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ABSTRACT

During a 2007 expedition dedicated to study kelp diversity within the Magellanic region, specimens of *Lessonia* Bory with corrugate, yellowish blades were found along the Beagle Channel. This type of corrugated *Lessonia* is uncommon, with exception of two species from New Zealand. Three possible hypotheses for its origin were explored in this study: an introduction of *L. adamsiae* Hay or *L. corrugata* Lucas from New Zealand by trans-oceanic dispersal; a hybridization between Chilean *Lessonia* species and *Macrocystis pyrifera* C.Agardh, particularly abundant in the region; or the existence of a new, undescribed species. Except for their corrugate blades, the individuals displayed short cylindrical stipes, digitate holdfasts, abundant mucilage cavities in the cortex, and reproductive sori on the proximal part of the blades, all traits typical of the genus *Lessonia*. Molecular phylogenies based on mitochondrial (*atp*8-*trn*S spacer, *cox*1, *cox*3) and nuclear (ITS1) markers revealed that this corrugate form of *Lessonia* is neither the result of hybridization with *Macrocystis* C.Agardh nor an introduction of Australasian corrugate species, *L. corrugata* or *L. adamsiae*, but has a close affinity with *L. flavicans* Bory. Field prospection in five sites along the Beagle Channel indicated that the species occurs in shallow depth (4-6 m), as dense populations of 100% of corrugated individuals, never intermixed with populations of *L. flavicans*. The possibility of an incipient speciation process in the region is discussed.

RÉSUMÉ

Populations d'un nouveau morphotype de corrugate Lessonia *Bory dans le canal Beagle, écorégion subantarctique de Magellan : un cas possible de spéciation en cours.*

Au cours d'une expédition de 2007 consacrée à l'étude de la diversité du kelp dans la région de Magellan, des spécimens de *Lessonia* Bory aux lames ondulées et jaunâtres ont été trouvés le long du canal Beagle. Ce type de *Lessonia* ondulé est peu commun, à l'exception de deux espèces de Nouvelle-Zélande. Trois hypothèses possibles concernant son origine ont été explorées dans cette étude : une introduction de *L. adamsiae* Hay ou *L. corrugata* Lucas de Nouvelle-Zélande par dispersion transocéanique ; une hybridation entre l'espèce chilienne *Lessonia* et *Macrocystis* pyrifera C.Agardh, particulièrement abondante dans la région ; ou l'existence d'une nouvelle espèce non décrite. À l'exception de leurs lames ondulées, les individus présentaient des stipes cylindriques courts, des crampons digitaux, d'abondantes cavités de mucilage dans le cortex et des sores de reproduction sur la partie proximale des lames, tous des traits typiques du genre *Lessonia*. Des phylogénies moléculaires basées sur des marqueurs mitochondriaux (*atp*8-*trn*S spacer, *cox*1, *cox*3) et nucléaires (ITS1) ont révélé que cette forme ondulée de *Lessonia* n'est ni le résultat d'une hybridation avec *Macrocystis* C.Agardh ni d'une introduction d'espèces ondulées d'Australasie, *L. corrugata* ou *L. adamsiae*, mais qu'elle a une étroite affinité avec *L. flavicans* Bory. La prospection sur le terrain dans cinq sites le long du canal Beagle a indiqué que l'espèce est présente à faible profondeur (4-6 m), sous forme de populations denses de 100 % d'individus ondulés, jamais mélangés avec des populations de *L. flavicans*. La possibilité d'un processus de spéciation naissant dans la région est discutée.

INTRODUCTION

The sub-Antarctic ecoregion of Magellan, at the southern tip of South America, hosts some of the most unique pristine environments in the world, supporting diverse sub-Antarctic marine ecosystems with high levels of endemism (Rozzi *et al.* 2012). Coastal communities in this region are largely dominated by kelp species, including the Laminariales genera *Macrocystis* C.Agardh and *Lessonia* Bory, and the Fucale *Durvillaea* Bory. The tree-like *Lessonia* (Lessoniaceae) is characterized by large thalli (1-5 m), a growth pattern involving longitudinal splitting of the blade, discoid holdfasts or branched haptera, cylindrical stipes, ligulate or elongated blades with dentate

margins and a usually smooth surface where unilocular sporangia associated with paraphyses develop in extensive sori on both surfaces (Edding *et al.* 1994; Asensi & De Reviers 2009). This non-buoyant genus differs from the monospecific genus *Macrocystis* (Laminariaceae) mainly by the absence of pyriform to subglobose pneumatocyst subtending an undivided and denticulate lamina (Demes *et al.* 2009). *Durvillaea* (Durvillaeaceae) is distinguishable from *Lessonia* in having a unique gas-filled honeycomb structure within the blades, which gives strength and extreme buoyancy (Graiff *et al.* 2013), among other traits. A total of 11 species have been identified within the genus (see Guiry & Guiry 2018), with six species occurring in Chile: *L. berteroana* Montagne,
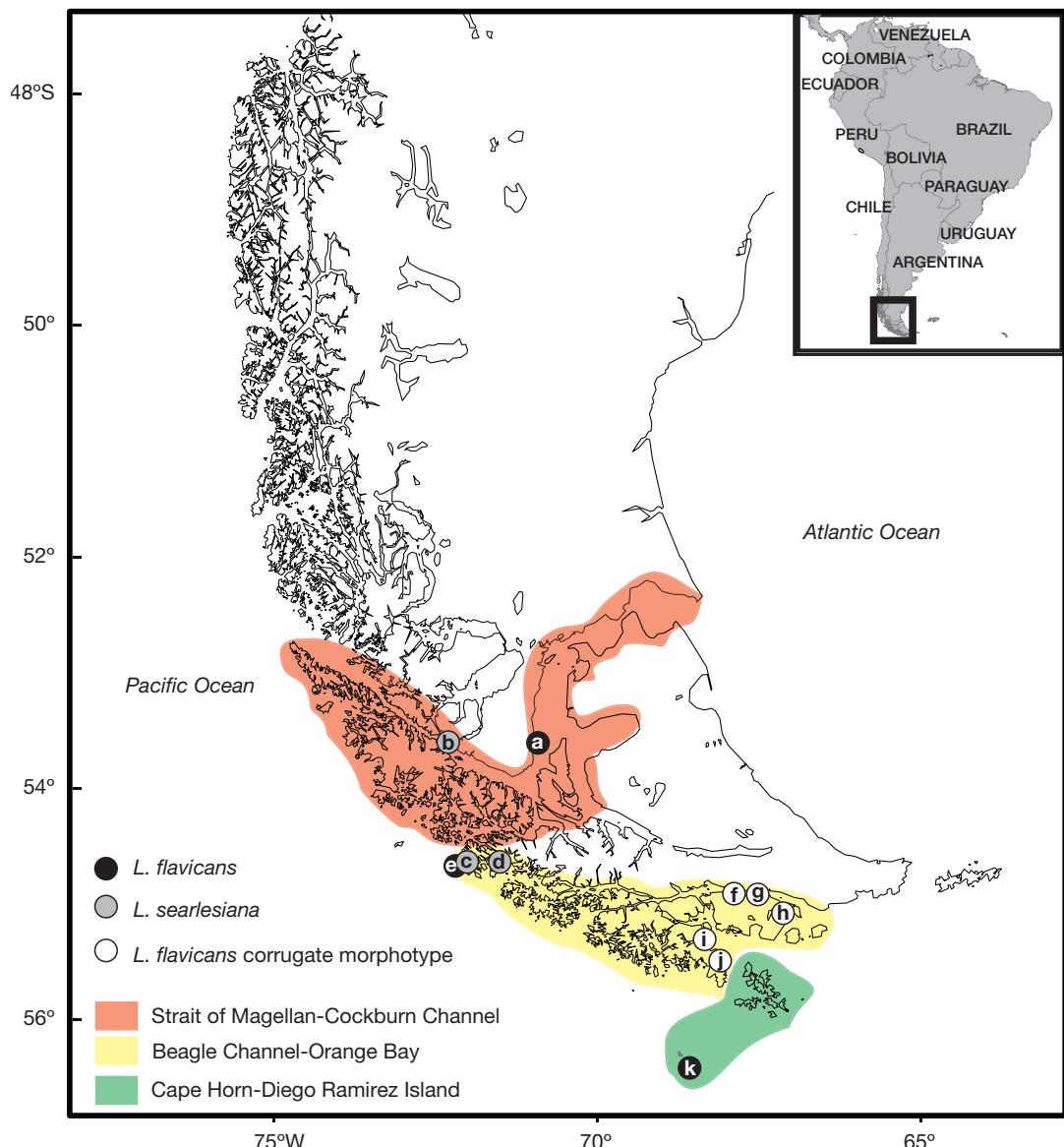
FIG. 1. — Map of the sub-Antarctic ecoregion of Magellan showing the collection sites of the corrugate morphotype of *Lessonia* Bory, *Lessonia flavicans* Bory, and *Lessonia searlesiana* Asensi & Reviers. Strait of Magellan-Cockburn channel: **a**, Fuerte Bulnes; **b**, Carlos III Island. Beagle channel-Orange Bay: **c**, London Island; **d**, Puerto Aguirre; **e**, London Island; **f**, Cormoran Bay; **g**, Paula Cove; **h**, Puerto Toro; **i**, Tekenika Bay; **j**, Orange Bay. Cape Horn-Diego Ramirez Island: **k**, Diego Ramirez Island.

L. flavicans Bory (that includes *L. vadosa* Searles as synonym), *L. nigrescens* Bory, *L. searlesiana* Asensi & Reviers (formerly as *L. flavicans sensu* Searles), *L. spicata* (Suhr) Santelices, and *L. trabeculata* Villouta & Santelices (Ramírez & Santelices 1991; Asensi & De Reviers 2009; González *et al.* 2012; Martin & Zuccarello 2012); and five in Australasia: *L. adamsiae* Hay, *L. brevifolia* J.Agardh, *L. corrugata* Lucas, *L. tholiformis* Hay, and *L. variegata* J.Agardh (Martin & Zuccarello 2012).

Morphological characteristics used to delimit species of *Lessonia* are the corrugation of frond surface, the marginal denticulation of fronds, the lacunate cortex, and shape of holdfasts (Edding *et al.* 1994; Asensi & De Reviers 2009). All species of *Lessonia* from southwest America have characteristic smooth blades (see Edding *et al.* 1994).

Sometimes, morphological features are unable to discriminate efficiently sister species, such as in the case of *L. berteroana* and *L. spicata* which lack diagnostic character (González *et al.* 2012) even though they are strongly genetically divergent (Tellier *et al.* 2009) and reproductively isolated (Tellier *et al.* 2011a). In all cases, however, distribution ranges do not overlap among these species. *Lessonia berteroana* and *L. spicata* show strict parapatry along the coast of Pacific South America, the former from Peru (17°S) to central Chile (30°S), the latter throughout central and southern Chile (29-41°S; Tellier *et al.* 2009). In the contact region (29-30°S), the two species were strictly segregated in space, showing lack of hybridization (Tellier *et al.* 2011a). *Lessonia trabeculata* is a subtidal species distributed from southern

TABLE 1. — Comparison of carbohydrate constituents in the corrugate *Lessonia* Bory and other species of the genus. Values are expresses in % dry weight.

Species name	D- mannitol	Alginic acid	Sulfated polysaccharides	Natural polysaccharides	References
L. searlesiana Asensi & Reviers	14.4	11.0	2.9	nd	Leal *et al.* 2008
L. nigrescens Bory	4.3	12.5	4.3	nd	Percival *et al.* 1983
L. trabeculata Villouta & Santelices	3.4	19.7	0.3	<0.2	Matsuhiro & Zambrano 1990
L. flavicans Bory	0.5	17.0	2.9	nd	Chandía *et al.* 2005
L. flavicans corrugate morphotype	3.2	15.9	3.2	nd	This study

Peru to Puerto Montt, southern Chile (~41°S) (Villouta & Santelices 1986). *Lessonia flavicans* and *L. searlesiana* co-occur in the southernmost tip of South America and the Falkland Islands, although their depth ranges do not overlap (*L. flavicans* occurs at 0.5-3 m, while *L. searlesiana* at 5-15 m) (Searles, 1978; Ramírez & Santelices 1991; Edding *et al.* 1994). These separate distribution ranges suggest that speciation in this genus is mostly linked to divergent selection based on ecological traits.

Several authors have studied *Lessonia* in southern Chile (e.g. Searles 1978; Villouta & Santelices 1986; Martin & Zuccarello 2012), however, further exploration and collections of *Lessonia* specimens are necessary, especially in the southernmost region of South America (archipelagic Magellanic region and the Cape Horn Biosphere Reserve) where macro-algal biodiversity is particularly diverse but poorly studied. During a 2007 expedition dedicated to study kelp diversity within the Magellanic region, a large monomorphic kelp population was noticed and registered in Navarino Island, close to Cape Horn, but could not be identified based on local floristic reports or monographic references (e.g. Searles 1978; Villouta & Santelices 1986; Asensi & De Reviers 2009). The specimen had typical *Lessonia* holdfast and thick, semi-rigid stipes, but the laminae were corrugated like *L. adamsiae* and *L. corrugata* in New Zealand, and yellowish like *Macrocystis*. Interestingly, Searles (1978) reported the existence of such a corrugate morphotype of *Lessonia* in the Ocasión Channel (54°37S, 71°42W), but no taxonomic classification was conducted since then. Further explorations, performed during ten years to achieve biodiversity and chemical analysis, helped to find other four sites colonized by the same morphotype, always observed in pure populations (i.e., populations with 100% of individuals with the same morphotype).

Several studies have showed genetic signatures of recent dispersal across vast oceanic distances (as among New Zealand and Chilean populations of macroalgae) of not buoyant species, presumably by attachment to floating substrata such as wood or buoyant macroalgae (Fraser *et al.* 2013; Boo *et al.* 2014). Most cases of such trans-oceanic dispersal from New Zealand to Chile seem to have occurred by the end of the last glacial period, as estimated from phylogeographic analyses (see Fraser 2016; Guillemin *et al.* 2016 for reviews on the subject). Therefore, one hypothesis regarding this morphotype is that it corresponds to an introduction of either *L. corrugata* or *L. adamsiae*, two species with similar frond morphology present in New Zealand. An alternative hypothesis is that the corrugate form is the result of a viable hybridization between

Lessonia and *Macrocystis*. Inter-genera hybridization seems easily achievable in Laminariales, at least in the laboratory (Druehl *et al.* 2005), while very few hybrids have been observed in nature, despite the co-occurrence of many kelp species in close parapatry (e.g. Tellier *et al.* 2011a). Because both *L. flavicans* and *L. searlesiana* are often in close contact with dense populations of *M. pyrifera* C.Agardh in the Magellanic region, there are opportunities for cross fertilization among gametophytes of each species. The existence of an intermediate phenotype between the two genera, combining blade characteristics of *Macrocystis* with stipe and holdfast characteristics of *Lessonia*, could result from such hybridization. Finally, there is a possibility that this morphotype corresponds to a different species, as it is the case in New Zealand for the two corrugated species *L. adamsiae* and *L. corrugata*. So far, intermediate morphology have not been observed and populations are either of dark smooth or yellowish corrugated blades.

The following objectives were addressed in the present study to determine the origin and taxonomic status of this *Lessonia* morphotype: 1) describe the morphology and anatomy of this morphotype of the South-American marine flora; 2) to test whether it corresponds to an introduction of either *L. corrugata* or *L. adamsiae* from New Zealand; 3) to test whether the corrugate, *Macrocystis*-like blades where the result of hybridization between the two genera; and 4) to examine the phylogenetic position and range distribution of this *Lessonia* morphotype not seen before.

MATERIAL AND METHODS

FIELD COLLECTIONS AND PREPARATION OF SAMPLES

A total of 179 specimens of corrugate morphotype *Lessonia*, *L. flavicans* and *L. searlesiana* were sampled during years 2007-2013 in 16 subtidal locations in the Magellanic region (i.e., Beagle Channel, Strait of Magellan, and Diego Ramirez archipelago) and the Falkland Islands, with 30-40 individuals collected per location (Fig. 1; Table 1). Samples of *L. trabeculata* (three individuals) were also collected in Coquimbo, northern Chile, to be used as outgourp in phylogenetic analyses (see below). The corrugated morphotype was found in three sites at Navarino Island: Cormoran Bay (54°53'53.21"S, 67°58'47.94"W), Paula Cove (54°56'11.18"S, 67°41'21.32"W) along the Beagle Channel, Puerto Toro (55°4'27.50"S, 67°4'27.36"W), and two sites at Hoste Islands: Orange bay (55°30'57.24"S, 68°3'41.04"W) and Tekenika Bay (55°19'35.40"S, 68°23'49.56"W), in the

HOLOTYPE
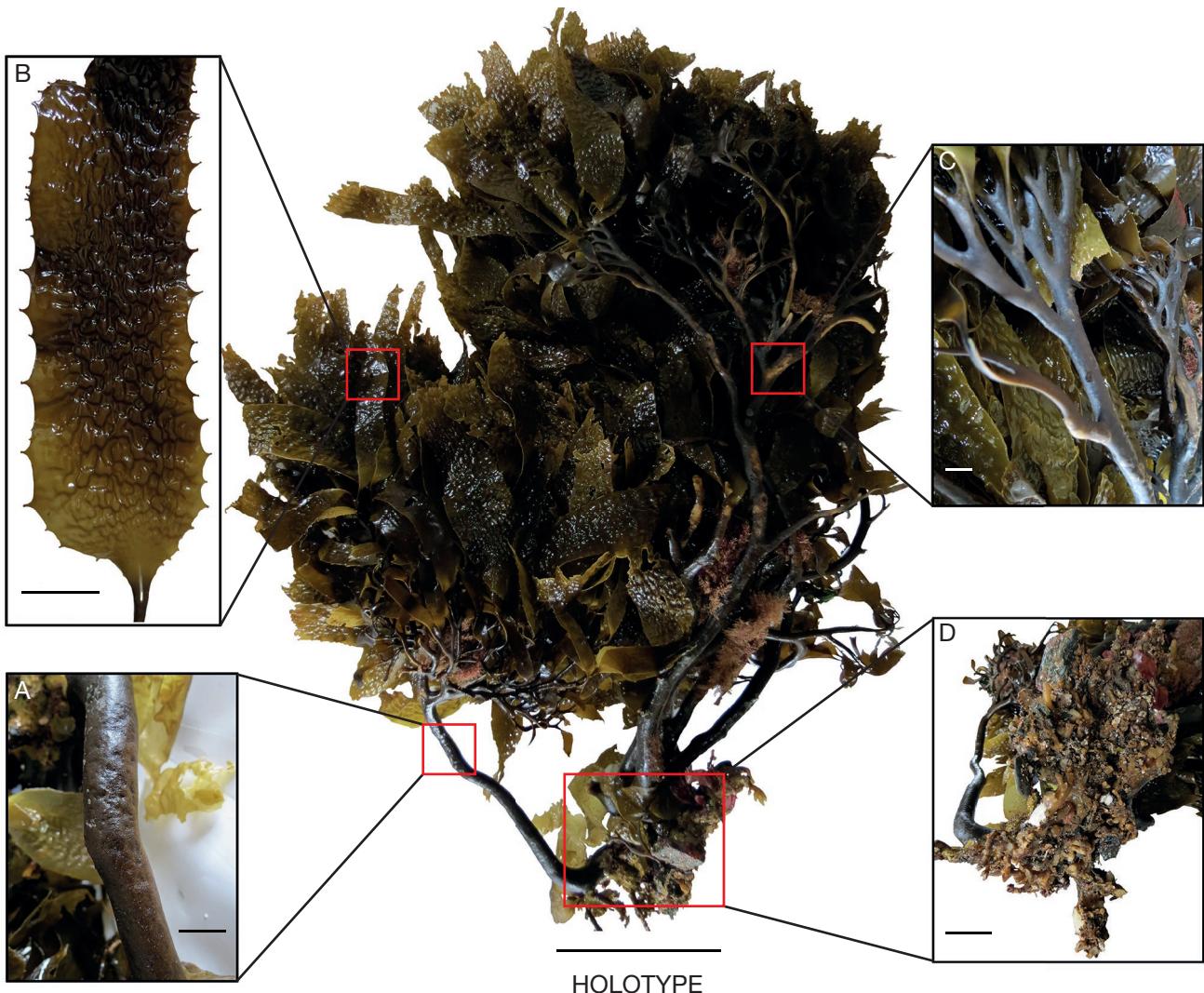
FIG. 2. — **A**, External morphology of the corrugate *Lessonia* Bory showing the brownish terete stipe; **B**, corrugated blades; **C**, that arise from the base of the blade and from dichotomously divided branches; **D**, the holdfast is rhizoidal in shape and composed mainly of fused haptera. Scale bars: holotype, 30 cm; A, C, 2 cm; B, 5 cm; D, 8 cm.

vicinity of Cape Horn (Fig. 1). Collection sites forms rocky terraces of rather steep slope that remain completely immersed during high tide (Pisano 1980). Seasonal fluctuation of temperature along Beagle Channel ranges from 10.5°C, 5.3°C, 2.5°C y 5.2°C in summer, autumn, winter, and spring, respectively (Santana *et al.* 2006), while the prevailing wind speeds range between 0 (as minimum value) and 19.8 ms^{-1}, occasionally reaching up to 27.5 m.s^{-1} (Santana *et al.* 2010). The photoperiod and irradiance during the austral winter (June – August) average 8 hours of light with a surface solar radiation of 22-23 Wm^{-2}, but it reaches 14 hours of light with solar radiation of 164-165 Wm^{-2} during austral summer (CEAZA-MET 2010, www.ceazamet.cl). The degree of wave exposure, presence of rivers or glaciers or steepness of the coast were determined qualitatively by simple observations. Individuals were randomly sampled along a 100-150 m transect by SCUBA diving from depth between 2 and 18 m. Each sample consisted of basal fragments from young and free of epiphytes blades. Eight to ten samples were immedi-

ately fixed in 4% formalin in seawater for later morphological examination, and 25-30 tissue samples (1-2 cm^2 each from different individuals) were dry preserved with silica gel beads in separate bags for later molecular analyses. Stipes and fronds were sectioned with razor blade and mounted in 70% glycerin. Quantitative characters, derived from the microscopic observations, represent average values with standard deviations obtained from approximately 25 measurements. Photographs were taken with a DP-71 camera (Olympus, Tokyo, Japan) attached to a BX-51 microscope (Olympus, Tokyo, Japan).

Voucher specimens are housed at the herbariums of the Laboratorio de Macroalgas Antárticas y Subantárticas, of the University of Magallanes, Punta Arenas, Chile, and of Department of Biology, Chungnam National University, Daejeon, Korea.

MOLECULAR ANALYSES
The phylogenetic analyses included 45 individuals of corrugate *Lessonia*, 50 *L. flavicans* (i.e., smooth blades)

TABLE 2. — DNA polymorphism indices of each population at the locus ITS1. Abbreviations: **n**, numbers of individual; **h**, number of haplotypes; **HD**, haplotype diversity; **k**, mean pairwise nucleotide difference; **π**, nucleotide diversity.

Species / Location	n	h	HD	k	π
Corrugate *L. flavicans*	45	4	0.13	0.178	0.0006
Typical *L. flavicans* Magellanic Region	50	4	0.16	0.326	0.00116
Typical *L. flavicans* Falkland Islands	29	14	0.894	4.419	0.01595
L. searlesiana Magellanic Region	50	24	0.859	5.807	0.01975
L. searlesiana Falkland Islands	5	3	0.7	3	0.0102

from the Magellanic region and 29 *L. flavicans* from Falkland Islands, 50 *L. searlesiana* from the Magellanic region, and 5 of *L. searlesiana* from the Falkland Islands (Table 1; Appendix 1). DNA extraction of *Lessonia* specimens followed Boo *et al.* (2011). PCR amplification of internal transcribed spacer 1 (ITS1) followed Yoon *et al.* (2001). Specific primers atp8SpF (5'-TGT ACG TTT CAT ATT ACC TTC TTT AGC-3') and atp8SpR (5'-TAG CAA ACC AAG GCT TTC AAC-3') were designed for the locus *atp*8-*trn*S spacer. Primers C1F101 (5'-CCA CHA AYC AYA AAG ATA TWG G-3') and C1R942 (5'-CCH CAN GTA AAC ATR TGR TGA GCC-3') were designed for cytochrome oxidase subunit 1 (*cox*1). The locus cytochrome oxidase subunit 3 (*cox*3) was amplified with primers C3F34 and R20 (Boo *et al.*, 2011). Sequencing of purified PCR products followed Boo *et al.* (2011) and was performed by Genotech (Daejeon, Korea). Each electropherogram output from each sample was edited using CHROMAS v.1.45 (Conor McCarthy, Australia). New generated sequences were deposited in GenBank (Table 1).

DATA ANALYSES

Phylogenetic relationships among *Lessonia* species and *Macrocystis pyrifera* were reconstructed using Bayesian Inference (BI) and Maximum Likelihood (ML) with two datasets of combined sequences, 1: ITS1 + *atp*8-*trn*S spacer, which included the corrugated morphotype of *Lessonia*, *L. flavicans*, *L. searleasiana*, *L. trabeculata*, *L. corrugate*, *L. adamsiae*, and *M. pyrifera*; and 2: *cox*1 + *cox*3, which included the corrugated morphotype of *Lessonia*, *L. flavicans*, *L. searleasiana*, *L. trabeculata*, and *M. pyrifera* (Table 1). The most appropriate model of evolution for the different partitions in each dataset was determined with the program PARTITIONFINDER2 (Lanfear *et al.* 2016) using a ML search conducted with PHYML (Guindon *et al.* 2010) and a "greedy" algorithm (Lanfear *et al.* 2012). The best partition schemes and their associated model of evolution were then integrated to the prior settings in the program MrBayes v.3.2.1 (Ronquist *et al.* 2012) for BI. Metropolis-coupled Markov Chain Monte Carlo (MC3) priors were 106 generations of two independent runs, two chains, sampling trees every 1000 generations. The burn-in period was identified by tracking the stability of highest likelihood values at each generation to determine whether they reached a plateau.

ML analyses were conducted with the program MEGA version 6 (Tamura *et al.* 2013) using models of evolution selected by MrAIC (Nylander 2004); Hasegawa-Kishino-Yano model (Hasegawa *et al.* 1985) with a gamma distribution (HKY + G), and Tamura-Nei (Tamura & Nei 1993) with a proportion of invariant sites (TN93 + I), respectively for *cox*1 + *cox*3 and ITS1 + *atp*8-*trn*S spacer combined datasets. Statistical support for each branch was obtained by 1.000 bootstrap.

The median-joining (MJ) network of ITS1 haplotypes from the Sub-Antarctic region was built using PopART (http://popart.otago.ac.nz; Bandelt *et al.* 1999). The DNA polymorphism of five groups (1, corrugate morphotype of *Lessonia* sp.; 2, typical *L. flavicans* from Magellanic region; 3, typical *L. flavicans* from Falkland Islands; 4, *L. searlesiana* from Magellanic region; and 5, *L. searlesiana* from Falkland Islands) was analyzed calculating the number of haplotype (h), the haplotype diversity (HD), the mean number of differences between pairs of sequences (κ), the nucleotide diversity (π) using DnaSP program (Librado & Rozas 2009). The differentiation among the five groups was estimated with the -analogues (Excoffier *et al.*, 1992) of Wright's (1965) -statistics, by using the software ARLEQUIN 3.0 (Excoffier *et al.* 2005). The null distribution of pairwise ST values under the hypothesis of no difference among populations was obtained by permutating haplotypes between populations. The P-value given by the test is the proportion of ST pseudo-values generated by random permutations that are larger than or equal to the observed value. The exact test of sample differentiation (Raymond & Rousset 1995) was run (with 20 000 Markov chain, 1000 dememorization steps) using this same software based on the haplotype frequency in the groups.

RESULTS

DISTRIBUTION RANGE

The distribution of the corrugate morphotype of *Lessonia* sp. was restricted to the southernmost part of the Magellanic region, on Navarino Island and Host Island (north and west of the Cape Horn, respectively) (Fig.1). It was absent from all sites visited in the western part of the Beagle Channel, and north of the Cockburn Channel, including the Strait of Magellan where only *Lessonia* species with typical smooth blades are present. The new morphotype of *Lessonia* was always present in dense monomorphic populations. Both *L. flavicans* and corrugate *Lessonia* morphotype were found reproductive at the same time of the year. In each site, populations of corrugate individuals formed a belt *c.* 5 m in width from the low-intertidal (e.g. 3 to 5 m depth at high tide) and always above populations of *Macrocystis pyrifera*.

MORPHOLOGICAL CHARACTERIZATION

The sporophytes of the corrugate morphotype are dendroid in shape (Fig. 2). Stipes are erect, brownish in color and consist of 3-12 terete (Fig. 2A). Blades are linear, lanceolate, 3.8-7.2 cm wide, with dentate margins and light brown to yellowish in color. Surface of blades is corrugated (Fig. 2B)
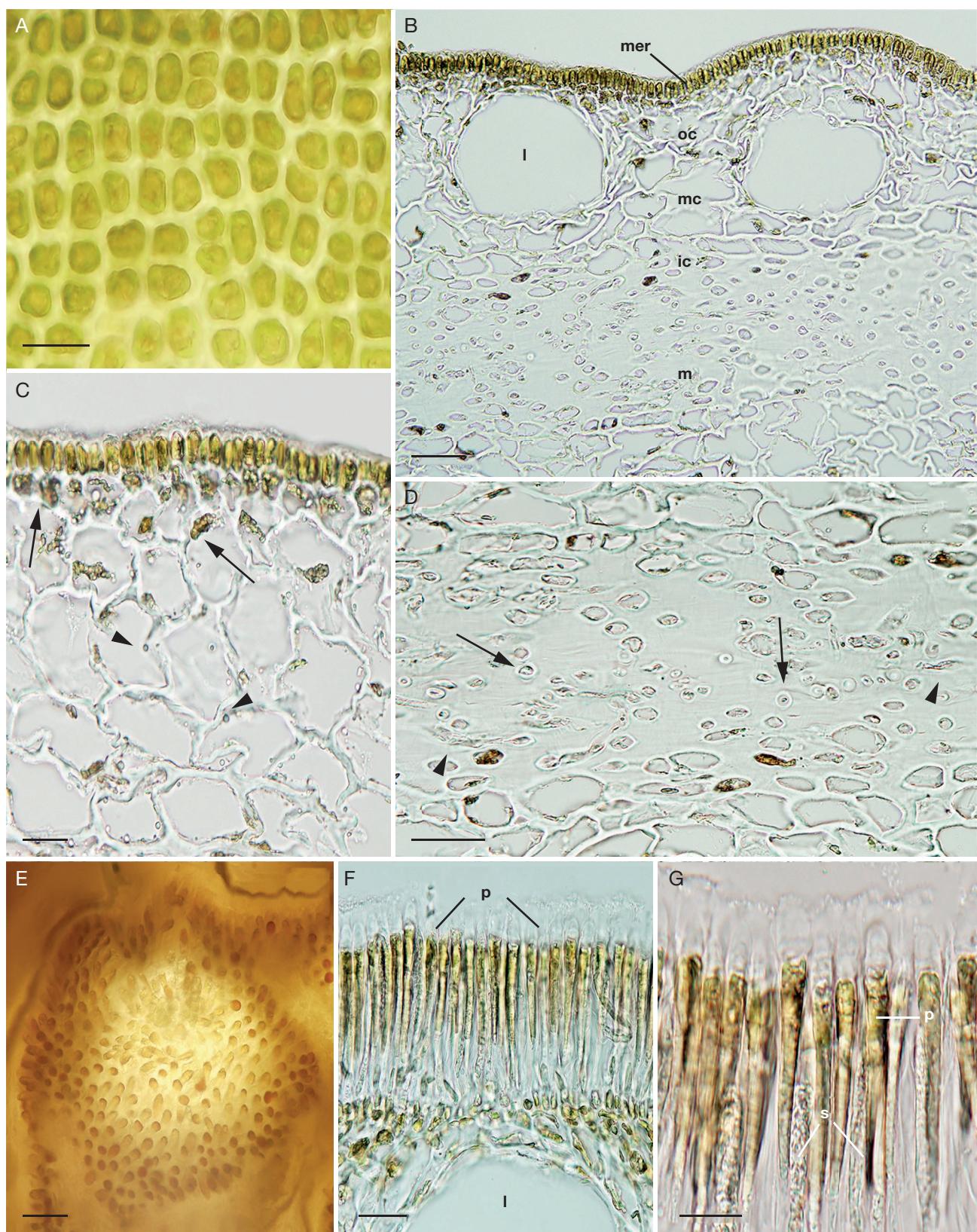
FIG. 3. — Internal morphology of the corrugate *Lessonia* Bory: **A**, small angular meristodermal cells on the surface of blade; **B**, transverse section through a sterile portion of the blade; **C**, detail of meristoderm and cortex showing cells with full (**right arrow**) and reduced (**left arrow**) protoplast and golden bodies in outer cortex and mid cortex; and rounded light refracting bodies (**arrowheads**); **D**, detail of medulla showing cylindrical cells (**arrow**) and some elongated filaments (arrowhead) immersed in a dense intercellular matrix; **E**, surface view of a sorus; **F**, transverse section through a sorus; **G**, detail of sorus . Abbreviations: **mer**, meristoderm; **oc**, outer cortex; **mc**, mid cortex; **ic**, inner cortex; **m**, medulla; **p**, paraphyses; **l**, part of the lacuna; **s**, sporangia. Scale bars: A, 15 μm; B, 50 μm; C, G, 20 μm; D, E, 40 μm; F, 30 μm.
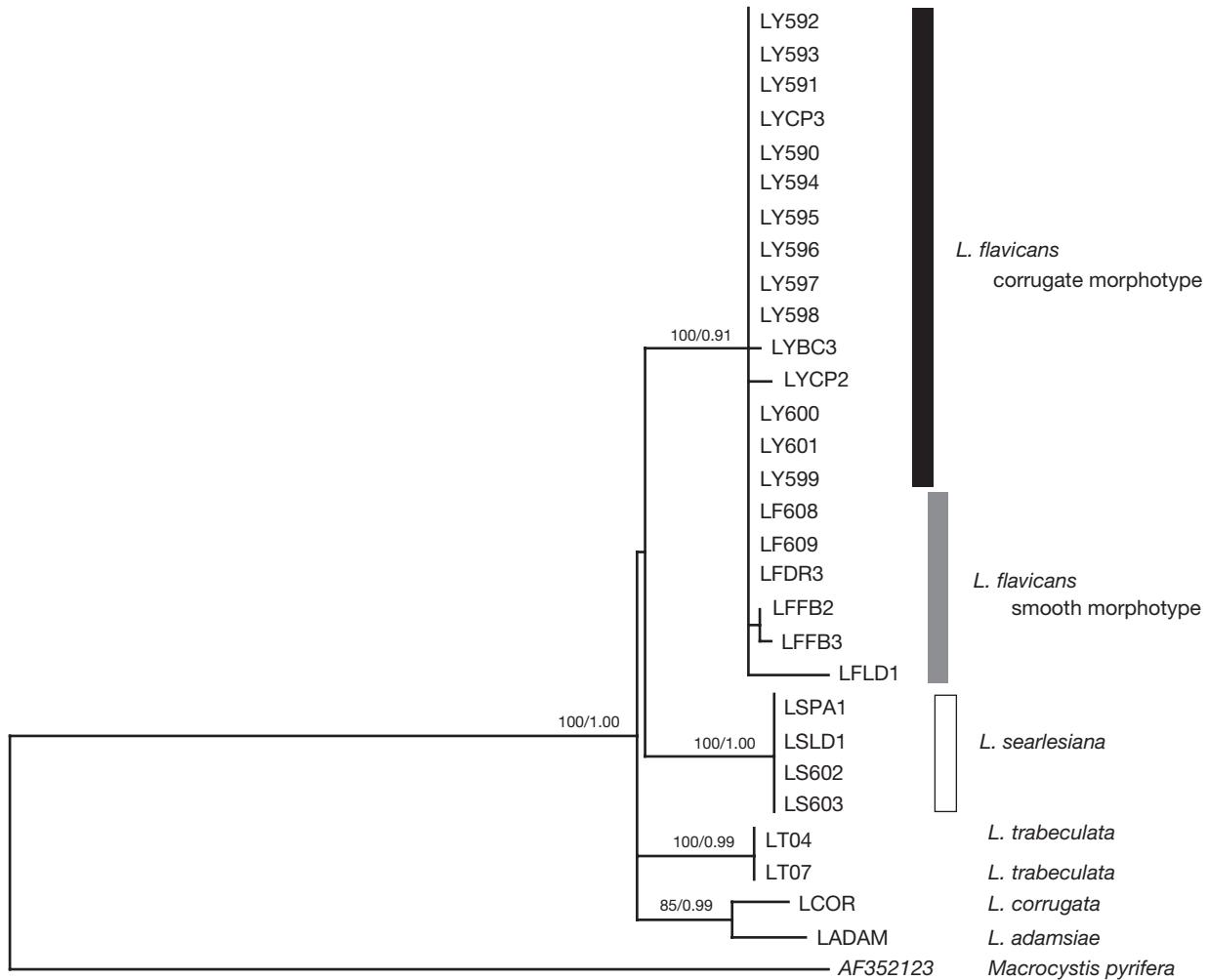
FIG. 4. — Phylogenetic tree based on concatenated ITS1 and *atp8-trn*S partial sequences (418 bp). Note that corrugated *Lessonia* Bory specimens are grouped with *Lessonia flavicans* Bory specimens which have smooth blade surface. First number on the branches refers to the bootstrap value determined from the ML phylogeny and the second is the posterior probability from the BI analysis. Scale bar: 0.05 substitution per site.

and these corrugations are irregular, similar to *M. pyrifera* and different from *L. corrugata* or *L. adamsiae* which have regular furrows more or less parallel to the axis of the mature blade. Opening angles of blades at the basal portion are variable, ranging from 30° to 56°. New blades are produced from a median longitudinal fissure that arises from the base of the blade and from dichotomously divided branches (three-eight times) (Fig. 2C). Sori are oval to irregular and occur within the proximal ⅔ of the blade. Holdfasts are 6-35 cm wide and 1-8 cm high, rhizoidal in shape when sporophytes are less than 40 cm in length. In larger individuals, the holdfast is composed of fused haptera in their superior portion and unfused and freely branching at the base. Haptera are generally 1-2 cm width (Fig. 2D).

Transverse sections revealed characteristics consistent with the genus *Lessonia*. The vegetative portions of blades are 596.9 ± 97.6 μm thick (all numbers reported as mean ± SE). Superficially, the meristoderm is composed of small angular cells, 4.12 ± 0.58 μm long by 4.05 ± 0.63 μm wide, which are heavily pigmented (Fig. 3A). Transversely, the meristoderm shows a single layer of pigmented and thick-walled palisade cells, 10.7 ± 1.3 μm high by 5.0 ± 0.7 μm wide (Fig. 3B). The cortex consists of three layers packed into a nine cells-thick wall. The outer cortex forms one (rarely two) layer of polygonal cells with an overall average width of 12.5 ± 3.1 μm. Most cortical cells have a protoplast with golden physode bodies, producing brownish blotches (Fig. 3C). However, these bodies are not restricted to outer cells. The mid cortex is 2-4 cells thick, 38.3 ± 11.9 μm in width and its polygonal cells, which increase gradually in size inward and lack plastids. But some cells contain a reduced protoplast and physode bodies (Fig. 3C). Additionally, the majority of mid cortex cells (as well as some outer cortical cells) contain small and rounded light refracting physodes associated with the cell wall (Fig. 3C). The inner cortex is composed of 1-2 layers of rectangular cells, 29.0 ± 9.3 μm thick, becoming smaller medially, and contain no protoplast (Fig. 3D).
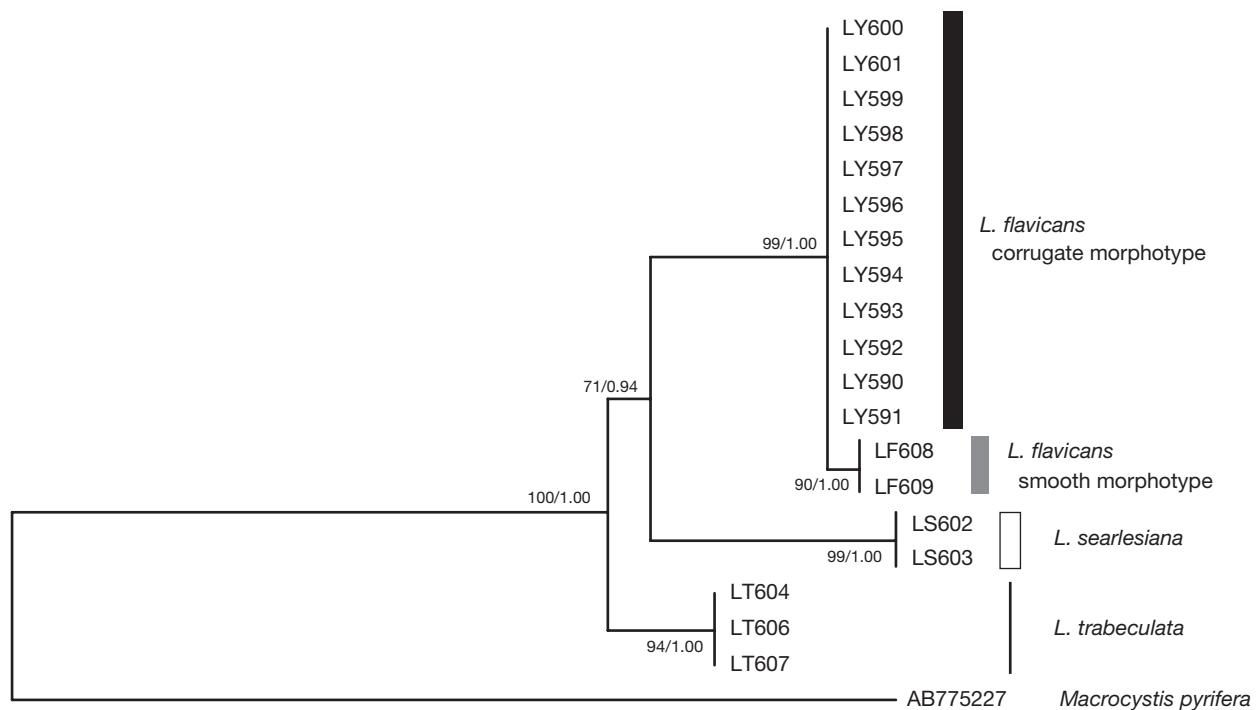
FIG. 5. — Phylogenetic tree based on concatenated *cox*1 and *cox*3 partial sequences (1176 bp). Note that corrugated *Lessonia* Bory specimens are grouped with *Lessonia flavicans* Bory specimens which have smooth blade surface. First number on the branches refers to the bootstrap value determined from the ML phylogeny and the second is the posterior probability from the BI analysis. Scale bar: 0.01 substitution per site.
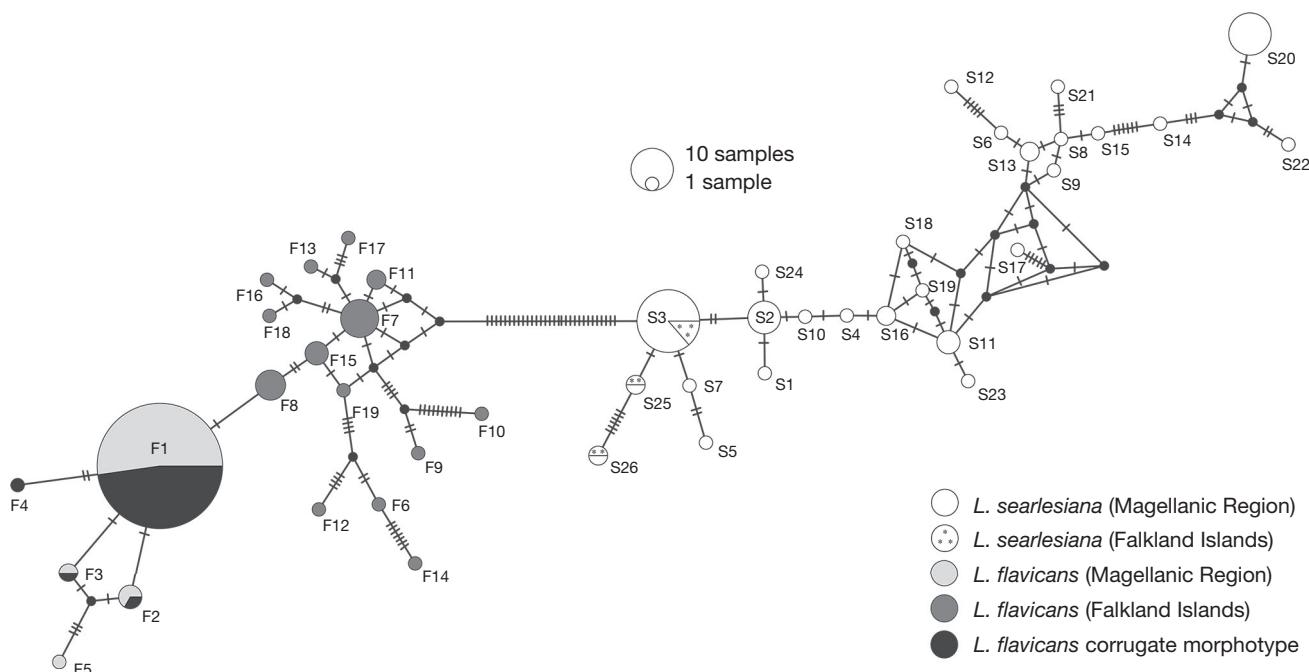
FIG. 6. — MJ network of ITS1 haplotypes of Magellanic *Lessonia* Bory, spp. individuals.

Table 3. — Genetic differentiation between pairs of populations at ITS1. Pairwise φ_{ST} values and their associated P-value.

Pairwise φ_{ST}\φ_{ST} P-value	Corrugate *L. flavicans*	Typical *L. flavicans* Magellanic Region	Typical *L. flavicans* Falkland Islands	*L. searlesiana* Magellanic Region	*L. searlesiana* Falkland Islands
Corrugate *L. flavicans*	–	0.613	<0.001	<0.001	<0.001
L. flavicans Magellanic Region	0	–	<0.001	<0.001	<0.001
L. flavicans Falkland Islands	0.500	0.508	–	<0.001	<0.001
L. searlesiana Magellanic Region	0.804	0.809	0.653	–	0.199
L. searlesiana Falkland Islands	0.961	0.956	0.655	0.034	–

Within the cortex, circular or oval mucilage-filled cavities (lacunae), with 171.03 ± 41.7 μm height by 202.4 ± 47.3 μm width, are spaced at intervals of 1-7 cortical cells (Fig. 3B). Lacunae occur equally on both sides of the blade and are bounded by compressed and thick-walled cortical cells. They have no multicellular lining and do not contain any internal projections or multicellular trabeculae. The medulla is composed of a dense intercellular matrix containing small cylindrical cells and a few non-pigmented, elongated filaments that run tangentially and longitudinally. The overall thickness of the medulla is 86 ± 8.7 μm.

Sporangial sori are formed on one side of the blade, are irregularly shaped and develop from the base to middle part of blades (Fig. 3E-G). The sori consist of elongated and club-shaped paraphyses that form a layer projecting above smaller and clavate sporangia (Fig. 3F, G). Paraphyses are 65.7±15.9 μm in length with bloated tips 8.5 ± 1.2 μm wide. Mature unilocular sporangia are 47.1 ± 6.3 μm long and 8.9 ± 1.3 μm wide (Fig. 3G).

MOLECULAR ANALYSES

The phylogenetic trees of combined datasets (ITS + *atp*8-*trn*S = 418 bp and *cox*1 + *cox*3 = 1176 bp) revealed that the corrugate morphotype of *Lessonia* grouped into a well-supported monophyletic clade with *L. flavicans* (Figs 4; 5), clearly separated from other *Lessonia* species. In these phylogenetic trees, the species *L. searlesiana* and *L. trabeculata* formed distinct and well-supported clades. The sequences of *Macrocystis pyrifera* in both analyses of combined datasets appeared as outgroup of the *Lessonia* clade. In the analysis of the combined dataset of ITS + *atp*8-*trn*S, the relationships between *Lessonia* species are unresolved, but all species are monophyletic clades, with the corrugate morphotype belonging to the *L. flavicans* clade.

The MJ network recovered four haplotypes of the corrugate *Lessonia* among the 45 sequences of ITS1 nuclear locus, three of which were shared with typical *L. flavicans* from Magellanic region (one of which was the dominant; 91%) and one haplotype appeared as singleton (Fig. 6). Populations of *L. flavicans* from the Magellanic region and the Falkland Islands did not share haplotypes and the polymorphism of the latter was much higher (see DNA polymorphism indices, Table 2). The exact test of global genetic differentiation at the ITS1 locus was significant (P <0.000001; 6000 markov steps). Pairwise φ_{ST} values revealed a significant genetic differentiation among the five populations, except between the corrugate and the typical *L. flavicans*, and as well as between *L. searlesiana* from Magellanic region and Falkland Islands (Table 3).

DISCUSSION

This study arose from field collections of *Lessonia* populations of a peculiar morphology in the southern part of the Magellan region. The presence of corrugate fronds in the genus *Lessonia* is known for two species of the South-West Pacific, namely *L. adamsiae* from New Zealand (Lucas 1931; Hay 1987; Womersley 1987) and *L. corrugata* from Tasmania (Womersley 1987). However, their corrugation is much more regular than what was observed in the Magellanic specimens. The genetic analyses of the present study, based on the nuclear genes ITS and *atp*8-*trn*S, confirmed that the corrugate morphotype in the Magellanic region does not correspond to an introduction of any of these New Zealand and Tasmanian species, as it formed a monophyletic clade with *L. flavicans* sequences, clearly divergent from *L. adamsiae* and *L. corrugata* clades. *Lessonia* specimens with corrugate laminae have been reported in the region by Searles (1978), who considered the possibility of a hybrid between *Macrocystis pyrifera* and *L. searlesiana* or *L. nigrescens*. He however noticed, as we do, that the internal anatomy is mostly characteristic of *L. flavicans*, including the presence of lacunae and the absence of *Macrocystis* features such as specialized phloem tissue and interconnected mucilage ducts. Our genetic analyses further confirm that the corrugate laminae are not the result of hybridization with *M. pyrifera*, as both nuclear and mitochondrial markers showed that each species belongs to different and highly divergent lineages.

Morphologically, the corrugate morphotype shares many features with *L. flavicans*, including abundant mucilage cavities in the cortex, blades with dentate margins, short cylindrical stipes, digitate holdfasts, and same reproductive periods. However, reproductive sori were found only on one side and in the proximal part of the blades, whereas they differentiate on both sides in *L. flavicans*.

Overall, these results suggest two possible origins of the corrugate morphotype: phenotypic plasticity due to environmental conditions in the sites where it is observed, or some recent or on-going speciation process from *L. flavicans*. There is no reported plasticity in any *Lessonia* species regarding blade surface, and so far, species are described either as smooth or corrugate along their entire range distribution. The only case of blade surface polymorphism is known from *Macrocystis laevis* (Hay 1986), which has smooth blades instead of the usually corrugate form in other *Macrocystis* taxa. *Macrocystis laevis* is however no longer recognized as a species but as a morphological variant of *M. pyrifera* (Demes *et al.* 2009; Macaya & Zuccarello 2010a). The *M. laevis*-type of individuals is rare

and generally reported as isolated individuals. No habitat or region is known to host populations of this *Macrocystis* morph, suggesting that it might correspond to abnormal development, possibly resulting from some mutational disorder. The corrugate morphotype of *Lessonia* is found restricted to the southernmost part of the region of Magellan, essentially south of the Beagle Channel (Fig. 1). However, it is not exclusive of this region as populations of typical *L. flavicans* are also observed, sometimes in close vicinity with small populations of corrugate *Lessonia* (less than 10 km in Navarino Island) but not intermixed (AM, JO, SR and SF, personal observations). Our observation so far could not detect differences in micro-habitat between typical *L. flavicans* and the corrugate morphotype, such as wave exposure, presence of rivers or glaciers or steepness of the coast, among other environmental factors. Neither there was intermediate forms of the corrugation, which could have been expected if trait expression (i.e., "smoothness") was correlated the environment. Blades were either smooth, with no sign of any relief on their surface, or clearly corrugated, with sulcus of regular depth all along the blade. Thus, there is no support for a plastic expression of blade surface morphology.

Corrugate yellowish laminae is the exclusive morphological trait that distinguishes this morphotype of *Lessonia* with 100% identification accuracy, with 100% of the individuals belonging to this type. Exclusion of sister species is common during the speciation process, especially when reproductive barriers are evolving (Coyne & Orr 2004). Such reproductive barriers have been reported specifically in the contact region between *L. spicata* and *L. berteroana*, leading to complete exclusion at the local scale (Tellier *et al.* 2009, 2011a). In this case, a strong selection against hybrid formation is acting in the region where both species are found in strict parapatry, as evidenced by changes in sex ration (Oppliger *et al.* 2011) and increased asexual reproduction (Oppliger *et al.* 2012), as well as the presence of mitochondrial heteroplasmy (Tellier *et al.* 2011b) specifically in the region of parapatry. This kind of speciation process, often called "budding speciation" (Gottlieb 2004), occurs when sister species diverge from each other via small, locally isolated peripheral populations. It is expected to generate a unique signature that, early in the speciation process, should consist in overlapping or adjacent ranges of sister species with very different sizes (i.e., asymmetric ranges) and different realized niche breadths (Funk & Omland 2003; Gottlieb 2004; Grossenbacher *et al.* 2014; Anacker & Strauss 2014). The observation of a smaller range in recently diverged species in such peripatric speciation is common in plants (see reviews from Grossenbacher *et al.* 2014 and Anacker & Strauss 2014). The restricted distribution in small and isolated populations of the corrugate morphotype is clearly supporting a scenario of budding speciation in *Lessonia* of the Beagle Channel. However, possible ecological differentiation and reproductive isolation between the corrugate and typical *L. flavicans* morphotypes have yet to be characterized. However, the fully segregated distributions of both entities and restricted range to southern part of the archipelago strongly suggest that an incipient speciation process is occurring in the Magellanic region.

Is the corrugate versus smooth blade surface a diagnostic character? We must consider the possibility that it is an evolutionarily convergent phenotype as it seems to have previously arisen at least once in the genus. According to the phylogeny of *Lessonia*, *L. adamsiae* and *L. corrugata* form a clade of corrugate species that have diverged from *L. brevifolia* about 2.8 million years ago, and then split in two new clades 2 million years ago (Martin & Zuccarello 2012). Therefore, the evolution of a corrugate form associated with speciation occurred somewhere in between these two events. The evolution of blade surface texture in the *L. flavicans* group seems to have evolved much more recently, as genetic divergence is not observed yet between morphotypes. Moreover, it is apparently associated with the evolution of other traits, such as the position of the sori on the blades.

The present study also highlighted a significant genetic divergence between populations of *L. flavicans* from the Magellanic region and the Falkland Islands. Such differentiation has been observed for other seaweeds in the Chilean sub-Antarctic ecoregion, including *M. pyrifera* (Macaya & Zuccarello 2010b) and *Gigartina skottsbergii* (Billard *et al.* 2015). It was also reported for invertebrates (e.g. the isopod *Serolis paradoxa* Fabricius; Leese *et al.* 2008; the limpet *Nacella mytilina* Helbling; González-Wevar *et al.* 2016), fishes (e.g. the Patagonian Toothfish; Rogers *et al.* 2006) or marine mammals (e.g. the sea lion *Otaria flavescens* Shaw; Hoffman *et al.* 2016). In addition, considerable genetic diversity (e.g. populations of both *Bostrychia intricata* Montagne and *Adenocystis utricularis* (Bory) Skottsberg) was observed in high-latitude subantarctic islands and Chilean fiordland, regions that are thought to have been affected by ice at the Last Glacial Maximum (Fraser *et al.* 2013). In contrast, other examples report an absence of genetic differentiation (e.g. Rocha-Olivares *et al.* 1999; González-Wevar *et al.* 2013) and conclude a high connectivity between the peri-Antarctic islands and the continent. The scenario might be a combination of past connectivity (i.e., during glacial periods or for post-glacial recolonization from glacial refugia) and present isolation, as suggested by the presence of private alleles in the Falkland Islands even when a common ancestral allele is shared among the different regions (see for example Billard *et al.* 2015). So far, the corrugate morphotype of *Lessonia* was not detected in the Falkland Islands, and strictly belongs to the Patagonian sub-clade of *L. flavicans*. This further supports the idea that it is not an ecotype that evolves locally from typical smooth morphotypes, but more likely a new taxon that is colonizing the southernmost Patagonia, but not (yet) the Falkland Islands.

In conclusion, the corrugate morphotype of *Lessonia* observed in some monomorphic populations of the southernmost part of the Magellanic region is clearly distinguishable from every other *Lessonia* species, either for those found in the region or in New Zealand and Tasmania (*L. adamsiae* and *L. corrugata*) which also have corrugated blades. No genetic divergence from *L. flavicans* has been detected with the nuclear and mitochondrial markers used in this study. The absence of plasticity of blade surface's texture in the *Lessonia* genus, and the clear spatial segregation and restricted geographic range distribution of the corrugate morphotype suggest a recent evolutionary divergence.

Further analyses are required to determine gene flow and whether this phylogeographic contrast reflects differing dispersal abilities (e.g. rafting ability, dissimilar buoyancy of gametes, etc.) or some other ecological factor, and test for eventual reproductive barriers between the between corrugated and typical *L. flavicans*.

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APPENDIX

APPENDIX 1. — List of species used in the phylogenetic analyses, information on collection, and accession numbers in GenBank (sequences generated in the present study are shown in bold).

Species, Collection information	Sample	GenBank Accession N°			
		ITS1	Atp8S	COI	COIII
Macrocystis pyrifera Linnaeus	Macro	AF352123	HQ336482	AB775227	AB775244
Lessonia adamsiae Hay	Ladam	GU593799	KX023377	–	–
Lessonia corrugata Lucas	Lcor	AY857902	KX023382	–	–
Lessonia trabeculata Villouta & Santelices					
Northern Chile, Coquimbo	LT604	MK764611	MK764546	MK764573	MK764592
Northern Chile, Coquimbo	LT606	–	–	MK764574	MK764593
Northern Chile, Coquimbo	LT607	MK764612	MK764547	MK764575	MK764594
Lessonia flavicans corrugate morphotype					
Navarino I., Cormoran Bay	LYBCor3	MK764613	MK764548	–	–
Navarino I., Paula Cove	LYCPa2	MK764614	MK764549	–	–
Navarino I., Paula Cove	LYCPa3	MK764615	MK764550	–	–
Hoste I., Orange Bay	LY590	MK764616	MK764551	MK764576	MK764595
Hoste I., Orange Bay	LY591	MK764617	MK764552	MK764577	MK764596
Navarino I., Puerto Toro	LY592	MK764618	MK764553	MK764578	MK764597
Navarino I., Puerto Toro	LY593	MK764619	MK764554	MK764579	MK764598
Hoste I., Tekenika Bay	LY594	MK764620	MK764555	MK764580	MK764599
Hoste I., Tekenika Bay	LY595	MK764621	MK764556	MK764581	MK764600
Hoste I., Tekenika Bay	LY596	MK764622	MK764557	MK764582	MK764601
Hoste I., Tekenika Bay	LY597	MK764623	MK764558	MK764583	MK764602
Hoste I., Tekenika Bay	LY598	MK764624	MK764559	MK764584	MK764603
Hoste I., Tekenika Bay	LY599	MK764625	MK764560	MK764585	MK764604
Hoste I., Tekenika Bay	LY600	MK764626	MK764561	MK764586	MK764605
Hoste I., Tekenika Bay	LY601	MK764627	MK764562	MK764587	MK764606
Lessonia flavicans Bory					
Magellan strait, Fuerte Bulnes	LF608	MK764628	MK764563	MK764588	MK764607
Magellan strait, Fuerte Bulnes	LF609	MK764629	MK764564	MK764589	MK764608
Magellan strait, Fuerte Bulnes	LFFBuln2	MK764630	MK764565	–	–
Magellan strait, Fuerte Bulnes	LFFBuln3	MK764631	MK764566	–	–
Cape Horn, London I.	LFLdo1	MK764632	MK764567	–	–
Cape Horn, Diego Ramirez I.	LFDRami3	MK764633	MK764568	–	–
Lessonia searlesiana Asensi & De Reviers					
Magellan strait, Carlos III Island	LS602	MK764634	MK764569	MK764590	MK764609
Magellan strait, Carlos III Island	LS603	MK764635	MK764570	MK764591	MK764610
Cape Horn, London I.	LSLondo1	MK764636	MK764571	–	–
Cape Horn, Puerto Aguirre	LSPAgui1	MK764637	MK764572	–	–