A new record of kelp *Lessonia spicata* (Suhr) Santelices in the Sub-Antarctic Channels: implications for the conservation of the “huiro negro” in the Chilean coast

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The Katalalixar National Reserve (KNR) lies in an isolated marine protected area of Magellan Sub-Antarctic channels, which represent an important area for marine biodiversity and macroalgal conservation. The present study is the first report of the species *Lessonia spicata*, “huiro negro”, in the Magellan Sub-Antarctic channels. This finding has implications for macroalgal biogeography and conservation concerns in the Chilean coast. In the ecological assessments of the KNR in 2018 we found populations of *L. spicata*, specifically on rocky shores of Torpedo Island and Castillo Channel. The morphological identification and molecular phylogeny based on nuclear (ITS1) sequences revealed that these populations of *Lessonia* are within the lineage of *L. spicata* of central Chile. This report increases the species richness of kelps for the Magellan Sub-Antarctic Channels from 2 to 3 confirmed species (*L. flavicans*, *L. searlesiana* and *L. spicata*), and it also extends the southern distribution range of *L. spicata*. This species has high harvest demand which is moving towards southern Chile, thus these populations should be considered as essential for macroalgal conservation in high latitudes of South America.
A new record of kelp *Lessonia spicata* (Suhr) Santelices in the Sub-Antarctic Channels: implications for the conservation of the “huiro negro” in the Chilean coast

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Abstract

The Katalalixar National Reserve (KNR) lies in an isolated marine protected area of Magellan Sub-Antarctic channels, which represent an important area for marine biodiversity and macroalgal conservation. The present study is the first report of the species *Lessonia spicata*, “huiro negro”, in the Magellan Sub-Antarctic channels. This finding has implications for macroalgal biogeography and conservation concerns in the Chilean coast. In the ecological assessments of the KNR in 2018 we found populations of *L. spicata*, specifically on rocky shores of Torpedo Island and Castillo Channel. The morphological identification and molecular phylogeny based on nuclear (ITS1) sequences revealed that these populations of *Lessonia* are within the lineage of *L. spicata* of central Chile. This report increases the species richness of kelps for the Magellan Sub-Antarctic Channels from 2 to 3 confirmed species (*L. flavicans*, *L. searlesiana* and *L. spicata*), and it also extends the southern distribution range of *L. spicata*. This species has high harvest demand which is moving towards southern Chile, thus these populations should be considered as essential for macroalgal conservation in high latitudes of South America.
Introduction

*Lessonia* Bory (Laminariales, Phaeophyceae) is one of the most conspicuous brown macroalgal genera that inhabit the littoral to sublittoral zone of rocky coasts (~20 m depth) in temperate-cool waters of the South Pacific Ocean (Cho *et al.*, 2006; Martin & Zuccarello, 2012). There are currently records of 11 species of the genus *Lessonia* that are taxonomically accepted, distributed along the coasts of South America, New Zealand, Tasmania, and Sub-Antarctic islands (Cho *et al.*, 2006). These species have major ecological roles in the structure of benthic marine communities (Villouta & Santelices, 1984; Vásquez & Santelices, 1984), and are commercially exploited for the extraction of alginic acid (Steneck *et al.*, 2002). *Lessonia* species are one of the most characteristic and abundant macroalgae (12°S to 56°S) that inhabit the rocky shores of the Chilean coast (17° to 56°S) (Searles, 1978; Avila *et al.*, 1985; Villouta & Santelices, 1986; Vásquez *et al.*, 1998; Tellier *et al.*, 2011; Martin & Zucarello, 2012; Mansilla *et al.*, 2014). Currently, six species have been recorded in Chile: *Lessonia nigrescens* Bory, *Lessonia berteroana* Montagne, *Lessonia spicata* Suhr, *Lessonia trabeculata* Villouta & Santelices, *Lessonia searlesiana* Asensi & De Reviers and *Lessonia flavicans* Bory (Guiry & Guiry, 2018). A recent morphological and molecular analysis showed that the species distributed from Peru (17°) to Puerto Montt (41°), commonly known as *L. nigrescens*, is actually two cryptic species; the populations distributed from Peru (17°S) to central Chile (30°S) correspond to *L. berteroana* Montagne, and those occurring from central Chile (29°S) to Puerto Montt (41°S) correspond to *L. spicata* (Suhr) Santelices (González *et al.*, 2012; Vega, 2016). However, *L. nigrescens* is still a valid species, because no material of the referred species has been found near its type locality, Cape Horn.

The huiro negro kelps, which include *L. berteroana* and *L. spicata*, are heavily exploited and represent almost 70% of the kelp biomass landed annually (Vega *et al.*, 2014). This economic activity is mainly practiced in northern Chile (18-32°S), through a complex productive chain with high social impact and low added value (Vásquez, 2008). *L. berteroana* and *L. spicata* are exported as a natural commodity to more than 20 countries mainly due to their alginate, which has high economic value (Westermeier *et al.*, 2019). Thus populations of huiro negro have economic interest along Chilean coasts, being essential to generate a stewardship from a local and large scale.
The Katalalixar National Reserve (KNR) is a national reserve area created in 1983. KNR comprises 674,500 ha and is located in a remote zone next to the village of Tortel. This area includes a wilderness temperate rainforest with a complex ecosystem of islands and fjords (Bell et al., 2007). The offshore area (western side) of the Magellan Sub-Antarctic Channels is one of the few places of the Magellan Biogeographic Province (MBP) (43°-56°S; Camus, 2001) that has not been explored systematically by scientific expeditions (Gorny & Zapata, 2018) (Fig. 1a, b). KNR is located at the southern limit of the Humboldt Current System (HCS). The HCS is a key component of the general oceanic circulation in the eastern South Pacific, being one of the most productive marine ecosystems on the earth (Thiel et al., 2007). HCS is originated in southern Chile between 42-48°S and is characterized by a northward flow in front of South American coasts with a strong upwelling of cool nutrient-rich waters (Silva et al., 2009). The origin of the HCS induced a large-scale redistribution of biota, and nowadays plays a key role in the biogeography of the South Pacific (Camus, 2001). Thus KNR provides an enormous opportunity to understand the taxonomic composition and biogeography of macroalgae that inhabit the southern boundary of the Humboldt Current (Camus, 2001; Thiel et al., 2007).

The present study contributes the first report of the species L. spicata in the Magellan Sub-Antarctic Channels. The distribution of this was thought to be limited to 41°S, but appears to be extended south of the Golfo de Penas (46° 59'-47° 40'S). Continuing survey studies will be necessary to understand the occurrence patterns of populations of L. spicata in the MBP.

**Materials & Methods**

Three individuals of *Lessonia spicata* were collected in the intertidal zone of Torpedo Island and Castillo Channel (Fig. 1a, b) in July, 2018. The specimens were air-dried and then pressed on herbarium sheets for morphological observation and molecular analysis. The Chilean Hydrographic and Oceanographic Service of the Navy (N° 13270/24/337), approved field sampling.

External and internal morphological observations were made. The anatomical observations were performed by sectioning with a razor and staining with 1% aqueous aniline blue acidified with 1% diluted HCl, and mounted in 70% glycerin. Photomicrographs were taken with a Canon Powershot S5 IS camera attached to a BX 51 Olympus microscope (Canon USA, Melville, New York, USA; Olympus Corp., Tokyo, Japan, respectively). A total of 15 replicates from the three
individuals were selected for measurement of cortical cell diameter following Gonzalez et al., (2012); means and standard deviations were calculated. Samples of other species occurring in the Sub-Antarctic region (*L. flavigans* and *L. searlesiana*) were also analyzed for comparative purposes. Voucher specimens were deposited in the herbarium of University of Magallanes, Punta Arenas, Chile.

**Molecular analyses**

Genomic DNA was extracted from ~ 5 mg of dried thallus ground in liquid nitrogen using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s protocol. The PCR primers for the ITS were ITSP1-ITS Ri (Tai et al., 2001; Martin & Zucarello, 2012) and KP5- KG4 (Lane et al., 2006). Polymerase chain reaction products were purified using a NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) and commercially sequenced (Macrogen, Seoul, South Korea). The electropherograms were edited using the Chromas v1.45 software (McCarthy, 1998) and the new generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/).

A total of 34 ITS sequences (731bp) were included in the construction of the phylogeny: 31 sequences belonging to the genus *Lessonia* and three outgroups, *Cymathaire triplicata* (Postels & Ruprecht) J. Agardh, *Ecklonia cava* Kjellman and *Macrocystis pyrifera* (Linnaeus) C. Agardh (Table 1). Sequences were aligned using the MUSCLE algorithm in MEGA5 v.6.06 software using the default settings (Tamura et al., 2013).

The phylogenetic analysis was constructed using Maximum likelihood (ML) and Bayesian inference (BI) analyses. The program PartitionFinder (Lanfear et al., 2012) were used to choose the best-fitting nucleotide substitution model under the Bayesian Information Criterion. The general time reversible nucleotide substitution model with a gamma distribution and a proportion of invariable sites (GTR + Γ+ I) was selected as the best substitution model. ML analysis was performed with the RAxML HPC-AVX program (Stamatakis, 2014) implemented in the raxmlGUI 1.3.1 interface (Silvestro & Michalak, 2012) with the statistical support obtained by 1000 bootstrap replications. BI was performed with the MrBayes v. 3.2.5 software (Ronquist et al., 2012) using Metropolis-coupled Markov Chain Monte Carlo (MC³). The inference of Bayesian posterior probability (BPP) was inferred following Calderon & Boo (2017).
The neighbor joining analysis was performed in MEGA5 v.6.06 with the default settings software, using 1000 bootstrap replicates.

**Results**

This is the first confirmed report of *L. spicata* in the Sub-Antarctic region, extending its distribution to the south by seven degrees of latitude (Fig. 2a). The sporophytes collected in the two localities have cylindrical stipes, flattened toward the beginning of the blades, with a regular, almost dichotomous long lanceolate blade with a spike (Fig. 2b-e).

**Internal anatomy**

Our specimens showed several layers of cortical tissue with cells of smaller diameter compared to *L. searlesiana* (Fig. 3b, e, h) and *L. flavicans* (Fig. 3c, f, i), moreover no lacunas were observed in our samples, unlike *L. flavicans* (Fig. 3c, h). The medulla was composed of elongated medullary cells with filamentous elements (Fig. 3g). The internal anatomy was composed of a narrow cortex (Fig. 3a), with cortical cell diameter of $25.91 \pm 2.90$ for the individual 1, $28.22 \pm 2.10$ for individual 2, and $27.02 \pm 2.27$ for the individual 3 (Table 2).

**Phylogenetic analysis**

The ITS phylogeny placed our specimens within the lineage of *L. spicata* of central Chile (Fig. 4a). The phylogenetic trees constructed by ML and BI had the same topology except for the phylogenetic position of *L. corrugata* and *L. variegata* from northeastern South Is. The three specimens analyzed consistently formed a strongly supported clade with sequences of *L. spicata* (97% for ML and 0.96 for BPP) collected in Chile; having to *L. berteroana* and *L. trabeculata* as sister taxa. The cladogram was consistent with the phylogenetic tree (Fig 4b). Variable sites occurred at 201 positions (27.5%), and 123 positions (16.8%) were parsimoniously informative. Intraspecific divergence of *L. spicata* from three different populations ranged between 0.0% and 0.2% (0–3 bp). *L. spicata* differed by 0.8%–1.0% from *L. berteroana* and by 1.1%–1.3% from *L. trabeculata*. *L. variegata* is a non-monophyletic species complex of four different species.

**Discussion**
We confirm here the presence of *L. spicata* both morphologically and genetically, whose individuals correspond to the central Chile lineage described by González *et al.*, (2012). Morphologically these features correspond to those described for *L. spicata* by Suhr (1839) and González *et al.*, (2012). These values also agree with those mentioned by González *et al.*, (2012) for *L. spicata*. Genetically our phylogeny is consistent with those of previous studies that show *Lessonia* as a monophyletic lineage (Lane *et al.*, 2006, Martin & Zucarello, 2012).

*Lessonia* species are a characteristic component of benthic ecosystems in this region (Searles, 1978; Martin & Zucarello, 2011). We highlight two aspects about the importance of this report of *L. spicata* for this area: a) we increase the knowledge of the species richness of kelps for the Sub-Antarctic Channels, and (b) this species has a strong extraction activity which we hypothesize that will move southward in the near future, therefore these populations should be properly preserved in order to prevent high risk of human impact.

The name *L. spicata* was proposed because it was the oldest name available to assign the lineage of central Chile, populations between 29° and 43°S. However, *L. spicata* would be a provisional name mainly because no representative specimens of *L. nigrescens* have been found near the type locality Cape Horn. Therefore, if the true *L. nigrescens* belongs to one of the lineages already described or to a new one, this name would have priority (González *et al.*, 2012). In the MBP *L. nigrescens* has been recorded not only for Cape Horn; Searles (1978) reported a population in the Trinidad Channel (Puerto Alert 49° 53.6 'S), and two others in the Aysén region (Searles, 1978). Puerto Alert is 126 km south of Castillo Channel where we found the population of *L. spicata*. Therefore, it is likely that Searles’ records (1978) correspond to populations of *L. spicata*. Finally, it is important to mention that, like González *et al.*, (2012), in recent expeditions to the Diego Ramirez and Cape Horn archipelago—which are related to the characterization of the Diego Ramirez-Drake Passage Marine Park (Rozzi *et al.*, 2017)—we have not found populations of *L. nigrescens*, only individuals of *L. flavicans* (Rozzi *et al.*, 2017). Therefore, in the absence of biological material from the type locality the status of *L. nigrescens* is still in doubt, and the lineage of central Chile that now extends south of 43°S should continue to be named as *L. spicata*.

Several bio-geographical breaks have been described along the coast of Chile (Santelices & Menesses, 2000; Tellier *et al.*, 2009; Fraser *et al.*, 2010); one of the most relevant for many taxa is at 42°S (Brattström & Johansen, 1983; Lancellotti & Vázquez, 1999; Valdovinos *et al.*, 2003). For macroalgae and particularly for kelp species such as *Durvillaea antarctica*, a marked
196 divergence is present south of 43°S, where populations between 49° and 55°S are genetically
different from the rest of the populations occurring in the Chilean coast (32°S and 43°S) (Fraser
et al., 2010). These authors suggested that although D. Antarctica has a high dispersion capacity
due to its buoyancy (rafting), it could only colonize free coasts, since it would have limited
potential to increase gene flow between established populations. Therefore, it is interesting that
although L. spicata has a low dispersal capacity in comparison to D. antarctica (Oppliger et al.,
2012), since it does not have the buoyancy capacity, there is a single genetic unit in the individuals
collected in this study and individuals from the central zone of Chile. L. spicata must have some
physiological adaptations which allowed it to colonize and inhabit areas of high latitudes. In this
sense, this species has been described as a perennial seaweed and has not been found in the "bank
of microscopic forms" in the Chilean central coast (boulders and water from tidal pools)
(Santelices et al., 1995; Santelices et al., 2002). However, it has been observed that microscopic
form of L. spicata can survive up to 90 days in total darkness and propagules can germinate in
total absence of light (Santelices et al., 2002). This high capacity for tolerance to darkness could
be a key strategy to colonize new areas with a significant seasonal changes in daylight hours and
luminosity (Photosynthetically Active Radiation) during the winter period (Ojeda et al., 2019).

Nevertheless, future studies and a greater number of samples along the Chilean coast (mainly the
area between 41°- 48°S) will help to elucidate its biogeographic history and how much structure
and connectivity the populations of L. spicata present throughout their distribution (29°S to 48°S).

The harvesting pressure on the genus Lessonia has increased alarmingly along the Chilean
cost, so we should take a precautionary approach to potential harvesting of L. spicata in its austral
distribution range. L. berteroana (sister species of L. spicata) is currently the most exploited
seaweed in South America; the main landings are in northern Chile (Westermeier et al., 2019).

Lessonia is socially important in this region because many artisanal fishers depend directly or
indirectly on its harvest (Vega et al., 2014). However, high demand, lack of oversight and harvest
methods have created a concerning scenario for kelp forests (Vega et al., 2014; Westermeier et al.,
2019). The extraction of L. spicata in southern Chile began in 2012, and its extractive pressure has
been moving southward, mainly between 33° and 41°S (SERNAPESCA, 2019). In the Chilean
Los Lagos Region (41°S), between 2014 and 2017 landing increased from 494 to 747 dry tons of
L. spicata (SERNAPESCA, 2019). This gradual increase should draw attention to kelp forest
conservation, since there is evidence on sustainability problems that Lessonia populations have
experimented and their biodiversity in northern Chile (Vega et al., 2016). This concern acquires significant relevance if we consider that the Magellan Sub-Antarctic Channels are the austral distribution range of L. spicata, where kelp forest populations are important for sustainability of small-scale fisheries (e.g. king crab; Cárdenas et al., 2007), indigenous traditions (Ojeda et al., 2018) and terrestrial and marine biodiversity (Darwin, 1839; Rosenfeld et al., 2014).

Conclusion

Despite the geographical distance and the presence of important biogeographic breaks (41° and 46°S), our results confirm that the individuals collected in the coastal zone of the Katalalixar Reserve are the species Lessonia spicata. The strong morphological and genetic evidence are indicating that the individuals analyzed are associated with the lineage of central Chile, and the populations of L. spicata would inhabit the area exposed to the Pacific.

With diverse industrial uses, including providing phycocolloids in the form of alginate L. spicata is a potentially important economic resource in the Chilean coast. However, with extractive pressure moving to the south, caution is needed given that this kelp serves not only as a habitat for many animals but also as a spawning ground for some benthic (e.g., gastropods) species.

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References

Asensi A, Reviers B. 2009. Illustrated catalogue of types of species historically assigned to Lessonia (Laminariales, Phaeophyceae) preserved at PC, including a taxonomic study of three South-American species with description of L. searlesiana sp. nov. and a new lectotypification of L. flavicans. Cryptogamie Algologie 30: 209-49.
Ávila M, Hoffmann A, Santelices B. 1985. Interacciones de temperatura, densidad de flujo fotónico y fotoperíodo sobre el desarrollo de etapas microscópicas de Lessonia nigrescens (Phaeophyta, Laminarilales). Revista Chilena de Historia Natural 58: 71-82.

Bell NE, Pedersen N, Newton AE. 2007. Ombronesus stuvensis, a new genus and species of the Ptychoniaceae (Bryophyta) from south west Chile. TAXON 56(3): 887-896. DOI: 10.2307/25065870

Brattström H, Johanssen A. 1983. Ecological and regional zoogeography of the marine benthic fauna of Chile: Report no. 49 of the Lund University Chile Expedition 1948-49. Sarsia 68(4): 289-339.

Camus A. 2001. Biogeografía marina de Chile continental. Revista Chilena de Historia Natural 74(3): 587-617. DOI 10.4067/S0716-078X2001000300008.

Calderon MS, Boo SM. 2017. The Phyllophoraceae (Gigartinales, Rhodophyta) from Peru with descriptions of Acletoa tarazonae gen. & sp. nov. and Gymnogongrus caespitosus sp. nov.. Phycologia 56(6): 686-696.

Cárdenas CA, Cañete JI, Mansilla A. 2007. Podding of juvenile king crabs Lithodes santolla (Molina, 1982) (Crustacea) in association with holdfasts of Macrocystis pyrifera (Linnaeus) C. Agardh, 1980. Investigaciones Marinas 35: 105-110.

Cho G, Klochkova NG, Krupnova TN, Boo SM. 2006. The reclassification of Lessonia laminarioides (Laminariales, Phaeophyceae): Pseudolessonia gen. nov. Journal of Phycology 42: 1289–1299. DOI 10.1111/j.1529-8817.2006.00280.x.

Darwin C. 1839. Voyages of the Adventure and Beagle. Vol. III. Journals and Remarks. 1832-1836. London: Henry Colburn.

Fraser CI, Thiel M, Spencer HG, Waters JM. 2010. Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. BMC Evolutionary Biology 10: 203. DOI 10.1186/1471-2148-10-203.

González A, Beltrán J, Hiriart-Bertrand L, Flores V, Reviers B, Correa JA, Santelices B. 2012. Identification of cryptic species in the Lessonia nigrescens complex (Phaeophyceae, Laminariales). Journal of Phycology 48(5): 1153-1165. DOI 10.1111/j.1529-8817.2012.01200.x.

Gorny M, Zapata-Hernández G. 2018. On the bathymetric and latitudinal distributions of Coelorinchus cf. cookianus (Gadiformes: Macrouridae): First record documented with a
remotely operated vehicle (ROV) in interior waters of the Campana Archipelago in central Patagonia, Chile (48°S). *Anales del Instituto de la Patagonia* 46(2): 29-36. DOI /10.4067/S0718-686X2018000200029.

Guiry MD, Guiry GM. 2019. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 07 Apr 2019.

Lancellotti DA, VASQUEZ JA. 1999. Biogeographycal patterns of benthic macroinvertebrates in the Southeastern Pacific littoral. *Journal of Biogeography* 26: 1001-1006. DOI 10.1046/j.1365-2699.1999.00344.x.

Lane CE, Mayes C, Druehl LD, Saunders GW. 2006. A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *Journal of Phycology* 42: 493-512. DOI 10.1111/j.1529-8817.2006.00253.x.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695-1701. DOI 10.1093/molbev/mss020.

Mansilla A, Ávila M, Ramírez M, Rodríguez JP, Rosenfeld S, Ojeda J, Marambio J. 2013. Macroalgas marinas bentónicas del submareal somero de la ecorregión Subantártica de Magallanes, Chile. *Anales del Instituto de la Patagonia* 41: 49-62. DOI 10.4067/S0718-686X2013000200004.

Martin P, Zuccarello GC. 2012. Molecular phylogeny and timing of radiation in *Lessonia* (Phaeophyceae, Laminariales). *Phycological Research* 60: 276-287. DOI 10.1111/j.1440-1835.2012.00658.x.

McCarthy C. 1998. Chromas version 1.45. School of Health Science, Griffith University, Southport, Queensland Australia. http://www.technelysium.com.au/chromas.html.

Ojeda J, Rozzi R, Rosenfeld S, Contador T, Massardo F, González-Calderón J, Mansilla A. 2018. Interacciones bioculturales del pueblo yagán con las macroalgas y moluscos: una aproximación desde la filosofía ambiental de campo. *Magallania* 46: 155-181. DOI 10.4067/S0718-22442018000100155.

Ojeda J, Marambio J, Rosenfeld S, Contador T, Rozzi R, Mansilla A. 2019. Seasonal changes of macroalgae assemblages on the rocky shores of the Cape Horn Biosphere Reserve, Sub-Antarctic Channels, Chile. *Aquatic Botany* 157:33-41. DOI.org/10.1016/j.aquabot.2019.06.001
Oppliger VL, Correa JA, Engelen AH, Tellier F, Vieira V, Faugeron S, Valero M, Gomez G, Destombe C. 2012. Temperature Effects on Gametophyte Life-History Traits and Geographic Distribution of Two Cryptic Kelp Species. *PLoS ONE* 7(6): e39289. DOI 10.1371/journal.pone.0039289.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer version 1.6. http://beast.bio.ed.ac.uk/Tracer.

Ronquist F, Teslenko M, Van Der Mark P, Ayres D, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542. DOI 10.1093/sysbio/sys029.

Rosenfeld S, Ojeda J, Húne M, Mansilla A, Contador T. 2014. Egg masses of the Patagonian squid *Doryteuthis* (Amerigo) *gahi* attached to giant kelp (*Macrocystis pyrifera*) in the sub-Antarctic ecoregion. *Polar Research* 33: 1-5. DOI 10.3402/polar.v33.21636.

Rozzi R, Massardo F, Mansilla A, Squeo FA, Barros E, Contador T, Frangopulos M, Poulin E, Rosenfeld S, Goffinet B, González-Weaver C, MacKenzie R, Crego RD, Viddi F, Naretto J, Gallardo MR, Jiménez JE, Marambio J, Pérez C, Rodriguez JP, Méndez F, Barroso O, Rendoll J, Schütter E, Kennedy J, Convey P, Russell S, Berchez F, Sumida PYG, Rundell P, Rozzi A, Armesto J, Kalín-Arroyo M, Martinic M. 2017. Parque Marino Cabo de Hornos - Diego Ramírez. Technical report to the proposal for creation of the Cape Horn - Diego Ramírez Marin Park. Punta Arenas, Chile: Ediciones Universidad de Magallanes. https://issuu.com/umag9/docs/ebook_low_parque_marino_umag_fin_di

Santelices B, Meneses I. 2000. A reassessment of the phytogeographic characterization of Temperate Pacific South America. *Revista Chilena de Historia Natural* 73(4): 605-614. DOI 10.4067/S0716-078X2000000400005.

Santelices B, Aedo D, Hoffmann AJ. 2002. Banks of microscopic forms and survival to darkness of propagules and microscopic stages of macroalgae. *Revista Chilena de Historia Natural* 75: 547-555.

Santelices B, Hoffmann AJ, Aedo D, Bobadilla M, Otaíza M. 1995. The bank of microscopic forms on disturbed boulders and stones in tide pools. *Marine Ecology Progress Series* 129: 215-228.
Searles RB. 1978. The genus Lessonia Bory (Phaeophyta, Laminariales) in Southern Chile and Argentina. British Phycological Journal 13: 361-381. DOI 10.1080/00071617800650421.

SERNAPESCA 2019. Informe Final Control Cuota Algas Pardas I, III, IV y VII año 2018; Huiro Negro (Lessonia berteroana/spicata), Huiro Palo (Lessonia trabeculata), Huiro Macro (Macrocystis pyrifera) y Cochayuyo (Durvillaea antarctica). Unidad de Control de Cuotas, Subdirección De Pesquería, Chile. http://www.sernapesca.cl/sites/default/files/informe_final_2018_bentonicos_algas_iii_y_iv.pdf

Silvestro D, Michalak I. 2012. RaxmlGUI: a graphical frontend for RAxML. Organisms Diversity and Evolution 12: 335-337. DOI 10.1007/s13127-011-0056-0.

Silva N, Rojas N, Fedele A. 2009. Water masses in the Humboldt Current System: Properties, distribution, and the nitrate deficit as a chemical water mass tracer for Equatorial Subsurface Water off Chile. Deep-Sea Research II 56: 992-1008.

Stamatakis A. 2014. RAxML Version 8: a tool for phylogenetic analysis and postanalysis of large phylogenies. Bioinformatics. DOI 10.1093/bioinformatics/btu033.

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation 29(4): 436-459. DOI 10.1017/S0376892902000322.

von Suhr JN. 1839. Beiträge zur Algenkunde. [Nº3]. Flora 22: 65–75.

Tai V, Lindstrom SC, Saunders GW. 2001. Phylogeny of the Dumontiaceae (Gigartinales, Rhodophyta) and associated families based on SSU rDNA and internal transcribed spacer sequence data. Journal of Phycology 37: 184-96. DOI 10.1046/j.1529-8817.2001.037001184.x.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729. DOI 10.1093/molbev/mst197.

Tellier F, Meynard AP, Correa JA, Faugeron S, Valero M. 2009. Phylogeographic analyses of the 30° S south-east Pacific biogeographic transition zone establish the occurrence of a sharp genetic discontinuity in the kelp Lessonia nigrescens: vicariance or parapatry. Molecular Phylogenetics and Evolution 53: 679-93. DOI 10.1016/j.ympev.2009.07.030.
Tellier F, Vega JMA, Broitman BR, Vasquez JA, Valero M, Faugeron S. 2011. The importance of having two species instead of one in kelp management: the Lessonia nigrescens species complex. *Cahiers de Biologie Marine* 52(4): 455-465.

Thiel M, Macaya EC, Acuña E, Arntz WE, Bastias H, Brokordt K, Camus PA, Castilla JC, Castro LR, Cortés M, Dumont CP, Escribano R, Fernández M, Gajardo JA, Gaymer CF, Gomez I, González AE, González HE, Haye PA, Illanes JE, Iriarte JL, Lancellotti DA, Luna-Jorquera G, Luxoro C, Manriquez PH, Marin V, Muñoz P, Navarretes SA, Perez E, Poulin E, Sellanes J, Sepúlveda HH, Stotz W, Tala F, Thomas A, Vargas CA, Vasquez JA, Vega JMA. 2007. ‘The Humboldt current system of northern and centracl Chile Oceanographic processes, ecological interactions and socioeconomic feedback’, *Oceanography and Marine Biology*, 45, pp. 195–344.

Valdovinos C, Navarrete SA, Marquet PA. 2003. Mollusk species richness in the southeastern Pacific: why are there more species towards the pole? *Ecography* 26: 139-144. DOI 10.1034/j.1600-0587.2003.03349.x.

Vásquez JA, Camus PA, Ojeda FP. 1998. Diversidad, estructura y funcionamiento de ecosistemas rocosos del norte de Chile. *Revista Chilena de Historia Natural* 71: 479-499.

Vásquez JA, Santelices B. 1984. Comunidades de macroinvertebrados en discos adhesivos de *Lessonia nigrescens* Bory (Phaeophyta) en Chile central. *Revista Chilena de Historia Natural* 57: 131-154.

Vásquez, JA. 2008. Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. *Journal of Applied Phycology* 20(5): 457-467. DOI 10.1007/s10811-007-9308-y.

Vega JMA, Broitman BR, Vásquez JA. 2014. Monitoring the sustainability of *Lessonia nigrescens* (Laminariales, Phaeophyceae) in northern Chile under strong harvest pressure. *Journal of Applied Phycology* 26(2): 791-801. DOI 10.1007/s10811-013-0167-4.

Vega JMA. 2016. Fauna asociada a discos de adhesión del complejo *Lessonia nigrescens*: ¿Es un indicador de integridad ecológica en praderas explotadas de huiro negro, en el norte de Chile?. *Latin American Journal of Aquatic Research* 44(3): 623-637. DOI 10.3856/vol44-issue3-fulltext-21.

Vega JMA, Asorey CM, Piaget N. 2016. Asociación *Scurria-Lessonia*, indicador de integridad ecológica en praderas explotadas de huiro negro *Lessonia berteroana* (ex L. nigrescens) en
el norte de Chile. **Revista de Biología Marina y Oceanografía** 51(2): 337-345. DOI 10.4067/S0718-19572016000200011

Villouta E, Santelices B. 1984. Estructura de la comunidad submareal de *Lessonia* (Phaeophyta, Laminariales) en Chile norte y central. **Revista Chilena de Historia Natural** 57: 111-122.

Villouta E, Santelices B. 1986. *Lessonia trabeculata* sp. nov. (Laminariales, Phaeophyta), a new kelp from Chile. **Phycologia** 25: 81-86. DOI 10.2216/i0031-8884-25-1-81.1.

Westermeier R, Murúa P, Patiño J, Manoli, G, Müller DG. 2019. Evaluation of kelp harvest strategies: recovery of *Lessonia berteroana* (Phaeophyceae, Laminariales) in Pan de Azucar, Atacama, Chile. **Journal of Applied Phycology** 31: 575-585. DOI 10.1007/s10811-018-1500-8.

Yoon HS, Lee JY, Boo SM, Bhattacharya D. 2001. Phylogeny of Alariaceae, Laminariaceae, and Lessoniaceae (Phaeophyceae) based on plastid-encoded RuBisCo spacer and nuclear-encoded ITS sequence comparisons. **Molecular Phylogenetics and Evolution** 21: 231–42.

Zuccarello GC, Martin P. 2016. Phylogeography of the *Lessonia variegata* species complex (Phaeophyceae, Laminariales) in New Zealand. **Algae** 31(2): 91-103. DOI 10.4490/algea.2016.31.4.15.
Figure 1

Collection points of *Lessonia spicata*. Map showing the location of Katalalixar National Reserve (KNR) in central Patagonia in the oceanic margin of the Campana Archipelago.
Figure 2

Distribution of *Lessonia spicata* (interspersed bars), showing its previously known distribution (solid bars). Habitat of specimen collected in both sites (Torpedo Island and Castillo Channel)

Distribution of *Lessonia spicata* (interspersed bars), showing its previously known distribution (solid bars). We included the Chilean biogeographical classification of Camus (2001). b) Habitat of specimen collected in Torpedo Island (LMS000001). c) Discoid holdfasts of specimen collected in Torpedo Island (LMS000001), d) Blades of specimen collected in Torpedo Island (LMS000001), e) Habitat of specimen collected in Castillo Channel (LMS000002, LMS000003).
Manuscript to be reviewed
Cross section of the medial part of mature fronds of Lessonia species who inhabit the Sub-Antarctic channels.

Cross section of the medial part of mature fronds of *Lessonia spicata* collected in the Katalalixar Reserve (Fig. 3a, d and g), of *L. searlesiana* from Fuerte Bulnes (Fig. 3b, e and h) and *L. flavicans* from Horn Island (Fig. 3c, f and i); mer = meristoderm, co = cortex and me = medulla, l= lacuna.
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Figure 4

Phylogenetic tree of ITS sequences obtained by maximum likelihood (ML) inference.

a) Phylogenetic tree of ITS sequences obtained by maximum likelihood (ML) inference. ML bootstrap values (≥ 50%) and Bayesian posterior probabilities (≥ 0.90) are indicated next to branches. b) Cladograms of ITS sequences obtained by the neighbor joining (NJ) method. Bootstrap values (≥ 50%) are indicated next to branches. The sequence for taxa in bold was generated in this study.
Table 1 (on next page)

List of species used in DNA analyses, information on collections and accession numbers in GenBank

List of species used in DNA analyses, information on collections and accession numbers in GenBank (sequences generated in the present study are shown in bold). ¹ Martin & Zuccarello (2012), ² Lane et al. (2006), ³ Yoon et al. (2001).
Table 1. List of species used in DNA analyses, information on collections and accession numbers in GenBank (sequences generated in the present study are shown in bold). 1 Martin & Zuccarello (2012), 2 Lane et al. (2006), 3 Yoon et al. (2001).

| Species                   | Collection site                                      | Voucher code | ITS          |
|---------------------------|------------------------------------------------------|--------------|--------------|
| LesA                      | Torpedo island, Aysen, Chile                        | MN061669     |              |
| LesB                      | Channel Castillo, Aysen, Chile                       | MN061670     |              |
| LesC                      | Channel Castillo, Aysen, Chile                       | MN061671     |              |
| Lessonia adamsiae         | South Promontory, The Snares, New Zealand           |              |              |
| LesA                      | Tahi, The Snares, New Zealand                       | A626         | GU593802     |
| Lessonia adamsiae         | San Marcos, Tarapaca, Chile                          | B858         | GU593781     |
| LesA                      | Smoothwater Bay, Campbell Is., New Zealand           | B859         | GU593782     |
| Lessonia brevifolia       | New Zealand                                          | A548         | GU593803     |
| Lessonia brevifolia       | Antipodes, New Zealand                               | A973         | GU593804     |
| Lessonia brevifolia       | Perseverance Harbour, Campbell Is., New Zealand      | B926         | GU593805     |
| LesA                      | Gov. Is. Reserve, Tasmania, Australia                | A985         | GU593794     |
| LesA                      | Skeleton Pt., Tasmania, Australia                   | C057         | GU593795     |
| LesA                      | Rookery Bay, East Falkland, Falkland Islands        | A634         | GU593786     |
| LesA                      | Punta Arenas, Patagonia, Chile                       | B985         | GU593789     |
| Lessonia spicata (as L. nigr. IA lineage) | La Pampilla, Coquimbo, Chile | A581         | GU593775     |
| Lessonia spicata (as L. nigr. IA lineage) | Bahia Mansa, Osorno, Chile | B719         | GU593780     |
| LesA                      | Wharf reef, Owenga, Chatham Is., New Zealand        | A518         | GU593797     |
| LesA                      | Wharekauri, Chatham Is, New Zealand                 | A532         | GU593798     |
| LesA                      | Punihuil, Chiloe Is, Chile                          | B715         | GU593783     |
| LesA                      | Punihuil, Chiloe Is, Chile                          | B716         | GU593784     |
| LesA                      | North Cape, Northland, New Zealand                  | A557         | GU593808     |
| LesA                      | Maitai Bay, Northland, New Zealand                  | B129         | GU593809     |
| LesA                      | The Sailors Grave, Coromandel, New Zealand          | B312         | GU593810     |
| LesA                      | South Bay, Kaikoura, New Zealand                    | A138         | GU593817     |
| LesA                      | New Wharf, Kaikoura, New Zealand                    | A606         | GU593818     |
| LesA                      | Curio Bay, Catlins, New Zealand                     | A434         | GU593820     |
| Species                    | Location                                      | Accession | Reference |
|---------------------------|-----------------------------------------------|-----------|-----------|
| Lessonia variegata (as L. variegata lineage S) | Causet Cove, Doubtful Sound, New Zealand     | C154      | GU593821^1 |
| Lessonia variegata (as L. variegata lineage W) | Princess Bay, Wellington, New Zealand         | A001      | GU593811^1 |
| Lessonia variegata (as L. variegata lineage W) | Cape Palliser, Wairarapa, New Zealand         | A613      | GU593815^1 |
| Lessonia variegata (as L. variegata lineage W) | Riversdale Beach, Wairarapa, New Zealand      | A025      | GU593816^1 |
| Cymathaere triplicata     | Whiffen Spit, Sooke, BC, Canada               | AY857884^2 |          |
| Macrocystis pyrifera      | California, USA                               | AF319037^3 |          |
| Ecklonia cava             |                                               | GU593773^1 |          |
Table 2 (on next page)

Morphological measurements (mean ± SE) of individuals collected in Torpedo Island and Castillo Channel.

External morphological data: TL = Thallus length (cm), DD = Disc diameter, NS = Number of stipes. Internal morphological data: DC = Diameter of cortical cells.
**Table 2.** Morphological measurements (mean ± SE) of individuals collected in Torpedo Island and Castillo Channel. External morphological data: TL = Thallus length (cm), DD = Disc diameter, NS = Number of stipes. Internal morphological data: DC = Diameter of cortical cells.

| Individual | Site          | TL    | DD  | NS  | DC          |
|------------|---------------|-------|-----|-----|-------------|
| 1          | Torpedo Island| 68    | 10  | 9   | 25.91 ± 2.90| This study |
|            | Castillo      |       |     |     |             |
| 2          | Channel       | 166   | 21  | 6   | 28.22 ± 2.10| This study |
|            | Castillo      |       |     |     |             |
| 3          | Channel       | 55    | 5   | 13  | 27.02 ± 2.27| This study |
|            |               |       |     |     |             |
| **Average**|               | 96.33 ± 60.68 | 12 ± 8.19 | 9.33 ± 3.51 | 27.05 ± 1.15 | This study |

*L. spicata*

| Site  | TL ± SE | DD | NS | DC ± SE |
|-------|---------|----|----|---------|
| Maintencillo | 150 ± 13.3 | -  | -  | 25.7 ± 1.4 (González et al. 2012) |
| Matanzas | 160 ± 5.0  | -  | -  | 27 ± 1.6 (González et al. 2012) |
| Calfuco | 120 ± 7.2  | -  | -  | 30 ± 1.8 (González et al. 2012) |