoot) J.V. Lamouroux

phological differences from *D. ligulata* (and from each other), we propose reduction to subspecific rank (see Table 4 and New Combinations section).

NEW COMBINATIONS

Desmarestia ligulata* [subsp. *ligulata*] f. *distans (C. Agardh) **comb. nov**. A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine

Basionym and early description: *Sporochnus ligulatus* var. *distans* C. Agardh (1824) in *Systema Algarum* p. 261.

Desmarestia ligulata* subsp. *muelleri (M.E. Ramírez et A.F. Peters) **comb. nov**. A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine

Basionym and early description: *Desmarestia muelleri* M.E. Ramírez et A.F. Peters (1993) in *Canadian Journal of Botany* 70: p. 2442, figs. 12, 15, 34, 42, 43.

Desmarestia ligulata* subsp. *gayana (Montagne) **comb. nov.** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine

Basionym and early description: *Desmarestia gayana* Montagne (1852) in *Flora Chileana, Plantas cellulares 2, Algas* in Gay, C. (ed.) *Historia fisica y politica de Chile* 8: p. 243, pl. 14, fig. 1.

Japanese ligulate Desmarestia. The multigene analyses of all isolates available for the clade of bladed and sulfuric acid containing taxa confirmed the early branching of Japanese ligulate *Desmarestia*, which had previously been referred to as *D. ligulata* (e.g., Okamura 1907-9, 1936, Yoshida 1998), from *D. ligulata* of other regions. None of the markers (SSU, ITS, *cox*1, *psa*A, and *rbc*L) used in the present study suggested inclusion of Japanese ligulate *Desmarestia* in the clade containing *D. ligulata* from Europe, i.e., the area of the type, as well as from all other regions of the area of distribution of this species. Despite being morphologically similar to material from outside Japan, Japanese ligulate *Desmarestia* is also physiologically different: (i) gametogenesis in Japanese specimens is under short-day photoperiodic control (Nakahara 1984), whereas no effect of photoperiod was detected in gametogenesis of strains of *D. ligulata* from western Canada (Peters and Müller 1986) and South America (Ramirez and Peters 1992); (ii) gametophytes of two different isolates from Hokkaido showed an upper survival temperature limit (USL) 1.5°C–2.9°C higher than *D. ligulata* gametophytes from Western Canada, Chile, New Zealand, Argentina, and Brittany (Peters and Breeman 1992). This higher survival limit may help Japanese ligulate *Desmarestia* to occur in a region with comparatively high summer temperatures (up to ~25°C). *Desmarestia viridis*, which is also present in Japan (van Oppen et al. 1993), has a similar, high USL. *D. aculeata*, with a ~5°C lower USL, does not occur in Japan and is only found further North (Lüning 1984, Peters and Breeman 1992).

Furthermore, chromosome counts gave different results for Japanese ligulate *Desmarestia* (n = 52–56; Nakahara 1984) and western Canadian *D. ligulata* (44 ± 4; Peters and Müller 1986). Taken together, the physiological and genetic separation of Japanese ligulate *Desmarestia* from *D. ligulata* sensu stricto sug-

gests that the Japanese entity must be recognized as a different species. The highly branched thallus found both in the Japanese entity and in *D. ligulata* sensu stricto, may represent the original morphology of ligulate *Desmarestia*.

Still, open questions remain regarding ligulate *Desmarestia* from the cold seas of the North-west Pacific. The case of *Desmarestia kurilensis* Yamada (Yamada 1935) unfortunately has to remain unresolved. The type specimen available at SAP did not yield DNA suitable for PCR, and the type locality (Urup, one of the central Kuril Islands) is practically inaccessible for phycological studies. Ligulate *Desmarestia* from the east coast of Korea clustered within the *D. dudresnayi* clade, next to the entity previously called *D. patagonica* from Chile (Fig. 4). As of now, we have no indication for the presence of *D. japonica* in Korea.

***Desmarestia japonica* H. Kawai, T. Hanyuda, D.G. Müller, E.C. Yang, A.F. Peters, & F.C. Küpper sp. nov.**

Thalli sporophytici annui 0.6–1 (-2) m alti, 2–6 (-20) mm lati, pallide olivaceo-brunnei sed cum aciditate cellularum laesi viridescenti, ex haptero conico vel complanato orientes; axe primario plerumque prominente trichothallico pseudoparenchymato, costa instructo, gignente ramos oppositos distichos determinati incrementi, bis vel ter ramificatos; ramis ultimis brevibus et in juventute dentatis propter filamenta terminalia incrementi trichothallici. Rami stipitati et ad basin apicemque attenuati; thallus in sectione constans e cellula magna centrali axiali circumcincta filamentis rhizoideis interioribus, cellulis medullariis magnis incoloribus, et 1 vel 2 stratis cellularum peripheralium parvarum chloroplastos multos discoideos sine pyrenoidibus continentium. Zoidangia unilocularia in strato peripherale immersa. Thalli gametophytici minuti filamentosi, ramis uniseriatis, monoecii oogami.

Sporophytic thalli annual, 0.6–1 (-2) m tall, 2–6 (-20) mm wide, light olive brown in color, becoming greenish when damaged by cellular acidity, arising from a conical or flattened holdfast; main axis usually prominent, trichothallic, pseudoparenchymatous, with midrib, giving rise to opposite, distichous branches of limited growth branched to two to three times; ultimate branches short and dentate with terminal filaments of trichothallic growth when young. Branches stipitate and attenuate at base and apex; in section thallus composed of a large central axial cell surrounded by inner rhizoidal filaments, large, colorless medullary cells, and one to two layers of small, peripheral cells containing many discoid chloroplasts without pyrenoids. Unilocular zoidangia embedded in peripheral layer. Gametophytic thalli minute, uniseriate branched filamentous, monoecious, and oogamous.

D. dudresnayi *and* D. herbacea. A further focus of the present work was the broad-bladed taxa and particularly *D. dudresnayi* which is a rare species occurring in western Europe from Scotland to Galicia, with isolated populations in Portugal (Bàrbara et al. 2006), the Mediterranean, particularly (Messina, Italy; Drew and Robertson 1974) and Isle of Alborán (Rindi and Cinelli 1995). The specimens of *D. dudresnayi* we collected from the type locality were smaller than the individuals from Galicia (see Bàrbara et al. 2004). Nevertheless, gametophytes from both localities were monoecious and morphologically similar. Their ITS sequences were similar indicating that the individuals from both localities belong to the same species. Both populations of *D. dudresnayi* sampled consisted of unbranched as well as sparsely branched individuals, consistent with previous reports (Sauvageau 1925, Drew and Robertson 1974). In the herbarium of the Natural History Museum at Paris (PC), about one-third of the specimens of *D. dudresnayi*, that have been collected on French coasts, are branched (Table 2), with up to eight laterals (mode = 2). The laterals were connected to the main blade by a flattened stipe as in the type of *D. dudresnayi* (Léman 1819, Fig. 4; see below). In other species of ligulate *Desmarestia*, stipes of branches are terete (see figures in Anderson 1985, Ramirez and Peters 1992).

According to our multigene analyses, the clade formed by *D. dudresnayi and D. herbacea* is phylogenetically separated from *D. ligulata* (Fig. 4). Within this clade, *D. dudresnayi* forms a subclade of taxa, which have sparsely branched or unbranched thalli and usually broad blades. Gametophytes of *D. dudresnayi* are monoecious like those of *D. ligulata*. The different timings required for gametogenesis in the same culture conditions provided additional evidence that there is a biological separation of *D. dudresnayi* and *D. ligulata*, supporting their taxonomic separation based on sporophyte morphology (Léman 1819, Sauvageau 1925). A study about the recognition of oligoguluronates as defense elicitors in brown algae provided chemotaxonomic support for this notion: While sporophytes of *D. dudresnayi* (strain CCAP 1306/1) recognized these cell wall degradation products, reacting with an oxidative burst reminiscent of *Laminaria* species, sporophytes of *D. aculeata* and *D. ligulata* did not (Küpper et al. 2002). The hypothesis that *D. dudresnayi* represents a growth form of *D. ligulata* is thus rejected.

Peters and Breeman (1992) hypothesized that *D. dudresnayi* belongs to a group of taxa which are similar (possibly conspecific) to South African *D. firma*, the latter in our molecular analyses being represented by a clade comprising two isolates of *D. firma* from South Africa as well as *D. herbacea, D. latissima, D. munda, D. firma,* and *D. peruviana* from the Pacific coast of the Americas. This clade is genetically closer to *D. dudresnayi* than *D. ligulata* (Fig. 4) but all isolates had dioecious gametophytes while those of *D. dudresnayi* were monoecious. Although the genetic basis of the difference between monoecism and dioecism in brown algae is

not known, we conclude that *D. dudresnayi* is a species separate from the clade with dieocious gametophytes. As all the dioecious taxa were genetically as similar to each other as the different isolates of *D. ligulata*, we propose to merge them in a single species, *D. herbacea* (Turner) Lamouroux, which is the oldest valid name. Its type (BM 000562739; fig. 19 in Anderson 1985) is clearly from a broad-bladed entity. However, the South African population appears to be slightly separated genetically as well as geographically, and we retain them as subspecies *firma*. The same reduction is proposed for the small and narrow-bladed, i.e., morphologically different, *D. peruviana*. Based on our limited samples, the northeast Pacific taxa *D. latissima* and *D. munda* deserve no taxonomic separation from *D. herbacea*; in our opinion, *D. latissima* is a growth form from the highly sheltered waters of Puget Sound (Washington, USA) and *D. munda* is another synonym of *D. herbacea*.

Due to its morphological similarity, *D. mexicana* Dawson (Dawson 1944) from Southern California, of which we did not have any samples, is considered to belong to the same species (Pedroche et al. 2008). *D. herbacea* is possibly also the correct identification for the deep-water ligulate *Desmarestia* reported as *D. munda* (Silva 1966) and as *D. ligulata* (Graham et al. 2007) from Galapagos respectively. Whether *D. tropica*, also described from Galapagos, is another peculiar form or subspecies of *D. herbacea* remains to be examined.

The closest relatives of our European isolates of *D. dudresnayi* according to our sequence analyses are samples originally identified as *D. patagonica* Asensi (Chile) and *D. tabacoides* (our new samples from Korea as well as the old isolate from California), which are all isolates with monoecious gametophytes. We had no cultures from our samples from Korea but Japanese *D. tabacoides* was shown to be monoecious (Nakahara and Nakamura 1971). In contrast to *D. dudresnayi*, where branched (Léman 1819) as well as unbranched (Montagne 1842, as *D. pinnatinervia* Montagne; Crouan and Crouan 1852, as *D. dudresnayi* forma *simplex*; Sauvageau 1925) thalli have been reported (also see above), no branched specimens are known from either *D. patagonica* (Asensi and Gonçalves 1972, Pinto 1989, Ramirez and Peters 1992, Asensi and Küpper 2012) or *D. tabacoides*. On the other hand, unbranched sporophytes of *D. dudresnayi* and *D. patagonica* are indistinguishable in size and morphology (compare our Fig. 2 with the figures in Asensi and Gonçalves 1972, Ramirez and Peters 1992). Due to monoecism of *D. dudresnayi*, *D. patagonica,* and *D. tabacoides* we have not attempted cross-fertility experiments. The genetic distances among our samples of *D. dudresnayi*, *D. patagonica*, and *D. tabacoides* are comparable to those among different samples of *D. ligulata* and we thus propose to merge the unbranched to little branched broad-bladed taxa in *D. dudresnayi* and to reduce *D. tabacoides* and *D. patagonica* to subspecies. The latter treatment may also be justified for unbranched ligulate *Desmarestia* from Tristan da Cunha (South Atlantic; described as *D. sivertsenii* Baardseth (Baardseth 1941) and from the northeast Pacific where it is described as *D. foliacea* (Pease 1917, 1920). Our isolate of unbranched *Desmarestia* from California, previously identified as *D. tabacoides* (Peters et al. 1997) is slightly genetically different from our new Korean sample and possibly represents *D. foliacea*. Two specimens from Friday Harbor (Washington, USA; type locality of *D. foliacea*), kindly sent to us by Brian Wysor, were morphologically similar to unbranched *D. dudresnayi*.

The literature knows two different spellings of the specific epithet of *D. dudresnayi*, honoring the first collector of the alga, Guy du Dresnay (1770–1837; Dizerbo 1965). The longer spelling, *dudresnayi*, was used in the protologue by Léman (1819). In fact, Léman provided the name as *D. dudresnay*, containing an automatically correctable error; see Anderson 1985, footnote on p. 438. The shorter epithet, *dresnayi*, was employed by Lamouroux (1824) and many subsequent authors (Cotton 1912, Newton 1931, Dizerbo 1965, Chapman 1972b, Drew and Robertson 1974, Fletcher 1987, Rindi and Cinelli 1995, Bàrbara and Cremades 1996, Guiry and Nic Dhonncha 2003). Others, such as Sauvageau (1925), Hamel (1931-39), Anderson (1985) and Bàrbara et al. (2004, 2005, 2006) used the longer epithet. As rule 60.1 of the Code of Botanical Nomenclature imposes that the original spelling of the name is retained, *dudresnayi* is the correct epithet.

The fate of the holotype specimen of *D. dudresnayi*, a branched individual with two small and two large laterals, is obscure (Chapman 1972b). Anderson (1985) designated an unbranched specimen collected at the type locality by du Dresnay and now housed in Lamouroux's collection in Caen (CN) as lectotype. There is, however, a drawing of the holotype in the volumes of plates belonging to Bory de Saint Vincent's Dictionnaire des Sciences Naturelles, which were published separately from the protologue between 1816 and 1829. This drawing featuring a branched individual was erroneously referred to as plate number 43 by Sauvageau (1925). In fact, plate number 43 contains either *Alisma plantago* or a set of figures of small fungi, and later authors (e.g., Chapman 1972b) have apparently not seen the drawing. In the libraries of Leiden University and the Natural History Museum, Paris, we located the figure, hand numbered as "40" and in "Volume II" of the plates, in the series on acotyledones. Below the figure, which corresponds exactly to the protologue, the name is provided in the short spelling, as "*Desmarestia dresnayi* (Lamx)" [or "*dresnavi*"]. A watercolor featuring the holotype was found by Chantal Billard in the Lenormand herbarium at Caen but the holotype itself is still missing. To our opinion, the watercolor should be

regarded as iconotype (Fig. 6). As details of branching are important characteristics of *D. dudresnayi* it would still be useful to locate the holotype.

NEW COMBINATIONS

***Desmarestia dudresnayi* subsp. *patagonica* (Asensi)** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine **comb. nov.**

Basionym and early description: *Desmarestia patagonica* Asensi in Asensi, A.O & Gonçalves Carralves, M. (1972) in *Darwiniania* 17: p. 378, fig. 1.

***Desmarestia dudresnayi* subsp. *tabacoides* (Okamura)** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine **comb. nov.**

Basionym and early description: *Desmarestia tabacoides* Okamura (1908) in *Icones of Japanese algae* 1: p. 187, pl. 38, figs 1–4, pl. 39, figs 9–13.

***Desmarestia dudresnayi* subsp. *foliacea* (V.A. Pease)** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine **comb. nov.**

Basionym and early description: *Desmarestia foliacea* V.A. Pease (1920) in *Puget Sound Marine Biological Station Publication* 2: p. 322, 342, pl. 58, figs 5–10, pl. 61, figs 1–5.

***Desmarestia dudresnayi* subsp. *sivertsenii* (Baardseth)** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine **comb. nov.**

Basionym and early description: *Desmarestia sivertsenii* Baardseth (1941) in: *Results of the Norwegian Scientific Expedition to Tristan da Cunha 1937–1939:* 9: p. 28, figs 10, C–E, 12.

***Desmarestia herbacea* subsp. *firma* (C. Agardh)** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine **comb. nov.**

FIG. 6. Iconotype of *Desmarestia dudresnayi*, water color of the holotype from Lenormand's herbarium in Caen, France.

Basionym and early description: *Sporochnus herbacea* var. *firma* C. Agardh (1824) in *Systema Algarum*, p. 261.

***Desmarestia herbacea* subsp. *peruviana* (Montagne)** A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine **comb. nov.**

Basionym and early description: *Desmarestia peruviana* Montagne (1839) in *Plantes Cellulares, Algae, Florula Boliviensis stirpes novae et minus cognitae* in: d'Orbigny, A. (ed.): *Voyage dans l'Amérique Méruidionale* Vol. 7, Botanique (2): p. 35, pl. 5, fig. 3.

DNA barcoding. In this study, *cox*1 pairwise distance values for Desmarestiales within species and between species, ranged from 0% to 1.2% and >2.4% respectively. These values were comparable to 29 species from 20 genera of phaeophycean taxa reported by McDevit and Saunders (2009) at 0%–0.46% and >3% respectively. Desmarestiales sequence diversity was similar to those of *Laminaria* (0%–0.5%, >2.9%) and *Saccharina* (0%–1.2% and >2.1%). The only anomalous patterns in genetic diversity were *Macrocystis integrifolia* and *M. pyrifera,* which had overlapping intra and interspecies ranges, compared to other Laminariales. Recent results have indicated these species should in fact be reduced to the one *M. pyrifera* (Demes et al. 2009, Macaya and Zuccarello 2010). Our results indicate that *cox*1 is an excellent barcode marker for Desmarestiales, predicting almost all of the species groups of the multigene phylogenetic analysis. *Desmarestia japonica* had over four times larger sequence divergence compared to all other *Desmarestia* species and therefore warrants placement in a different species group and confirms results of systematic studies. ITS barcoding correctly identified species grouping, although with much less resolution than *cox*1 as genetic distances were smaller with greater than 1.0% PWD separating species. However, the ITS marker crucially lacks resolution and there is only 0.2% separating species and genus. The genetic distances for *Desmarestia* ITS barcodes were similar to those of *Saccharina latissima* and Laminariales, whose species cut-off was greater than 1% (McDevit and Saunders 2010). The lack of species/genus separation was also observed for *S. latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl et G.W. Saunders, where the biogeographical boundaries established using *cox*1-barcodes had collapsed using ITS-barcodes, with the authors speculating introgression as the cause (McDevit and Saunders 2010). It is possible that a lack of resolution in the ITS barcodes of Desmarestiales have occurred for similar reasons. For example *D. japonica*, a separate species, showed partial species level affinity with some but not all members of the unbranched to little-branched Desmarestiales, a sister taxon to the monophyletic *D. ligulata* group. By contrast, the same Japanese specimen showed less similarity to the *D. ligulata* group. In summary, *cox*1 performs well as a DNA barcoding marker in Desmarestiales, whilst ITS is more prone to error due to lack of resolution.

Conclusions and outlook. Our data suggest retention of four species of sulfuric-acid containing ligulate *Desmarestia*. We recognized co-occurring and morphologically dissimilar forms, or geographically separated populations, as subspecies, if they showed highly similar sequences.

From a geographical point of view, all North Atlantic taxa of *Desmarestia* have been reviewed, with the exception of the Moroccan *D. tingitana* Hamel, which is well branched but has broader blades than typical *D. ligulata* (Hamel 1931-39). The systematics of the Southern Hemisphere taxa have also been largely clarified (Moe and Silva 1977, 1981, 1989, Anderson 1985, Ramirez and Peters 1992, Peters et al. 1997, 2000). Population genetic and ecological approaches are now desirable to find out what provokes the profound morphological differences among South American *D. ligulata*, *D. ligulata* subsp. *gayana* and *D. ligulata* subsp. *muelleri*, and between *D. herbacea* and *D. herbacea* subsp. *peruviana*. All materials studied so far originate from drift collections where these taxa were found together – yet it is unclear whether they actually occur in the same or different habitats. The North Pacific Ocean still poses open taxonomic questions, and both terete and ligulate taxa of *Desmarestia* are still in need of a comprehensive revision – the present work only provided a start.

Desmarestia remains a fascinating genus of brown algae, from an ecological, physiological, developmental, and esthetic perspective. Even though a defense function of sulfuric acid accumulation (a unique feature of *Desmarestia* species among all brown algae) against grazers such as sea urchins has been demonstrated (Pelletreau and Muller-Parker 2002), the underlying physiology and biochemistry of the process is only poorly understood. So far, *D. dudresnayi* is the only member of this genus for which oligoalginate recognition and an oxidative burst could be demonstrated (Küpper et al. 2002). Despite the observation of Saenko et al. (1978) of an iodine content of 0.12% and a bromine content of 0.13% dry weight in *D. viridis* from the sea of Okhotsk, virtually nothing is known about the halogen metabolism of *Desmarestia* species in general – even though it is tempting to speculate that they resemble other, morphologically complex brown algae such as kelps (Küpper et al. 2008) in that respect. Also, gametophytes of *D. viridis, D. ligulata,* and *D. herbacea* as well as *D. ligulata* sporophytes turned out to be susceptible to the oomycete pathogen *Eurychasma* (Müller et al. 1999), but in general, the pathologies of Desmarestiales remain poorly studied. Furthermore, Motomura and Sakai (1984) had found that both iron and boron control gametogenesis in *Desmarestia*. Altogether, these are intriguing facets, warranting further, in-depth study of the life of this peculiar brown algal genus.

ACKNOWLEDGMENTS

This work was supported by the BK21 Global Internship Program (Bio Brain Center for Daedock R&D Innopolis, Chungnam National University) funded by the Korea Research Foundation to ECY, an access grant to the UK Natural Environment Research Council (NERC, UK) Molecular Bioanalytics Facility (MGF-154), core funding to the Culture Collection of Algae and Protozoa (Oceans 2025 NF3) from the Natural Environment Research Council, and with fellowships from Studienstiftung des deutschen Volkes (German Academic Merit Foundation) and the European Commission to FCK (Marie Curie Fellowship, Program MAST-III). This work also received funding from the MASTS pooling initiative (The Marine Alliance for Science and Technology for Scotland) and their support is gratefully acknowledged. Furthermore we acknowledge the support of the European Community research infrastructure action under the FP7 'capacities' specific program ASSEMBLE (grant no. 227788). MASTS is funded by the Scottish Funding Council (grant reference HR09011) and contributing institutions. Thanks are due to the curator of CN, Chantal Billard, for searches in the herbaria of Chauvin, Lamouroux and Lenormand, and to the curator of PC, Bruno de Reviers, for providing access to specimens and retrieval of old literature. We would also like to thank Martin Sayer, Elaine Azzopardi and Hugh Brown of the UK National Facility for Scientific Diving for supporting our collections and surveys of *Desmarestia dudresnayi* at Dunstaffnage (Oban, Scotland).

Agardh, C. A. 1824. *Systema Algarum.* Berling, Lund 312 pp.

Anderson, R. J. 1982. The life history of *Desmarestia firma* (C. Ag.) Skottsb. (Phaeophyceae, Desmarestiales). *Phycologia* 21:316–22.

Anderson, R. J. 1985. Morphological and taxonomic relationship among branched, ligulate members of the genus *Desmarestia* (Phaeophyceae, Desmarestiales), with particular reference to South African *D. firma*. *Can. J. Bot.* 63:437–47.

Anderson, R. J. & Velimirov, B. 1982. An experimental investigation of the palatability of kelp bed algae to the sea urchin *Parechinus angulosus* LESKE. *P.S.Z.N.I: Mar Ecol.* 3:357–73.

Asensi, A. O. & Gonçalves, M. 1972. Una nueva especie de alga parda: *Desmarestia patagonica* Asensi sp. nov. (Phaeophyta-Desmarestiales). *Darwiniana* 17:378–83.

Asensi, A. O. & Küpper, F. C. 2012. Seasonal periodicity and reproduction of brown algae (Phaeophyta) at Puerto Deseado (Patagonia). *Bot. Mar.* 55:217–28.

Baardseth, E. 1941. The marine algae of Tristan da Cunha. *Results of the Norwegian Scientific Expedition to Tristan da Cunha 1937–38* 9:1–173.

Bàrbara, I. & Cremades, J. 1996. Seaweeds of the Ría de A Coruña (NW Iberian Peninsula, Spain). *Bot. Mar.* 39:371–88.

Bàrbara, I., Cremades, J., Calvo, S., Lopez-Rodriguez, M. C. & Dosil, J. 2005. Checklist of the benthic marine and brackish Galician algae (NW Spain). *An. Jar. Bot. Madr.* 62:69–100.

Bàrbara, I., Cremades, J. & Veiga, A. J. 2004. A floristic study of a maërl and gravel subtidal bed in the Arousa ría (Galicia, Spain). *Botanica Complutensis* 28:35–46.

Bàrbara, I., Diaz, P., Cremades, J., Peña, V., Lopez-Rodriguez, M. C., Berecibar, E. & Santos, R. 2006. Catálogo gallego de especies amenazadas y lista roja de las algas bentónicas marinas de Galicia. *Bol. Inf. Soc. Esp. Ficol.* 35:9–19.

Chapman, A. R. O. 1972a. Morphological variation and its taxonomic implications in the ligulate members of the genus *Desmarestia* occurring on the west coast of North America. *Syesis* 5:1–20.

Chapman, A. R. O. 1972b. Species delimitation in the filiform, oppositely branched members of the genus *Desmarestia* Lamour. (Phaeophyceae, Desmarestiales) in the northern hemisphere. *Phycologia* 11:225–31.

Coleman, A. W., Suarez, A. & Goff, L. J. 1994. Molecular delineation of species and syngens in volvocacean green algae (Chlorophyta). *J. Phycol.* 30:80–90.

Cotton, A. D. 1912. Marine Algae. A biological survey of Clare Island in the county of Mayo, Ireland and of the adjoining district. *Proc. Royal Irish Acad.* 31 sect. 1:1–178.

Crouan, P. L. & Crouan, H. M. 1852. Algues marines du Finistere (Exsiccata). *Brest* 1–3:1–404.

Dawson, E. Y. 1944. The marine algae of the Gulf of California. *Allan Hancock Pacific Expeditions* 3:189–432., Plates 31–77.

Demes, K. W., Graham, M. H. & Suskiewicz, T. S. 2009. Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: the giant kelp, *Macrocystis* (Laminariales, Phaeophyceae), is a monospecific genus. *J. Phycol.* 45:1266–9.

Dizerbo, A. H. 1965. *Desmarestia dresnayi* Lamour ex Leman in France and Spain. *Br. Phycol. Bull.* 2:504.

Draisma, S. G. A., Peters, A. F. & Fletcher, R. L. 2003. Evolution and taxonomy in the Phaeophyceae: effects of the molecular age on brown algal systematics. *In* Norton, T. A. [Ed.] *Out of the Past. Collected Reviews to Celebrate the Jubilee of the British Phycological Society.* The British Phycological Society, Belfast, pp. 87–102.

Drew, E. A. & Robertson, W. A. A. 1974. Direct observation of *Desmarestia dresnayi* Lamour. ex Leman in the British Isles and in the Mediterranean. *Br. Phycol. J.* 9:195–200.

Fletcher, R. L. 1987. *Fucophyceae (Phaeophyceae).* British Museum (Natural History), London, 359 pp.

Gouy, M., Guindon, S. & Gascuel, O. 2012. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* 27:221–224.

Graham, M. H., Kinlan, B. P., Druehl, L. D., Garske, L. E. & Banks, S. 2007. Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *Proc. Natl. Acad. Sci. USA* 104:16576–80.

Guiry, M. D. & Guiry, G. M. 2012. AlgaeBase. World-wide electronic publication. National University of Ireland, Galway. Available from http://www.algaebase.org

Guiry, M. D. & Nic Dhonncha, E. 2003. AlgaeBase Version 2.0. National University of Ireland, Galway.

Hamel, G. 1931–39. *Pheophycees de France.* Wolf, Paris, 431 pp.

Hebert, P. D. N., Ratnasingham, S. & deWaard, J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Royal Soc. B Biol. Sci.* 270:S96–9.

Katoh, K., Kumah, K., Toh, H. & Miyata, T. 1995. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* 33:511–8.

Kawai, H., Hanyuda, T., Draisma, S. G. A. & Müller, D. G. 2007. Molecular phylogeny of *Discosporangium mesarthrocarpum* (Phaeophyceae) with a reinstatement of the order Discosporangiales. *J. Phycol.* 43:186–94.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–20.

Kucera, H. & Saunders, G. W. 2008. Assigning morphological variants of *Fucus* (Fucales, Phaeophyceae) in Canadian waters to recognized species using DNA barcoding. *Botany* 86:1065–79.

Küpper, F. C., Carpenter, L. J., McFiggans, G. B., Palmer, C. J., Waite, T. J., Boneberg, E. M., Woitsch, S. et al. 2008. Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proc. Natl. Acad. Sci. USA* 105:6954–8.

Küpper, F. C., Müller, D. G., Peters, A. F., Kloareg, B. & Potin, P. 2002. Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of the sporophytes of Laminariales. *J. Chem. Ecol.* 28:2057–81.

Lamouroux, J. V. F. 1824. Desmarestie. *In* Bory de Saint-Vincent, J. B. G. M. [Ed.] *Dictionnaire classique d'historie naturelle*, Vol. 5, Paris.

Lane, C. E., Lindstrom, S. C. & Saunders, G. W. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Mol. Phylogenet. Evol.* 44:634–48.

Le, S. Q., Lartillot, N. & Gascuel, O. 2008. Phylogenetic mixture models for proteins. *Philos. Trans. R. Soc. B Biol. Sci.* 363:3965–76.

Léman, D. S. 1819. Desmarestia. *In* de Saint-Vincent, B. [Ed.] *Dictionnaire des Sciences Naturelles*, Vol. 13. F. G. Levrault, Strasbourg & Paris, pp 104–6.

L'Hardy-Halos, M. T. 1972. Recherches en scaphandre autonome sur le peuplement végétale de l'infralittoral rocheux: La Baie de Morlaix (Nord Finistère). *Bull. Soc. Sci. Bretagne* 47:177–92.

Lüning, K. 1984. Temperature tolerance and biogeography of seaweeds: the marine algal flora of Helgoland (North Sea) as an example. *Helgolãnder Meeresunters.* 38:305–17.

Lüning, K. 1990. *Seaweeds – Their Environment, Biogeography, and Ecophysiology.* John Wiley & Sons Inc., New York, Chichester, Brisbane, Toronto, Singapore.

Macaya, E. C. & Zuccarello, G. C. 2010. DNA barcoding and genetic divergence in the giant kelp *Macrocystis* (Laminariales). *J. Phycol.* 46:736–42.

Marin, B., Palm, A., Klingberg, M. & Melkonian, M. 2003. Phylogeny and taxonomic revision of plastid containing Euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist* 154:99–145.

McClintock, M., Higinbotham, N., Uribe, E. G. & Cleland, R. E. 1982. Active, irreversible accumulation of extreme levels of H_2SO_4 in the brown algae, *Desmarestia. Plant Physiol.* 70:771–4.

McDevit, D. C. & Saunders, G. W. 2009. On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. *Phycol. Res.* 57:131–41.

McDevit, D. C. & Saunders, G. W. 2010. A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. *Phycologia* 49:235–48.

Moe, R. L. & Silva, P. C. 1977. Sporangia in the brown algal genus *Desmarestia* with special reference to Antarctic *D. ligulata. Bull. Jpn. Soc. Phycol.* 25(Suppl.):159–67.

Moe, R. L. & Silva, P. C. 1981. Morphology and taxonomy of *Himantothallus* (including *Phaeoglossum* and *Phyllogigas*), an Antarctic member of the Desmarestiales (Phaeophyceae). *J. Phycol.* 17:15–29.

Moe, R. L. & Silva, P. C. 1989. *Desmarestia antarctica* (Desmarestiales, Phaeophyceae), a new ligulate Antarctic species with an endophytic gametophyte. *Plant System. Evol.* 164:273–83.

Montagne, C. 1842. Troisième centurie de plantes cellulaires éxotiques nouvelles. *Sér.* 2:241–82.

Motomura, T. & Sakai, Y. 1984. Regulation of gametogenesis of *Laminaria* and *Desmarestia* (Phaeophyta) by iron and boron. *Jpn. J. Phycol. (Sorui)* 32:209–15.

Müller, D. G., Küpper, F. C. & Küpper, H. 1999. Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae). *Phycol. Res.* 47:217–23.

Nakahara, H. 1984. Alternation of generations of some brown algae in unialgal and axenic cultures. *Sci. Pap. Inst. Algol. Res. Fac. Sci. Hokkaido Univ.* 7:77–194, pl. I–XII.

Nakahara, H. & Nakamura, Y. 1971. The life history of *Desmarestia tabacoides* Okamura. *Bot. Mag.* 84:69–75.

Newton, L. 1931. *A Handbook of the British Seaweeds.* British Museum (Natural History), London.

Okamura, K. 1907-9. *Icones of Japanese Algae.* Kazamashobo, Tokyo.

Okamura, K. 1936. *Nippon Kaisoshi.* Uchida Rokakuho, Tokyo.

van Oppen, M. J. H., Olsen, J. L., Stam, W. T. & Wiencke, C. 1993. Arctic-Antarctic disjunctions in the benthic seaweeds *Acrosiphonia arcta* (Chlorophyta) and *Desmarestia viridis/willii* (Phaeophyta) are of recent origin. *Mar. Biol.* 115:381–6.

Pease, V. A. 1917. North Pacific coast species of *Desmarestia. Publications of the Puget Sound Marine Station* 1:383–94.

Pease, V. A. 1920. Taxonomy and morphology of the ligulate species of the genus *Desmarestia*. *Publications of the Puget Sound Marine Station* 2:313–67.

Pedroche, P. F., Silva, P. C., Aguilar Rosas, L. E., Dreckmann, K. M. & Aguilar Rosas, R. 2008. *Catálogo de las algas benthónicas del Pacífico de México II. Phaeophycota*. Universidad Autónoma Metropolitana and University of California Berkeley, Mexicali & Berkeley, pp. [i-viii], i-vi, 15-146.

Pelletreau, K. N. & Muller-Parker, G. 2002. Sulfuric acid in the phaeophyte alga *Desmarestia munda* deters feeding by the sea urchin *Strongylocentrotus droebachiensis*. *Mar. Biol.* 141:1–9.

Peters, A. F. & Breeman, A. M. 1992. Temperature responses of disjunct temperate brown algae indicate long-distance dispersal of microthalli across the tropics. *J. Phycol.* 28:428–38.

Peters, A. F. & Breeman, A. M. 1993. Temperature tolerance and latitudinal range of brown algae from temperate Pacific South America. *Mar. Biol.* 115:143–50.

Peters, A. F. & Müller, D. G. 1986. Life-history studies - a new approach to the taxonomy of ligulate species of *Desmarestia* (Phaeophyceae) from the Pacific coast of Canada. *Can. J. Bot. Rev. Can. Bot.* 64:2192–6.

Peters, A. F., Ramirez, M. E. & Rülke, A. 2000. The phylogenetic position of the subantarctic marine macroalga *Desmarestia chordalis* (Phaeophyceae) inferred from nuclear ribosomal ITS sequences. *Polar Biol.* 23:95–9.

Peters, A. F., van Oppen, M. J. H., Wiencke, C., Stam, W. T. & Olsen, J. L. 1997. Phylogeny and historical ecology of the Desmarestiaceae (Phaeophyceae) support a southern hemisphere origin. *J. Phycol.* 33:294–309.

Phillips, N., Burrowes, R., Rousseau, F., de Reviers, B. & Saunders, G. W. 2008. Resolving evolutionary relationships among the brown algae using chloroplast and nuclear genes. *J. Phycol.* 44:394–405.

Pinto, R. 1989. Caracterización de la flora algológica del area de Iquique, Norte de Chile. *Vultur* 1:1–16.

Ramirez, M. E., Müller, D. G. & Peters, A. F. 1986. Life history and taxonomy of two populations of ligulate *Desmarestia* (Phaeophyceae) from Chile. *Can. J. Bot. Rev. Can. Bot.* 64:2948–54.

Ramirez, M. E. & Peters, A. F. 1992. The South American species of *Desmarestia* (Phaeophyceae). *Can. J. Bot. Rev. Can. Bot.* 70:2430–45.

Rindi, F. & Cinelli, F. 1995. Contribution to the knowledge of the benthic algal flora of the Isle of Alborán, with notes on some little-known species in the Mediterranean. *Crypt. Algol.* 16:103–14.

Saenko, G. N., Kravtsova, Y. Y., Ivanenko, V. V. & Sheludko, S. I. 1978. Concentration of iodine and bromine by plants in the seas of Japan and Okhotsk. *Mar. Biol.* 47:243–50.

Sasaki, H., Kataoka, H., Murakami, A. & Kawai, H. 2004. Inorganic ion compositions in brown algae, with special reference to sulfuric acid ion accumulations. *Hydrobiologia* 512:255–62.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Proc. Royal Soc. Lond. B* 360:1879–88.

Sauvageau, C. 1925. A propos de la rencontre du *Desmarestia Dudresnayi* Lamx. dans le golfe de Gascogne. *Rev. Algol.* 1:1–13.

Schiff, J. A. 1962. Sulfur. *In* Lewin, R. A. [Ed.] *Physiology and Biochemistry of Algae*. Academic Press, New York, pp. 239–46.

Silberfeld, T., Leigh, J. W., Verbruggen, H., Cruaud, C., de Reviers, B. & Rousseau, F. 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the "brown algal crown radiation". *Mol. Phylogenet. Evol.* 56: 659–74.

Silva, P. C. 1966. Status of our knowledge of the Galapagos benthic marine algal flora prior to the Galapagos International Scientific Project. *In* Bowman, R. I. [Ed.] *The Galapagos*. University of California Press, Berkeley, CA, pp. 149–56.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–90.

Starr, R. C. & Zeikus, J. A. 1987. UTEX - the culture collection of algae at the University of Texas at Austin. *J. Phycol.* 23(Suppl.):1–47.

Stegenga, H., Bolton, J. J. & Anderson, R. J. 1997. Seaweeds of the South African west coast. *Contrib. Bolus Herb.* 18:1–655.

Stolpe, U., Wiencke, C., Clayton, M. N. & Lehmann, H. 1991. Life history, morphology and development of a ligulate *Desmarestia* species from southernmost Chile. *Br. Phycol. J.* 26:235–45.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731–9.

Taylor, W. R. 1945. Pacific marine algae of the Allan Hancock expeditions to the Galápagos Islands. *Allan Hancock Pacific Expeditions* 12:1–528.

Wiencke, C., Clayton, M. N. & Langreder, C. 1996. Life history and seasonal morphogenesis of the endemic Antarctic brown alga *Desmarestia anceps* Montagne. *Bot. Mar.* 39:435–44.

Wiencke, C., Clayton, M. N. & Schulz, D. 1995. Life history, reproductive morphology and development of the Antarctic brown alga *Desmarestia menziesii* J. Agardh. *Bot. Acta* 108:201–8.

Yamada, Y. 1935. Marine algae from Urup, the middle Kuriles, especially from the vicinity of Iema Bay. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido Imperial University* 1:1–26.

Yoon, H. S., Hackett, J. D. & Bhattacharya, D. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl Acad. Sci. USA* 99:11724–9.

Yoshida, T. 1998. *Marine Algae of Japan*. Uchida Rokakuho Publishing Co. Ltd., Tokyo, pp. 1–1222.