

The importance of having two species instead of one in kelp management: the *Lessonia nigrescens* species complex

Florence TELLIER^{1,2,3,4}, J. M. Alonso VEGA¹, Bernardo R. BROITMAN^{1,4}, Julio A. VASQUEZ¹,
Myriam VALERO^{2,3} and Sylvain FAUGERON⁴

(1) *Facultad de Ciencias del Mar & CEAZA, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile*
Phone: +56-51-209943, Fax: +56-51-209812, E-mail: ftellier@gmail.com

(2) *UPMC Univ Paris 6, UMR 7144, Equipe BEDIM, LIA "DIAMS", Station Biologique de Roscoff,*
Place Georges Teissier, 29682 Roscoff cedex, France

(3) *CNRS, UMR 7144, Equipe BEDIM, LIA "DIAMS", Station Biologique de Roscoff, Place Georges Teissier,*
29682 Roscoff cedex, France

(4) *Departamento de Ecología, Center for Advanced Studies in Ecology and Biodiversity, LIA "DIAMS", Facultad de*
Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile

Abstract: Natural resource management requires extensive knowledge of the biology of the organisms and their responses to ecological and anthropogenic processes. While species are commonly used as management units, the presence of cryptic species – morphologically indistinguishable, but with different biological characteristics – may hinder the design of appropriate management and conservation measures. Thus, correctly assigning individuals to species is of particular importance and challenges current management approaches in seaweeds, which are highly plastic morphologically. After a synthesis of the species definitions, we show an example of the challenges of managing cryptic species with the Chilean kelp *Lessonia nigrescens*. We review phylogenetic, biological and ecological evidence indicating that this economically important intertidal species is composed of two cryptic species. In the face of increasing global demand for kelp, an intensified impact of harvesting pressure is observed around the region where both cryptic species find their northern and southern edge of their geographic distributions, around 30°S. We recommend special management strategies targeting harvested areas around such range edge populations. Our synthesis highlights the importance of having two species that are ecologically and genetically different. In addition, the cryptic species may diverge in biochemical composition, opening new perspectives for the alginates' industry. The capacity of conservation and management programs to correctly distinguish new species is of particular importance as numerous cryptic species are constantly being discovered in seaweeds, including kelps.

Résumé : De l'importance d'avoir deux espèces au lieu d'une pour la gestion de la ressource : le complexe spécifique *Lessonia nigrescens*. La gestion des ressources naturelles requiert une connaissance approfondie de la biologie des organismes et de leurs réponses aux processus écologiques et anthropiques. Alors que l'espèce est communément utilisée comme unité de gestion, la présence d'espèces cryptiques – non distinguables par leur morphologie, mais ayant des caractéristiques biologiques différentes – peut entraver la conception de mesures de gestion et conservation appropriées. Par conséquent, l'affectation correcte des individus à une espèce est particulièrement importante, et remet en question les approches actuelles de gestion des algues marines, qui ont une grande plasticité phénotypique. Après une synthèse des

définitions des espèces, nous présentons un exemple de défi soulevé par la gestion d'espèces cryptiques, à travers le cas de la Laminariale (kelp) du Chili, *Lessonia nigrescens*. Nous passons en revue les preuves phylogénétiques, biologiques et écologiques qui indiquent que cette algue intertidale, de grande importance économique, est composée de deux espèces cryptiques. Face à la croissance actuelle de la demande mondiale de kelp, une intensification de la pression de récolte est observée autour de la région où les deux espèces cryptiques ont leur limite de distribution nord et sud, autour de 30°S. Pour ces zones de récolte, nous recommandons l'application de stratégies de gestion particulières aux populations de limite d'aire. Notre synthèse souligne l'importance d'avoir deux espèces génétiquement et écologiquement différentes. D'autre part, les espèces cryptiques peuvent présenter des différences biochimiques, ouvrant de nouvelles perspectives pour l'industrie des alginates. La capacité des programmes de conservation et de gestion à distinguer correctement de nouvelles espèces est particulièrement importante car de nouvelles espèces cryptiques sont constamment découvertes chez les algues marines, et en particulier chez les kelps.

Keywords: Brown seaweed • Cryptic species • *Lessonia* • Resource management • Species concept

Introduction

In the face of increasing challenges to the conservation and sustainable management of natural resources, there is an increasing demand for the development of adequate science-based strategies (see for example in forests: Agrawal et al., 2008, in herbaceous plant and invertebrate: Bonin et al., 2007, in fisheries: Beddington et al., 2007 and in coastal marine resources: Gelcich et al., 2010). The development of appropriate management and conservation plans remains challenging as it requires extensive information transfer between the scientific community, policymakers and socio-economic actors. One of the most widely agreed concepts among policymakers is the use of individual species to quantify biodiversity, to develop regulations on resource exploitation, and to identify the factors threatening populations. The International Union for the Conservation of Nature (IUCN) uses categorization of species in threat categories (Red Lists), and most national and international laws and treaties regulating trade and transit are based on species. Examples of such conservation policies focusing on species are the U.S. Endangered Species Act and the Habitat Directive of the European Union, or the Convention on International Trade in Endangered Species (CITES), for commerce regulation (see Mace, 2004 for further details).

How many species?

Why it is an important question for the stakeholders? In Chile, the harvesting and collecting of coastal marine resources (both invertebrates and seaweeds) are also based on species (Gelcich et al., 2010). Nevertheless, cases of mismatch between the biological unit (species) and the management units are commonly found. For instance, the

species geographic range does not always correspond to a single administrative region and therefore different management strategies may be applied along the species range. Stakeholders may also be more interested in some regions of the species range, because of availability of the resource or specific traits of those populations. Nevertheless, the future of the population is strongly dependent on the degree of connectivity with other populations from the same species, and on the degree of divergence among populations. As a consequence, a geographic unit may be an ill-suited management unit, as it considers only a section of the species distribution.

Why is it a challenge and a debate for biologists? The lack of consensus in the scientific community of about “what a species is” and the numerous debates on the question raise confusion for policymakers, who require simple and clear decision-making units, independent of the scientific debate. Two main aspects illustrate the difficulties involved in a simple scientific definition of species. First, while species delimitation relies on a classification into discrete units, speciation is a continuous process during which new species are originated from an ancestral one. The mismatch between the process and the product is confusing for conservation and management. Second, there is a plethora of species definitions within the scientific community (Hey, 2001; Coyne & Orr, 2004). They differ in the criteria used for species delimitation, the research goals for which they are used and in their potential application for conservation and management purposes. There is an ongoing debate between conservation biologists, systematists and evolutionary biologists for the concept of Evolutionary Significant Unit (ESU) that was proposed by Ryder (1986) to define the minimal unit for conservation management. The definition takes into account both the history of

populations as revealed by molecular analysis and adaptive differences indicated by life history and other ecological information. Even if it integrates the contributions of both genetics and ecology, the ESU concept is criticized because it is too subjective as it relies largely – or scarcely – on genetics (see Crandall et al., 2000) and cannot be universally adopted in practice. We will develop three of the commonly used species concepts: the Biological Species Concept (BSC), the Phylogenetic Species Concept (PSC) and Ecological Species Concepts (ESC). The BSC focuses on reproductive isolation to define species, chiefly the mechanisms that limit hybridization and therefore maintain species integrity when co-occurring. The PSC emphasizes on the evolutionary history (at a larger temporal scale) that led to the origin of the new species, which is inferred from phylogenetic relationships between species. The ESC uses ecological characteristics, such as habitat requirements, geographic distributions or demographic characteristics, to distinguish species.

The three sets of criteria outlined above dominate the species concept, yet a fourth set based on the morphological attributes is also widely used. Well before Linnaeus, morphology has been the dominant approach to classify species (Winker, 2005). It is relatively easy to implement by non-specialists, particularly under field conditions commonly faced by conservation and management practitioners. Nevertheless, phenotypic variation does not always allow the clustering of individuals into discrete categories and morphological identification may lead to taxonomic confusion. It is also frequent to detect strong morphological differences between organisms belonging to a single species based on other species concepts, which can be driven by environmental factors (i.e. phenotypic plasticity) and/or by genetic factors (i.e. local adaptation, sexual dimorphism). Cryptic species are another common case of mismatch between morphological characterization of species and biological species (or any other species concept); while species are morphologically indistinguishable, they are reproductively isolated and have independent evolutionary histories (Bickford et al., 2006).

Marine seaweeds present a major taxonomic challenge, because traditional morphological classification has shown to be limited by the few morphological characters available for identification, high phenotypic plasticity and the presence of numerous cryptic species (Knowlton, 2000). For example, kelps (Laminariales, Phaeophyceae) show important phenotypic plasticity in response to environmental heterogeneity, such as wave-exposure in *Ecklonia radiata* (Wernberg et al., 2003; Wing et al., 2007), or local environment in *Macrocystis pyrifera* (Demes et al., 2009). In the case of *M. pyrifera*, phenotypic plasticity has led to taxonomic confusion, with the former recognition of morphs as distinct species (see Demes et al., 2009 for

discussion of evidence for lumping species). While species lumping has been done formally (*Macrocystis* sp.) or have been suggested (*Undaria* sp., Uwai et al., 2007), genetic studies have revealed incomplete speciation in *Alaria* sp. (Lane et al., 2007) and between *Saccharina latissima* (Linnaeus) isolates (McDevit & Saunders, 2010). Taxonomic incongruence has also been reported at higher taxonomic levels (genus or families) by phylogenetic studies (Lane et al., 2006). In fact, Lane et al. (2006) found the *Laminaria* genus to be heterogeneous and led to the transfer of most Japanese *Laminaria* species to the *Saccharina* genus, including the highly cultivated kelp *Saccharina (Laminaria) japonica* (Lane et al., 2006).

Why is it an important consideration for management and conservation purposes? Independent of the species definition used for kelp or other macroalgae, resource stakeholders commonly avoid recognizing new species to maintain the commercial identity of raw materials or elaborated products. The case of *Saccharina (Laminaria) japonica* is particularly illuminating. One of the most important cultivated seaweed in the world (Bixter & Porse, 2011) is formally recognized as *Saccharina japonica* since the work of Lane et al. (2006), but most scientific publications still use *Laminaria* (and not *Saccharina*) in the title. A database query of Web of Knowledge publications including “*Saccharina japonica*” or “*Laminaria japonica*” in the title and published between 1st January and 25th October 2010 gave only 4 results for *Saccharina*, but 15 publications for *Laminaria* (one publication included both names; proceedings abstracts were excluded of the search).

As a second example, the alginate market registers three kelp species of the *Lessonia* genus: *L. nigrescens* Bory de Saint-Vincent, 1826, *L. trabeculata* Villouta & Santelices, 1986 and *L. flavicans* Bory de Saint-Vincent, 1826, as all being harvested from Northern Chile to Southern Peru (Bixter & Porse, 2011). However, *L. flavicans* is not currently harvested and, more importantly, does not occur in Northern Chile, but has a geographic distribution restricted to the very southernmost part of the Chilean and Argentine coasts (Villouta & Santelices, 1986, but see also de Reviers & Asensi, 2009). It seems that “*L. flavicans*” from the alginate market corresponds either to *L. nigrescens* or *L. trabeculata*, but it remains considered as a distinct product on the marketplace.

An adequate taxonomic classification of kelp species will allow a more accurate characterization of extracted molecules and potentially the identification of new biomolecules, such as alginic acid of clinical grade (Zimmermann et al., 2005). Natural products of such high aggregated value require accurate species identification and dedicated management plans. In addition to the constant search for new products, another challenge concerns new uses of products already established in the market such as

alginates or algal flour. High-quality alginate extracts are mainly used in the food and pharmaceutical markets, and emergent applications range from medical and human nutrition to specialized feedstock for aquaculture, agriculture and animal breeding (Vásquez, 2008; Bixter & Porse, 2011). Thus, the large potential for new industrial and biomedical uses of products derived from kelp populations that are already highly exploited deserves a closer examination of their origin and taxonomic status. Accurate taxonomy is particularly important when co-existing species have differential ratios of alginic acids. As an example, in Chilean kelps, *Lessonia nigrescens* has a high proportion of β -D-mannuronate (M-alginate, ~ 60%), while *L. trabeculata* stipes have an extremely high content of α -L-guluronate (G-alginate, ~ 90%; Venegas et al., 1993; Draget et al., 2002).

Results

Case study: the Lessonia nigrescens species complex

We will illustrate some of the challenges faced by policymakers and natural resource stakeholders through the case of *Lessonia nigrescens*, where two cryptic species have been identified (Tellier et al., 2009). After presenting the context of the study, we will review the lines of evidence justifying splitting the formerly recognized entity and outline its consequences for conservation and management.

Biological, sociological and economic contexts

According to the current taxonomic description and geographic distribution (for complete references, see AlgaeBase and Guiry & Guiry, 2010), *Lessonia nigrescens* Bory inhabits the intertidal rocky shore along Peruvian and Chilean coasts, from 17°S to 56°S (Cape Horn). Within its distribution range, other kelp taxa are present in the shallow subtidal habitat: *Lessonia trabeculata* between 12°S and 42°S, *Macrocystis pyrifera*, and other species from the *Lessonia* genus south of 42°S (Villouta & Santelices, 1986; Edding et al., 1994; Hoffman & Santelices, 1997; de Reviers & Asensi, 2009; Tellier et al., 2009).

Along most of this region, *L. nigrescens* inhabits the lower intertidal zone, where it is the dominant taxon in biomass and cover. The stand width and the abundance of this taxon vary depending on the rock inclination and on the wave exposure (Ojeda & Santelices, 1984; Santelices & Ojeda, 1984; Hoffman & Santelices, 1997). South of 30°S of latitude, *L. nigrescens* can be found in coexistence with the bull kelp *Durvillaea antarctica*, particularly in wave-exposed environments (Santelices et al., 1980).

L. nigrescens species, as other kelps, are considered bio-

engineer species (*sensu* Jones et al., 1994) as they play a major role in structuring intertidal rocky shores communities (Hoffman & Santelices, 1997). They form real forests (*sensu* Steneck et al., 2002) where numerous invertebrate species inhabit and recruit (see for example Cancino & Santelices, 1984; Vásquez & Santelices, 1984), including high-value invertebrates species such as *Concholepas concholepas* and *Loxechinus albus* (Gelcich et al., 2010; Vásquez, 2007).

Lessonia sp., including *L. nigrescens* and *L. trabeculata*, appear misidentified as “flavicans” in the trade market, as presented above. It presently accounts for 28% of the world kelp harvest, being the second most important collected and harvested kelp after the *Saccharina* (*Laminaria*) species (Bixter & Porse, in press). Historically, the landing biomass of *Lessonia* sp. has increased (Vásquez, 2008), particularly during the last decade (1999-2009). During this short period, the proportion of the world market accounted for by *Lessonia* sp. shifted from 12% in 1999 to 28% in 2009. This trend will probably continue in the future, as these species are considered as the most attractive and versatile kelps for industrial use (Bixter & Porse, 2011).

Along the Chilean coasts, *L. nigrescens* is harvested through traditional techniques consisting in either removing entire holdfasts, cutting stipes of living individuals, or collecting beached natural mortality of and then drying the algae outdoors (Figs 1 & 2, Vásquez, 2008). Chilean traditional fishermen are organized into artisanal fisher associations (unions cooperatives) that target high value invertebrates and kelp species into collectively allocated benthic management units known as MEARB (Management and Exploitation Area of Benthic Resources, see Gelcich et al., 2010 for more details, see also Frangoudes, 2011, for a comparison among countries). Since 2004, in the face of increasing global demand for alginates, the Chilean authorities established the prohibition of direct kelp extraction outside MEARB's (source: SERNAPESCA, Servicio Nacional de Pesca, www.sernapesca.cl, Vásquez, 2008).

Phylogenetic species

An increasing number of cryptic species have been recently revealed using molecular approaches, particularly through the Barcoding of Life initiative (Hebert et al., 2003), which consists of using a very short genetic sequence from a standard part of the genome to distinguish species. The barcoding approach, although relatively easy for non-specialists and for large samples screenings, has some drawbacks. For instance, the use of only a few molecular markers (generally one or two, e.g. in animals: Hebert et al., 2003, in seaweeds: McDevit & Saunders, 2009, in plants: Starr et al., 2009) often leads to the confusion between the evolutionary history of the species and that of the molecular

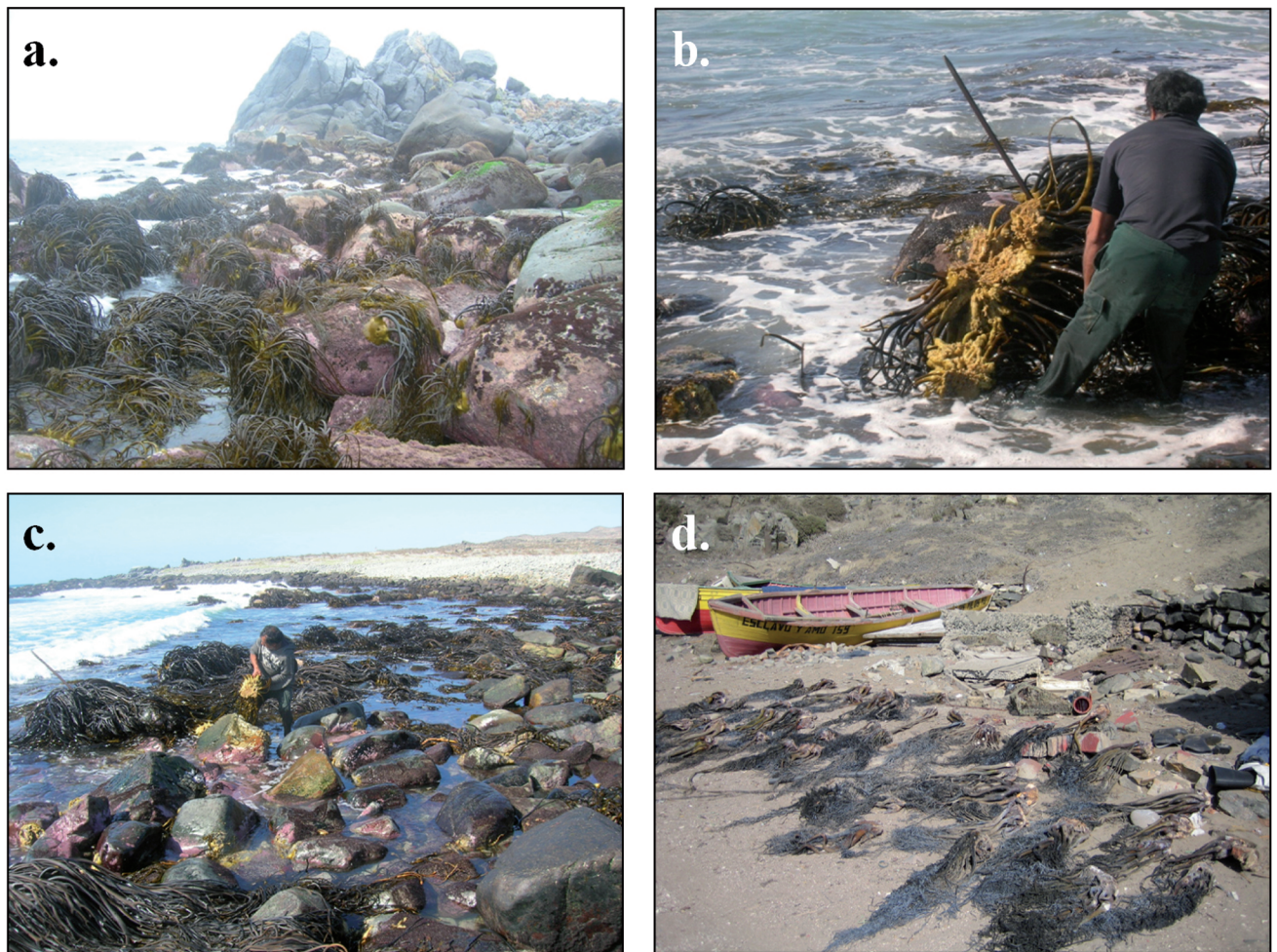


Figure 1. Illustration of the kelp harvesting in Chile. **a.** Kelp population during a low tide. **b. & c.** Artisanal fisherman harvesting the kelp by cutting the individual holdfast of the alga. **d.** Algae drying in the coastal desert, prior to selling to third-men. This last photograph is from the Southern species of *Lessonia nigrescens* (location at 31°S), while the three former are from the Northern species of *L. nigrescens* (locations at 28°S (a) and 29°S (b, c)). Photos from A. Vega & F. Tellier.

Figure 1. Illustration de la récolte de Laminariales (kelps) au Chili. **a.** Exemple d'une population de kelps à marée basse. **b. & c.** Pêcheurs artisanaux récoltant une kelp en rompant le disque de l'algue. **d.** Algues séchant dans le désert côtier, avant d'être vendues à un intermédiaire. Cette dernière photographie est de l'espèce Sud de *Lessonia nigrescens* (site localisé à 31°S), tandis que les précédentes sont de l'espèce Nord de *L. nigrescens* (sites localisés à 28°S (a) et 29°S (b, c)). Photos : A. Vega et F. Tellier.

marker (Ballard & Rand, 2005). Hence, the combination of molecular markers from distinct genomic compartments (nucleus, mitochondria and chloroplast) is required to confirm the cohesion of a species or to show the presence of several phylogenetic species. Barcoding of multiple markers has been used recently to resolve species identities for kelp taxa with a strong within-species structure (*Alaria*: Lane et al., 2007, *Saccharina latissima*: McDevit & Saunders, 2010).

A phylogenetic study of *L. nigrescens* revealed two strongly divergent lineages with a divergence of the same order of magnitude than between other species of the genus *Lessonia* (Tellier et al., 2009). In addition, because the four

molecular markers used were congruent and came from distinct molecular compartments (nucleus, mitochondria and chloroplast), it is highly likely that this pattern is reflecting the species' rather than the marker's history (Tellier et al., 2009). Thus, according to the PSC concept, two cryptic species exist within *L. nigrescens*.

Biological species

Testing for true biological species requires addressing smaller spatial and temporal scales than phylogenetic studies, focusing on the interfertility between conspecific individuals and on the degree of reproductive isolation between heterospecific individuals. To be considered inter-

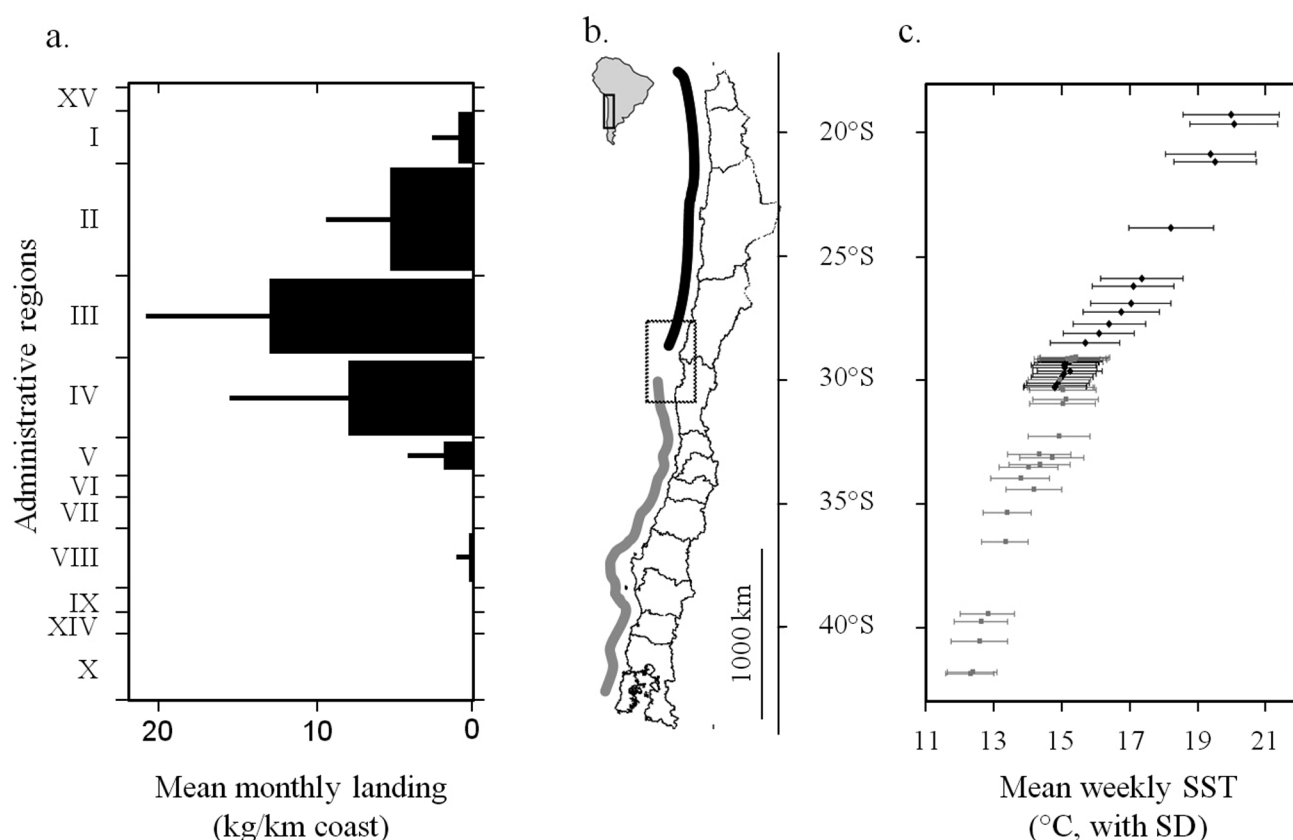


Figure 2. Harvesting pressure on *Lessonia* sp. along the Chilean coasts, geographic distribution of the two cryptic species of *Lessonia nigrescens* and environmental characteristics (here the Sea Surface Temperature, SST). **a.** Mean monthly landing of *Lessonia* sp. for each administrative Chilean region. Bar length indicates the landing quantity (expressed in kg per km of coastline), while bar width is proportional to the region geographic extent (roughly equivalent to the regional coastline length) – the volume of the bar indicates therefore the total landing volume of that region. **b.** Geographic distribution of the two cryptic species of *L. nigrescens* along the Chilean coast. The black and the grey bars along the coastline indicate the distribution of the cryptic species according to Tellier et al. (2009): in black the Northern species and in grey the Southern species. The box represents the region enlarged in Figure 3. **c.** Mean Sea Surface Temperature for each location for which the species has been identified. Long term records (26 years: 1982-2008) were obtained from Advanced Very-High Resolution Radiometer (AVHRR) satellite imagery (Casey & Cornillon, 1999). Each value corresponds to a previously characterized population (Tellier et al., 2009 & 2011), composed of individuals either from Northern species (in black) or from Southern species (in grey). Means are indicated with standard deviations.

Figure 2. Pression de récolte de *Lessonia* sp. le long des côtes chiliennes, distribution géographique des deux espèces cryptiques de *Lessonia nigrescens* et caractéristiques environnementales (dans le cas présent, la température de surface de l'eau, SST). **a.** Moyenne de la quantité récoltée par mois de *Lessonia* sp. pour chaque région administrative chilienne. La longueur des barres indique la quantité totale récoltée (exprimée en kg par km de côte) et la largeur de la barre est proportionnelle à l'étendue géographique de la région (équivalent quasiment à la longueur de côte régionale) – le volume de la barre indique donc le volume total récolté dans la région. **b.** Distribution géographique des espèces cryptiques de *L. nigrescens* le long de la côte chilienne. Les traits noir et gris le long de la ligne de côte indiquent la distribution des deux espèces cryptiques selon Tellier et al. (2009) : en noir l'espèce Nord et en gris, l'espèce Sud. Le rectangle représente la région agrandie dans la Figure 3. **c.** Température moyenne de la surface de la mer pour chaque endroit où l'espèce a été identifiée. Les données correspondent aux enregistrements sur le long terme (26 ans, 1982-2008) du système d'imagerie « Advanced Very-High Resolution Radiometer » (AVHRR) (Casey & Camillon, 1999). Chaque valeur correspond à une population déjà caractérisée (Tellier et al., 2009 & 2011), composée d'individus soit de l'espèce Nord (en noir), soit de l'espèce Sud (en gris). Sont indiquées les moyennes et les déviations standards.

fertile, interspecific hybrids have to be fertile allowing some gene flow between the two hybridizing species.

To quantify the extent of interspecific gene flow between the two cryptic species of *L. nigrescens*, a population

genetics approach was used (Tellier et al., 2011). The geographic region studied was limited by the complete spatial segregation of the two species, i.e. the absence of sympatry; and the gene flow study therefore considered

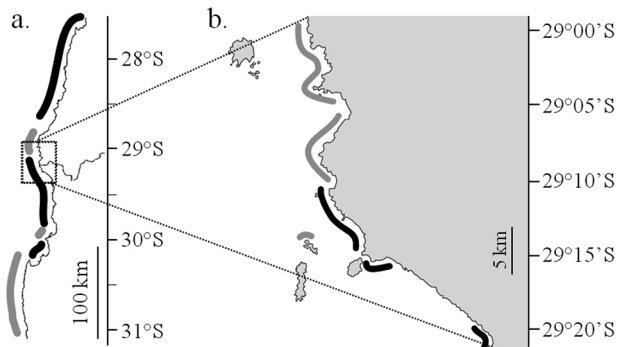


Figure 3. Geographic distribution of the two cryptic species of *L. nigrescens* along the Chilean coast. **a.** At the scale of the transition zone and **b.** At the scale of a contact region. As in Figure 2, the black and the grey bars along the coastline indicate the species distribution: Northern species in black, Southern species in grey (according to Tellier et al., 2011, and aggregated zones: F. Tellier, unpublished data). Note that each location is inhabited by only one or the other species.

Figure 3. Distribution géographique des deux espèces cryptiques de *L. nigrescens* le long de la côte chilienne. **a.** A l'échelle de la zone de transition et **b.** A l'échelle de la région de contact. Comme dans la Figure 2, les traits noirs et gris le long de la ligne de côte indiquent la distribution des espèces : l'espèce Nord en noir, l'espèce Sud en gris (selon Tellier et al., 2011, et F. Tellier, données non publiées pour les informations ajoutées). Chaque site est composé seulement d'individus de l'une ou l'autre des deux espèces.

populations immediately adjacent (parapatric distribution). The molecular markers used were four microsatellite loci, which are markers of high resolution and have the advantage to be transmitted by both parents to the offspring. All the alleles were diagnostic to one or the other species, without shared alleles between species, and all individual genotypes were pure genotypes (all alleles from only one species). These clear-cut results indicated that no hybrids are formed in natural conditions, maintaining the genetic cohesion of each species. Therefore, the two phylogenetic species are reproductively isolated in natural conditions, and can be considered as true biological species.

Ecological species

The two cryptic *L. nigrescens* species identified present contrasted distribution ranges: while one is located between 16°S (Southern Peru) and 30°S, the second is present from 29°S to 42°S (Tellier et al., 2009 & 2011, Fig. 2b). Waiting for a formal taxonomic description (but see below), we will name these species as “Northern species” and “Southern species” respectively. In the overlapping part of their distribution ranges (i.e. between 29°S and 30°S), a fine scale study showed that the two species never co-occurred within a single site. Hence, the overlapping region is

composed of a mosaic of pure Northern populations and pure Southern populations, and the species maintain their spatial segregation (Fig. 3a & b).

Additional evidence indicates that the two species not only differ in their geographic distribution, but also more generally in their ecological characteristics. A reciprocal transplantation experiment in the overlapping region suggested differential, species-specific responses to microenvironmental factors (Tellier, 2009). Additional studies have shown that microscopic stages of the two species have contrasting tolerances and demographic responses to thermal stresses (Oppliger, 2010; Oppliger et al., 2011). Thermal stress is of particular relevance as the two species have contrasting distributions along the latitudinal gradient of oceanic temperature. Figure 2c shows the strong increase in mean Sea Surface Temperatures (SST) north of 30°S when considering long-time records.

Interestingly, all these studies suggested that the two species differ not only in their response to environmental factors, but also in their intrinsic (genetic) demographic dynamics. Further studies will be necessary to address the exact environmental factors acting at the range edges of the two species, with a particular emphasis on population dynamics and productivity, which are key elements to inform policymaking.

Morphological species

No published work is currently available addressing potential morphological differences among the two species of *L. nigrescens*. However, the definition of cryptic species, undistinguishable by morphological criteria, raises a difficulty for ecologists and phycologists (Bickford et al., 2006). In fact, the formal description of a new nominal species requires a morphological characterization and if diagnostic morphological features are revealed, it would ease species identification in the field and provide management plans with a tool that could be readily implemented in the field. However, because of their high phenotypic plasticity, contrasting distributional ranges and the absence of sympatric sites, such study remains particularly challenging.

Discussion: Consequences for management and conservation

Threats of managing indiscriminately the two species as a single one

The application of a common management strategy to cryptic species may increase the risk of overexploitation and degradation or loss of the habitat provided by engineer

species, such as kelps (Coleman & Williams, 2002). As suggested previously (see above), the two *L. nigrescens* species have demonstrated different population dynamics and tolerance to environmental stress. Further studies would be necessary to determine if they also differ in other attributes, such as those maintaining connectivity among local populations or productivity.

The knowledge of ecological and demographic differences may allow the identification of major threats to the persistence of the species. In fact, because the two species are occupying different ecological niches, they are not interchangeable. If a major disturbance affects one species; the second may not be able to colonize the empty area neither through natural recolonization nor by artificial restoration techniques (Correa et al., 2006).

The presence of two distinct species implies also a cautious protocol when moving living material from one place to the other, as it has been reported in Northern Chile (S. Faugeton, pers. obs.). The replenishment of local populations either by transplanting young individuals or by using “seeds” (in fact very small juveniles) may be compromised if it involves the wrong species, unadapted to the local conditions. Nowadays, because this transfer is likely to be very local (among geographically close populations) and at a small (artisanal) scale, the probability of mixing the two species remains very low, except in the Coquimbo Region (IV Region) where both species are found in close proximity (Figs 2b & 3a).

Two species instead of one: recommendations

We have shown that the Southern *Lessonia* species is particularly sensitive to increases in temperature (Oppliger, 2010). This may be the main factor that limits its northern distribution edge where abrupt shifts in sea surface temperature regimes and upwelling intensity are observed (Tapia et al., 2009; Wieters et al., 2009). In addition, marginal populations (i.e. the populations that are at the range margins, in the contact zone between 27–30°S) show evident signatures of processes that are affecting the demography of both species, such as biased sex-ratios (Oppliger et al., 2011) and increased vegetative reproduction (Oppliger, 2010). Demographic differences can be explained by the fact that they are living in the margin of their respective suitable habitat and the selective pressures limiting hybridization of the two species in this contact region. The demographic consequences are likely to be expressed through higher sensitivity to local perturbations, such as harvesting, and a reduced capacity to recover from local extinction. Although these predictions need to be evaluated in the field, dispersal capacity in *Lessonia* spp. is limited, as evidenced by the slow natural recolonization of populations impacted by El Niño Southern Oscillation (ENSO) events (Martinez et al., 2003)

throughout northern Chile, or by historical point-source pollution (Medina et al., 2005), which interrupted population connectivity since early 1940s and lead to a 40km disruption of the other way continuous natural stands (Faugeron et al., 2005).

Most of the kelp harvesting in Chile is carried out in the arid and semiarid region between 18°S and 32°S, which provides reliable open-air drying of kelp individuals (Vásquez, 2008), highlighting the need for a cautious management approach in the region. In addition, several thermopower projects are being considered in the region and an extensive survey would be necessary to monitor the potential impacts on *L. nigrescens* species. In California, the thermal outfall of a power-generating station has induced the local replacement of two temperature-sensitive kelp species by the more tolerant kelp *Macrocystis pyrifera* (Schiel et al., 2004). The local conditions have been compared to a permanent El Niño-like event, with stronger effects on the species having their geographic range edge closer to the station (Schiel et al., 2004).

The existence of two species is therefore a concern because these are in contact in one of the most harvested regions in Chile. The edge of the geographic range is a particular place where several ecological and evolutionary processes can exert strong pressure on the local populations of both species, potentially limiting their demographic tolerance to harvest. Management strategies should therefore not only take into consideration the ecological differences between the two species and their different evolutionary histories (if ESU is to be used as management criteria), but also the fact that the contact region is particular and should deserve special attention.

Future directions

In order to increase our knowledge of the biology of the two species of *L. nigrescens* and accurately manage the kelp resource, we recommend the characterization of the population dynamics as a necessary step to set recommendations on “when to harvest” to maximize productivity without affecting the stability of the resource. The hypervariable molecular markers (microsatellites) developed may also be useful to measure the effect of harvesting pressure on genetic diversity and to control the effects of management measures (see Valero et al., 2011), for example, using natal identification of harvested individuals. Natal identification through traceability studies is particularly valuable for species characterized by very strong population structure (Tellier et al., 2009). Habitat restoration experiments, transplanting individuals into locations historically devastated by pollutants, may also benefit from the use of microsatellite markers as it would help to design the level of genetic diversity that is adequate for long term local population persistence.

Finally, the presence of two species, instead of one, opens new perspectives on the commercial and medical uses of the biomolecules extracted from them: because the two species are strongly divergent in their genetics and ecology, we may expect distinct gene expression patterns that could translate into different high-value products and uses. Comparative studies have revealed chemical differences among closely related species, such as *L. nigrescens* and the subtidal *L. trabeculata*, but also between other *Lessonia* species (Campos-Vallette et al., 2010) and between *Durvillaea* species (Kelly & Brown, 2000).

Having “two species instead of one” when implementing management and exploitation plans shows us an unexpected scenario for public authorities and stakeholders. Although we used as an example the case of *L. nigrescens*, similar situations of “having two instead of one” are probably a common issue in the context of management of kelp resources. Nevertheless, the clear-cut situation observed in *L. nigrescens* is probably an advantage when transferring scientific knowledge to stakeholders and to the society, compared to less clear cases, i.e. the species that are still *in the process of speciation*, hence not completely reproductively isolated, as suggested for Japanese kelp complexes. To explain something like “having one-and-half instead of one” is certainly a challenge for the scientific community.

More broadly, the existence of cryptic species, by challenging our understanding of biodiversity, requires criteria alternative to morphology and phylogeny to be considered. We have shown here that careful evaluation of ecological differences may be far more relevant to management success than the delimitation of categorical management units. In the context of global change, temperature increase and ocean acidification, the actual capacity of species to cope with the environmental variability is one of the major questions faced by scientists and managers and conservation practitioners. The evolutionary capacity to adapt to the changing environment is another aspect that needs to be studied. Specific adaptations may lead to speciation. Therefore, the study of the origin and maintenance of cryptic species may give essential clues for predicting long term conservation and management strategies.

Acknowledgments

We dedicate this manuscript as homage to Paula Ayerdi, who was taken from us on the 2010 Chilean tsunami; she actively participated in field and lab experiments and contributed with valuable discussions before her life was cut short. We deeply thank our respective labs for help during various stages of the research: J. Correa/S. Faugeron lab, ex-EGPM team, ChangoLab, LabEco and LADIMO

teams. Importantly, this synthesis work was motivated by the fruitful discussions we had with members of these research teams and with Ecokelp teams. We also acknowledge A. Atlan, B. Kloareg and E. Poulin, J. Correa, E. Petit and D. Jollivet for their constructive comments. We also are grateful to two anonymous reviewers for constructive comments on earlier versions of the manuscript. F. Tellier was supported by a doctoral grant from the French Ministry of Education and Research (MERNT) and by a grant from the “Collège Doctoral Franco-Chilien”. A. Vega was supported by a doctoral grant from the Gobierno Regional de Coquimbo, Chile and the Facultad de Ciencias del Mar, Universidad Católica del Norte, Chile. This study is part of the research programs FONDECYT 1060493, FONDECYT 1070417, FONDAP 1501-0001 Program 7, ANR ECOKELP (ANR 06 BDIV 012), Pôle Mer Bretagne, “ARCUS” of the French Ministry of Foreign Affairs, and the Laboratoire International Associé “Dispersal and Adaptation of Marine Species” (LIA DIAMS). Additional support from FONDECYT 3110051 and from PROJECT HYPERLAB High Yield and Performance Stem Cell Laboratory FP7-223011 is also acknowledged.

References

- Agrawal A., Chhatre A. & Hardin R. 2008.** Changing governance of the world's forests. *Science*, **320**: 1460-1462.
- Ballard J.W.O. & Rand D.M. 2005.** The population biology of mitochondrial DNA and its phylogenetic implications. *Annual Review of Ecology and Systematics*, **36**: 621-642.
- Beddington J.R., Agnew D.J. & Clark C.W. 2007.** Current problems in the management of marine fisheries. *Science*, **316**: 1713.
- Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker K., Ingram K.K. & Das I. 2006.** Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, **22**: 148-155.
- Bixter H.J. & Porse H. 2011.** A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, **23**: 321-335.
- Bonin A., Nicolè F., Pompanon F., Miaud C. & Taberlet P. 2007.** Population adaptive index: A new method to help measure intraspecific genetic diversity and prioritize populations for conservation. *Conservation Biology*, **21**: 3, 697-708.
- Campos-Vallette M.M., Chandia N.P., Clavijo E., Leal D., Matsuhira B., Osorio-Roman I.O. & Torres S. 2010.** Characterization of sodium alginate and its block fractions by surface-enhanced Raman spectroscopy. *Journal of Raman Spectroscopy*, **41**: 758-763.
- Cancino J. & Santelices B. 1984.** Ecological importance of holdfasts of *Lessonia nigrescens* Bory Phaeophyta in Central Chile. *Revista Chilena de Historia Natural*, **57**: 23-33.
- Casey K.S. & Cornillon P. 1999.** A comparison of satellite and in

- situ-based sea surface temperature climatologies. *Journal of Climate*, **12**: 1848-1863.
- Coleman F.C. & Williams S.L. 2002.** Overexploiting marine ecosystem engineers: potential consequences for biodiversity. *Trends in Ecology and Evolution*, **17**: 40-44.
- Correa J.A., Lagos N.A., Medina M.H., Castilla J.C., Corda M., Ramirez M., Martinez E., Faugeton S., Andrade S., Pinto R. & Contreras L. 2006.** Experimental transplants of the large kelp *Lessonia nigrescens* (Phaeophyceae) in high-energy wave exposed rocky intertidal habitats of northern Chile: Experimental, restoration and management applications. *Journal of Experimental Marine Biology and Ecology*, **335**: 13-18.
- Coyne J.A. & Orr H.A. 2004.** *Speciation*. Sinauer Associates: Sunderland, MA, USA. 545 pp.
- Crandall K.A., Bininda-Emonds O.R.P., Mace G.M. & Wayne R.K. 2000.** Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, **15**: 290-295.
- de Revers B. & Asensi A. 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 a description of *L. searlesiana* sp. nov. and a new lectotypification of *L. flavicans*. *Cryptogamie Algologie*, **30**: 209-249.
- 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. *Journal of Phycology*, **45**: 1266-1269.
- Draget K.I., Smidsrod O. & Skjåk-Bræk G. 2002.** Alginates from algae. In: *Biopolymers, Polysaccharides II: Polysaccharides from Eukaryotes* (S. De Baets, E.J. Vandamme, A. Steinbüchel ed), Vol. 6, pp. 215-244. Wiley-VCH: Weinheim.
- Edding M., Fonck E. & Machiavello J. 1994.** *Lessonia*. In: *Biology of Economic Algae* (I. Akatsuka ed), pp 40-436. SPB Academic Publishing: The Hague.
- Faugeton S., Martínez E.A., Correa J.A. & Billot C. 2005.** Long-term copper mine waste disposal in northern Chile associated with gene flow disruption of the intertidal *Lessonia nigrescens*. *Marine Ecology Progress Series*, **288**: 129-140.
- Frangoudes K. 2011.** International comparison of seaweeds management. *Cahiers de Biologie Marine*, **52**: 517-525.
- Gelcich S., Hughes T.P., Olsson P., Folke C., Defeo O., Fernandez M., Foale S., Gunderson L.H., Rodriguez-Sickert C., Scheffer M., Steneck R.S. & Castilla J.C. 2010.** Navigating transformations in governance of Chilean marine coastal resources. *Proceedings of the National Academy of Sciences of the USA*, **107**: 16794-16799.
- Guiry M.D. & Guiry G.M. 2010.** *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://algaebase.org>; searched on 24 October 2010.
- Hebert P.D.N., Ratnasingham S. & deWaard J.R. 2003.** Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B* (Suppl.), **270**: S96-S99.
- Hey J. 2001.** The mind of the species problem. *Trends in Ecology and Evolution*, **16**: 326-328.
- Hoffman A. & Santelices B. 1997.** *Marine flora of central Chile*, Ediciones Universidad Católica de Chile: Santiago, 434 pp.
- Jones C.G., Lewton J.H. & Shachak M. 1994.** Organisms as ecosystem engineers. *Oikos*, **69**: 373-386.
- Kelly B.J. & Brown M.T. 2000.** Variations in the alginate content and composition of *Durvillaea antarctica* and *D. willana* from southern New Zealand. *Journal of Applied Phycology*, **12**: 317-324.
- Knowlton N. 2000.** Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia*, **420**: 73-90.
- Lane C.E., Mayes C., Druehl L.D. & Saunders G.W. 2006.** A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic reorganization. *Journal of Phycology*, **42**: 493-512.
- 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. *Molecular Phylogenetics and Evolution*, **44**: 634-648.
- Mace G.M. 2004.** The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B*, **354**: 711-719.
- Martínez E.A., Cárdenas L. & Pinto R. 2003.** Recovery and genetic diversity of the intertidal kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after El Niño 1982/83. *Journal of Phycology*, **39**: 504-508.
- 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. *Phycological Research*, **57**: 131-141.
- McDevit D.C. & Saunders G.W. 2010.** DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. *Phycologia*, **49**: 235-248.
- Medina M., Andrade S., Faugeton S., Lagos N., Mella D. & Correa J.A. 2005.** Biodiversity of rocky intertidal benthic communities associated with copper mine tailing discharges in northern Chile. *Marine Pollution Bulletin*, **50**: 396-409.
- Ojeda F. & Santelices B. 1984.** Ecological dominance of *Lessonia nigrescens* (Phaeophyta) in central Chile. *Marine Ecology Progress Series*, **19**: 83-91.
- Oppliger L.V. 2010.** Reproduction at the range limits of Laminariales at the Chilean and European coasts. PhD thesis, Université Pierre et Marie Curie & Pontificia Universidad Católica de Chile, 173 pp.
- Oppliger L.V., Correa J.A., Faugeton S., Beltrán J., Tellier F., Valero M. & Destombe C. 2011.** Sex-ratio variation in the *Lessonia nigrescens* complex (Laminariales, Phaeophyceae): Effect of latitude, temperature and marginality. *Journal of Phycology*, **47**: 5-12.
- Ryder O.A. 1986.** Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution*, **1**: 9-10.
- Santelices B., Castilla J.C., Cancino J. & Schmiede P. 1980.** Comparative ecology of *Lessonia nigrescens* and *Durvillaea antarctica* (Phaeophyta) in Central Chile. *Marine Biology*, **59**: 119-132.
- Santelices B. & Ojeda F.P. 1984.** Recruitment, growth and survival of *Lessonia nigrescens* (Phaeophyta) at various tidal

- levels in exposed habitats of central Chile. *Marine Ecology Progress Series*, **19**: 73-82.
- Schiel D.R., Steinbeck J.R. & Foster M.S. 2004. Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology*, **85**: 1833-1839.
- Starr J.R., Naczi R.F.C. & Chouinard B.N. 2009. Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). *Molecular Ecology Resources*, **9** (Suppl. 1): 151-163.
- Steneck R.S., Graham M.H., Bourque B.J., Corbett D., Erlandson J.M., Estes J.A. & Tegner M.J. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, **29**: 436-459.
- Tapia F., Navarrete S.A., Castillo M., Menge B., Castilla J.C., Largier J., Wieters E.A., Broitman B.L. & Barth J.A. 2009. Thermal indices of upwelling effects on inner-shelf habitats. *Progress in Oceanography*, **83**: 278-287.
- Tellier F. 2009. Etude des processus de spéciation associés à une zone de transition biogéographique : exemple de l'algue brune *Lessonia nigrescens*. PhD thesis, Université Pierre et Marie Curie & Pontificia Universidad Católica de Chile, 384 pp.
- Tellier F., Meynard A.P., Correa J.A., 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**: 679-693.
- Tellier F., Tapia J., Faugeron S., Destombe C. & Valero M. 2011. The *Lessonia nigrescens* species complex (Laminariales, Phaeophyceae) shows strict parapatry and complete reproductive isolation in a secondary contact zone. *Journal of Phycology*, **47**: 894-903.
- Uwai S., Arai S., Morita T. & Kawai H. 2007. Genetic distinctness and phylogenetic relationships among *Undaria* species (Laminariales, Phaeophyceae) based on mitochondrial *cox3* gene sequences. *Phycological Research*, **55**: 263-271.
- Valero M., Destombe C., Mauger S., Ribout C., Engel C.R., Daguin-Thiébaud C. & Tellier F. 2011. Using genetic tools for sustainable management of kelps: a literature review and the example of *Laminaria digitata*. *Cahiers de Biologie Marine*, **52**: 467-483.
- Vásquez J.A. 2007. Ecology of *Loxechinus albus*. *Developments in Aquaculture and Fisheries Science*, **37**: 227-241.
- Vásquez J.A. 2008. Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. *Journal of Applied Phycology*, **20**: 457-467.
- Vásquez J.A. & Santelices B. 1984. Comunidades de macro-invertebrados en discos adhesivos de *Lessonia nigrescens* Bory (Phaeophyta) en Chile central. *Revista Chilena de Historia Natural*, **52**: 131-154.
- Venegas M., Matsuhira B. & Edding M.E. 1993. Alginate composition of *Lessonia trabeculata* (Phaeophyta: Laminariales) growing in exposed and sheltered habitats. *Botanica Marina*, **36**: 47-51.
- Villouta E. & Santelices B. 1986. *Lessonia trabeculata* sp. nov. (Laminariales, Phaeophyta), a new kelp from Chile. *Phycologia*, **25**: 81-86.
- Wernberg T., Coleman M., Fairhead A., Miller S. & Thomsen M. 2003. Morphology of *Ecklonia radiata* (Phaeophyta: Laminariales) along its geographic distribution in south-western Australia and Australasia. *Marine Biology*, **143**: 47-55.
- Wieters E.A., Broitman B.R. & Branch G.M. 2009. Benthic community structure and spatiotemporal thermal regimes in two upwelling ecosystems: Comparisons between South Africa and Chile. *Limnological Oceanography*, **54**: 1060-1072.
- Wing S.R., Leichter J.J., Perrin C., Rutger S.M., Bowman M.H. & Cornelisen C.D. 2007. Topographic shading and wave exposure influence morphology and ecophysiology of *Ecklonia radiata* (C. Agardh, 1817) in Fiordland, New Zealand. *Limnological Oceanography*, **52**: 1853-1864.
- Winker K. 2005. Sibling species were first recognized by William Derham (1718). *Auk*, **122**: 706-707.
- Zimmermann H., Zimmermann D., Reuss R., Feilen P.J., Manz B., Katsen A., Weber M., Ihmig F.R., Ehrhart F., Gessner P., Behringer M., Steinbach A., Wegner L.H., Sukhorukov V.L., Vásquez J.A., Schneider S., Weber M.M., Volke F., Wolf R. & Zimmermann U. 2005. Towards a medically approved technology for alginate-based microcapsules allowing long-term immunoisolated transplantation. *Journal of Materials Science: Materials in Medicine*, **16**: 491-501.