Phylogeny, phylogeography and population connectivity of *Lessonia* (Phaeophyceae)

Cover: *Lessonia variegata* in the lower intertidal of Moa Point, Wellington.

Phylogeny, phylogeography and population connectivity of *Lessonia* (Phaeophyceae)

by

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Abstract

The brown algal genus *Lessonia* is distributed in the Southern Hemisphere where it can form dominant kelp beds on the exposed rocky shores of New Zealand, South America and Tasmania. Its disjunct distribution within the West Wind Drift contrasts with the view that it is a poor disperser. Apart from studies in Chile, where it is an economically important genus, little is known about *Lessonia* and in some areas even the number of species is unknown. Using different genetic markers I examined the phylogeny, phylogeography, and the connectivity of populations in *Lessonia*. Using the literature, species affiliations and nomenclatural problems have been investigated. Combining the sequences of three mitochondrial, one chloroplast and two nuclear markers, a supermatrix approach was used to investigate the phylogenetic relationship and the timing of speciation for all known *Lessonia* species. The Australasian *Lessonia* species form a clade within a paraphyletic grouping of South American species. Radiation in *Lessonia* occurred about 5 Mya at the beginning of the Pliocene and rapid radiation took place in Australasia 3.5 Mya. The data also revealed cryptic species within a *L. variegata* species complex. Further analysis within the Australasian clade, using mitochondrial (atp8-sp) and chloroplast (rbc-sp) markers and wider sampling (469 individuals from 57 sample sites) supported four cryptic species and revealed localized distribution for all Australasian lineages. Genetic breaks between *Lessonia* lineages corresponded well to known biogeographic regions and could be correlated to the geographic structure of New Zealand at the end of the Pliocene. The Cook Strait region was analysed more closely with newly developed microsatellite markers to test the influence of geographic breaks (Cook Strait and Palliser Bay) on the connectivity of populations.

The results suggested that connectivity depends on the width of unsuitable habitat, and within inner Cook Strait it is facilitated by sometimes strong tidal flows that create turbulences and unique current patterns. The results implied that rafting is an important mean of dispersal. The study of the early literature on *Lessonia* supported the new lectotypification of *L. flavicans* but revealed that *L. frutescens* and possibly *L. ovata* (supported by images of rediscovered herbarium material) are synonymous to *L. searlesiana* and as the older epithets they should have priority. Suggestions have been made for the lectotypification of *L. fuscescens* and *L. ovata*. In general *Lessonia* shows non-overlapping distribution in Australasia but overlapping distribution in South America. Despite being a poor disperser, indicated by fine scale genetic structure, *Lessonia* is also able to connect populations over wide areas of unsuitable habitats.

für Waltraud und Ludwig

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Chapter 1

Introduction & overview

1.1 Thesis structure

For this thesis I studied the phylogeny of the genus *Lessonia*, the phylogeography of Australasian *Lessonia* species, and the connectivity of populations using Cook Strait as a case study area. The approaches are hierarchical so that the results of one topic serve as the basis for the next. Experience has shown that phylogenetic methods improve if the phylogeny of the next highest level is known (e.g., for the choice of the outgroup). Also when local genetic patterns have to be interpreted it is helpful to know whether they derive from a monophyletic grouping or not. Therefore this dissertation is not only divided into three taxonomic levels for the main data chapters but also chronologically ordered into themes that allowed me to go from global to local while using information gained at higher levels.

Lessonia is introduced in Chapter 1, starting with reviewing early and recent descriptions and the known biogeography of *Lessonia* as the conceptional framework of this study (Section 1.2); this is followed by explaining the biology of *Lessonia* with a focus on its dispersal abilities based on different phases of its heteromorphic life cycle as a background for diverse dispersal scenarios (Section 1.3). Before I started, a taxonomic reorganisation at the family level (Lane *et al.* 2006), fundamentally changed our knowledge of sister taxa to *Lessonia*. The genera *Eisenia*, *Ecklonia* and *Egregia* form now, together with *Lessonia*, the family

Lessoniaceae and are considered the primary source for outgroups (Chapter 1.4). In Chapter 2 I report on the global phylogeny of *Lessonia* investigating almost all known *Lessonia* species using molecular markers from mitochondrial, chloroplast, and nuclear DNA. In this study I first recognised four cryptic species within *Lessonia variegata*. Also new is the discovery of a monophyletic Australasian group, comprising the species endemic to New Zealand (*L. variegata*, *L. brevifolia*, *L. adamsiae*, *L. tholiformis*) and Tasmania (*L. corrugata*). This Australasian group is investigated more closely in Chapter 3 using the mitochondrial *atp8*-spacer region and a method (SSCP) for fast and cost-efficient screening of a large dataset. The distribution of all Australasian species is mapped and compared to known biogeographic and phylogeographic breaks. Chapter 4 is written as a technical note to introduce new microsatellite markers that I developed specifically for *Lessonia* to address questions on a finer local scale. This is finally done in Chapter 5 where the Cook Strait seaway is tested for its influence on the connectivity of populations. In Chapter 6, I address a nomenclatural problem that persisted for the southern South American *Lessonia* species, which had led to a multiplication of names and taxonomic confusion. Chapter 7 provides a summary and outlines directions for future research.

All data Chapters (Chapter 2 to 5) are written in a style to be submitted to a scientific journal for publication.

-Chapter 1: Introduction and overview

-Chapter 2: Martin, P., & Zuccarello, G, C,. Molecular phylogeny and timing of radiation in *Lessonia* (Phaeophyceae, Laminariales). *Journal of Phycology*. (submitted)

- Chapter 3: Phylogeography of *Lessonia* (Phaeophyceae, Laminariales) in Australasia
- Chapter 4: Characterisation of microsatellites in the Southern Hemisphere kelp, *Lessonia variegata* (Phaeophyceae, Laminariales)
- Chapter 5: Connectivity between populations of *Lessonia variegata* (Phaeophyceae, Laminariales) across Cook Strait, New Zealand
- Chapter 6: Taxonomic confusion in *Lessonia* from the Falkland Islands
- Chapter 7: Summary and directions for future research

1.2 *Lessonia* – First descriptions and biogeography

Lessonia Bory, 1825:p.23 as *Lessonia flavicans*. *Caule subarboreo cylindrico, ramis compressis, foliis ovato-linearibus, subdenticulatis flavicantibus*. [Species description also validating the genus (ICBN Art. 42)]

Lessonia is a member of the family Lessoniaceae Setchell & Gardner 1925:591 within the order Laminariales Migula 1909:243. The genus *Lessonia* was never formally described but the description of the single species of *Lessonia flavicans* (see above) also validated the genus (Art. 42 ICBN, Searles 1978). An emended description of *Lessonia* is: “Thallus 1-4 m high, perennial. Holdfast discoid, divided and becoming conical, or ligulate, attached by branched haptera, with the stipe branched from the base or higher, often twisted, with each branch bearing a blade. Blades simple, 0.5-2 m long and 1-5 cm wide, ligulate to irregularly elongate, flat, margins denticulate, surface smooth to rugose or corrugate. Growth by longitudinal splitting of the blades, resulting in dichotomous branching of stipe. Blade and stipe with a central, filamentous medulla, extensive cortex of relatively small cells with or without mucilage cavities, and outer layer of meristoderm. Spores produced in unilocular sporangia with paraphyses in extensive sori, produced on both surfaces of blades. Gametophytes dimorphic, dioecious, oogamous, branched uniseriate filaments” (Guiry & Guiry 2011).

Currently nine species are recognized within *Lessonia* (Table 1.1), however, the species have been often confused with each other in the literature because of misleading and entangled descriptions and different interpretations of the original

diagnoses. The latest attempt to clarify the situation has been made by Asensi & de Reviers (2009) who created an illustrated catalogue of type species preserved at the Muséum National d'Histoire Naturelle, Paris, France (PC). Their main changes have been a new lectotypification of *L. flavicans* and the description of a new species *L. searlesiana* based on herbarium material. In the following I will introduce all known *Lessonia* species, their synonyms and distribution while a critical review of the works of *Lessonia* authorities and others that have influenced our knowledge about this genus will be given in Chapter 6.

Lessonia is the only genus of the Laminariales that is solely distributed in the Southern Hemisphere (Bolton 2010) between 12°S and 56°S. Four species are currently recognised in South America, five in Australasia, and two unknown species are reported from the Kerguelen and Heard Islands (Hay 1987, Edding *et al.* 1994). The South American species are *Lessonia flavicans* Bory 1825, *L. nigrescens* Bory 1826, *L. ovata* Hook.f.&Harv., 1847, and *L. trabeculata* Villouta & Santelices 1986 [but see also Searles (1978) for species recognition before 2009 and Asensi & de Reviers (2009) for a revision on species nomenclature of *L. flavicans* and *L. vadosa*; here I adopt the suggestions made by Asensi & de Reviers (2009) but with *L. searlesiana* as a synonym to the earlier described *L. ovata*]). In Australasia we recognise *L. variegata* J. Agardh 1894, *L. brevifolia* J. Agardh 1894, *L. corrugata* Lucas 1931, *L. adamsiae* Hay 1987, and *L. tholiformis* Hay 1989. Species reported from Kerguelen and Heard Islands are *L. nigrescens* and *L. ovata* on Heard (Dickie 1876), *L. flavicans* (as *L. fuscescens*) on Kerguelen (Dickie 1876 & 1879), Delepine (1963) reported that there are maybe two species of *Lessonia* on Kerguelen, and Papenfuss (1964) reported *L. fuscescens* and *L. nigrescens* on Heard and *L.*

fuscescens on Kerguelen. Even though the species on Kerguelen and Heard Islands are reported as *L. nigrescens*, *L. ovata* or *L. fuscescens* it is more likely that these are undescribed species as *Lessonia* is a poor disperser (Faugeron *et al.* 2005) and thus species ranges are restricted. That *Lessonia* is believed to be a poor disperser is in relation to the seaweeds that have floating structures. Dispersal of spore aggregates, parts of fronts, or detached algae have been shown (in other algae by e.g., Norton 1992 and Sliwa *et al.* 2006) to be not efficient for long distance dispersal. *Lessonia* does not have any special floating abilities or other abilities for long distance dispersal and would thus appear to be a poor disperser. However, specimens that are attached to other vehicles might be able to travel long distances. Other vehicles might be other algae that are known for long distance dispersal, for example *Macrocystis* and *Durvillaea antarctica*. Both algae grow in close distance to *Lessonia* and it is known that they can grow on top of each others holdfast (especially *Durvillaea antarctica* has been found to grow on top of other algae as shown in Fig. 1.11).

The distribution range of the South American species is more or less overlapping, with the intertidal *L. nigrescens* occupying the widest range from 12°S in Peru (Martinez, 1999), around Cape Horn with the Diego Ramirez Islands at 56°S as the southernmost record (Kuehnemann 1970 in Edding *et al.*, 1994), to Punta Atlas in Argentina (Barrales & Lobban, 1975) (Fig. 1.1). The northern distribution limit in the Pacific is highly dependent on environmental disturbances such as El Niño, which can result in a disappearance of *Lessonia* populations as far south as 26°S, as occurred after the 1982-83 El Niño event (Edding *et al.*, 1994). The wipe-out effect due to high temperature is buffered in areas of coastal upwelling and is not as

significant for the subtidal *L. trabeculata*. A recent survey on *L. nigrescens* populations from Chiloe (~42°S) to the north of its distribution range revealed the existence of two genetically distinct cryptic species (Tellier *et al.* 2009). Unfortunately no *L. nigrescens* was included from its type locality (Cape Horn) thus it is unsure whether all genetic variation is covered. If *L. nigrescens* from the type locality differs from the IA-lineage (intermediate area lineage as used by Tellier *et al.* 2009) to an extent normally found between species, then *L. berteroana* Montagne, 1842 could be resurrected from its synonymous status with *L. nigrescens* into a valid species status. *L. berteroana* has narrower, acute blades (not as wide as in *L. nigrescens*), a filamentous cortex with clusters of ramified filaments (rather than a homogenous cortex), elongated epidermal cells, and a more northern distribution (Asensi & de Reviers 2009). As the type locality of *L. berteroana* lays in the Z30 zone (see Tellier *et al.* 2009) it belongs most likely to either the Z30 or IA-lineage but not the northern lineage, which has to be described. Further investigations are needed to clarify and describe the status of species within this complex.

The subtidal *L. trabeculata* shares its range with the intertidal *L. nigrescens* on the Pacific coast of South America from as far north as 14°S (Fernandez, 1999), and south to 42°S (Villouta & Santelices, 1986) (Fig. 1.1 & 1.2). Variation in the size of populations from the northern range of *L. trabeculata* can occur in La Niña years, when grazer abundance is strongly increased (Vega *et al.*, 2005).

The southernmost distribution of *L. flavicans* is the Beagle Channel from where it is found north until 49°S on the Chilean coast and to 47°44'S (Puerto

Deseado) on the Argentinian coast, and east to the Falkland Islands (Searles, 1978) (Fig. 1.3).

L. ovata shares its north-eastern (Puerto Deseado, Argentina), eastern (Falkland Islands) and northwestern (Canal Trinidad) limit with *L. flavicans*, but is found further south all around Cape Horn (Searles, 1978) (Fig. 1.3). *L. ovata* might occur in two different morphotypes (Fig. 1.6).

In southern Chile *L. nigrescens* co-occurs with *L. flavicans* and *L. ovata* (Fig. 1.4), however, *L. nigrescens* is absent from the Falkland Islands (Fig. 1.5).

Skottsberg's (1907, 1921) reports of *L. nigrescens* on the Falkland Islands are misleading as his concept of *L. nigrescens* was synonymous to *L. vadosa* (Chapter 6). Searles (1978) excluded the records of *L. nigrescens* from the Falkland Island (Hooker & Harvey 1845, p.152 [wrong citation in Searles 1978; should be Hooker & Harvey 1847, p.458]; Skottsberg 1907, p.72; and Cotton 1915, p.166), indicating misidentification in all cases. The affiliation of species in South America seems to be difficult because of overlapping species ranges in synchrony with overlapping morphologies and the occurrence of probably two or more undescribed species (Searles, 1978; Ramirez and Santelices, 1991). The intertidal habitat, occupied in South America by *L. nigrescens*, might be occupied on the Falkland Islands by a morphotype of *L. flavicans* (Fig. 1.7A); whereas two additional morphotypes might occur in the subtidal habitat (Fig. 1.5 & 1.7B-F).

In contrast to the overlapping distribution of the South American *Lessonia* (Fig. 1.1-5) the distribution of *Lessonia* in Australasia is localized and shows high endemism (Fig. 1.8 & 1.9).

Lesssonia corrugata is endemic to Tasmania (Australia), *L. variegata* to the three main Islands of New Zealand (North Is, South Is, and Stewart Is), *L. tholiformis* to the Chatham Islands, *L. adamsiae* to the Snares, and *L. brevifolia* to the four Sub-Antarctic Islands of New Zealand (Auckland Islands, Campbell Islands, Antipodes Islands, and Bounty Islands). However, Schiel and Hay observed dense clumps of presumably *L. brevifolia* at a site at Five Fingers Peninsula (Fiordland; South Is.) where otherwise *L. variegata* was dominant (Schiel & Hickford, 2001). This is the only record of co-occurrence in *Lesssonia* sp. in Australasia.

1.2.1 Distinguishing features of *Lesssonia* species and their synonyms

1.2.1.1 Australasian species

Lesssonia adamsiae Hay, 1986:295ff endemic to The Snares was named after Nancy Adams, a well known New Zealand phycologist, and is different to other species by conspicuously corrugated blades (which look similar to the corrugation found in *Macrocystis* and differ markedly from the corrugation associated with *L. corrugata*) and a high number of predominantly flattened branches (up to 100) that emerge from the holdfast. The base is a massive, asymmetrical coalescence of holdfast, basal branches and haptera, which is otherwise only known from *L. trabeculata*.

Lesssonia brevifolia J. Agardh, 1894:88 was described from the Auckland Island as being a dwarf species that is scarcely one foot (30cm) long. In the diagnosis Agardh (1894) states that the length of the blades are scarcely four times longer than their breadth. In the narrative he mentioned a length of 4-5 inches (10-13cm) and that all blades show an erose apex structure and that no “fruit-bearing” sori have been seen. As the blades of this species develop normal length comparable with *L. variegata* (per. obs.) it is speculative that the specimen Agardh based his description

on had lost the upper part of the blade below the sori, which would give them the appearance of a short bladed form. *L. brevifolia* differs from other Australasian *Lessonia* by the small number of branches that emerge from the base (1-2(4)) and a more or less hapteroid holdfast of mainly unfused and freely branching haptera (Hay 1987). The species is now known from the Auckland Islands, Campbell Islands, Bounty Islands, and Antipodes Islands (Hay 1987, Adams 1994) where it might be found to a depth of 30m (Hay *et al.* 1985).

Lessonia corrugata Lucas, 1931:410 is endemic to Tasmania and easy to distinguish by its corrugated blades. It is the only Australasian species that is not found in New Zealand.

Lessonia tholiformis Hay, 1989:461ff is only known from the Chatham Islands. Young individuals might have corrugated leaves like *L. adamsiae* but the species is easy to distinguish from other *Lessonia* by its unique dome-shaped holdfast (hence *tholiformis*=form of a tholus {in ancient Greek architecture, a circular building with a conical roof}).

Lessonia variegata J. Agardh, 1877:6 was first mentioned by J. Agardh while listing the marine algae of New Zealand. He only mentioned that *L. variegata* had lacunae and did not provide any other diagnosis of this species; this however was done by Laing (1894) who validated J. Agardh as the authority. *L. variegata* is found in the subtidal to a depth of 18m (Schiel 1990) in exposed rocky shores of the North Island, South Island, and Steward Island (Hay 1987, Adams 1994). Cryptic speciation within a *L. variegata* species complex was recently detected (Chapter 2) with a unique distribution of each of the four lineages (Chapter 3). The type locality is Wellington (Lyall Bay), where it has striated blades (thus *variegata*).

No synonyms are known for any Australasian *Lessonia* but they have been listed as *L. fuscescens* (Hooker 1867, Dickie 1879), *L. flavicans* (Papenfuss 1964, Hay *et al.* 1985), or *L. variegata* on the Sub-Antarctic Islands (Papenfuss 1964, Hay *et al.* 1985) before they were described as distinct species.

1.2.1.2 South American species

Lessonia flavicans Bory, 1825:23 was the first *Lessonia* species described; successive descriptions made by Bory de Saint-Vincent in 1825, 1826 and 1828 of the same species but with different epithets (which all translate to yellowish) has led to confusion about its affiliation (see Chapter 6) and until recently a different species was associated with this name (i.e., the species definition after Searles (1978) was generally accepted). The old and new understanding of *L. flavicans* is of a big, tree-like alga with narrow blades and less wide blade base angle compared to *L. ovata* or *L. searlesiana* and **with cortical lacunae** in blade sections. The species is found at some distance from the shore and, when fully grown, it might have only one stem. Its appearance is as depicted in the ‘Atlas’ of the ‘Voyage autour du monde’ (Bory de Saint-Vincent 1828), ‘Flora Antarctica’ (Hooker & Harvey 1847) and ‘Illustrationes algarum’ (Postels & Ruprecht 1840). [This description does not entirely conform with the description made by Asensi & de Reviers (2009) but it is conform to the first descriptions made by d’Urville and Bory de Saint-Vincent in 1825, 1826 and 1828; it is also conform to the new lectotype of *L. flavicans* and to a species found at the Falkland Islands.) Synonymy: *Lessonia fuscescens* Bory, 1826; *Lessonia frutescens* Skottsberg, 1907; *Lessonia nigrescens* f. *lacunifera* Skottsberg, 1921; *Lessonia vadosa* Searles, 1978; Misapplied names: *L. nigrescens sensu* Skottsberg

Lessonia nigrescens Bory, 1826:322, should be regarded as a species complex with at least two cryptic species (Tellier *et al.* 2009). It has an easy to identify and unique holdfast with tightly fused haptera to build a hemispherical mound where up to 20 branches protrude. Branches and blades have a dark colour (therefore *nigrescens*). *L. nigrescens* is the only *Lessonia* species to be found in the intertidal. Synonyms: *Laminaria ramosissima* Lamour. & Chauv. (nomen herbariorum ? in Bory de Saint-Vincent 1825); *Chordaria spicata* Suhr, 1839; *Laminaria scissa* Suhr, 1841; *Lessonia suhrii* J. Agardh, 1841 [nom. illeg. Art. 55 ICBN]; *Lessonia berteriana* Mont., 1842; *Lessonia binderi* Sonder (nom. herb. ? in Kuetzing 1849); *Lessonia nigrescens* f. *montagnei* Skottsberg, 1921.

Lessonia ovata Hook.f.&Harv. 1847:459 has short dichotomously branched stipes with short branches, which are further divided; the petiole broadens into an ovate or linear-ovate, olive to darkish green, thin-skinned lamina. The lamina are often very broad (6-40cm), which gives the blade an oval (*ovata*) shaped base. Blade base angle is broader than in *L. flavicans*. **No lacunae** are present in stipe or blade. Synonyms: *Lessonia searlesiana* Asensi & de Reviers, 2009 Misapplied names: *Lessonia frutescens sensu* Searles.

Lessonia searlesiana Asensi & de Reviers, 2009:209ff is most likely a synonym of *L. ovata*, see this entry and Chapter 6.

Lessonia trabeculata Villouta & Santelices, 1986:81ff is found subtidally from 0.5-20m and shares its distribution range with the intertidal *L. nigrescens*. It has a massive, asymmetrical and irregularly shaped holdfast where many branches emerge at the base still tightly packed. In transverse sections of the blade, lacunae with elongated, multicellular filaments (*trabeculae*) are visible under the

microscope. It was often confused with *L. flavicans* but as they do not have any overlapping distribution, it is easy to detect.

Taxonomic confusion has been addressed by many authorities of *Lessonia* (Searles 1978, Villouta & Santelices 1986, Asensi & de Reviers 2009), however, no satisfying solution has been found yet and work is still needed.

1.2.2 Figures and Tables

Fig. 1.1 Distribution of *L. nigrescens* and *L. trabeculata*.

Fig. 1.2 Vertical distribution of *Lessonia* species in central Chile. *L. nigrescens* (black) shares the intertidal with *Durvillaea antarctica* (orange); *L. trabeculata* (white) is found in the subtidal (0.5-20m) above and between *Macrocystis pyrifera* (green).

Fig. 1.3 Distribution of *L. ovata* (= *L. flavicans sensu* Searles) and *L. flavicans*.

Fig. 1.4 Vertical distribution of *Lessonia* species in south Chile (Patagonia). *L. nigrescens* (black) shares the intertidal with *Durvillaea antarctica* (orange); *L. flavicans* (yellow) is found in the subtidal (0.5-2 (15) m) above a belt of *Macrocystis pyrifera* (green) whereas *L. ovata* (brown) is found below the *Macrocystis* belt (2-20). It is possible that *L. flavicans* might become bigger or as big as *L. ovata* (see Fig. 1.5 and photographs in Fig. 1.6 & 1.7).

Fig. 1.5 Vertical distribution of *Lessonia* species at the Falkland Islands. *L. flavicans* (yellow) can develop into huge algae several meters in height (up to 8m with trunks as thick as a human thigh (d'Urville 1825)) and is found in the subtidal (0.5-15m) above and between *Macrocystis pyrifera* (green) whereas *L. ovata* (brown) is usually found deeper (?-20) and might develop in big algae 4m in height. No *L. nigrescens* is found at the Falkland Islands but *L. flavicans* might have extended its range into the intertidal, where it is found between *Durvillaea antarctica* (orange). In the intertidal *L. flavicans* shows a slightly different morphology with round and more flexible stipes that look similar to the stipes of the intertidal *L. nigrescens* from the mainland (see photographs in Fig 1.6 & 1.7).

Fig. 1.6 Pictures of *Lessonia ovata* on the Falkland Islands (A-C) and Patagonia (D-F), showing different morphologies. The pictures on the Falkland Islands were taken at a depth of 20m. (Photos provided by K. Neely (A-C), and C. Cardenas (D-F)).

Fig. 1.7 Pictures of *Lessonia flavicans* on the Falkland Islands, showing different morphologies. A) intertidal together with *Durvillaea antarctica*. A single intertidal *L. flavicans* is shown in the insert. Note that even if the stipes appear similar to the stipes of *L. nigrescens*, the holdfast morphology is different to the one found in *L. nigrescens*, B-F) subtidal morphologies showing exceptionally big thalli extending above the canopy (D). (Photos taken by C. Fraser (A), or given by K. Neely (B-F)).

Fig. 1.8 Distribution of *Lessonia* in Australasia showing restricted distribution with no overlapping species ranges.

Fig. 1.9 Distribution of *Lessonia* in Australasia. *Lessonia* (white) is found in the subtidal below *Durvillaea antarctica* (orange) till 10-20m, depending on the species. *Macrocytis pyrifera* (green).

Table 1.1 Morphological, anatomical and habitat characteristics of nine *Lessonia* species including their synonyms and misapplied names.

Features	<i>L. brevifolia</i>	<i>L. adamsiae</i>	<i>L. tholiformis</i>	<i>L. variegata</i> *	<i>L. corrugata</i>	<i>L. ovata</i>	<i>L. nigrescens</i> *	<i>L. trabeculata</i>	<i>L. flavicans</i>
Size	up to 2.5m	up to 3m	up to 1.5m	1-1.5m	1-1.5m	up to 4m	up to 4m	up to 2.5m	up to 3m and more
Habitat	subtidal 2-25m	subtidal 1-22m+	subtidal >10m	subtidal 1-15m	subtidal 1-10m	subtidal 2-20m	intertidal +1 to -1m	subtidal 0.5-20m	shallow subtidal 0.5-2m (15)
Distribution	Australasian					South American			
	N.Z. Sub-Antarctic Islands	Snares I., NZ	Chatham Is., NZ	N.Z. North Island, South Island, Stewart Island	Tasmania, AUS	magellanic South America, southern Patagonia; Falkland Islands	south from Lima (Peru, ~12°S) along the Chilean coast, around Cape Horn (including Diego Ramirez Islands, ~56°S), till Punta Atlas (Argentina, ~45°S); not on Falkland	south of ~14°S in Peru till Chiloe (~42°S)	magellanic South America, Patagonia north of Beagle Channel, Falkland Is.

Table 1.1 continued

Blades	<i>L. brevifolia</i>	<i>L. adamsiae</i>	<i>L. tholiformis</i>	<i>L. variegata</i> *	<i>L. corrugata</i>	<i>L. ovata</i>	<i>L. nigrescens</i> *	<i>L. trabeculata</i>	<i>L. flavicans</i>
Shape	ovalate	linear, fragile	linear lanceolate	linear lanceolate	linear ensiform	linear-ovalate with a wide blade base angle	linear	linear or linear lanceolate	linear lanceolate
Margins & general appearance	glandulated, with microscopic superficial verrucosities	dentated, with little teeth 15-30mm distant	teeth up to 5mm long and tipped with a stiff, dark, oval knob; spaced at 15-25mm intervals	distantly papilated; surface smooth or longitudinally wrinkled	with alternate spines, 5mm long with broad base; surface rugose with 5 or more parallel longitudinal ribs	denticulated	finely dentated	smooth or dentated	dentated
Corrugated / smooth	smooth	corrugated	smooth (juv. rugulose)	smooth	corrugated	smooth	smooth	smooth	smooth
Long (cm)	9.8-12.3cm	50-150cm	50-70cm	60cm	91cm	17-86(100)cm	(-)	(-)	(8)12-68(89)cm
Width (cm)	narrow 2-(3)9cm	broad 7-11cm	narrow 5-7(10)cm	v. narrow 1-4(7)cm	v. narrow 3-5cm	v. broad 6-40cm	v. narrow 1-4cm	narrow 1-12cm; or 0.9-8.6 in undivided, 1.7-12.4 in splitting blades	narrow 2-(6)9cm
Thickness (µm)	600-690	350-400	500-1100	530-650	430-570	v. thin; 300-400	-	-	400-900

Table 1.1 continued

	<i>L. brevifolia</i>	<i>L. adamsiae</i>	<i>L. tholiformis</i>	<i>L. variegata</i> *	<i>L. corrugata</i>	<i>L. ovata</i>	<i>L. nigrescens</i> *	<i>L. trabeculata</i>	<i>L. flavicans</i>
Pigmentation	uniform; dark brown	uniform	irregular brownish flecks	striated flecked; light yellow to olive-yellow or olive-green	uniform; olive with a greenish tinge	uniform; dark brown	uniform; dark green, almost blackish	uniform; brown	uniform; brown
Lustre dried	suede-like	shiny		shiny	shiny	shiny	shiny	shiny(?)	suede-like
width of medulla (µm)	80-130	50-60	100	80-90	150-170	up to 25	up to 100	up to 50	up to 50
No. cell rows in meristoderm	1	1	1	1	1	1-2	2-3	2-3	1-2
No. cell layers in cortex	7-10 (-)	4-6 (-)	~8 polygonal cells	7-10 (-)	6-8 (-)	5-8 rectangular cells	10-16 polygonal cells	7-15 polygonal cells	5-8 polygonal cells
Cortex lacunate / solid	lacunate	lacunate	lacunate	lacunate	lacunate	solid	solid	lacunate, with trabeculae	lacunate
Paraphyses length (µm) width (µm)	(-) (-)	(-) (-)	(70-80) (8-9) at tip	80 with a hialine hat; 20 wide	(-) (-)	54.4-64.7 5.6-9	18.1-75.4 1.3-5.7	42-59 5-8	52.7-66 5.5-7.8
Sporangia length (µm) width (µm)	(-) (-)	50-60 6-7	(50-65) (9-11)	50-65 10	(-) (-)	34.8-46.4 8.7	43.5-55.1 5.8-8.7	43 ± 4.7 5.8-8.7	29-43.5 4.4-7.3

Table 1.1 continued

Branches	<i>L. brevifolia</i>	<i>L. adamsiae</i>	<i>L. tholiformis</i>	<i>L. variegata</i> *	<i>L. corrugata</i>	<i>L. ovata</i>	<i>L. nigrescens</i> *	<i>L. trabeculata</i>	<i>L. flavicans</i>
No. emerging from base or holdfast of mature algae	1-2(4)	commonly 5-15	10-25	up to 100	100+	1 1-2	up to 20	1-29(47)	1-2(4 or more in young algae)
No. emerging from holdfast of juvenile algae (<20 cm)	1	1	(-)	1	1	1	numerous	1(?)	1
Flexibility	rigid	rigid	rigid	flexible	v. flexible	rigid	flexible	rigid	rigid
Cross section shape	oval-terete	terete at base, oval distally	oval or rounded, flattened near blades	oval or irregular at base, flattened distally	subterete at base, flattening to the top	oval-terete	terete at base, flattening to the top	oval-terete terete of subteret, flattening to the top	oval-terete
Max. branch width (mm)	~ 40	60	20-40	20	20	50	40	(-)	(-)
Cortex lacunate / solid	lacunate	lacunate	large cavities	solid	solid	solid	solid	lacunate; lacunae with trabeculae	spherical or ellipsoidal lacunae
Branching pattern	dichotomous (3-4 dichotomies)	dichotomous (4-5 dichotomies)	dichotomous	forking from the base, 6-7 times at narrow angles; twisted branches	dichotomous (6 dichotomies), cuneate widening of the stem below each dichotomy	dichotomous or subdich. branched	dichotomously branched	dichotomous or subdich. in the uppermost portions; 3-4 times furcate to mid portions	dichotomous or subdich. branched; uneven branching might lead to v. tall and thick trunks (8m?)

Table 1.1 continued

Holdfast or base	<i>L. brevifolia</i>	<i>L. adamsiae</i>	<i>L. tholiformis</i>	<i>L. variegata</i> *	<i>L. corrugata</i>	<i>L. ovata</i>	<i>L. nigrescens</i> *	<i>L. trabeculata</i>	<i>L. flavicans</i>
Appearance of mature algae	more-or-less hapteroid; stoloniferous	massive, asymmetrical, a coalescence of holdfast, basal branches & haptera	semi-solid dome or mound formed by overlapping layers of flattened, lobed haptera (similar to <i>L. nigrescens</i>); building a conical mass	massive, woody, brittle; dichotomously branched haptera; spreading; circular outline	strong and scutiform, spreading	dissected, with unfused dichotomously branched haptera; more-or-less hapteroid	massive, conical or hemi-spherical mound of tightly fused haptera	massive, asymmetric and irregularly shaped, a coalescence of holdfast, basal branches & haptera; 13-20cm height; wenn young haptera are free or only partially fused	dissected, with unfused dichotomously branched haptera; more-or-less hapteroid
Buttresses	none	tall, rounded buttresses	none	buttresses rib-like	none	none	none	rounded buttresses	none

1.3 *Lessonia* – Biology and dispersal

Algae are very important in all marine, freshwater, and some terrestrial systems around the world. They are primary producers and a leading source of free oxygen. However, algae are not monophyletic and this dissertation is restricted to the Phaeophyceae (brown algae) and in particular to one genus of the Laminariales (kelp), namely *Lessonia* (strap kelp).

The Laminariales are a significant floristic component of the intertidal and subtidal zones on almost any rocky coast in temperate and polar seas. They are referred to as being the most structurally complex of all algae as they are divided into lamina, stem, and holdfast (Bold and Wynne 1978). Kelp communities create forest-like environments on the sea floor where they offer structure (*Lessonia* can form canopies but would in most areas belong to the understory), habitat (e.g., for epi- or endophytes), shelter (e.g., for fish recruits), and food (e.g., copepods, isopods, and sea urchins, but also Paua (*Haliotis* sp.) and Butterfish (*Odax* sp.)) to marine life (e.g., Dayton 1985, Lobban & Harrison 1994, Francis 2001, Nelson 2005). The Laminariales are characterized as being parenchymatous with several different cell layers (medulla, cortex, and meristoderm), growing in length by an intercalary meristem, having anisogamous reproduction, and a heteromorphic (D^h) life cycle (Fig 1.10), involving a macroscopic sporophyte and microscopic, dioecious gametophytes (e.g., Bold and Wynne 1978, Womersley 1987). *Lessonia* sporophytes, as in many other Laminariales, are perennial (Adams 1994), with a major growing phase during summer (Tala *et al.* 2004, Nelson 2005, Schwarz *et al.* 2006) and peak reproduction of gametophytes in winter (Tala *et al.* 2004, Schwarz *et al.* 2006), followed by peak sporulation of sporophytes in spring/early summer

(Schwarz *et al.* 2006). Extensive sori form in the middle of each blade. When ripe, spores are released in clumps of mucus (Markey & Wilce 1976, Toth 1976, Hoffmann & Camus 1989), which usually sink to the bottom near the parent (Norton 1992, Dayton 1973, Reed *et al.* 1988, Forrest *et al.* 2000, Faugeron *et al.* 2005, Kusumo *et al.* 2006) and attach to the substrate where they grow into male and female gametophytes.

Dispersal can occur in short, medium, or long distances in many different ways (Fig. 1.10). As short-distance-dispersal (SDD) I understand the dispersal of propagules (spores and sperm). It is the main consensus that this form of dispersal is limited to a few meters (Norton 1992, Dayton 1973, Reed *et al.* 1988, Santelices 1990, Forrest *et al.* 2000, Faugeron *et al.* 2005, Kusumo *et al.* 2006), but the radius might increase if whole stands sporulate simultaneously (Norton 1992). In *Lessonia*, spores are released in large drops of mucus (Markey & Wilce 1976, Toth 1976, Hoffmann & Camus 1989) that usually have enhanced sinking rates (as polysaccharides are heavier than water) if not trapped in surface tension (Santelices 1990). The advantage of enhanced sinking rates in heterogeneous environments is that spores will more likely find suitable habitat in the neighbourhood of the parent than further away (Moorjani & Jones 1972). Higher sinking rates seem to be also an adaptation to exposed environments (Taylor & Schiel 2003). An often used argument against successful fertilisation after enhanced dispersal of spores is the dilution effect and the requirement that male and female gametophytes have to be in high numbers and close proximity (i.e., $>10/\text{mm}^2$; Reed 1990a) for successful fertilisation as female pheromones and male sperms are limited in their ability to disperse (e.g., Faugeron *et al.* 2005). This constraint is more valid for expanding populations but

less for the connectivity of existing populations, and spores have been detected 4km downstream of the source populations, and might travel even further (Reed 1988). A requirement for settlement, however, is the stickiness of the spore that decreases with time and thus also constrains the dispersal distance (Santelices 1990, Taylor & Schiel 2003).

Dispersal is also possible via drifting of detached individuals or fertile thallus fragments. In contrast to floating, drifting is used here for detached algae that are or became negatively buoyant and tumble over the sea floor in shallow water driven by tidal or wind-induced currents (Thiel & Gutow 2005a). Individuals of *Lessonia* are very hard to detach (pers. obs.); nevertheless, piles of detached algae are often seen washed up on beaches after heavy winter storms (e.g., Schiel & Foster 2006); the season coincides with the time of peak reproduction (see above). Not by chance, the first account of *Lessonia* translates: “Cast ashore by tempests, this algae oft aggregates huge dykes surpassing a yard in vertical stature, and if one were to step upon this seaborne effigy then that poor soul would become much entangled in an ankle deep mire of putrefaction, releasing an unendurable and malodorous assault on one’s olfactory senses, which one could only compare with that of a cauliflower in an advanced state of decomposition.” (d’Urville 1825). Drift was reported to cover 100 to 1000m/year in *Undaria pinnatifida*, another non-floating kelp (Forrest *et al.* 2000). Dispersal of algal fragments is likely to span the same distance but might occur more often (Forrest *et al.* 2000). Laminae grow all year round to compensate the distal loss of older tissue that is shredded by waves and currents (Tala & Edding 2005). If fronds are fertile the most likely area where the lamina breaks apart is below the sorus, leaving short blades behind. [This appearance might have led to the

description of *L. brevifolia* Agardh. *L. brevifolia* is a species endemic to the Sub-Antarctic Islands of New Zealand, however, the laminas are not short as described but have a length and appearance comparable to the laminas of *Lessonia variegata* (pers. obs.)]. Another form of medium-distance-dispersal (MDD) is saltation. Saltation means the dispersal of specimen growing on unstable substrate, like small rocks, by currents lifting the whole alga together with the rock and releasing it further downstream. Saltation was observed in *Undaria pinnatifida* where the dispersal distance was assumed to span 10 to 100m/year (Sliwa *et al.* 2006).

As *Lessonia* is negatively buoyant its only possibility for long-distance-dispersal (LDD) seems to be as gametophytes or sporophytes attached to other floating devices. Floating devices might be pumice, wood, plastic, etc. (Thiel & Gutow 2005a), but also other brown algae such as *Macrocystis pyrifera* (Skottsberg (1907) stated that his *L. frutescens* specimen was collected with a *Macrocystis* holdfast from shallow water) or *Durvillaea antarctica* (Fig. 1.11). These two algae grow in overlapping habitats together with *Lessonia* (Searles 1978, Edding *et al.* 1994) and are known for their floating abilities. An estimated 70 million rafts (20 million supporting a holdfast) of *Durvillaea antarctica* are afloat in the Southern Ocean at any given time (Smith 2002) and both kelp species, *Durvillaea* and *Macrocystis*, have each been found to share a single haplotype throughout the Southern Hemisphere (Macaya & Zuccarello 2010, Fraser *et al.* 2009). LDD via floating or rafting might cover distances from <100 km up to >5000 km (Thiel & Haye 2006). Theoretically free-drifting spores also have the ability of LDD. Amsler & Searles (1980) report colonization over a distance of at least 35km via spore dispersal (in Reed *et al.* 1992). Hoffmann & Camus (1989) reported that spores of *L.*

nigrescens were still able to germinate after four days in suspension. Amsler & Neushul (1991) state that the ability of some algal spores to photosynthesise might increase dispersal distance. Reed *et al.* (1992) found that some algal spores remain swimming up to 120h under photosynthetically saturating irradiance and will not necessarily die after they stopped swimming, as some germinated in suspension. His laboratory observations indicate that free-floating spores might develop into gametophytes, which are still able to settle, fertilize and grow into sporophytes (Reed *et al.* 1992). Positive phototaxis, which was found in some algal spores, also increases dispersal distance (Norton 1992). Spores might even get trapped in surface tension and remain floating (Santelices 1990). However despite these *in vitro* experiments, which can theoretically increase the possibility of connectivity and colonisation over long distances, the odds are low, as successful settlement decreases with distance, as mortality of the microscopic stages is very high, as planktonic grazing will decrease the chances of survival with time, and as successful fertilisation requires high densities (i.e., $>10/\text{mm}^2$) of gametophytes (Santelices 1990, Reed 1990a, Schiel & Foster 2006).

In general the ability of dispersal strongly affects the possibility of diversification and speciation of disjunct populations. For example, due to floating structures, the high dispersal abilities in *Macrocystis pyrifera* and *Durvillaea antarctica* have led, in both algae, to highly connected populations and for each species a single haplotype has been detected throughout the Southern Hemisphere on islands and continents connected by the West Wind Drift (Fraser *et al.* 2009, Macaya & Zuccarello 2010). In *Macrocystis* this has led to multiple morphological species being merged into a single morphologically plastic species, *M. pyrifera* (Coyer *et al.*

2001, Macaya & Zuccarello 2010). On the other hand, species that lack floating structures, have been reported to show genetic structure over short distances, e.g., *Lessonia nigrescens* (Martinez *et al.* 2003, Faugeron *et al.* 2005), *Ecklonia radiata* (Coleman *et al.* 2009), *Postelsia palmaeformis* (Kusumo *et al.* 2004 and 2006), and *Laminaria digitata* (Billot *et al.* 2003). The dispersal ability of a species is known to influence the connectivity and genetic structure of populations, but also the ability to colonize and speciate (e.g., Ross *et al.* 2009). Thus knowledge of the dispersal ability is helpful for the interpretation of questions asked in a phylogeographic and population genetic framework.

1.3.1 Figures

Fig. 1.10 Possible dispersal mechanisms in *Lessonia*. The different modes of dispersal include dispersal of propagules (spores and sperm), which is associated with the life cycle and mainly spans distances between 0-10m (e.g., Norton 1992), however, spores have been detected 4km downstream of the source populations (Reed 1988). Saltation was investigated in *Undaria pinnatifida* where it was accounted for dispersal between 10-100m/year (Sliwa *et al.* 2006). In the same study dispersal of drifting algae and algal fragments was assumed to span 100-1,000m/year. Rafting of gametophytes and sporophytes attached to floating devices might allow dispersal over distances <100km to >5,000km (Thiel & Haye 2006). Possible rafts for *Lessonia* are the two floating species *Macrocystis pyrifera* and *Durvillaea antarctica*, which have overlapping habitats with *Lessonia*.

Fig. 1.11 *Durvillaea antarctica* (orange in insert) overgrowing a *Lessonia nigrescens* (black in insert) holdfast. (Photo taken by C. Fraser).

1.4 Lessoniaceae – New molecular taxonomy and phylogeny

Taxonomy is originally based on morphological and/or anatomical features and in many cases these are good tools to predict natural inheritance. As taxonomic hypotheses improve with the complexity of the feature(s) used, morphology often fail to resolve a natural lineage in taxa with high morphological plasticity (Lane *et al.* 2007) or where morphological traits are few (Zuccarello & West 2003). In some of these cases, genetics can help to find the true phylogenetic relationships.

Setchell & Gardner (1925) were the first to recognize the Lessoniaceae among five other families within the order Laminariales. The advanced and morphologically more complex families (Alariaceae, Laminariaceae, and Lessoniaceae or ALL-complex) have remained uncontested in their species composition until Fain *et al.* (1988), who were the first to use molecular techniques to the systematics of Laminariales, found that the Lessoniaceae were polyphyletic. Half a decade later Saunders & Druehl (1993) commenced the reorganization of the families based on sequence data with a revision of the Alariaceae. Little by little every single family of the ALL-complex has found to be polyphyletic (Druehl *et al.*, 1997; Kawai & Saskia, 2000; Yoon *et al.*, 2001) and has finally led to a substantial taxonomic re-organization (Lane *et al.* 2006) (Table 1.2).

In the Lane *et al.* (2006) survey, the Lessoniaceae Setchell & Gardner was subject to the most fundamental changes with *Lessonia* being the only genus remaining in the family. The genera *Dictyoneuropsis* and *Dictyoneurum* were transferred to the new proposed family Costariaceae. Most genera, i.e., *Macrocystis*, *Nereocystis*, *Pelagophycus* and *Postelsia*, were transferred to the Laminariaceae and

later also *Lessonia laminarioides*, which was previously known as the only *Lessonia* species from the Northern Hemisphere, was reclassified as *Pseudolessonia laminarioides* (Cho *et al.* 2006) and likewise placed within the Laminariaceae. Prior to the changes of Lane *et al.* (2006), Saunders and Dreuhl (1993) recommended moving *Lessoniopsis* to the Alariaceae and emended the description of this group. *Ecklonia*, *Egregia*, and *Eisenia* formally placed within the Alariaceae, due to the presence of sporophylls (*Ecklonia*, which has no sporophylls, has, according to the definition made by Setchell & Gardner, the potential for sporophylls as lateral outgrowths of the blade arising from the transition zone, also, the authors state that early stages of *Ecklonia* are like those of *Eisenia*), were moved to the Lessoniaceae, as was *Eckloniopsis* formally a member of the Laminariaceae, as *Eckloniopsis radicata* persistently nests within *Ecklonia* (Saunders & Dreuhl 1993, Yoon *et al.* 2001, Lane *et al.* 2006). Lane *et al.* (2006) suggested reviewing the status of *Eckloniopsis* in favour of a single genus *Ecklonia*. The new assemblage of genera within the Lessoniaceae is not supported in any other molecular study known to me (Dreuhl *et al.* 1997, Boo *et al.* 1999, Kawai & Sasaki 2000, Yoon *et al.* 2001, Phillips *et al.* 2008, McDevit & Saunders 2009) and it seems that *Egregia* is the reason. The faster evolving sequences of *Egregia*, and long-branch attraction, have also been a problem for Lane *et al.* (2006), but due to a large dataset in terms of species number and number of molecular markers, they were able to give statistical support to this assemblage. Within the Lessoniaceae, three groups were recognized, two are monogeneric (*Lessonia* and *Egregia*) and one combines the genera *Eisenia*, *Ecklonia*, and *Eckloniopsis*. Due to the difficult placement of *Egregia*, the *Ecklonia* clade was chosen to be the primary source for outgroup selection for *Lessonia* phylogeny. Another possible outgroup became apparent through the work of Lee *et*

al. (2008), who catalogued Northern and Southern Hemisphere brown algae while using the barcoding gene COI, and who found a close relationship between *Lessonia* and *Laminaria ephemera*. In Lane *et al.*'s (2006) study, *L. ephemera* was found nested within other *Laminaria* species. At the very least these contrasting results reveal how little we still know about kelp systematics.

The major reorganizations made by Lane *et al.* (2006) have required a critical editing of the genus descriptions. Lane *et al.* (2006) described the new proposed Costariaceae as follows:

Costariaceae Lane, Mayes, Druehl *et* Saunders: Members of the Laminariales that generally have a flattened stipe but are occasionally terete, and either a perforated or reticulated blade, or both. Type genus: *Costaria*; additional genera: *Agarum*, *Dictyoneurum* and *Thalassiophyllum*.

The Alariaceae have been earlier revised by Saunders & Druehl (1993):

Alariaceae Setchell & Gardner 1925 emend. Saunders & Druehl 1993: Fronds consisting of a simple vegetative lamina with a central midrib or thickening, terminating a simple or dichotomously branched primary stipe. Sporophylls developing from the intercalary meristem situated at the stipe-lamina transition zone, distichously arranged on the primary stipe. Other features as characteristic of the order Laminariales. Type genus: *Alaria*; additional genera: *Lessoniopsis*, *Pleurophycus*, *Pterygophora*, and *Undaria*.

No revision, however, was made for the Laminariaceae and Lessoniaceae as they both circumscribe a morphologically diverse assemblage of taxa that are not as

easily defined as the previous groups. Yet, based on the generic composition after Lane *et al.* (2006) the description of the Lessoniaceae could be amended to:

Lessoniaceae Setchell & Gardner 1925:591,621: Holdfast branched hapteroid; blades without midrib or central thickening; no sporophylls arising from the primary stipe (i.e., between holdfast and the intercalary meristem or, in other words, below the first vegetative frond). From here the Lessoniaceae are best described as two different entities. A) *Lessonia*, *Ecklonia* (including '*Eckloniopsis*'), and *Eisenia*: lacking pneumatocysts, a round to terete and overall solid, not hollow stipe (with the exception of *Ecklonia maxima* and some *Ecklonia kurome* specimens); the transition zone might give rise to splitting (*Lessonia*) or outgrows (*Eisenia*); distribution bipolar (*Ecklonia*, *Eisenia*) or solely in the Southern Hemisphere (*Lessonia*; unique among all Laminariales). Sporophylls arising from the primary blade (*Eisenia*) or if absent (*Lessonia*, *Ecklonia*) sporangia might develop in the middle of vegetative blades; B) *Egregia*: multiple, flattened stipes bearing pneumatocysts and sporophylls that develop randomly among the vegetative bladelets along the whole length of the stipe margin above the primary part of the stipe (the sporophylls in *Egregia* are believed to be due to parallel evolution analogous to any other sporophylls and are a distinguishing feature for *Egregia*); *Egregia* is the only Lessoniaceae that is found solely in the Northern Hemisphere.

1.4.1 Tables

Table 1.2 Taxonomic changes of the Laminariales over time.

Taxonomy of the Laminariales Oltmanns after Setchell & Gardner 1925	Taxonomy of the Laminariales Migula 1909:243 (formally often referred to Kylin 1919) as listed in Systema Naturae 2000	Taxonomy of the ALL-group after Lane et al. (2006)
Lessoniaceae Setchell & Gardner 1925:591,621 (splitting at the transition zone between the stipe and the blade)	Lessoniaceae	Lessoniaceae Post. & Rupr. (...)
Tribe: Lessoniaceae Setchell	<i>Lessonia</i> Bory, 1825	<i>Lessonia</i>
<i>Dictyoneurum</i> Rupr., 1852	<i>Dictyoneurum</i>	
<i>Nereocystis</i> Post. and Rupr., 1840	<i>Nereocystis</i>	
<i>Postelsia</i> Rupr., 1852	<i>Postelsia</i>	
Tribe: Macrocystaceae Kuetzing (lim. mut.)		
<i>Macrocystis</i> C.A. Agardh, 1820	<i>Macrocystis</i>	
<i>Pelagophycus</i> Aresch., 1881	<i>Pelagophycus</i>	
Tribe: Lessoniopseae Setchell		
<i>Lessoniopsis</i> Reinke, 1903	<i>Lessoniopsis</i>	
	<i>Dictyoneuropsis</i> G.M. Smith, 1942	
		<i>Ecklonia</i>
		<i>Eckloniopsis</i>
		<i>Egregia</i>
		<i>Eisenia</i>
Laminariaceae Reichenb. [single blades, either simple or longitudinally split but never with the splits extending into the transition zone.]	Laminariaceae (the accepted authority is Bory, 1827:63)	Laminariaceae Post. & Rupr. (...)
Tribe: Laminariaceae Bory (lim. mut.)		
<i>Laminaria</i> Lamour., 1813 (in part)	<i>Laminaria</i> Lamouroux, 1813, nom. cons.	<i>Laminaria</i>
Tribe: Cymathaeaceae Setchell and Gardner		

Table 1.2 continued

<i>Pleurophycus</i> Setchell & Saunders ex J.Tilden, 1900	<i>Pleurophycus</i>	
<i>Cymathaere</i> J. Agardh., 1868	<i>Cymathaere</i>	<i>Saccharina</i> (resurrected) Stackh., 1809 [++ <i>Cymathaere japonica</i> , <i>Hedophyllum</i> , <i>Kjellmaniella</i>]
Tribe: Agareae Kuetz. (lim. mut)		
<i>Costaria</i> Greville, 1830	<i>Costaria</i>	
<i>Thalassiphyllum</i> Post. & Rupr., 1840	<i>Thalassiphyllum</i>	
<i>Agarum</i> (Bory) Post. & Rupr.	<i>Agarum</i> Bory, 1826, nom. cons.	
Tribe: Hedophyllaea Setchell		
<i>Hedophyllum</i> Setchell, 1901	<i>Hedophyllum</i>	<i>Hedophyllum</i> see <i>Saccharina</i>
<i>Arthrothamnus</i> Rupr., 1848	<i>Arthrothamnus</i>	<i>Arthrothamnus</i>
	<i>Costularia</i> C.B. Clarke, in Thiselton-Dyer, 1898	
	<i>Feditia</i> Yu. Petrov & I. Gusarova, in I. Gusarova & Yu. Petrov, 1972	
	<i>Kjellmaniella</i> Miyabe in Okamura, 1902	
	<i>Phyllariella</i> Y.E. Petrov & V.B. Vozzhinskaya, 1966	
	<i>Streptophyllum</i> K. Miyabe & M. Nagai, in Nagai, 1940	<i>Streptophyllopsis</i> ("based on available information" but was not sequenced)
		<i>Macrocystis</i>
		<i>Nereocystis</i>
		<i>Pelagophycus</i>
		<i>Postelsia</i>
		<i>Pseudolessonia</i> G. Y. Cho, N. G. Klochkova, T. N. Krupnova et Boo, 2006

Table 1.2 continued

Alariaceae Setchell & Gardner, 1925:591, 633 [with sporophylls]	Alariaceae	Alariaceae Post. and Rupr. (sporophylls that derive from the stipe, but <i>Pleurophycus</i>)
Tribe: Alarieae Setchell		
<i>Pterygophora</i> Rupr. 1852	<i>Pterygophora</i>	<i>Pterygophora</i>
<i>Alaria</i> Greville 1830, nom. cons.	<i>Alaria</i>	<i>Alaria</i>
Tribe: Ecklonieae Setchell	<i>Ecklonia</i> J.W. Hornemann, 1828	
<i>Eisenia</i> Aresch., 1876	<i>Eisenia</i>	
Tribe: Egregieae Setchell		
<i>Egregia</i> Aresch., 1876	<i>Egregia</i>	
	<i>Eckloniopsis</i> K. Okamura, 1927	
	<i>Undaria</i> W.F.R. Suringar, 1873	<i>Undaria</i>
		(<i>Undariella</i>)
		Lessoniopsis (added by Saunders and Druehl 1993b)
		<i>Pleurophycus</i>
Chordaceae Dumort.	Chordaceae	Chordaceae
<i>Chorda</i> Stackh., 1797	<i>Chorda</i>	<i>Chorda</i>
	<i>Halosiphon</i> Jaasund, 1957	
	Pseudochordaceae	Costariaceae C.E. Lane, Mayes, Druehl et G.W. Saunders fam. nov. (Members of the Laminariales that generally have a flattened stipe but are occasionally terete, and either a perforated or reticulated blade, or both.)
		<i>Costaria</i>
	Phyllariaceae	<i>Agarum</i>
	<i>Phyllaria</i> (A. Le Jolis) J. Rostafinski, 1877	<i>Dictyoneurum</i>
	<i>Phyllariopsis</i> E.C. Henry & G.R. South, 1987	(<i>Dictyoneuropsis</i>)
	<i>Saccorhiza</i> Bachelot de la Pylaie, 1830, nom. cons.	<i>Thalassiphyllum</i>

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Chapter 2

Molecular Phylogeny and timing of radiation in *Lessonia* (Phaeophyceae, Laminariales)

2.1 Abstract

The genus *Lessonia* is distributed solely in the Southern Hemisphere, with four species found in South America and five in Australasia. My goal was to determine the evolutionary relationships between the *Lessonia* species of the two disjunct areas. I combined plastid, nuclear and mitochondrial markers in a comprehensive dataset from several individuals per known species. Furthermore, for some species I added samples from multiple populations to take account of their widespread distribution over known bioregions. The results suggested that Australasian *Lessonia* form a monophyletic group and that South American species form a paraphyletic group. Delimitations of the accepted species are highly supported except for *L. variegata* and *L. nigrescens*. I showed for the first time four lineages for the New Zealand endemic *Lessonia variegata* with an unexpected high level of genetic differentiation that justifies a split into four species. Molecular clock analysis suggested that *Lessonia* is young with a Southern Hemisphere radiation in the Pliocene more recent than 5 Mya. A sudden radiation took place in Australasia ca. 3.5 Mya when almost all Australasian species diverged within a time frame of only 35 000 years.

Key Index words: Australasia, cryptic species, glaciation, Laminariales, *Lessonia*, molecular clock analysis, New Zealand, Phaeophyceae, phylogeny, South America

Abbreviations: atp8-sp, spacer region between atp-dehydrogenase subunit 8 and t-RNA serine; CTAB, Cetyltrimethylammonium Bromide; HPD, highest posterior density; ITS, nuclear marker covering the internal transcribed spacers (1 and 2) between the large and small-ribosomal RNA subunits, and the 5.8S ribosomal RNA; MJ, median joining; MP, maximum parsimony; My, million years; Mya, million years ago; nd6, a partial sequence of the gene coding for NADH dehydrogenase subunit 6; NJ, neighbour joining; rbc-sp, spacer between the large and short subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene; trnW-sp, the spacer between the t-RNA's for tryptophan and isoleucine;

2.2 Introduction

The molecular phylogeny of the kelps (Laminariales) has received much attention, due to their ecological importance and charismatic character (Saunders & Druehl 1992, Lane *et al.* 2006, McDevit & Saunders 2009). Supported by molecular evidence, the old morphologically based familial relationships of the Laminariales were fundamentally changed by Lane *et al.* (2006). In their comprehensive study, no other family was rearranged to the same extent as the Lessoniaceae (Setchell & Gardner 1925), with only *Lessonia* left in its original family. Lane *et al.* (2006) proposed a new grouping consisting of *Lessonia*, *Eisenia*, *Ecklonia* (with *Eckloniopsis*), and *Egregia* (all, except *Lessonia*, were formally placed within the Alariaceae). *Egregia* however often does not group with other Lessoniaceae *sensu* Lane *et al.* (Lane *et al.* 2006, Phillips *et al.* 2008, McDevit & Saunders 2009) with only *Lessonia* and its sister group of *Ecklonia* and *Eisenia* forming a consistent clade. *Lessonia* is the only genus in this group that is solely distributed in the Southern Hemisphere (Lane *et al.* 2006).

Despite being a poor disperser (Faugeron *et al.* 2005), *Lessonia* is found disjunct in Australasia and South America on exposed rocky shores, between 12°S and 56°S (Lucas 1931, Papenfuss 1964, Barrales & Lobban 1975, Searles 1978, Hay 1987 and 1989, Fernandez *et al.* 1999, Martinez 1999). 32 species of *Lessonia* have been described of which nine are currently recognized (Edding *et al.* 1994): four in South America (*L. flavicans* Bory, *L. nigrescens* Bory, *L. trabeculata* Villouta & Santelices, and *L. ovata* Hook.f. & Harv.), and five in Australasia (*L. adamsiae* Hay, *L. brevifolia* J.Agardh, *L. corrugata* Lucas, *L. tholiformis* Hay, and *L. variegata* J.Agardh); one to two poorly known species have been reported from Kerguelen and

Heard Island (Delepine 1963, Papenfuss 1964) but are not properly described. Since its original description, the species designation of the South American *L. flavicans* was ambiguous and it has led to extremely entangled synonyms (Agardh 1878). Despite the latest attempt by Asensi & de Reviers (2009) the issue is still unresolved, as only specimen preserved at the Muséum National d'Histoire Naturelle were considered in their survey. However I follow Asensi & de Reviers (2009) suggestion for the designation of *L. flavicans* Bory but I will use the older epithet *L. ovata* Hook.f. & Harv as synonym to the newly described species *L. searlesiana* Asensi & de Reviers (see Chapter 6).

The divergence ages of the species of *Lessonia* is not known. Lüning & tom Dieck (1990) suggested that “the present-day cold-water algal floras must have evolved as a response to [the two major] climatic deterioration[s] ... at the Eocene/Oligocene boundary [35 million years ago (Mya)], and in the Miocene (15-10 Mya)”. This view is supported by estimations based on molecular clock analysis using a mutation rate for kelp cpDNA of $0.2 - 0.4\% \text{My}^{-1}$ that places the time of the radiation within the Laminariaceae between 8.5 to 34 Mya (Druehl & Saunders 1992), but more likely between 16 and 20 Mya (Saunders & Druehl 1992) and the radiation within the genus *Laminaria* between 15-19 Mya (Stam *et al.* 1988). That the divergence estimates of Stam *et al.* (1988) and Saunders & Druehl (1992) were the same but for different taxonomic levels (family level and genus level respectively) was later corrected by the resurrection of *Saccharina* (Lane *et al.* 2006), which split *Laminaria* into two genera and revealed that Stam *et al.* (1988) actually investigated familial and not generic radiation. A recent estimate of molecular-derived divergence times, calibrated using diatom *psbA* (Hoarau *et al.*

2007), sets the time of divergence between two laminariales families (Alariaceae and Laminariaceae) between 10-15 Mya and the mutation rate of the RuBisCo spacer between 0.26 and 0.40%My⁻¹ (Tellier *et al.* 2009).

Multiple studies have shown that morphological plasticity in the Laminariales has led to an accumulation of many synonyms (Lane *et al.* 2007 [*Alaria*], Uwai *et al.* 2007 [*Undaria*], Demes *et al.* 2009, and Macaya & Zuccarello 2010 [*Macrocystis*]); whereas other studies have revealed cryptic species (Sasaki & Kawai 2007 [*Chorda sp.*], Tellier *et al.* 2009 [*Lessonia nigrescens* Bory]). With the increase of molecular studies in brown algal systematics, the number and relationships of species is expected to change with morphological species merged and cryptic species discovered.

The molecular relationships among the disjunct *Lessonia* species and the timing of their divergence are still unknown but are important for further biogeographic interpretations. The presence of cryptic species has not been investigated for the Australasian *Lessonia*, even though cryptic species have been found in Chile (Tellier *et al.* 2009) and knowledge about biodiversity is central for ecological management and conservation. The aim of this study is to gain a better understanding of the phylogenetic relationships of the Southern Hemisphere genus *Lessonia*, especially in using molecular data to delimit species, uncover cryptic species, and estimate the timing of divergence within this genus.

2.3 Material and Methods

2.3.1 Sampling

A total of 48 individuals of *Lessonia* were collected from 29 sites throughout the distribution range of the nine recognized species (Fig. 2.1, Table S 2.1). Widespread species like *L. variegata*, *L. nigrescens*, and *L. trabeculata* have been collected from distant sites. Tissue (2-3 cm²) was collected from the base of a blade, where the blade is youngest and free of epiphytes, patted dry and subsequently sealed in bags with silica gel. All southern South American *Lessonia* are named as indicated by their collectors or for C156 and C160 determined as *L. vadosa* based on obvious lacunae in the cortex of the blade (Searles 1978). The sample A634 was used in a previous study as *L. flavicans* (Lane *et al.* 2006). To ensure that a majority of genetic variation is captured, I surveyed 440 additional specimens from 35 additional populations in Australasia, using the atp8-Sp region, the most variable of the markers (Chapter 3). In the same way, confidence in capturing genetic variation of South American species was given through comparison with a sample rich survey undertaken by Tellier *et al.* (2009), who sampled 127 individuals from 36 locations.

2.3.2 DNA extraction, amplification, and sequencing

DNA extraction was performed using a modified CTAB protocol (Zuccarello & Lokhorst 2005).

PCR amplifications were performed in 20μL containing 1μL DNA, 1x buffer (New England BioLabs, Ipswich, MA, USA), 200nM dNTP, 0.4% BSA, 2.0mM Mg²⁺, 0.32U *Taq* polymerase (New England BioLabs), and 0.17nM of each primer. Five genetic markers were used: three mitochondrial markers; the spacer region

between adenosine-tri-phosphate dehydrogenase subunit 8 and t-RNA serine (atp8-Sp), the spacer between the t-RNA's for tryptophan and isoleucine (trnW-Sp), and a partial sequence of the gene coding for NADH dehydrogenase subunit 6 (nd6); one chloroplast marker, the spacer between the large and short subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (rbc-Sp); and one nuclear marker covering the internal transcribed spacers (1 and 2) between the large and small-ribosomal RNA subunits, and the 5.8S ribosomal RNA (ITS). The markers have been selected for independency and to cover all three algal genomes (mitochondrion, chloroplast, and nuclear) and a variety of marker variability (genes and spacer regions) to ideally increase the phylogenetic signal in a combined dataset. For atp8-Sp and trnW-Sp I used primers from Voisin *et al.* (2005). For all other markers I used primers as in Lane *et al.* (2006), Peters & Ramirez (2001), and Tai *et al.* (2001), some of which were modified (Table S 2.2). The reverse primer for nd6 was newly developed to match a *Lessonia* specific binding site (n6R-5' TAGATTACGACCTCCYTGAC 3'). PCR conditions for each marker were as summarized in Fig. S 2.1.

PCR products were purified with ExoSAP-IT (USB, Cleveland, Ohio, USA) and commercially sequenced (Macrogen Inc., Seoul, South Korea).

2.3.3 Data analysis

Sequence assembly was performed with forward and reverse sequences in the 'STADEN package' (Staden *et al.* 2002) using the implementable programs PHRED (Ewing & Green 1998), PHRAP (Green unpublished [<http://bozeman.genome.washington.edu/phrap.docs/phrap.html>]), and GAP4 (Bonfield *et al.* 1995). All sequences were aligned with ClustalW 1.4 (Thompson *et*

al. 1994) and subsequently refined by eye in BioEdit ver. 7.0.9.0 (Hall 1999). The five alignments were used in a combined dataset (“matrix-I”; main dataset) to calculate neighbour joining (NJ) trees in MEGA 4.0 (Tamura *et al.* 2007), maximum parsimony (MP) trees with PAUP* 4.0 (Swofford 2002), maximum likelihood (ML) trees with PHYML v2.4.4 (Guindon & Gascuel 2003), and Bayesian trees with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The compatibility between the datasets was tested with the incongruence-length difference test (Farris *et al.* 1995) implemented in PAUP* (Table S 2.3). To test sensitivity and dependency of the results to variations in the alignment, I performed a second alignment using different underlying algorithms. Therefore I aligned the ingroup for each marker region with MUSCLE v. 3.8 (Edgar 2004), ambiguously aligned regions were removed with GBLOCKS 0.91b (Castresana 2000). Before the filtered sequences were concatenated I aligned the outgroup taxa to the data using MUSCLE and deleted insertions in the outgroup. The second supermatrix was called matrix-II and was only constructed to verify the results, which are based on the first dataset (matrix-I). Testing different alignments is especially useful when using hypervariable markers such as spacer regions to avoid unanticipated and unintentional bias in the data. The variability of the markers and their degree of saturation has been tested while plotting corrected distances against uncorrected distances (Verbruggen & Theriot 2008) (Table 2.1, Fig. S 2.2). Sequence divergences (p-distances and HKY-distances (Hasegawa *et al.* 1985)) between species and lineages were calculated using PAUP*.

With reference to the taxonomic study of Lane *et al.* (2006) on the Laminariales, I chose *Ecklonia* as outgroup. To reduce uncertainties to the status of characters, I applied a multiple outgroup approach with one specimen each from *E.*

radiata (C.Agardh) J.Agardh and *E. cava* Kjellman. To ensure independency to the outgroup selection, I tested the stability of the topology while applying additional genera to the outgroup. The tested taxa were *Eisenia bicyclis* (Kjellman) Setchell, *Macrocystis pyrifera* (Linnaeus) C.Agardh, *Undaria pinnatifida* (Harvey) Suringar, and *Laminaria ephemera* Setchell. I also tested the possibility of rooting the phylogenetic trees without outgroups (i.e., mid-point rooting or rooting under a molecular clock assumption). Both approaches require clock-like evolution to some extent but in the case of midpoint rooting, only the two most divergent lineages are required to have evolved at the same rate (Verbruggen & Theriot 2008). Clock-like evolution was tested in PAUP*. The NJ tree was calculated under a LogDet (Tamura & Kumar 2002) model of sequence evolution with 10000 bootstrap replications.

An unweighted MP tree was calculated with 1000 heuristic search replicates and TBR branch-swapping. Bootstrap values (Felsenstein 1985) were calculated using ten random additions under the heuristic search method.

Likelihood settings were calculated with Modeltest 3.7 (Posada & Crandall 1998). The best fit model selected by AIC was the GTR+I+G model. ML bootstraps were calculated with 500 replicates (in PHYML; BIC=-7872).

The alignments for the Bayesian analysis (in MrBayes) were partitioned by marker regions and in case of the nd6 additionally by codon position. The partition strategy was selected against two other strategies (single partition or one partition for each marker) based on its Bayes Factor (log BF) (Table S 2.4) using the implement in TRACER v.1.5. GTR+I+G was applied to all partitions allowing for separate values for each partition. The overall rate was allowed to be different across partitions. Five million generations were analysed in each of the two four-chain runs.

Trees were sampled every thousand generations. Stationary was visualised in TRACER v.1.5 (Rambaut & Drummond 2007) to adjust the burn-in period before the trees have been summarized.

Divergence times were calculated in BEAST v.1.5.2 (Drummond & Rambaut 2007) using an uncorrelated log-normal distribution for a relaxed clock model. I tested three calibration strategies that were based on different assumptions of divergence time between laminarialean taxa. The three different settings (A-C) were run, each time using only one of these calibration strategies. Priors for the calibration strategies were: A) a uniform distribution for the tmrca of *Ecklonia* and *Lessonia* between 8.5-34 Mya, based on within-family radiation (Druehl & Saunders 1992; however Saunders & Druehl (1992) favour radiation between 16 and 20 Mya); B) a uniform distribution for the time of the most recent common ancestor (tmrca) between *Ecklonia* and *Lessonia* between 15-19 Mya, based on the within-family radiation of Laminariaceae (Stam *et al.* 1988, with taxonomic changes after Lane *et al.* 2006, and similar to Saunders & Druehl 1992); C) estimates of mutation rates calibrated to the divergence time between *Alaria* and *Laminaria* between 10 to 15 Mya [Tellier *et al.* 2009 following Hoarau *et al.* (2007), who introduced a heterokont molecular clock for *psbA*]. Beside the estimate for *rbc- Sp* acquired from Tellier *et al.* (2009) I calculated mutation rates for the *atp8- Sp* region, *trnW- Sp* region, *nd6*-gene, and nuclear ITS (Appendix S1.E) to be used as priors (ucld.mean) in calibration strategy C.

Additional priors used in all three calibration strategies were as follows. The substitution models and clock models were unlinked; whereas the trees were linked to the *nd6* partition tree (the tree likelihood with *nd6* linkage was the highest, even

though no other linkage was significantly worse). Substitution models and partitions were applied as described for the Bayesian tree. To account for the species-level phylogeny I used the Yule prior to be shared by all tree models. The starting tree was randomly generated. The MCMC chain length was set to 15 million and the parameters were logged every 500 generation resulting in 30,000 logged trees. For each calibration strategy three runs were performed, to allow for different starting points, and subsequently combined with LogCombiner v1.5.4. TreeAnnotator v1.5.4 (both programs are available in BEAST v1.5.4 package) was used to produce a single target tree with all information summarized. This tree was finally refined in FigTree v1.2.3 (<http://tree.bio.ed.ac.uk/software/figtree/>) to create a publishable output. If the posterior values would not level in all runs or if the posterior values were significantly different between runs, I extracted the best 25% of the trees (based on their posterior probabilities) after the runs had been combined to be summarized in TreeAnnotator v1.5.4.

Differences in the topological reconstruction (i.e., the root placement) have been tested with TREEFINDER version of October 2008 (Jobb 2008).

Base composition was calculated in MEGA 4.0 to construct a matrix of pair wise differences in GC content. This was used to assess the amount of compositional heterogeneity between the lineages.

2.4 Results

The mtDNA *atp8*-Sp yielded a dataset of 153bp (gaps removed) and was the most variable of the markers with 45 (29.4%) variable (v) sites out of which 37 were parsimony informative (p.i.). Gaps were removed from all the datasets and thus yielded datasets of: 227bp (56 (24.7%) v/ 52 p.i.) for *trnW*-Sp; the first 860bp (126 (14.7%) v/ 115 p.i.) of the *nd6*-gene; 331bp (12 (3.6%) v/ 11 p.i.) for *rbc*-Sp; and 709bp (95 (13.4%) v/ 86 p.i.) for ITS. Within 126 variable sites of the *nd6*-gene 32 were at the first codon position, 17 at the second and 77 at the third. None of the markers used showed signs of saturation but for the 3rd position of the *nd6*-gene in the outgroup comparison, the ingroup however was unaffected by saturation (Fig. S 2.2).

The incongruence-length test, used to evaluate compatibility between datasets, yielded a p-value = 0.06. Cunningham (1997) suggested that p-values > 0.01 indicate that combining the data will generally improve the accuracy of the phylogeny. The combined dataset had a length of 2280bp (Table 2.1).

Species delimitations were overall well supported in all tree inference methods (Bayesian, ML, MP, and NJ) of the combined dataset (Fig. 2.2), except for *L. variegata* and *L. nigrescens*. The data revealed four clades of *L. variegata* from New Zealand, all of which had high support (Fig. 2.2). The species epithet, based on the variegation of the blades, has only been found in the Wellington lineage [*L. variegata*/W, lineage named after the type locality of *L. variegata* (Agardh 1878)]. Despite intensive sampling I could not find distinctive variegation in the other lineages, which had uni-coloured yellowish to brownish blades. Although

preliminary studies have revealed possible morphological characters to distinguish the other three lineages (Chapter 7), the value of these characters is uncertain based on lacking information about the morphological variation within lineages. All lineages have been found to be restricted to certain areas around New Zealand with almost no overlap (Chapter 3). The sequence divergence between each lineage of *L. variegata* and its closest relative was of equal magnitude to the sequence divergence of other species (Table S 2.5). The smallest sequence divergence between species in Australasia was 1.9% between *L. adamsiae* and *L. variegata*-southern lineage (/S) and 2% between *L. adamsiae* and *L. corrugata*.

The data supported the split of *L. nigrescens* into two species as proposed by Tellier *et al.* (2009) (Fig. 2.2). Based on their finding of spatial separation I adopted their lineage abbreviations for the northern lineage (N) and the Intermediate Area lineage (IA).

Samples identified as either *L. vadosa* or *L. flavicans* had only minor sequence differences (0.13%; comparison of specimen A634 and C156), while there was major divergence of *L. vadosa* samples between different localities. For Example the level of differentiation between *L. vadosa* from the Falkland Islands, with or without the *L. flavicans* sample, and *L. vadosa* from the mainland was 0.69% and 0.72%, respectively.

The placement of the root between *L. trabeculata* and *L. nigrescens* was not well supported (topology Fig. 2.2 vs. topology Fig. S 2.3). Root placement could affect phylogenetic relationships of the ingroup and evolutionary interpretations. Thus I tested whether the alignment effected topology (matrix-I vs. matrix-II), whether outgroup selection effected root position (e.g., due to long branch effect),

and whether alternative rooting methods that do not require an outgroup (molecular clock rooting or mid-point rooting) changed tree topology. I also tested the fit of the ingroup alignment to three alternative topologies. Both supermatrices (matrix-I and matrix-II) had the same length but the ingroup alignments differed in 19 positions (0.8%). The differences resulted in an ML topology for matrix-II where the root was between *L. trabeculata* and *L. vadosa* (TopoB, Fig. S 2.3). However, in neither tree, whether it was calculated based on matrix-I or matrix-II (Fig. 2.2 or Fig. S 2.3), was the root position supported and other ingroup relationships were not altered.

Additional outgroups that are more distantly related to both *Lessonia* and *Ecklonia* (Table S 2.5) were used to test root stability and tree topology with matrix-I [*Eisenia bicyclis*, *Macrocystis pyrifera*, *Undaria pinnatifida* (data not shown), and *Laminaria ephemera* (Fig. S 2.4)]. In all approaches, but the NJ-LogDet-calculation with *Laminaria ephemera* (Fig. S 2.4), the root position and tree topology was consistent to TopoA (Fig. 2.2); however, support of dichotomies was best when using only *Ecklonia*.

Clock-like evolution was rejected (Table S 2.6). Thus rooting under a molecular clock model should be treated with some caution. The two most divergent taxa (*L. nigrescens*/N and *L. variegata*/K, Table S 2.5) also evolved at different rates. However, phylogenetic trees rooted with a molecular clock assumption or at the midpoint resulted in a third topology with the root position between South America and Australasia (TopoC), defining two monophyletic groups. Within these clades, clock-like evolution was accepted (Table S 2.6).

The ingroup data of both datasets have been tested for their fit to the three alternative topologies (TopoA-C), revealing a best fit to TopoA (Table S 2.7). In

TopoA, the South American *Lessonia* species form a paraphyletic assemblage with a group of Australasian species nested within it and *L. nigrescens* as sister to all other *Lessonia*. Recalculating the phylogenetic reconstruction with *L. nigrescens* as the only outgroup yielded highest support values for the relationships of the South American lineages (Fig. S 2.5). In a cascaded order *L. trabeculata* branches off first followed by *L. flavicans*. The Australasian *Lessonia* were monophyletic and strongly supported with a sister relationship to *L. flavicans* from South America. The branching order between Australasian taxa could not be well resolved. The only exception was a supported relationship between *L. corrugata* from Tasmania and *L. adamsiae* from The Snares (Fig. 2.2 and S 2.5).

The average amount of compositional heterogeneity was low (average difference in GC content = 0.3753%, Table S 2.8) limiting causes of systematic error. The trees calculated under the three different calibration strategies showed all topology TopoB, which might be an artefact of using a clock assumption (even a relaxed clock) as phylogeny and divergence dates were coestimated under this approach (Drummond *et al.* 2006). Divergence times differ between all three calibrations, whereas strategy A is significantly different to strategy C, the strategies B and C greatly overlap in their 95% HPD (highest posterior density) of divergence times including each the mean of the respective other strategy (Fig. S 2.6). Using root heights as priors the mean divergence time between *Ecklonia* and *Lessonia* was found at the lower range of the priors [10 Mya or 16 Mya for strategy A (8.5-34 Mya) or B (15-19 Mya) respectively]. Using mutation rates as priors, the root height between *Ecklonia* and *Lessonia* was calculated at around 18 Mya. The mean of first speciation within *Lessonia* was calculated to have occurred 2.4 Mya, 3.9 Mya, or 4.6

Mya according to calibration strategy A, B, and C, respectively and radiation in Australasia started around 1.8 Mya, 2.9 Mya, or 3.4 Mya.

2.5 Discussion

We used *Ecklonia* as outgroup based on findings by Lane *et al.* (2006). However, *Ecklonia* shows great genetic distance to the ingroup and the last common ancestor was calculated to have lived 18 Mya, which is even higher than the divergence time estimated between families within the Laminariales. Tellier *et al.* (2009) estimated the divergence time between *Laminaria digitata* (Laminariaceae) and *Alaria crassifolia* (Alariaceae) between 10 and 15 Mya.

The closest relative of the tested outgroups has been *Laminaria ephemera* (HKY mean distance was 0.14 vs. 0.16 between *Ecklonia* and *Lessonia*), which supports findings by Lee *et al.* (2008) who catalogued northern and Southern Hemisphere brown algae while using the barcoding gene CO1. In contrast a relationship between *L. ephemera* and *Lessonia* was not found by Lane *et al.* (2006) who, investigating higher level relationships, included multiple markers and several species of Laminariaceae (including *L. ephemera*) and Lessoniaceae in their analysis to show that these two groups were clearly distinct. Gene tree incongruence is often explained by hybridisation or incomplete lineage sorting. In this case incomplete lineage sorting is likely to play a minor role as *Lessonia* and *Laminaria* have diverged a long time ago. Thus the close genetic distance between *Lessonia* and *L. ephemera* on one side and the distinction of *Lessonia* and *L. ephemera* into two different groups on the other side might be a signature of ancient hybridisation and reticulated evolution can not be excluded for *Lessonia*. Studies have shown that

hybridisation may occur above species level within the Laminariales (Druehl *et al.* 2005 and references herein) but few studies have proposed ancient hybridisation as a mode of speciation within the Laminariales (Zhang *et al.* 2009). New methods have been proposed to detect hybridisation that can distinguish hybridisation from incomplete lineage sorting (Holland *et al.* 2008) or to assess the contribution of hybridisation to gene tree incongruence (Meng & Kubatko 2009). Whether *Lessonia* is derived from ancient hybridisation is not known however it could influence the tree topology, thus I tested different combinations of outgroups, which all revealed a TopoA topology (*L. nigrescens* as sister to all other *Lessonia*) except for the ML-tree of the *Ecklonia/L. ephemera* combination, which had a TopoB topology (*L. nigrescens* and *L. trabeculata* as sister to all other *Lessonia*). Rooting without outgroup (e.g., midpoint rooting or molecular clock rooting) is not optimal for the dataset but has resulted in a third topology (TopoC) with the root between South American and Australasian *Lessonia*. Regardless, all three topologies have been tested for their fit to the datasets, using a topology test method implemented in treefitter (Table S 2.7). Even though this method supported topology TopoA none of the other two topologies had statistical support for exclusion and thus the root position could not be determined with confidence.

The results suggest that *Lessonia* is a monophyletic lineage, as shown by Lane *et al.* (2006). While Lane *et al.* (2006) only analysed four species, I sampled all known species with multiple samples from disparate locations. The status of all species is supported with high support values except for *L. variegata* and *L. nigrescens*.

We show for the first time four distinct and highly supported lineages that suggest non-monophyly for *L. variegata*. The sequence divergences between these lineages are greater than distances between some accepted species. This and the high support for each lineage, indicates that these four lineages should be regarded as four species, as they meet the criteria of phylogenetic species (Mishler & Theriot 2000, Wheeler & Platnick 2000). Similar findings were made by Tellier *et al.* (2009) in South America who likewise concluded species status for two *L. nigrescens* lineages, which is confirmed by my data. Further investigation of morphological characters to eventually describe and distinguish the lineages of *L. variegata* or alternatively show the cryptic nature of these lineages is needed (but see Chapter 7 for a preliminary analysis of four morphological characters).

Relationships at the backbone of the Australasian lineages had no support in any of the tree building methods. A NJ-LogDet analysis was added to verify these results as this method has been found to retrieve true relationships even if other methods fail (Jermiin *et al.* 2004). The accuracy of this method however depends on the difference in GC content between lineages and the relationship of internal to terminal branch length. As the data showed only minor difference in GC content (mean difference = 0.38%), recovery of the correct phylogeny with the NJ-LogDet method was expected to be high (Jermiin *et al.* 2004). The failure of this method could be due to the internal branch lengths being less than 1% of the terminal branches. Given approximately 3 million years of evolution for the terminal branches (see dating analysis) then the radiation in Australasia would have taken place in a time frame of less than 30,000 years indicating a sudden radiation (Fig. 2.3).

The results show a monophyletic origin of the Australasian flora. The only supported relationship among Australasian *Lessonia* was found between *L. adamsiae*, from The Snares (48°01'S 166°32'E), and *L. corrugata* that is endemic to Tasmania. The placement of the Tasmanian species within New Zealand lineages might imply westward dispersal from The Snares to Tasmania from a last common ancestor between *L. adamsiae* and *L. corrugata* (Fig. 2.2 and S 2.5). However, additional analysis revealed possible hybrid-speciation of *L. adamsiae* derived from *L. corrugata* and *L. variegata*/S (southern lineage) ancestors (Chapter 3).

No major differences have been found between the samples from the Falkland Islands (0.18%). One (A634) was previously used in other studies as *L. ovata* (= *L. flavicans sensu* Searles) (Lane *et al.* 2006) and the other two were identified by me as *L. flavicans* (= *L. vadosa sensu* Searles) based on the characteristic lacunae. The maximum difference (0.79%) found between samples from the Falkland Islands and *L. vadosa* samples from Chile (Punta Arenas) represents rather the difference between distantly sampled populations of a widespread species than the difference between two species. However, it was the highest within group divergence found in this study. For comparison, the divergence within other widespread species was 0.75% and 0.53% for *L. nigrescens*/IA and *L. trabeculata*, respectively. I conclude that there is either not enough genetic differentiation between *L. flavicans* and *L. ovata* or the samples have been misidentified (i.e., lacunae are not an unambiguous identifying characteristic or the samples in GenBank are mislabelled (see Table 7.1). As there is not enough evidence for the former and no possibility to re-examine the sample (A634) collected as *L. flavicans sensu* Searles, I treat sample A634 as a wrongly identified *L. flavicans*

specimen, as its haplotypes match those identified as *L. flavicans* from the Falkland Islands (Note: *L. flavicans sensu* Searles is different to *L. flavicans* in the way it is used here (see Chapter 6)).

The results suggest that the South American species form a paraphyletic grade with *L. nigrescens* in a sister relationship to all other *Lessonia* species and *L. flavicans* as a sister to a terminal Australasian clade. The basal placement of the three South American lineages might suggest an origin of the genus in South America, where three speciation events were followed by a single dispersal to Australasia and a subsequent Australasian radiation (after Sanmartin *et al.* 2007). An alternative hypothesis (based on a nested ancestral area interpretation as a consequence of unidirectional dispersal after Cook & Crisp 2005) would suggest that New Zealand is the ancestral area. Dispersal events from New Zealand to South America, following the Antarctic Circumpolar Current, would have preceded the radiation in New Zealand and would have led to the formation of the South American species via repeated colonisation. It has been argued by Crisp & Cook (2005) that phylogeographic interpretations that are based on tree topology alone are simplistic and not reliable. This topic needs further investigation.

Using a relaxed molecular clock assumption the resulting trees always resembled TopoB. My confidence in dating speciation events is higher for the Australasian grade than for the splitting events of the South American species, as, other than the former, the later change with the topology (TopoA vs. TopoB). With root heights as priors, the mean divergence time between *Ecklonia* and *Lessonia* was found at the lower range of the priors [10 Mya for strategy A (8.5-34 Mya) or 16 Mya for B (15-19 Mya); both strategies are based on within family splitting events].

Using mutation rates as priors, the root height (tmrca of *Ecklonia* and *Lessonia*) was calculated at around 18 Mya. This is at first sight higher than expected given that the mutation rates are based on the splitting time (between families) of 10 and 15 Mya (Tellier *et al.* 2009 using the divergence between *Laminaria* and *Alaria*). However, given the high genetic distance between *Ecklonia* and *Lessonia*, which is at a level with distances between families rather than within families (Table S 2.5) a root height at the upper range of the divergence spectrum, as in calibration strategy C, seems to be more plausible. Therefore I further discuss the data based on hypotheses C alone (Fig. 2.3).

The Laminariales are believed to have evolved in the Pacific Ocean in the Northern Hemisphere as a response of climate deterioration (i.e., cooling) during the Tertiary, followed by antitropical dispersal in the late Pliocene (Estes & Steinberg 1988 and 1989, Lüning & tom Dieck 1990, and Lindberg 1991). This dispersal would have occurred during the first bipolar glaciation of the Cenozoic, which started 2.7 – 3 Mya (Shackleton *et al.* 1984). New evidence of synchronized bipolar cooling of the two Hemispheres since the Miocene might have allowed for antitropical dispersal of the coldwater adapted algae during episodic cooling cycles that started ~20 My (DeConto *et al.* 2008) or even 35 My (Moran *et al.* 2006) earlier than previously thought. The differences are enormous and have the potential to challenge our knowledge about antitropical dispersal in brown algae. The rearrangement of genera within the Lessoniaceae (Lane *et al.* 2006) has led to a different global distribution pattern for this family, which is either bipolar (*Ecklonia* and *Eisenia*) or restricted to the Southern Hemisphere (*Lessonia*). *Egregia* is the only genus within this grouping that is solely found in the Northern Hemisphere but only

associated weakly with the remaining genera (Lane *et al.* 2006). A Southern Hemisphere radiation of Lessoniaceae after an earlier antitropical split from a common ancestor can be an alternative hypothesis to a Northern Hemisphere radiation of all Laminariales as was proposed by Estes & Steinberg (1988) and Lüning & tom Dieck (1990). In any case the possibility of *Lessonia* (Southern Hemisphere only) having evolved through hybridisation between ancestors of *Ecklonia* (bipolar) and *Laminaria* (Northern Hemisphere only) would be hard to explain in a phylogeographic framework. However the timing of speciation through hybridisation might have been any time between 5 Mya (the first divergence event of *Lessonia* species) and 18 Mya (trmca of *Ecklonia* and *Lessonia*).

The data (calibration strategy C) suggests that a differentiation within *Lessonia* started less than 5 Mya (Fig. 2.3). This matches the beginning of the Pliocene when gradual cooling of the warmer Miocene had created a climate similar to today's El Nino events (Molnar & Cane 2007, Bonham *et al.* 2009). Given the solely Southern Hemisphere distribution of *Lessonia* antitropical dispersal must have occurred latest in the late Miocene or early Pliocene, but might have been earlier depending on the theory of Lessoniaceae radiation. Biotic interchange of marine taxa during the early Pliocene with a north to south asymmetry was proposed earlier and summarized by Lindberg (1991).

The analysis further suggests that a sudden radiation of *Lessonia* species took place between 3 to 3.5 Mya. And even if *Lessonia* populations have been present in the Southern Hemisphere at least since the early Pliocene they might not have been widely distributed until their radiation. The increased availability of an alternative

food source might have triggered adaptation of limpets and marine mammals to herbivory as described by Estes & Steinberg (1988).

The timing of South American speciations should be treated with some caution as the lineages did not evolve clock-like (I used an uncorrelated log-normal distribution for the relaxed clock model to accommodate that) and as the timing also depends on the topology. Whereas a topology test favoured TopoA as the best fit to the data (Table S 2.7), the relaxed phylogenetics model in BEAST resulted in a TopoB tree. However, the calculations revealed a simultaneous speciation event to the radiation in Australasia between the South American *L. nigrescens* and *L. trabeculata* (3.4 Mya). The calculation of the splitting event between the two *L. nigrescens* lineages resulted a value between 1.367 - 2.840 Mya (with a mean of 2.066 Mya), which roughly coincides with the beginning of a worldwide unstable climate and the dawn of rapid oscillating glaciations at the Pliocene/Pleistocene boundary (Rapp 2009). However, my estimates are higher than the earlier proposed 0.250 - 1.698 Mya for the split between the two *L. nigrescens* lineages (Tellier *et al.* 2009). This can be explained the rooting differences between our surveys, as Tellier *et al.* (2009) used the same calibration I used in strategy C but *L. corrugata* as outgroup for the South American group (thus they assumed a TopoC topology).

In conclusion, my data shows that the South American species form a paraphyletic grade including a terminal monophyletic Australasian group. Four supported evolutionary lineages were detected in *Lessonia variegata*, indicating that this taxon consists of four species, which has not been reported before. This could have important implications for ecological management and conservation. *Lessonia* might have derived from ancient hybridisation between an early member of the

Lessoniaceae and an ancestor of *Laminaria ephemera*. The first speciation took place in *Lessonia* 3.8 – 5.5 Mya marking the time of the latest possible antitropical dispersal from north to south; although earlier dispersal cannot be excluded. In Australasia, a rapid radiation started 3.5 Mya (2.9 – 4.1 Mya), leading to seven of the eight recent Australasian species.

2.6 Figure and Tables

Fig. 2.1 Distribution and sample sites of *Lessonia*. a) Global distribution of *Lessonia*. b) sample sites in Australasia and c) in southern South America. Sample ID given as four ID numbers (see Table S 2.1 for exact locations) followed by species designation; ad = *L. adamsiae* (The Snares), br = *L. brevisfolia* (Campbell Is. & Antipodes), co = *L. corrugata* (Tasmania), fl = *L. flavicans sensu* Searles (Falkland), ni = *L. nigrescens* (Chile), th = *L. tholiformis* (Chatham Is.), tr = *L. trabeculata* (Chile), va = *L. flavicans* (southern Chile & Falkland), K = *L. variegata*/K (northeastern South Is.), N = *L. variegata*/N (northern North Is.), S = *L. variegata*/S (southern South Is.), W = *L. variegata*/W (central New Zealand).

2. Molecular phylogeny and timing

Fig. 2.3 Bayesian tree (TopoB) of a relaxed clock model of the combined dataset (matrix-I) following calibration strategy C with mutation rates as priors. Bars and numbers in brackets showing 95% of the highest posterior density of the node height (indicating the tmrca of adjacent branches); mean node age is given as a vertical number; support values are posterior probabilities. Scale axes in Mya. Figure shows speciation within *Lessonia* (<5 Mya) and a sudden radiation in the Australasian grade (ca. 3.5 - 3 Mya).

Table 2.1 Marker and dataset characteristic (matrix-I). Table shows the length of the marker regions, the mixture of variability in the markers used (variable sites (v) and parsimony informative sites (p.i.) in ingroup comparison), and amount of saturation (including outgroup). mt = mitochondrial, cl = chloroplast, nuc = nuclear, bp = base pair. ¹ Test of variability after Koblmueller *et al.* 2006 (< 10% low, 10 – 20% high, > 20% hyper). ² Saturation test after Verbruggen & Theriot 2008 (see also Fig. S 2.2a-g). ³ for p.i. only.

marker	DNA	bp	v (bp)	v (%)	p.i. (bp)	p.i. (%)	variability ¹	saturation as slope ²	
atp8-Sp	mt	153	45	29.4	37	24.2	hyper	0.8501	weak
trnW-Sp	mt	227	56	24.7	52	22.9	hyper	0.8182	weak
nd6-gene	mt	860	126	14.7	115	13.4	high	0.9086	weak
1 st		287	32	11.1	28	9.8	high (low ³)	0.9381	weak
2 nd		287	17	5.9	15	5.2	low	0.9691	weak
3 rd		286	77	26.9	72	25.2	hyper	0.7209	intermediate
rbc-Sp	cl	331	12	3.6	11	3.3	low	0.9539	weak
ITS	nuc	709	95	13.4	86	12.1	high	0.9388	weak
ITS1		314	69	22.0	64	20.4	hyper	0.9199	weak
5.8S		160	1	0.6	1	0.6	very low	-	-
ITS2		235	25	10.6	21	8.9	high (low ³)	0.8722	weak
combined		2280	334	14.6	301	13.2	high	-	-

2.7 References

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2.8 Supplementary

2.8.1 Figures

Fig. S 2.1 PCR profiles for the fife markers used. Touchdown PCR was used for atp8-Sp and trnW-Sp; a simple PCR profile was used for nd6-gene, rbc-Sp, and ITS.

Fig. S 2.2 Slope of saturation curves for detecting saturation within a marker region. Dashed line indicates the no saturation slope (i.e., uncorrected distances equal corrected distances). All markers, except the 3rd position of the nd6-gene, are slow evolving and weak saturated. The 3rd codon position of the nd6-gene is medium fast evolving with intermediate saturation (after Verbruggen and Theriot 2008) (see also Table 1).

Fig. S 2.3 Bayesian topology (TopoB) using the second alignment (matrix-II). Scale bar = substitutions per site. Sample abbreviations according to sample ID's from Table S1. Support values are Bayesian posterior probabilities, MP bootstraps, and NJ LogDet bootstraps, respectively. “*” represents 100% support, whereas “-” = < 0.95 posterior probability or < 70% bootstrap support, except for the red numbers that support the root position. Only Bayesian and NJ resulted in a TopoB topology, however, without support. The MP-tree had an unsupported TopoA topology. Abbreviations as in Fig. 2.

Fig. S 2.4 NJ-LogDet topology (TopoB) of the combined dataset (matrix-I) with *Laminaria ephemera* included to the outgroup. Scale bar = substitutions per site. Sample abbreviations according to sample ID's from Table S1 and as described in Fig. 2. Support values are ML bootstraps, MP bootstraps, and NJ LogDet bootstraps, respectively. "*" represents 100% support, whereas "-" = < 0.95 posterior probability or < 70% bootstrap support.

Fig. S 2.6 Node age differences between root positions and calibration strategies. Notice that the y-axis for the root height is different to the y-axis for the ingroup node heights. Graphic shows that the node heights within Australasia are nearly independent from the root position (except for root C cal-C) but vary if a different calibration strategy is used. Solid line root A, dotted line rootB, dashed line root C. Blue calibration strategy A, brown calibration strategy B, black calibration strategy C.

2.8.2 Tables

Table S 2.1 Species identification, sample ID, location with geographic information (if available), and GenBank accession numbers. Collector if not authors in parentheses. ~ indicates that exact GPS location of the sampled population was not recorded but retrieved from Google Earth ver. 5.1. The *L. flavicans* specimen (A635) was also used in a previous study by Lane *et al.* (2006); however I suspect it to be misidentified *L. vadosa* specimen (see text).

Species	Sample ID	Location (collectors if not authors)	GenBank accession numbers	
<i>E. cava</i>	B701		atp8-Sp:	GU593723
			trnW-Sp:	GU593923
		Lat:	nd6:	GU593823
		Long:	rbc-Sp:	GU593873
		(C. Lane)	ITS:	GU593773
<i>E. radiata</i>	A106	Paia Point, Kaikoura, NZ	atp8-Sp:	GU593724
			trnW-Sp:	GU593924
		Lat: 42°29'22.43"S	nd6:	GU593824
		Long: 173°31'27.05"E	rbc-Sp:	GU593874
			ITS:	GU593774
<i>L. adamsiae</i>	A614	Tahi, The Snares, NZ	atp8-Sp:	GU593749
			trnW-Sp:	GU593949
		Lat: ~48°03'42.42"S	nd6:	GU593849
		Long: ~166°30'59.99"E	rbc-Sp:	GU593899
		(L. Hunt)	ITS:	GU593799
<i>L. adamsiae</i>	A615	Tahi, The Snares, NZ	atp8-Sp:	GU593750
			trnW-Sp:	GU593950
		Lat: ~48°03'42.42"S	nd6:	GU593850
		Long: ~166°30'59.99"E	rbc-Sp:	GU593900
		(L. Hunt)	ITS:	GU593800
<i>L. adamsiae</i>	A616	Tahi, The Snares, NZ	atp8-Sp:	GU593751
			trnW-Sp:	GU593951
		Lat: ~48°03'42.42"S	nd6:	GU593851
		Long: ~166°30'59.99"E	rbc-Sp:	GU593901
		(L. Hunt)	ITS:	GU593801
<i>L. adamsiae</i>	A626	South Promontory, The Snares, NZ	atp8-Sp:	GU593752
			trnW-Sp:	GU593952
		Lat: ~48°0'54.38"S	nd6:	GU593852
		Long: ~166°34'40.55"E	rbc-Sp:	GU593902
		(L. Hunt)	ITS:	GU593802
<i>L. brevifolia</i>	A548	Smoothwater Bay, Campbell Is., NZ	atp8-Sp:	GU593753
			trnW-Sp:	GU593953

		Lat: ~52°32'38.87"S	nd6:	GU593853
		Long: ~169°15'9.09"E	rbc-Sp:	GU593903
		(D. Neale)	ITS:	GU593803
<i>L. brevifolia</i>	A973	Antipodes, NZ	atp8-Sp:	GU593754
			trnW-Sp:	GU593954
		Lat: ~49°41'12.94"S	nd6:	GU593854
		Long: ~178°46'13.05"E	rbc-Sp:	GU593904
		(C. Fraser)	ITS:	GU593804
<i>L. brevifolia</i>	B296	Perseverance Harbour, Campbell Is., NZ	atp8-Sp:	GU593755
			trnW-Sp:	GU593955
		Lat: ~52°33'40.07"S	nd6:	GU593855
		Long: ~169°13'24.55"E	rbc-Sp:	GU593905
		(C. Fraser)	ITS:	GU593805
<i>L. corrugata</i>	A985	Bicheno, Tasmania, Tas	atp8-Sp:	GU593744
			trnW-Sp:	GU593944
		Lat: ~41°52'21.99"S	nd6:	GU593844
		Long: ~148°18'38.34"E	rbc-Sp:	GU593894
		(C. Boedeker)	ITS:	GU593794
<i>L. corrugata</i>	C057	Skeleton Pt., Tasmania, Tas	atp8-Sp:	GU593745
			trnW-Sp:	GU593945
		Lat: 41°14'54.93"S	nd6:	GU593845
		Long: 148°19'46.11"E	rbc-Sp:	GU593895
			ITS:	GU593795
<i>L. nigrescens</i>	A581	La Pampilla, Coquimbo, Chile	atp8-Sp:	GU593725
IA lineage			trnW-Sp:	GU593925
		Lat: 29°55'58.00"S	nd6:	GU593825
		Long: 71°20'15.09"W	rbc-Sp:	GU593875
		(E. Macaya-Horta)	ITS:	GU593775
<i>L. nigrescens</i>	A584	La Pampilla, Coquimbo, Chile	atp8-Sp:	GU593726
IA lineage			trnW-Sp:	GU593926
		Lat: 29°55'58.00"S	nd6:	GU593826
		Long: 71°20'15.09"W	rbc-Sp:	GU593876
		(E. Macaya-Horta)	ITS:	GU593776
<i>L. nigrescens</i>	A587	La Pampilla, Coquimbo, Chile	atp8-Sp:	GU593727
IA lineage			trnW-Sp:	GU593927
		Lat: 29°55'58.00"S	nd6:	GU593827
		Long: 71°20'15.09"W	rbc-Sp:	GU593877
		(E. Macaya-Horta)	ITS:	GU593777
<i>L. nigrescens</i>	B717	Bahia Mansa, Osorno, Chile	atp8-Sp:	GU593728
IA lineage			trnW-Sp:	GU593928
		Lat: 40°33'53.33"S	nd6:	GU593828
		Long: 73°44'2.33"W	rbc-Sp:	GU593878

		(E. Macaya-Horta)	ITS:	GU593778
<i>L. nigrescens</i>	B718	Bahia Mansa, Osorno, Chile	atp8-Sp:	GU593729
IA lineage		Lat: 40°33'53.33"S	trnW-Sp:	GU593929
		Long: 73°44'2.33"W	nd6:	GU593829
		(E. Macaya-Horta)	rbc-Sp:	GU593879
			ITS:	GU593779
<i>L. nigrescens</i>	B719	Bahia Mansa, Osorno, Chile	atp8-Sp:	GU593730
IA lineage		Lat: 40°33'53.33"S	trnW-Sp:	GU593930
		Long: 73°44'2.33"W	nd6:	GU593830
		(E. Macaya-Horta)	rbc-Sp:	GU593880
			ITS:	GU593780
<i>L. nigrescens</i>	B858	San Marcos, Tarapaca, Chile	atp8-Sp:	GU593731
northern lineage		Lat: 21°6'54.26"S	trnW-Sp:	GU593931
		Long: 70°7'34.14"W	nd6:	GU593831
		(E. Macaya-Horta)	rbc-Sp:	GU593881
			ITS:	GU593781
<i>L. nigrescens</i>	B859	San Marcos, Tarapaca, Chile	atp8-Sp:	GU593732
northern lineage		Lat: 21°6'54.26"S	trnW-Sp:	GU593932
		Long: 70°7'34.14"W	nd6:	GU593832
		(E. Macaya-Horta)	rbc-Sp:	GU593882
			ITS:	GU593782
<i>L. tholiformis</i>	A517	Wharf reef, Owenga, Chatham Is, NZ	atp8-Sp:	GU593746
		Lat: 44°1'25.21"S	trnW-Sp:	GU593946
		Long: 176°22'7.87"W	nd6:	GU593846
		(W. Nelson)	rbc-Sp:	GU593896
			ITS:	GU593796
<i>L. tholiformis</i>	A518	Wharf reef, Owenga, Chatham Is, NZ	atp8-Sp:	GU593747
		Lat: 44°1'25.21"S	trnW-Sp:	GU593947
		Long: 176°22'7.87"W	nd6:	GU593847
		(W. Nelson)	rbc-Sp:	GU593897
			ITS:	GU593797
<i>L. tholiformis</i>	A532	Wharekauri, Chatham Is, NZ	atp8-Sp:	GU593748
		Lat: ~43°42'15.18"S	trnW-Sp:	GU593948
		Long: ~176°34'54.12"W	nd6:	GU593848
		(W. Nelson)	rbc-Sp:	GU593898
			ITS:	GU593798
<i>L. trabeculata</i>	B715	Punihuil, Chiloe Is, Chile	atp8-Sp:	GU593733
		Lat: 41°55'25.96"S	trnW-Sp:	GU593933
		Long: 74°0'14.46"W	nd6:	GU593833
		(E. Macaya-Horta)	rbc-Sp:	GU593883
			ITS:	GU593783

<i>L. trabeculata</i>	B716	Punihuil, Chiloe Is, Chile	atp8-Sp:	GU593734
			trnW-Sp:	GU593934
		Lat: 41°55'25.96"S	nd6:	GU593834
		Long: 74°0'14.46"W	rbc-Sp:	GU593884
		(E. Macaya-Horta)	ITS:	GU593784
<i>L. trabeculata</i>	B834	Rio Seco, Tarapaca, Chile	atp8-Sp:	GU593735
			trnW-Sp:	GU593935
		Lat: 21°0'3.99"S	nd6:	GU593835
		Long: 70°9'55.70"W	rbc-Sp:	GU593885
		(E. Macaya-Horta)	ITS:	GU593785
(<i>L. ovata</i>)	A634	Rookery Bay, East Falkland, Falkland Islands	atp8-Sp:	GU593736
			trnW-Sp:	GU593936
		Lat: ~51°42'20.00"S	nd6:	GU593836 =AY857931
		Long: ~57°48'15.00"W (C. Lane)	rbc-Sp:	GU593886 =AY851543
			ITS:	GU593786 =AY857900
<i>L. sp</i> determined as <i>L. flavicans</i>	C156	Surf Bay, East Falkland, Falkland Islands (intertidal)	atp8-Sp:	GU593737
			trnW-Sp:	GU593937
		Lat: 51°41'31.04"S	nd6:	GU593837
		Long: 57°46'7.94"W	rbc-Sp:	GU593887
		(C. Fraser)	ITS:	GU593787
<i>L. sp</i> determined as <i>L. flavicans</i>	C160	Surf Bay, East Falkland, Falkland Islands (intertidal)	atp8-Sp:	GU593738
			trnW-Sp:	GU593938
		Lat: 51°41'31.04"S	nd6:	GU593838
		Long: 57°46'7.94"W	rbc-Sp:	GU593888
		(C. Fraser)	ITS:	GU593788
<i>L. flavicans</i>	B985	Punta Arenas, Patagonia, Chile	atp8-Sp:	GU593739
			trnW-Sp:	GU593939
		Lat: ~53°11'22.90"S	nd6:	GU593839
		Long: ~70°52'21.84"W	rbc-Sp:	GU593889
		(C. Cardenas)	ITS:	GU593789
<i>L. flavicans</i>	B986	Punta Arenas, Patagonia, Chile	atp8-Sp:	GU593740
			trnW-Sp:	GU593940
		Lat: ~53°11'22.90"S	nd6:	GU593840
		Long: ~70°52'21.84"W	rbc-Sp:	GU593890
		(C. Cardenas)	ITS:	GU593790
<i>L. flavicans</i>	B987	Punta Arenas, Patagonia, Chile	atp8-Sp:	GU593741
			trnW-Sp:	GU593941
		Lat: ~53°11'22.90"S	nd6:	GU593841
		Long: ~70°52'21.84"W	rbc-Sp:	GU593891

		(C. Cardenas)	ITS:	GU593791
<i>L. flavicans</i>	B988	Punta Arenas, Patagonia, Chile	atp8-Sp:	GU593742
		Lat: ~53°11'22.90"S	trnW-Sp:	GU593942
		Long: ~70°52'21.84"W	nd6:	GU593842
		(C. Cardenas)	rbc-Sp:	GU593892
			ITS:	GU593792
<i>L. flavicans</i>	B989	Punta Arenas, Patagonia, Chile	atp8-Sp:	GU593743
		Lat: ~53°11'22.90"S	trnW-Sp:	GU593943
		Long: ~70°52'21.84"W	nd6:	GU593843
		(C. Cardenas)	rbc-Sp:	GU593893
			ITS:	GU593793
<i>L. variegata</i> lineage N	A556	North Cape, Northland, NZ	atp8-Sp:	GU593756
		Lat: ~34°24'8.36"S	trnW-Sp:	GU593956
		Long: ~173°2'5.64"E	nd6:	GU593856
		(W. Nelson)	rbc-Sp:	GU593906
			ITS:	GU593806
<i>L. variegata</i> lineage N	A557	North Cape, Northland, NZ	atp8-Sp:	GU593758
		Lat: ~34°24'8.36"S	trnW-Sp:	GU593958
		Long: ~173°2'5.64"E	nd6:	GU593858
		(W. Nelson)	rbc-Sp:	GU593908
			ITS:	GU593808
<i>L. variegata</i> lineage N	B129	Maitai Bay, Northland, NZ	atp8-Sp:	GU593759
		Lat: 34°49'36.54"S	trnW-Sp:	GU593959
		Long: 173°24'47.88"E	nd6:	GU593859
			rbc-Sp:	GU593909
			ITS:	GU593809
<i>L. variegata</i> lineage N	B210	Outer South Head, Omapere, NZ	atp8-Sp:	GU593757
		Lat: 35°32'16.28"S	trnW-Sp:	GU593957
		Long: 173°21'55.67"E	nd6:	GU593857
			rbc-Sp:	GU593907
			ITS:	GU593807
<i>L. variegata</i> lineage N	B312	The Sailors Grave, Coromandel, NZ	atp8-Sp:	GU593760
		Lat: 36°57'39.33"S	trnW-Sp:	GU593960
		Long: 175°50'41.36"E	nd6:	GU593860
		(J. Buchanan)	rbc-Sp:	GU593910
			ITS:	GU593810
<i>L. variegata</i> lineage W	A001	Princess Bay, Wellington, NZ	atp8-Sp:	GU593761
		Lat: 41°20'42.36"S	trnW-Sp:	GU593961
		Long: 174°47'15.89"E	nd6:	GU593861
			rbc-Sp:	GU593911
			ITS:	GU593811
<i>L. variegata</i>	A002	Princess Bay, Wellington, NZ	atp8-Sp:	GU593762

lineage W			trnW-Sp:	GU593962
		Lat: 41°20'42.36"S	nd6:	GU593862
		Long: 174°47'15.89"E	rbc-Sp:	GU593912
			ITS:	GU593812
<i>L. variegata</i> lineage W	A003	Princess Bay, Wellington, NZ	atp8-Sp:	GU593763
		Lat: 41°20'42.36"S	trnW-Sp:	GU593963
		Long: 174°47'15.89"E	nd6:	GU593863
			rbc-Sp:	GU593913
			ITS:	GU593813
<i>L. variegata</i> lineage W	A025	Riversdale Beach, Wairarapa, NZ	atp8-Sp:	GU593766
		Lat: 41°6'29.73"S	trnW-Sp:	GU593966
		Long: 176°4'10.55"E	nd6:	GU593866
			rbc-Sp:	GU593916
			ITS:	GU593816
<i>L. variegata</i> lineage W	A500	Whites Bay, Marlborough Sounds, NZ	atp8-Sp:	GU593764
		Lat: 41°23'17.96"S	trnW-Sp:	GU593964
		Long: 174°3'23.28"E	nd6:	GU593864
			rbc-Sp:	GU593914
			ITS:	GU593814
<i>L. variegata</i> lineage W	A613	Cape Palliser, Wairarapa, NZ	atp8-Sp:	GU593765
		Lat: 41°36'48.47"S	trnW-Sp:	GU593965
		Long: 175°17'32.66"E	nd6:	GU593865
			rbc-Sp:	GU593915
			ITS:	GU593815
<i>L. variegata</i> lineage K	A138	South Bay, Kaikoura, NZ	atp8-Sp:	GU593767
		Lat: 42°25'33.45"S	trnW-Sp:	GU593967
		Long: 173°41'6.25"E	nd6:	GU593867
			rbc-Sp:	GU593917
			ITS:	GU593817
<i>L. variegata</i> lineage K	A606	New Wharf, Kaikoura, NZ	atp8-Sp:	GU593768
		Lat: 42°24'47.17"S	trnW-Sp:	GU593968
		Long: 173°42'8.22"E	nd6:	GU593868
		(R. Dunmore)	rbc-Sp:	GU593918
			ITS:	GU593818
<i>L. variegata</i> lineage K	A779	Cape Campbell, NZ	atp8-Sp:	GU593769
		Lat: 41°43'46.18"S	trnW-Sp:	GU593969
		Long: 174°16'19.08"E	nd6:	GU593869
			rbc-Sp:	GU593919
			ITS:	GU593819
<i>L. variegata</i> lineage S	A434	Curio Bay, Catlins, NZ	atp8-Sp:	GU593770
		Lat: 46°39'38.85"S	trnW-Sp:	GU593970
			nd6:	GU593870

		Long: 169°6'29.45"E	rbc-Sp:	GU593920
			ITS:	GU593820
<i>L. variegata</i> lineage S	C154	Causet Cove, Doubtful Sound, NZ	atp8-Sp:	GU593771
		Lat: 45°17'47.63"S	trnW-Sp:	GU593971
		Long: 166°54'33.01"E	nd6:	GU593871
		(W. Nelson)	rbc-Sp:	GU593921
			ITS:	GU593821
<i>L. variegata</i> lineage S	C155	Shelter Islands, Doubtful Sound, NZ	atp8-Sp:	GU593772
		Lat: 45°16'13.54"S	trnW-Sp:	GU593972
		Long: 166°53'29.26"E	nd6:	GU593872
		(W. Nelson)	rbc-Sp:	GU593922
			ITS:	GU593822
<i>Laminaria</i> <i>ephemera</i>	C202	Salmon Banks (FHL), Washington, USA	atp8-Sp:	
		Lat: -48°26'10"N	trnW-Sp:	
		Long: 123°02'20"W	ITS:	
<i>L. ephemera</i>	(used to complete the sequences missing in C202)	Botanical Beach, Port Renfrew, BC, Canada (C. Lane)	nd6:	AY857924
			rbc-Sp:	AY851557

Table S 2.2 Primer characteristics and their coded PCR-profiles (see Fig. S1). Primers in bold were developed for this study. The original primer was put in brackets if only slight modifications were made. ^a after Voisin *et al.* 2005; ^b after Lane *et al.* 2006; ^c after Peters & Ramirez 2001; ^d after Tai *et al.* 2001.

ATP8-spacer region (~172bp)		Length	%GC
atp8 ^a	tgtacgtttcatattaccttctttagc	27bp	33
atp8R ^a	tagcaaaccaaggctttcaac	21bp	43
PCR-profile	Td VI 55/(5)33x		
trnW-spacer region (~238bp)			
trnW ^a	gggggttcaaaccctctctt	20bp	50
trnW-R ^a	cctacattgttagcttcagagaa	24bp	37
PCR-profile	Td VI 55/(5)33x		
nd6 gene (1159bp)			
KM1 ^b	tcttattgaaagtttagggcc	21bp	38
n6iR (KM2N^b)	tctataaaccacatttrattta	22bp	18
n6i (KM5^b)	tcttttagtttagtrtctta	20bp	20
n6R	tagattcagcagctccytgac	21bp	48
PCR-profile	nd6 50°/35x		
rbc-spacer (363bp)			
rbcLSS (KR5^b)	acttcaacagatacacctga	20bp	40
rbcS97R ^c	catctgtccattctacactaac	22bp	41
PCR-profile	rbc 45°/35x		
ITS (~850bp)			
ITSP1 ^d	ggaaggagaagtcgtaacaagg	22bp	50
KP5 ^b	acaacgatgaagaacgcag	19bp	47
ITSRi (KIR1 ^b)	ttcaaagttttgatgattcac	21bp	29
KG4 ^b	ctttctccgcttagttatatg	23bp	39
PCR-profile	ITS 50°/31x		

Table S 2.3 Partition-homogeneity test or Incongruence Length Test (PAUP*) with heuristic search (nreps=100; TBR). A test to verify whether different datasets can be combined into a supermatrix. Cunningham (1997) suggested that p-values > 0.01 indicate that combining the data will generally improve the accuracy of the phylogeny. If p-values are < 0.001 the accuracy of the combined data would suffer relative to the individual partitions. The combination of the marker datasets into a supermatrix (matrix-I) is favoured.

Dataset: matrix-I

Test including outgroup (B701Ec, A106Er)

p=0.06

Pairwise test of Homogeneity

p-values	atp8	trnW	nd6	rbc	ITS
atp8	-	0.59	0.89	0.68	0.28
trnW		-	0.15	0.75	0.01
nd6			-	0.69	0.03
rbc				-	0.98
ITS					-

Test without outgroup

p=0.03

Pairwise test of Homogeneity

p-values	atp8	trnW	nd6	rbc	ITS
atp8	-	0.51	0.87	0.78	0.11
trnW		-	0.14	0.89	0.01
nd6			-	0.71	0.01
rbc				-	1.00
ITS					-

Table S 2.4 Partition strategy test (Tracer v1.5). BF > 20 is strong support for favoured model. Nst=6 Rates=invgamma ngammacat=8 [was applied separately for all partitions]. The partition of the supermatrix by marker regions and in case of the nd6-gene additionally by codon position is favoured.

	single partition	marker	marker + codon pos.
number of partitions	1	5	7
ln L	-7912.708	-7726.410	-7647.826
over 1000 bootstraps	-7932.395	-7747.549	-7670.276

BF	single partition	marker	marker + codon pos.
single partition	-		
marker	80.278	-	
marker + codon pos.	113.837	33.559	-

References: Newton & Raftery (1994) with modifications proposed by Suchard *et al.* (2001, MBE 18: 1001-1013)

Table S 2.5 HKY-distances (matrix-I) between and within lineages (PAUP*). F = Falkland Is, IA = intermediate area, K = Kaikoura, N = northern, P = Patagonia, S = southern, sc = species complex, W = Wellington. **Table S5a.** Ingroup comparison. **Table S5b.** Outgroup comparison

	<i>L. nig</i> /sc	<i>L. nig</i> /IA	<i>L. nig</i> /N	<i>L. tra</i>	<i>L. tra</i> /S	<i>L. tra</i> /N	<i>L. vad</i>	<i>L. vad</i> /F	<i>L. vad</i> /P	<i>L. cor</i>	<i>L. tho</i>	<i>L. ada</i>	<i>L. bre</i>	<i>L. var</i> /sc	<i>L. var</i> /N	<i>L. var</i> /W	<i>L. var</i> /K	<i>L. var</i> /S
<i>L. nigrescens</i> /sc	-																	
<i>L. nig</i> /IA	n/a	-																
<i>L. nig</i> /N	n/a	0.0232	-															
<i>L. trabeculata</i>	0.0249	0.0232	0.0302	-														
<i>L. tra</i> /S	0.0245	0.0227	0.0299	n/a	-													
<i>L. tra</i> /N	0.0258	0.0241	0.0308	n/a	0.0053	-												
<i>L. vadosa</i>	0.0366	0.0354	0.0401	0.0227	0.0225	0.0230	-											
<i>L. vad</i> /F	0.0354	0.0341	0.0391	0.0209	0.0209	0.0209	n/a	-										
<i>L. vad</i> /P	0.0373	0.0362	0.0407	0.0237	0.0234	0.0243	n/a	0.0071	-									
<i>L. corrugata</i>	0.0469	0.0466	0.0480	0.0376	0.0379	0.0370	0.0337	0.0325	0.0344	-								
<i>L. tholiformis</i>	0.0426	0.0415	0.0459	0.0329	0.0331	0.0326	0.0330	0.0319	0.0337	0.0349	-							
<i>L. adamsiae</i>	0.0403	0.0388	0.0450	0.0301	0.0304	0.0295	0.0287	0.0278	0.0292	0.0208	0.0281	-						
<i>L. brevifolia</i>	0.0461	0.0443	0.0514	0.0389	0.0392	0.0383	0.0375	0.0363	0.0383	0.0357	0.0345	0.0294	-					
<i>L. variegata</i> /sc	0.0443	0.0434	0.0470	0.0346	0.0348	0.0341	0.0350	0.0341	0.0356	0.0333	0.0325	0.0278	0.0359	-				
<i>L. var</i> /N	0.0447	0.0439	0.0470	0.0344	0.0341	0.0350	0.0350	0.0339	0.0356	0.0339	0.0322	0.0302	0.0360	n/a	-			
<i>L. var</i> /W	0.0429	0.0417	0.0463	0.0350	0.0356	0.0338	0.0349	0.0341	0.0353	0.0319	0.0332	0.0258	0.0337	n/a	0.0323	-		

Table S 2.5 continued

<i>L. var/K</i>	0.0512	0.0509	0.0520	0.0390	0.0395	0.0381	0.0395	0.0384	0.0401	0.0385	0.0367	0.0361	0.0425	n/a	0.0399	0.0380	-	
<i>L. var/S</i>	0.0395	0.0383	0.0431	0.0295	0.0297	0.0291	0.0310	0.0301	0.0314	0.0297	0.0276	0.0195	0.0333	n/a	0.0309	0.0284	0.0348	-
Overall mean within group	0.0123	0.0044	0.0004	0.0035	0.0000	n/a	0.0042	0.0013	0.0008	0.0026	0.0022	0.0005	0.0023	0.0265	0.0032	0.0012	0.0003	0.0032
Max. within group	0.0246	0.0075	0.0004	0.0053	0.0000	n/a	0.0079	0.0018	0.0013	0.0026	0.0031	0.0009	0.0026	0.0416	0.0040	0.0018	0.0004	0.0044

Table S2.5b. Outgroup comparison

	Lessonia	MpA005	UpA004	EbB699	LeC203	EcB701	ErA106
<i>Lessonia</i> (all)	-						
<i>Macrocystis pyrifera</i> A005	0.1592	-					
<i>Undaria pinnatifida</i> A004	0.1572	0.1601	-				
<i>Eisenia bicyclis</i> B699	0.1487	0.1545	0.1593	-			
<i>Laminaria ephemera</i> C203	0.1387	0.1604	0.1476	0.1469	-		
<i>Ecklonia cava</i> B701	0.1568	0.1645	0.1676	0.0535	0.1507	-	
<i>Ecklonia radiata</i> A106	0.1566	0.1627	0.1635	0.0537	0.151	0.0281	-

Table S 2.6 Molecular clock test (PAUP*). T1 = without clock; T2 =clock enforced. AA = Australasia, SA = South America. Molecular clock was rejected for all *Lessonia* but accepted for tests within geographic groups (p=0.001). Bonferroni was not applied as the p-values depend on the topology. For the root position see Table S 2.7.

	Clock test rootA	Clock test rootB	Clock test rootC
T1 (-ln L1)	6474.632	6474.632	6474.632
T2 (-ln L2)	6529.349	6518.997	6515.383
ntax	48	48	48
(T1-T2)·2	109.434	88.729	81.503
deg_freedom	48	48	48
p-value	4.365E-07 clock is rejected	1.578E-04 clock is rejected	9.755E-04 clock is rejected

	Clock test AA	Clock test SA rootA	Clock test SA rootB or C
T1 (-ln L1)	5637.302	4193.942	4193.942
T2 (-ln L2)	5657.455	4209.296	4214.176
ntax	30	19	19
(T1-T2)·2	40.307	30.709	40.468
deg_freedom	28	17	17
p-value	0.062 clock is accepted	0.022 clock is accepted	0.001 clock is accepted

Table S 2.7 Topology test (Paired Sites Tests in treefinder version of October 2008). No topology is rejected, but TopoA has the best support.

matrix-I (Lorigin-gXF)

	TopoA	TopoB	TopoC
ELW:	0.35	0.32	0.34
BP:	0.42	0.24	0.33
KH:	1.00	0.43	0.38
SH:	1.00	0.58	0.57
WSH:	1.00	0.58	0.57
AU:	0.61	0.37	0.55
Likelihood:	-6369.52	-6369.65	-6369.54
AIC:	13025.04	13025.30	13025.08
AICc:	13044.32	13044.58	13044.36
BIC:	13844.70	13844.96	13844.75
HQ:	13324.01	13324.27	13324.05
#Sites:	2280	2280	2280
#Param.:	143	143	143

matrix-II (mg-dataset)

	TopoA	TopoB	TopoC
ELW:	0.34	0.34	0.32
BP:	0.49	0.40	0.11
KH:	1.00	0.38	0.05
SH:	1.00	0.47	0.25
WSH:	1.00	0.47	0.11
AU:	0.67	0.50	0.24
Likelihood:	-6264.93	-6264.97	-6264.97
AIC:	12815.86	12815.93	12815.94
AICc:	12835.14	12835.22	12835.22
BIC:	13635.53	13635.60	13635.60
HQ:	13114.83	13114.91	13114.91
#Sites:	2280	2280	2280
#Param.:	143	143	143

Table S 2.8 Average difference in GC-content (matrix-I)

All	0.003753
within SA	0.003823
within AA	0.002395
between SA/AA	0.003777

2.8.3 Appendix

S1 Mutation rates

Estimation of mutation rates using HKY-distances (after Hoarau *et al.* 2007, and Tellier *et al.* 2009). HKY distances were calculated in PAUP*

A) psbA-gene divergence rate (as in Hoarau *et al.* 2007)

Table A 2.9.3.1 psbA-gene divergence rate (as in Hoarau *et al.* 2007); HKY corrected distances were used to account for saturation effect of the marker.

	[1]	[2]	[3]
<i>Haslea ostrearia</i> Y15074	-		
<i>Skeletonema costatum</i> Y15137	0.07545	-	
<i>Laminaria digitata</i> AY528849	0.18828	0.16428	-
<i>Alaria crassifolia</i> AY528847	0.18829	0.16428	0.01146
Divergence time between <i>Haslea</i> and <i>Skeletonema</i> = 65-87 My (Hoarau <i>et al.</i> 2007)			

B) psbA clock (using divergence rate and time between *Haslea* and *Skeletonema*)

$$0.07545/87 = 0.000867241 \text{ My}^{-1} = 0.087\% \text{ My}^{-1}$$

$$\text{to } 0.07545/65 = 0.001160769 \text{ My}^{-1} = 0.116\% \text{ My}^{-1}$$

C) Divergence time between *Laminaria* and *Alaria* based on psbA clock (after Tellier *et al.* 2009)

$$0.01146/0.001160769 \text{ My}^{-1} = 9.9 \text{ My}$$

$$0.01146/0.000867241 \text{ My}^{-1} = 13.2 \text{ My}$$

(Tellier *et al.* 2009 had calculated a divergence time of 10 - 15 My)

D) Calculation of divergence rates given the divergence time between *Laminaria* and *Alaria* of 9.9 to 13.2 Mya

D1) rbc-Sp divergence rate

Samples used:

Alaria esculenta AF109802 and *A. marginata* AF318959

Laminaria digitata AY851559 and *L. sinclairii* AY851558

Lessonia nigrescens B858N, *L. variegata*/N B210, and *L. variegata*/W A001

Table A 2.9.3.2 HKY corrected distance of rbc-Sp

	Ala	Lam	Les
Ala	-		
Lam	0.079	-	
Les	0.076	0.049	-

Average between group = 0.068 (Average used in Tellier *et al.* 2009 = 0.08)

Table A 2.9.3.3 Corrected divergence rate for rbc-Sp in My⁻¹

	min	max	mean
divergence rate	0.0051	0.0069	0.0060
mutation rate	0.0026	0.0034	0.0030

D2) atp8-Sp divergence rate

Samples used:

Alaria esculenta DQ841616 and DQ841615

Laminaria digitata DQ841608 and DQ841607

Lessonia nigrescens B858N, *L. variegata*/N B210, and *L. variegata*/W A001

Table A 2.9.3.4 HKY corrected distance of atp8-Sp

	Ala	Lam	Les
Ala	-		
Lam	0.229	-	
Les	0.299	0.258	-

Average between group = 0.262

Table A 2.9.3.5 Corrected divergence rate for atp8-Sp in My⁻¹

	min	max	mean
divergence rate	0.0197	0.0264	0.0230
mutation rate	0.0098	0.0132	0.0115

D3) trnW-Sp divergence rate

Samples used:

Alaria esculenta DQ841679 and DQ841680

Laminaria digitata DQ841669 and DQ841670

Lessonia nigrescens B858N, *L. variegata*/N B210, and *L. variegata*/W A001

Table A 2.9.3.6 HKY corrected distance of trnW-Sp

	Ala	Lam	Les
Ala	-		
Lam	0.128	-	
Les	0.206	0.162	-

Average between group = 0.165

Table A 2.9.3.7 Corrected divergence rate for trnW-Sp in My⁻¹

	min	max	mean
divergence rate	0.0124	0.0166	0.0145
mutation rate	0.0062	0.0083	0.0073

D4) nd6-gene divergence rate

Samples used:

Alaria esculenta AY878857, *A. marginata* AY878858, and *A. nana* AY878859

Laminaria digitata AY857921, *L. sinclairii* AY857920, and *L. saccharina* AB480844

Lessonia nigrescens B858N, *L. variegata*/N B210, and *L. variegata*/W A001

Table A 2.9.3.8 HKY corrected distance of nd6-gene

	Ala	Lam	Les
Ala	-		
Lam	0.121	-	
Les	0.142	0.155	-

Average between group = 0.139

Table A 2.9.3.9 Corrected divergence rate for nd6-gene in My^{-1}

	min	max	mean
divergence rate	0.0105	0.0140	0.0123
mutation rate	0.0052	0.0070	0.0061

D5) ITS divergence rate

Samples used:

Alaria crassifolia AF319001

Laminaria digitata AF319014

Lessonia nigrescens B858N, *L. variegata*/N B210, and *L. variegata*/W A001

Table A 2.9.3.10 HKY corrected distance of ITS

	Ala	Lam	Les
Ala	-		
Lam	0.062	-	
Les	0.067	0.100	-
Average between group = 0.076			

Table A 2.9.3.11 Corrected divergence rate for ITS in My^{-1}

	min	max	mean
divergence rate	0.0057	0.0076	0.0067
mutation rate	0.0029	0.0038	0.0033

E) settings used in BEAST for calibration strategy C

Priors:

atp8.meanRate: normal, mean=0.0115, stdev=0.0017

trnW.meanRate: normal, mean=0.0073, stdev=0.0010

nd6.meanRate: normal, mean=0.0061, stdev=0.0009

rbc.meanRate: normal, mean=0.0030, stdev=0.0004

its.meanRate: normal, mean=0.0033, stdev=0.0005

Chapter 3

Phylogeography of *Lessonia* (Phaeophyceae, Laminariales) in Australasia

3.1 Abstract

A goal of phylogeography is to relate phylogenetic patterns to potential historic and contemporary geographic isolating events. Here I investigated the distribution of Australasian *Lessonia*, an important habitat forming algal genus found on most rocky shores around New Zealand, its Sub-Antarctic Islands, and Tasmania.

Lessonia variegata, from the main islands of New Zealand, has recently been split into four species with previously undefined distributional boundaries. To a certain extent genetic breaks within a species may correlate to genetic breaks of other species. Breaks found more often in phylogeographic studies may denote boundaries of general biogeographic regions. My aim was (1) to compare the distribution of *Lessonia* with proposed bioregions and (2) to develop phylogeographic hypotheses to explain its present day distribution in Australasia. I used two markers, with different mutation rates to gain information of the phylogenetic history of *Lessonia*. The data revealed high phylogeographic structuring and reciprocal endemism for all Australasian *Lessonia*. I found localized distribution of all lineages within the *L. variegata* species complex. One lineage (*L. variegata*/N) is confined to the northern part of the North Island; *L. variegata*/W is found at the southeastern part of the North Island and, spanning Cook Strait, at the eastern part of Marlborough Sounds; *L. variegata*/K is endemic to the northeastern part of the South Island; and *L.*

variegata/S is restricted to the southern part of the South Island. No overlapping areas have been found. The data showed that genetic breaks in *Lessonia* do correlate to bioregions and highlight the importance of Cape Campbell at the northeast of the South Island, a well know phylogeographic break, as a barrier between adjacent North Island and South Island species. The species distribution also correlates to the geographic structuring of New Zealand during the Pliocene, 3.5 Mya.

Key Index Words: Australasia, bioregions, chloroplast marker, *Lessonia*, mitochondrial marker, New Zealand, Phaeophyceae, Phylogeography, Tasmania

Abbreviations: atp8-sp, spacer region between atp dehydrogenase subunit 8 and t-RNA serine; CTAB, Cetyltrimethylammonium Bromide; MJ, median joining; MP, maximum parsimony; My, million years; Mya, million years ago; rbc-sp, spacer between the large and short subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene; SSCP, Single stranded conformational polymorphism;

3.2 Introduction

Lessonia Bory is a very young genus within the Phaeophyceae. Recent molecular studies (Chapter 2) have shown that the Australasian *Lessonia* (*L. adamsiae* Hay, *L. brevifolia* J.Agardh, *L. corrugata* Lucas, *L. tholiformis* Hay, and *L. variegata* J.Agardh) are a monophyletic group and have diverged during a rapid radiation 3.5 – 3 million years ago (Mya). *L. variegata*, earlier believed to be a single species occupying the coasts of the three main islands of New Zealand, has been divided into a non-monophyletic species complex of four different lineages (Chapter 2). The full extent of their geographic distributions, however, is still unexplored.

New Zealand has had a complex geological history since being detached from Gondwana about 80 Mya (McLoughlin 2001). For the last 3.5My, the upper mean of the proposed time of speciation within *Lessonia* (Chapter 2), New Zealand was influenced by three major geological and climatic events: tectonic movements in conjunction with the Kaikoura Orogeny or Alpine Fault (King 2000); the changed sea levels and temperatures due to glacials and interglacials during the Pleistocene (Carter 2001); and volcanic activity especially in the lake Taupo area (Froggatt *et al.* 1986, Manville & Wilson 2004). Each of these events alone or in combination with other events might have influenced the distribution and speciation/extinction of *Lessonia* populations in New Zealand.

A review of 42 molecular studies on the phylogeography of coastal marine invertebrates and plants undertaken in New Zealand between 1980 and 2008, revealed a multitude of phylogeographic breaks (Ross *et al.* 2009 and references herein). To develop biogeographic classification schemes scientists search for overall

trends in taxa distribution (Shears *et al.* 2006, Ministry of Fisheries and Department of Conservation, 2008; hereafter called MPA 2008). One derived from a best fit on presence-absence data of macroalgae samples is given by Shears *et al.* 2006 (Fig. 3.1a). In their approach, *Lessonia variegata* was considered to be the only *Lessonia* species within the study area. Currently in use to protect New Zealand's marine habitats is a classification approach based on fourteen biogeographic regions (MPA 2008) (Fig. 3.1b). This scheme assumes that physical habitats and ecosystems, if separated by enough space (100s to 1000s of kms), will contain different biological communities due to a combination of broad-scale factors. Such factors may include water temperature, oceanography, current dynamics, large-scale latitudinal gradients, climate or barriers to dispersal. As biogeographic classification schemes derive from a multitude of different techniques and taxa they do not necessarily overlap with phylogeographic surveys of single taxa (Ross *et al.* 2009), even though there is a theoretical link. None of the phylogeographic studies reviewed by Ross *et al.* (2009) was fully congruent with the bioregions proposed by Shears *et al.* (2008) or the one used by governmental agencies (MPA 2008). A reason might be that mechanisms that shape species assemblages are different to those that lead to genetic substructure of a single widespread species, and/or the use of markers in phylogeographic studies that evolve too slowly to detect recent structuring (Ross *et al.* 2009). However, two breaks are of special interest as they have been found frequently: One is the biogeographic break at East Cape between the Portland and Northeastern bioregions where eddies are believed to obstruct connectivity between adjacent populations (Moore 1949 & 1961, Knox 1975, Nelson 1994, Walls 1995 and 2006, Francis 1996, Stevens & Hogg 2004, Shears *et al.* 2008). The other is the phylogeographic division between North and South Island lineages at Cape Campbell. Upwelling south of

Cape Campbell was initially postulated as the driving force (Apte & Gardner 2002, Waters & Roy 2004, Ayers & Waters 2005) but a recalibration of the divergence time showed that the genetic breaks were older than the upwelling zone (Goldstien *et al.* 2006). Changes in the hydrography of the Cook Strait region during the late Pleistocene as a consequence of fluctuating sea levels following the cycles of glaciation and deglaciation have been suggested as alternative causes (Goldstien *et al.* 2006). The phylogenetic break at Cape Campbell, which is indicated in the MPA (MPA 2008), was not evident in a recent classification scheme based on macroalgal data (Shears *et al.* 2008).

For the present study, populations from New Zealand's Sub-Antarctic Islands, the Chatham Islands and Tasmania/Australia have been sampled in addition to populations on the three main islands of New Zealand to cover the whole distribution range of *Lessonia* in Australasia. Only few molecular studies on the phylogeography of marine benthic taxa are known that incorporated the Sub-Antarctic Islands of New Zealand and the Chatham Islands (reviewed in Ross *et al.* 2009, e.g., Goldstien 2010). Species with a circum-Sub-Antarctic distribution and high dispersal ability have shown either no genetic diversity as in *Macrocystis pyrifera* (Macaya & Zuccarello 2010) or high genetic variability in New Zealand and its Sub-Antarctic Islands as in *Durvillaea antarctica* (Fraser *et al.* 2009a). Affinity of Chatham Islands and Bounty Island haplotypes to North Island haplotypes was evident in the later study, and in *Carpophyllum maschalocarpum* (Buchanan & Zuccarello 2010 submitted), the same haplotypes have been found on North Island and Chatham Islands. Even though the Chatham Islands are influenced from both the North and South Island (Wallis & Trewick 2009), they are considered an own

province, called Moriorian (Powell 1961). Powell (1961) also suggested Antipodean Province for the four Sub-Antarctic island groups, the Auckland Islands, Campbell Islands, Antipodes Islands, and Bounty Islands. The Snares, however, originally within the Antipodean Province, were later moved into the Forsterian Province covering the southern part of New Zealand from Otago Peninsula to Fiordland including Stewart Island (Fell 1953 in Powell 1961, Powell 1961, Knox 1975) and were finally dedicated an own province in the Marine Protected Areas Policy (MPA 2008).

Genetic structuring in the waters surrounding Tasmania has been found in the brown alga *Durvillaea potatorum* (Fraser *et al.* 2009b) showing two distinct lineages that might as well be reproductive isolated species corresponding to the Peronian (New South Wales) and Flindersian (west of Peronian) province, respectively.

The aim of this research is to correlate the distribution in *Lessonia* lineages to proposed bioregions and to study the distribution of *Lessonia* in Australasia. Here I investigate the distribution of all Australasian *Lessonia* with focus on the *L. variegata* species complex and discuss possible phylogeographic scenarios and speciation events. Therefore I compare the proposed timing of speciation events with the proposed timing of geological events as geological events might have triggered speciation. Knowledge of the distribution of *Lessonia* in Australasia might help to protect and conserve biodiversity, especially in the new discovered *Lessonia* species and their communities.

3.3 Material and Methods

3.3.1 Sampling

A total of 500 individuals of *Lessonia* were collected in the intertidal or subtidal while free diving at 57 sites in New Zealand and Tasmania, Australia (Table 3.1). Collection sites in New Zealand have been selected to span all phylogeographic regions as identified by Shears *et al.* (2008) (Fig. 3.1a). Tissue (2-3cm²) was collected near the transition zone at the base of the blade where the blade is youngest and free of epiphytes, patted dry and subsequently sealed in bags with silica gel.

3.3.2 DNA extraction, amplification, SSCP and sequencing

DNA extraction was performed with 0.5cm² of dried tissue using a modified CTAB protocol (Zuccarello & Lokhorst 2005). For fast and easy grinding I attached the microfuge pestle to a cordless drill.

PCR amplification and PCR conditions of the mitochondrial spacer region between adenosine-tri-phosphate dehydrogenase subunit 8 and t-RNA serine (atp8-Sp) and the chloroplast spacer between the large and short subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (rbc-sp) were performed as described in Chapter 2. The primers used have been reported in Voisin *et al.* (2005), Lane *et al.* (2006), and Chapter 2.

Different atp8-sp haplotypes among and within populations were separated by single strand conformation polymorphism (SSCP) (Zuccarello *et al.* 1999). The protocols for SSCP and silver staining are described in Buchanan & Zuccarello (2010 submitted) and Bassam *et al.* (1991), respectively. Known haplotypes were run as identifier on each gel. Band patterns that deviate from the patterns of the

known haplotypes were sequenced to recognize possible new atp8-sp haplotypes. Additionally, random atp8-sp samples were sequenced as a control for SSCP.

Sequencing was done commercially (Macrogen Inc. Seoul, South Korea) after the PCR products have been purified with ExoSAP-IT (USB, Cleveland, Ohio, USA). Atp8-sp sequences of 182 individuals were combined with SSCP patterns of 399 individuals for further calculations, and 81 individuals were processed with both methods to check SSCP reliability. Additionally 54 individuals were also sequenced with a marker for the rbc-sp region.

3.3.3 Data analysis

Forward and reverse sequences were assembled in 'STADEN package' (Staden *et al.* 2002) using the implementable programs PHRED (Ewing & Green 1998), PHRAP (Green unpublished [<http://bozeman.genome.washington.edu/phrap.docs/phrap.html>]), and GAP4 (Bonfield *et al.* 1995). The alignment was performed in BIOEDIT ver. 7.0.9.0 (Hall 1999) using CLUSTALW 1.4 (Thompson *et al.* 1994) and was subsequently refined by eye as suggested in Sinclair *et al.* (2005).

Median joining (MJ) networks for the whole datasets (atp8-sp and rbc-sp) and separately for each species (atp8-sp) were calculated with NETWORK ver. 4.5.1.6 (Bandelt *et al.* 1999). Full median networks (showing all possible reticulations) were calculated for the rbc-sp network and for the single species atp8-sp networks. A full median network is achieved when epsilon is greater or equal to the product of weighting and the maximum distance between the samples. However a full median network of the whole atp8-sp dataset would have been too memory extensive, thus a complex median network with epsilon=10 was calculated followed

by a maximum parsimony (MP) calculation (Polzin *et al.* 2003), to remove non-parsimonious links.

Isolation by distance was determined using mantel test function in ARLEQUIN 3.11 (Excoffier *et al.* 2005) to compare alternative dispersal routes around Tasmania for *L. corrugata*.

3.4 Results

Signs of saturation were not apparent in the atp8-sp region. All SSCP banding and sequencing results were concordant. In the case of *L. tholiformis*, I identified four haplotypes via sequencing, based on three variable sites; however the differences in SSCP banding were too small to allow unambiguous identification. Nevertheless there have been no difficulties separating these samples from other *Lessonia* species.

The slow-evolving cl-DNA rbc-sp yielded a dataset of 363bp. This marker showed almost only variation between species with eight variable sites and two deletions, and only one uninformative mutation within *L. variegata*/N. The rbc-sp haplotypes of *L. variegata*/S and *L. adamsiae* were identical thus the rbc-sp is not suitable to distinguish between all Australasian species. Also identical were the haplotypes of two specimens of *L. variegata*/N (B099 and B210) with the single rbc-sp haplotype of *L. variegata*/W. The remaining *L. variegata*/N specimens differ by a shared 32bp deletion from the *L. variegata*/W haplotype (Table 3.2).

The faster evolving mt-DNA atp8-sp yielded a dataset of 133bp. To increase resolution, I included 40bp of the adjacent atp8-gene and 17bp of the downstream trnS-gene into an extended dataset of 190bp. There was no (*L. adamsiae*, *L. brevifolia*) to little (*L. variegata*/S, and *L. variegata*/K; 1bp each) genetic variation in

the southern *Lessonia* species and highest variation in the northernmost species *L. variegata*/N (6bp). The other species showed moderate variation (Table 3.2).

A full median rbc-sp network for all *Lessonia* was achieved with epsilon = 70 showing a star shaped relationship (Fig. 3.2). A reduced median atp8-sp network of all *Lessonia* showed star like radiation in Australasia und was calculated with default settings (epsilon = 0) in NETWORK (Fig. 3.3).

A full median atp8-sp network (epsilon>30) of *L. variegata*/W haplotypes revealed that two close related haplotypes are found in distant populations within its distribution range and less related haplotypes were found in an intermediate zone (Fig. 3.4).

The full median atp8-sp network (epsilon>50) of *L. corrugata* haplotypes showed a straight line with absolutely no reticulations and the least related haplotypes found in close proximity at Ladies Bay (site 57) and Hobart (site 56; Fig. 3.5). According to the atp8-sp network of all Australasian *Lessonia* species (Fig. 3.3) a root in Fig. 3.5 was determined at mc1. Based on the network and the distribution of the *L. corrugata* haplotypes two dispersal hypotheses were tested. One simulating isolation by distance along a the east coast (H_{east}), the other one isolation by distance all around Tasmania assuming the area between Ladies Bay and Hobart to be a secondary contact zone (H_{west}). The mantel test results indicated a better support for the hypothesis H_{west} with $r^2_{\text{west}} = 0.93$, $p_{\text{west}} = 0.006$ and $r^2_{\text{east}} = 0.13$, $p_{\text{east}} = 0.307$.

All eight Australasian *Lessonia* species (as defined in Chapter 2) showed a non-overlapping distribution (i.e., endemism) (Fig. 3.6). *Lessonia variegata*/N is found in the “Raglan” and “Northeastern” bioregions (regions and provinces after

Shears *et al.* 2008, Fig. 3.1a) from Hokianga (site 1) around North Cape to Lottin Point/East Cape (site 16). A sharp genetic break was found at East Cape between Lottin Point and Horoera (site 16 and site 17) (~30 km apart) dividing the *L. variegata*/N from *L. variegata*/W. *Lessonia variegata*/W was found south of Horoera (site 17) all along the southeast coast of the North Island to Whites Bay (Marlborough Sounds / South Island; site 26), spanning Cook Strait. Southward *L. variegata*/K was solely found between Cape Campbell (site 27) and Paia Point (site 32; 17km south of Kaikoura). *Lessonia variegata*/S was found south from Tumbledown Bay (site 33; Banks Peninsula) to Doubtful Sound (site 39; Fiordland) including Stewart Island (site 38). *Lessonia brevifolia* is found on Auckland Islands (site 42), Campbell Islands (site 43), Antipodes Islands (site 44), and Bounty Islands (site 45), and *L. adamsiae* is endemic to The Snares (sites 40 and 41). *Lessonia tholiformis* and *L. corrugata* are endemic to the Chatham Islands (sites 46-50; Fig. 3.6) and to Tasmania (sites 52-57; Fig. 3.5), respectively.

3.5 Discussion

Recent research (Chapter 2) had split *L. variegata* into four species with yet unknown distribution limits. This survey evaluates the applicability of bioregions (Fig. 3.1) to the distribution of *Lessonia* species with emphasis to the *Lessonia variegata* species complex (Fig. 3.6) and tries to explain biogeographic distribution of all Australasian *Lessonia* species in a phylogeographic framework. For biogeographic interpretation, I used two markers with different mutation rates that allow insight into different time frames (Verbruggen & Theriot 2008) with a slow evolving marker (rbc-sp) for relationships between species (3.5-3 Mya) and a faster evolving marker (atp8-sp) for relationships within species (Chapter 2).

3.5.1 Distribution and Bioregions

Extensive sampling was undertaken to determine distribution limits and to assess correlation to proposed bioregions. The results show local endemism for all Australasian *Lessonia* (Fig. 3.5-3.6) including the four recently proposed cryptic species within the *Lessonia variegata* species complex (Chapter 2). Overall the genetic breaks or species boundaries found in *Lessonia* correspond well to the proposed biogeographic classifications after Shears *et al.* (2008) and MPA (2008).

L. variegata/N is found throughout the Northeastern bioregion but on the western boundary it was found as far south as Hokianga (site 1, Fig. 3.6). *Lessonia* populations have been reported as far south as Auckland region on the western coast of the North Island (Auckland Museum records). On the eastern side a sharp genetic break between *L. variegata*/N and *L. variegata*/W (sites 16 and 17, respectively) corresponds to the break between the Northeastern and Portland bioregion. This break has been identified in a multitude of biogeographic classification schemes (Moore 1949, Knox 1975, Walls 1995 and 2006, Nelson 1994, Francis 1996, Shears *et al.* 2008) and phylogeographic surveys (Apte & Gardner 2002, Stevens & Hogg 2004, Waters & Roy 2004, Ayers & Waters 2005), and is probably the most detected biogeographic break in the marine realm around New Zealand. The break is generally attributed to the permanent and anticyclonic East Cape Eddy that captures most of the south east flowing East Auckland current (Stanton *et al.* 1997) and is thus believed to be a significant barrier to gene flow (e.g., Stevens & Hogg 2004). Despite the retention effect of the East Cape Eddy, temperature differences between the warm subtropical East Auckland Current and the cool northward flowing

Wairarapa Current, which meet at East Cape (Chiswell 2000), might also maintain species boundaries.

L. variegata/W is found throughout Portland and the northern half of the Cook bioregion. The break between Portland and Cook bioregion was detected as a transition zone between two different *L. variegata*/W haplotypes (Fig. 3.4). In discrepancy to Shears *et al.* (2008) but in concordance to MPA (2008) I found a genetic break at Cape Campbell between *L. variegata*/W and *L. variegata*/K. In the Cook Strait area, only a combination of the two classification schemes seems to embrace the distribution of *Lessonia* lineages. Whereas Shears *et al.* (2008) divided the Cook Strait into a western and eastern region (Abel and Cook), MPA (2008) used a north to south division (North and South Cook Strait) (Fig. 3.1). However, with the addition of a break at Cape Campbell my data is better represented with a west to east division as no *Lessonia* have been found in Abel bioregion and *L. variegata*/W is found on both sides of Cook Strait. A break near Cape Campbell was previously found in a biogeographic survey of 375 reef fish (Francis 1996) as well as in several phylogeographic surveys (Apte & Gardner 2002; *Perna canaliculus*, Schnabel *et al.* 2000 and Stevens & Hogg 2004; southern limit of *Paracorophium lucasi*, Waters & Roy 2004, and Ayers & Waters 2005; *Patiriella regularis*, Goldstien *et al.* 2006; *Cellana ornata*). The actual location of the break, however, was unknown or differed between the taxa surveyed and so the explanations differed. Whereas some suggested upwelling as a possible differentiating force (e.g., Apte & Gardner 2002, Waters & Roy 2004, and Ayers & Waters 2005), or favoured currents and eddies (e.g., Stevens & Hogg 2004) others suggested that changes in the dynamic hydrography of Cook Strait triggered differentiation while dismissing upwelling as a possible reason

(Goldstien *et al.* 2006). A recalibration showed that the postglacial upwelling south of Cape Campbell originated long after the genetic differentiation found in *Perna canaliculus* and *Patiriella regularis* (Goldstien *et al.* 2006). Upwelling can not be stressed as a factor separating the two *Lessonia* lineages either, as *L. variegata*/K is found across the area of upwelling till Cape Campbell. Whether currents and eddies are a likely barrier is not known, but whatever the reason the break between *L. variegata*/W and *L. variegata*/K is supported by a natural border, which is the marshlands of two successive river deltas that separates the different lineages (along a distance of more than 40km).

L. variegata/K is solely found in the southern part of the Cook bioregion from Cape Campbell (site 27, Fig. 3.6) till Goose Bay (site 32). Whereas upwelling cannot explain the break between *L. variegata*/W and *L. variegata*/K it might be the reason for a unique haplotype within the *L. variegata*/K lineage that is only found at Cape Campbell. The southern limit of *L. variegata*/K corresponds to the break between Cook and Banks bioregion. Again, the distribution of *L. variegata*/K fits best to a combination of the biogeographic classification scheme after Shears *et al.* (2008) and MPA (2008), with a better fit to Shears *et al.* (2008) in the south and to MPA (2008) in the north.

L. variegata/S is distributed from Chalmers over Stewart Island to Fiordland bioregions. No *Lessonia* was found along the sandy areas that characterise the majority of the Banks bioregion but for a population on Banks peninsula (site 33). As this population is more than 200km north of its nearest neighbour, it is possibly better described as a satellite population than as the northern boundary of the species distribution (Fig. 3.6).

No *Lessonia* was found in this study on the west side of New Zealand (e.g., Westland, Buller, Able, and Raglan) despite records of scattered populations [Westland: Open Bay Island (Neale & Nelson 1998) (site R1, Fig. 3.1); Raglan: Maori Bay and O'Neill Bay (Auckland Museum records) (site R2)].

The other four species are endemic and confined to bioregions that are composed of one or more islands, i.e., *L. adamsiae* is endemic to The Snares, which is given its own bioregion after MPA (2008) but was before assigned to the Forsterian Province; *L. brevifolia* is restricted to the Sub-Antarctic Islands bioregion (Antipodes, Auckland Islands, Bounty Islands, and Campbell Islands); *L. tholiformis* is only found on the Chatham Islands; and *L. corrugata* is endemic to Tasmania.

The phylogeographic breaks in *Lessonia* generally correspond well to the proposed biogeographic schemes of both Shears *et al.* (2008) and the MPA (2008). In the Cook Strait area only a combination of the two classification schemes is supported by the distribution boundaries of *Lessonia*. The east to west division of the Cook Strait as proposed in Shears *et al.* (2008) corresponds better to the distribution of *Lessonia* than a north to south split as proposed in the MPA (2008). A break at Cape Campbell, on the other side, was not recognised by Shears *et al.* (2008) but was by the MPA (2008). In the MPA (2008) no biogeographic break was made to separate the sandy areas of Canterbury Bight and Pegasus Bay from the rocky coastline north of Pegasus Bay. This break was proposed by Shears *et al.* (2008) and also evident in *Lessonia*. An east to west division of the Cook Strait as in Shears *et al.* (2008), a break at Cape Campbell as in the MPA (2008), and a split north of Pegasus Bay as in Shears *et al.* (2008) would possibly be a beneficial combination of the two proposed classification schemes as it appears in the case of *Lessonia*.

3.5.2 Phylogeography

3.5.2.1 Ancient component (*relationships between species and speciation*)

My results reveal high phylogeographic structure and local endemism in *Lessonia*. All *Lessonia* species in Australasia, including the four species within the *L. variegata* species complex, are confined to a certain area with no overlapping in their distribution (Fig. 3.5-3.6). While there is high phylogeographic and phylogenetic structure there is little phylogenetic resolution in *Lessonia*. This is often coupled with star radiation (Fig. 3.2 and 3.3) and was found to be a common pattern in phylogeographic studies of terrestrial plants and animals in New Zealand (Wallis & Trewick 2009 and references herein). In the terrestrial realm, the tectonic uplift in the Pliocene and the resulting emergence of the Southern Alps was reasoned to have created conditions for rapid radiation (Wallis & Trewick 2009). In Chapter 2, I suggested that rapid radiation in the Australasian *Lessonia* that took place 3.5-3 Mya and the outline of Pliocene New Zealand might have offered suitable conditions (Fig. 3.7). The time of sudden radiation is often indicative of dramatic change in the history of a species e.g., the time of colonisation into a new area via long distance dispersal (Carranza *et al.* 2000, Waters & Roy 2004b, Thiel & Haye 2006). In the case of *Lessonia*, this might be as well the time it arrived in Australasia.

Standard bifurcation tree approaches are not able to deal with real star like evolutionary relationships, nor can trees account for internal haplotypes; here networks are able to give a more realistic representation (e.g., Sinclair *et al.* 2005). On the other side networks are normally not rooted which leads to lacking information about the direction of evolution. Here we could use the close relationship of the Australasian ingroup to the South American *L. vadosa* (Chapter 2)

to infer the root of the *atp8*-sp and *rbc*-sp networks. The networks (Fig. 3.2 and 3.3) revealed star like phylogeny in Australasia, which supports the assumption of rapid radiation (Hundertmark *et al.* 2003) in *Lessonia*. The *rbc*-network (Fig. 3.2) suggests an ancient relationship of *L. variegata*/N to an ancestral *L. variegata*/W haplotype, due to the low mutation rate of the *rbc*-marker (Chapter 2). Geographic isolation might have led to divergent evolution in *L. variegata*/N and *L. variegata*/W. The *rbc*-sp network suggests that *L. variegata*/W is ancestral to *L. variegata*/N. At the time of radiation in *Lessonia* (mid to late Pliocene), the central and southern part of the North Island was mainly submerged and a big ridge emerged through uplift possibly spanning from Cape Palliser to Cape Kidnappers (Fig. 3.7E, Lewis *et al.* 1994, Bunce *et al.* 2009). Whereas the west coast of this ridge has undergone dramatic changes, the east coast has not changed much since its emergence, resembling today the biggest part of the distribution area of *L. variegata*/W. *L. variegata*/W possibly has its origin on this ridge before it became later connected to northern North Island by bridging the Manawatu Strait through further tectonic uplift 1 Mya (Bunce *et al.* 2009; fig. S3). Today's distribution of *L. variegata*/W spans from East Cape (site 17, Fig. 3.6) to Whites Bay (site 26, southeast Marlborough Sounds). The East Cape Eddy would have maintained separation between the two North Island species even after the closure of the Manawatu Strait if it was not acting as a barrier already.

Apart from *L. variegata*/W on the east coast of the Marlborough Sounds, two additional *Lessonia* species are found on the South Island. One, *L. variegata*/K is endemic to the northeast from Cape Campbell to Paia Point. As pointed out above, peripatric speciation of *L. variegata*/W on an early Pleistocene island is likely thus

vicariance (e.g., formation of Cook Strait) has not created diversification between a possible common ancestor of *L. variegata*/W and /K, especially as the timing of radiation predates the timing of Cook Strait formation. Later during glacial periods of the Pleistocene, Cook Strait was closed and land emerged due to decreasing sea levels (e.g., Fleming 1979). Thus, without invoking passive rafting as a means of dispersal, the bridging of Cook Strait from north to south by *L. variegata*/W was possible during glacial periods given suitable habitat have had emerged with decreasing sea levels.

On its southern limit *L. variegata*/K is separated from *L. variegata*/S by a >350km gap of quaternary sediment. This gap formed by glacial outwash and aggradations with the retreat of the first ice age of the Pleistocene and has persisted during glacial and interglacial period ever since (Fig. 3.6-3.7B, Fleming 1979). Sandy beaches and soft sandstones are unsuitable habitat for *Lessonia* (personal observation) and no *Lessonia* was found in Pegasus Bay and Canterbury Bight. Based on a survey of *L. nigrescens*, Faugeron *et al.* (2005) suggested that the normal dispersal potential in *Lessonia* is low, with recruitments normally found in the vicinity of the parent. Thus the gap was probably big enough to prevent connectivity and enabled divergent evolution between the two South Island species.

Lessonia adamsiae shows relatedness to either *L. variegata*/S (rbc-sp, Fig. 3.2) or *L. corrugata* (atp8-sp, Fig. 3.3). However, the atp8-sp is a fast evolving marker whereas rbc-sp is slower evolving (Chapter 2) the latter thus revealing the older phylogenetic history. The use of both fast and slow evolving markers might help to pinpoint a more accurate understanding of historical events in the evolution of a species. A hypothesis derived from the combination of the two markers is that

the radiation of *Lessonia* in paleo-Australasia (starting ~3.5 Mya) also included colonisation of Tasmania, initiating peripatric speciation of *L. corrugata*. The finding that the slow evolving marker shows no difference between *L. adamsiae* and *L. variegata*/S, whereas the faster evolving marker shows a closer relationship between *L. adamsiae* and *L. corrugata* is interpreted here as a speciation event through hybridisation. In this scenario hybridisation took place between an ancestor of *L. variegata*/S on the one hand and *L. corrugata* specimen dispersing from Tasmania to New Zealand on the other hand. The hybridisation event would have been ancient (~2 Mya) with *L. adamsiae* on The Snares as the descendent.

The data show endemic status for all Australasian *Lessonia* species to their respective islands or regions. No overlapping distribution ranges have been found in Australasia, which is in strong contrast to the situation in South America and Falkland Islands (Searles 1978, Edding *et al.* 1994).

3.5.2.2 Recent component (phylogeographic structure within species)

The faster evolving atp8-sp was used on three examples (*L. corrugata*, *L. variegata*/W and *L. brevifolia*) to gain information on the phylogeographic structure within species.

Genetic divergence was also found within *L. corrugata*. A geographic mapping of the atp8-sp network, whose root position was determined using *L. adamsiae* as outgroup, revealed that the least related haplotypes are found in close proximity at Ladies Bay and Hobart (Fig. 3.5). A scenario previously described for the western clade of *Durvillaea potatorum* (Fraser *et al.* 2009b) can be used to explain this contradiction. Following Fraser *et al.* (2009b), a putative glacial refugium might have existed northwest off Tasmania where cold Sub-Antarctic

water was pushed further north by a deflected subtropical convergence translocating the northern range of the cold water adapted *L. corrugata*. Assuming that this refugium was the origin for all recent *L. corrugata* new postglacial coastal pathways and redirected currents might have allowed for two different dispersal routes around Tasmania: one going east through the reopened Bass Strait and then south following the East Australian current; the other along the west coast following the Zeehan current. The area between Ladies Bay and Hobart, where the least related haplotypes have been found in close proximity, is supposedly a secondary contact zone. To compare this hypothesis (H_{west}) with a correlation based only on the geographic distance between the populations on the east coast (e.g., without a connection between Ladies Bay and Skeleton Bay through Bass Strait) (H_{east}), I performed two different Mantel test analyses. The better fit of the correlation coefficient for H_{west} ($r^2_{\text{west}} = 0.93$, $p_{\text{west}} = 0.006$; $r^2_{\text{east}} = 0.13$, $p_{\text{east}} = 0.307$) supports the idea of a single north-western refugium (Fig. S 3.1). However hypothesis H_{west} has to be confirmed with a larger sample size. Ideally the predicted haplotypes mc1 and/or mc2 (Fig. 3.5) should be found in populations on the west side of Tasmania to verify this scenario.

While plotting *L. variegata*/W atp8-sp haplotypes on a map (Fig. 3.4), I found distant populations at East Cape and Whites Bay with closely related haplotypes, whereas in geographically intermediate populations I found haplotypes that are not directly related. This pattern can be explained with secondary contact of formerly separated populations. Since the middle Pleistocene (1 Mya), volcanic outfall regularly affected the west coast of the North Island (e.g., Froggatt *et al.* 1986, Newnham *et al.* 1999, Coleman *et al.* 2001, Manville & Wilson 2004).

Ignimbrite and/or tephra of eruptions in the Taupo volcanic zone might have created situations of reoccurring isolation for *Lessonia* populations at East Cape. Based on the data, I assume that isolation has caused the divergent evolution within *L. variegata*/W. Southward dispersal of derived East Cape-haplotypes driven by the East Cape Current and northward dispersal of Wellington-haplotypes driven by the Wairarapa Coastal Current has led to a contact zone at the border of two Biogeographic Provinces, Portland and Cook (Fig. 3.4).

Lessonia brevifolia most likely originated during radiation in Australasia as a result of peripatric speciation on one of New Zealand's Sub-Antarctic Islands; however the finding of no genetic variation between the distant islands (>700km between Campbell and Antipodes, which is the maximum of the shortest distances between islands) indicates a single glacial refugium. Model simulations (Chiswell 2009) would suggest the Auckland Islands as the most likely source for post glacial colonisation of Campbell Islands, Antipodes Islands, and Bounty Island.

3.6 Figures and Tables

Fig. 3.1 Biogeographic regions and *Lessonia* sample sites (red dots). Yellow dots denote sites where no *Lessonia* have been found. R1-3 indicate sites where *Lessonia* is reported in the literature or from museum records. a) Biogeographic regions of the three main islands of New Zealand after Shears *et al.* (2008) based on presence and absence of macroalgae. b) Biogeographic regions implemented in the Marine Protected Areas Policy (MPA 2008) including The Snares, the Sub-Antarctic Islands, and the Chatham Islands. Note the differences at Cook Strait area; whereas Shears *et al.* (2008) divided this area into a western and eastern region (Able and Cook) MPA (2008) divided this area into a northern and southern region (North and South Cook Strait). A break at Cape Campbell (at site 27) is not assigned by Shears *et al.* (2008).

Fig. 3.2 Full median network ($e=70$) of the *rbc-sp* region showing star like evolution and ancient relationships among Australasian *Lessonia*. The network is rooted on South American species (*L. nigrescens* (Ln), *L. trabeculata* (Ltr), and *L. vadosa* (Lvd)). All samples from *L. variegata*/S (Lv/S) and *L. adamsiae* (La) share the same haplotype as do *L. variegata*/W (Lv/W) and two samples of *L. variegata*/N (Lv/N) (see Table 3.1). Lb *L. brevifolia*, Lc *L. corrugata*, Lv/K *L. variegata*/K, and Lt *L. tholiformis*.

Fig. 3.3 Reduced median network of the *atp8*-sp region showing star like evolution within Australasian *Lessonia*. *L. adamsiae* (La) has an *atp8*-sp haplotype that is ancestral to *L. corrugata* (Lc) haplotypes. For the distribution of the haplotypes within Tasmania and New Zealand see Fig. 3.5 and 3.6 respectively. Lb *L. brevifolia*, Lt *L. tholiformis*, Lv/K *L. variegata*/K, Lv/N *L. variegata*/N, Lv/S *L. variegata*/S, and. Lv/W *L. variegata*/W.

Fig. 3.4 Haplotype distribution and *atp8*-sp network of *L. variegata*/W showing the relationship between haplotypes. Tripod indicates root position. Two closely related haplotypes (Lv/W and Lv/W2) are found geographically far apart at either the East Cape or throughout Cook biogeographic region. Recent southward dispersal of Lv/W3 and Lv/W4 haplotypes following the East Cape Current (ECC) is suggested. Lv/W haplotypes were found at sites 20 to 26 with increasing abundance in the Cook Strait area. Lv/W1 was only found at site 24. The East Cape Eddy (ECE) is thought to act as barrier for gene flow between Portland and Northeastern bioregion. The grey area in the centre of the North Island illustrates the cover of ignimbrite after the Kidnapper eruption 1 Mya (after Wilson *et al.* 1995). Site numbers correspond to the sites in Table 3.1.

Fig. 3.5 Sample sites, haplotype distribution map and atp8-sp network of *L. corrugata*. Tripod indicates root position (this network is part of an Australasian network showing only the *L. corrugata* haplotypes, thus it is rooted). Two unrelated haplotypes are found in close proximity at Ladies Bay and Hobart. Two dispersal hypotheses have been tested, H_{west} (blue) and H_{east} (red). The two tested matrices differ in the geographic distance (colour coded) based on the dispersal hypotheses. A mantel test correlation coefficient supports H_{west} (with $r^2_{west} = 0.93$, $p_{west} = 0.006$; $r^2_{east} = 0.13$, $p_{east}=0.307$). The haplotype distribution fits an explanation where out of a north western refugia *L. corrugata* dispersed at either side of Tasmania following the Zeehan Current or the Bass Strait Waters and East Australian current respectively (Bass Strait Current after Gibbs 1992).

Fig. 3.6 Atp8-sp haplotype distribution map. Circle size proportional to sample size (see size standard on the left) numbers in circles are sample site ID's. Purple line between site 27 (Cape Campbell) and 28 (Kekerengu) denotes area of upwelling. Species ranges are indicated by dotted lines.

Fig. 3.7 New Zealand at the Late Pliocene. Coloured lines show the current distribution of *Lessonia variegata* lineages. Green *L. variegata*/N, yellow/orange *L. variegata*/W, red *L. variegata*/K, and blue *L. variegata*/S. A) Plate movement ($\sim 30\text{km/My}$) leading to the Kaikoura Orogeny (King 2000) and

the opening of Cook Strait (Lewis *et al.* 1994, Bruce 2009). B) With the retreat of the first ice age of the Pleistocene, the Canterbury Plains have been formed through glacial outwash and aggradations. They persisted during any subsequent glacial and interglacial period (Fleming 1979). C) The lower part of the North Island was submerged during the Pleistocene (e.g., Fleming 1979; fig. 19). D) Northland was a chain of unconnected islands (e.g., Fleming 1979; fig. 19). E) The south eastern part of the North Island was a long stretched island, neither connected to the rest of the North Island nor to the South Island (Lewis *et al.* 1994, Bruce 2009). F) Banks peninsula was a volcanic island (e.g., Fleming 1979, Stevens *et al.* 1995). G) In the south, Stewart Island was connected with the South Island and The Snares were either connected or very close to the mainland (e.g., Stevens 1980; fig. 16.36). Not shown) The Sub-Antarctic Islands were all present; three were of volcanic origin and the youngest was active till 1 Mya (Peat 2003). The rocks of the Chatham Islands can be dated to 130 Mya, however, they have been submerged during the Pliocene till 2-3 Mya (Holt 2008).

Table 3.1 Sample sites with coordinates (Lat. And Long.) and number (N) of atp8-sp and rbc-sp haplotypes (H), respectively. X in LtX indicates that the concrete *L. tholiformis* haplotype is not known, due to insufficient resolution with SSCP.

			atp8-sp		rbc-sp	
Site		Lat. Long.	N	H	N	H
<i>L. variegata</i>/N (184)			184		13	
1	Outer South Head - Hokianga Harbour	35°32'16.28"S 173°21'55.67"E	29	29 Lv/N	1	1 Lv/W
2	Shipwreck Bay	35°10'20.57"S 173° 5'51.91"E	2	2 Lv/N		
3	Tapotupotu Bay	34°26'03.90"S 172°42'58.67"E	10	10 Lv/N		
4	Hooper Point	34°25'09.17"S 172°51'26.96"E	10	9 Lv/N, 1 Lv/N4	1	1 Lv/W
5	North Cape	~34°24'8.36"S ~173° 2'5.64"E	5	4 Lv/N, 1 Lv/N1	3	3 Lv/N
6	Maitai Bay	34°49'36.54"S 173°24'47.88"E	11	7 Lv/N, 4 Lv/NM	1	1 Lv/N
7	Stephenson Island	~34°57'46"S ~173°46'44"E	5	5 Lv/N	1	1 Lv/N
8	Cape Wiwiki	35°09'22.56"S 174°07'19.62"E	10	10 Lv/N		
9	Skull Bay - Poor Knights Islands (Tawhiti Rahi Is.)	35°28'7.06"S 174°44'7.69"E	9	9 Lv/N	1	1 Lv/N
9	Dutch - Poor Knights Islands (Aorangi Is.)	35°28'20.76"S 174°44'5.04"E	22	22 Lv/N		
9	Frasers Landing - Poor Knights Islands (Aorangi Is.)	35°29'0.54"S 174°44'41.16"E	8	8 Lv/N		
9	Magic Wall - Poor Knights Islands (Aorangi Is.)	35°29'18.54"S 174°44'13.62"E	11	11 Lv/N		
10	Dolphin Bay - Tutukaka	35°37'33.84"S 174°32'31.25"E	11	10 Lv/N, 1 Lv/N3	1	1 Lv/N
11	Coromandel - Wekarua Island	~36.8429S ~175.4173E	6	6 Lv/N		

12	Coromandel - Fletcher Bay	~36°28'27.28"S ~175°23'32.61"E	1	0 Lv/N, 1 Lv/N2	1	1 Lv/N2
13	Coromandel - Waikawau Bay	~36°36'0.64"S ~175°32'25.56"E	4	4 Lv/N		
14	Coromandel - The Sailors Grave	36°57'39.33"S 175°50'41.36"E	11	0 Lv/N, 4 Lv/N2, 6 Lv/N5, 1 Lv/NS	3	3 Lv/N
15	EastCape - Maraehako Bay	~37°43'29.99"S ~177°41'41.97"E	10	10 Lv/N		
16	EastCape - Lottin Point Road	~37°31'58.88"S ~178°10'20.61"E	9	9 Lv/N		
<i>L. variegata</i>/W (85)			85		14	
17	EastCape - Horoera	~37°38'16.10"S ~178°28'1.31"E	4	0 Lv/W, 2 Lv/W2, 2 Lv/W3	1	1 Lv/W
18	EastCape - Hautai Beach	37°38'23.02"S 178°28'40.22"E	4	0 Lv/W, 4 Lv/W2	2	2 Lv/W
19	Waimarama	39°47'5.50"S 176°59'59.30"E	3	0 Lv/W, 3 Lv/W3	1	1 Lv/W
20	Mataikona - Pop1	40°47'43.27"S 176°15'56.46"E	10	1 Lv/W, 9 Lv/W4		
20	Mataikona - Pop2	40°47'38.47"S 176°15'58.26"E	2	0 Lv/W, 2 Lv/W4		
21	Riversdale south	41° 6'29.73"S 176° 4'10.55"E	9	2 Lv/W, 7 Lv/W4	2	2 Lv/W
21	Riversdale Beach		3	1 Lv/W, 2 Lv/W4		
22	Cape Palliser	41°36'48.47"S 175°17'32.66"E	3	3 Lv/W	2	2 Lv/W
23	Breakers Bay - Wgtn	41°20'8.17"S 174°49'32.54"E	2	2 Lv/W		
23	Moa Point - Wgtn	41°20'32.00"S 174°48'33.00"E	8	8 Lv/W		
23	Princess Bay - Wgtn	41°20'42.36"S 174°47'15.89"E	3	3 Lv/W	3	3 Lv/W
23	Island Bay - Wgtn	41°20'57.39"S 174°45'49.06"E	2	2 Lv/W		

24	Makara Beach - Wgtn	41°12'58.11"S 174°42'9.89"E	5	4 Lv/W, 1 Lv/W1	1	1 Lv/W
25	Fighting Bay Pop1	41°18'43.44"S 174°12'25.26"E	4	4 Lv/W		
25	Fighting Bay Pop2	41°18.70796S 174°12.39924E	3	3 Lv/W		
25	Fighting Bay Pop3	41°18'46.62"S 174°11'59.52"E	3	3 Lv/W		
26	Whites Bay	41°23'17.96"S 174° 3'23.28"E	17	17 Lv/W	2	2 Lv/W
<i>L. variegata</i>/K (55)			55		5	
27	Cape Campbell	41°43'46.18"S 174°16'19.08"E	7	0 Lv/K, 7 Lv/K1	1	1 Lv/K
28	Kekerengu	42° 0'15.57"S 174° 0'43.77"E	4	4 Lv/K		
29	Waipapa	42°12'39.35"S 173°52'20.22"E	10	10 Lv/K		
30	New Wharf - Kaikoura	42°24'47.17"S 173°42'8.22"E	2	2 Lv/K	2	2 Lv/K
30	Seal Colony - Kaikoura	42°25'30.07"S 173°43'1.30"E	16	16 Lv/K	1	1 Lv/K
30	South Bay - Kaikoura	42°25'33.45"S 173°41'6.25"E	9	9 Lv/K		
31	3km north of Goose Bay	42°27'32.52"S 173°33'6.88"E	4	4 Lv/K		
32	Paia Point	42°29'22.43"S 173°31'27.05"E	3	3 Lv/K	1	1 Lv/K
<i>L. variegata</i>/S (73)			73		6	
33	Banks Pen. - Tumbledown Bay	43°49'46.26"S 172°43'39.78"E	1	1 Lv/S	1	1 Lv/S
34	Mapoutahi - Otago	45°44'06.58"S 170°37'02.82"E	12	12 Lv/S		
34	Tairoa Head - Otago	~45°46'59.95"S ~170°44'0.69"E	5	5 Lv/S		
35	Roaring Bay - Nugget Point	46°26'53.53"S 169°47'55.93"E	4	3 Lv/S, 1 Lv/S1	1	1 Lv/S
36	CurioBay	46°39'38.85"S	8	8 Lv/S	2	2 Lv/S

		169° 6'29.45"E				
37	Bluff	46°36'49.49"S 168°21'27.24"E	10	10 Lv/S		
38	Ackers Pt. Lighthouse - Stewart Is.	46°53'46.94"S 168° 9'49.82"E	10	10 Lv/S		
39	Causet Cove - Doubtful Sound	45°17'47.63"S 166°54'33.01"E	22	22 Lv/S	1	1 Lv/S
39	Shelter Islands - Doubtful Sound	45°16'13.54"S 166°53'29.26"E	1	1 Lv/S	1	1 Lv/S
<i>L. adamsiae</i> (18)			18		4	
40	South Promontory	~48°0'54.38"S ~166°34'40.55"E	8	8 La	1	1 Lv/S
41	Tahi	~48°03'42.42"S ~166°30'59.99"E	10	10 La	3	1 Lv/S
<i>L. brevifolia</i> (24)			24		4	
42	Monumental Island - Auckland Is.	~50°50'2.15"S ~165°55'7.13"E	1	1 Lb		
43	Smoothwater Bay - Campbell Is.	~52°32'38.87"S ~169°15'9.09"E	1	1 Lb	1	1 Lb
43	Perseverance Harbour - Campbell Is.		11	11 Lb	1	1 Lb
44	Antipodes Is.	~49°41'12.94"S ~178°46'13.05"E	4	4 Lb	1	1 Lb
45	Bounty Is.		7	7 Lb	1	1 Lb
<i>L. tholiformis</i> (43)			43		5	
46	Kaingaroa Harbour	43°43'46.10"S 176°16'11.15"W	5	5 LtX		
47	Wharekauri	~43°42'15.18"S ~176°34'54.12"W	10	3 LtX, 7 Lt1	1	1 Lt
48	Point Dorset (1) - Port Hutt	43°48'57.40"S 176°42'13.94"W	3	2 LtX, 1 Lt1	1	1 Lt
48	Point Dorset (2) - Port Hutt	43°49'54.73"S 176°45'23.05"W	1	1 LtX		
49	Hanson Point - Waitangi	43°56'43.08"S 176°33'41.20"W	5	4 LtX, 1 Lt1		
50	Wharf reef - Owenga	44°1'25.21"S	7	2 LtX, 4 Lt1,	2	2 Lt

		176°22'7.87"W		1 Lt2		
50	Te One Creek - Owenga	44°1'12.53"S 176°22'51.60"W	6	2 LtX, 3 Lt1, 1 Lt3		
50	Manukau Point	44°1'58.86"S 176°19'47.52"W	5	4 LtX, 1 Lt4		
51	<i>C. Lane</i>		1	0 LtX, 1 Lt1	1	<i>1 Lt</i>
<i>L. corrugata</i> (13)			13		2	
52	Skeleton Pt. - Binalong Bay	41°14'54.93"S 148°19'46.11"E	2	0 Lc1, 2 Lc3	1	<i>1 Lc2</i>
53	Four Mile Creek	41°32'25.74"S 148°17'9.53"E	1	0 Lc1, 1 Lc2		
54	Bicheno	41°52'29.77"S 148°18'40.88"E	2	0 Lc1, 2 Lc2	1	<i>1 Lc</i>
55	Remarkable Cave	43°11'19.48"S 147°50'40.58"E	2	2 Lc1		
56	Kingston Beach - Hobart	~42°59'4.12"S ~147°19'34.20"E	1	1 Lc1		
56	Blackman's Beach - Hobart	43°0'28.02"S 147°19'45.57"E	1	1 Lc1		
57	Ladies Bay - Southport	43°26'27.84"S 146°59'18.64"E	4	0 Lc1, 4 Lc4		

Table 3.2 Dataset characteristics; showing the number of individuals (N), the length of the marker region and number of variable sites; A) atp8-sp and B) rbc-sp. ¹ variable sites of the last 40bp of the atp8-gene; ² variable sites of the first 17bp of the trnS-gene.

A) atp8-sp	All	<i>L. var</i> /N	<i>L. var</i> /W	<i>L. var</i> /K	<i>L. var</i> /S	<i>L. ada</i>	<i>L. bre</i>	<i>L. cor</i>	<i>L. tho</i>
N	493	184	85	55	73	18	24	11	43
length	133bp	113bp	113bp	114bp	113bp	103bp	113bp	103bp	119bp
variable sites	27	2	4	1	1	-	-	3	3
atp8-gene ¹	5	3	-	-	-	-	-	2	-
trnS-gene ²	1	1	-	-	-	-	-	-	-
indel	13bp; 1bp; 6bp; 18bp; 16bp	12bp del (Lv/NM), 13bp ins (Lv/NS)	-	-	-	-	-	-	-
B) rbc-sp									
N	54	13	14	5	6	4	4	3	5
length	363bp	363bp	363bp	363bp	363bp	363bp	363bp	357bp	363bp
variable sites	8	1	-	-	-	-	-	-	-
indel	32bp; 6bp	32bp	-	-	-	-	-	-	-

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Chapter 4

Characterisation of microsatellites in the Southern Hemisphere kelp *Lessonia variegata* (Phaeophyceae, Laminariales)

4.1 Abstract

Lessonia variegata has recently been split into four species with a localized, non-overlapping distribution. One lineage, *L. variegata* Wellington lineage, is found on both the North Island and South Island of New Zealand. In order to investigate connectivity and population differentiation I developed ten microsatellite loci, which were tested on 150 samples from three populations. Seven markers were polymorphic, displaying 2-24 alleles per locus; one polymorphic marker was however, difficult to score and requires further optimisation. All eight tested markers were able to amplify across the four members of the *L. variegata* species complex, and differences in length were noted.

4.2 Introduction

Large brown algae of the order Laminariales (kelp) play an important role in cold temperate regions (Billot *et al.* 1998) where they form unique habitats and ecological niches for a variety of organisms (Edding *et al.* 1994, Schwarz *et al.* 2006). *Lessonia variegata* J. Agardh, which is found at exposed rocky coasts on the three main islands of New Zealand, has recently been split into four cryptic species (Chapter 2). The Wellington lineage (*L. variegata*/W) is distributed from the most eastern point of the North Island of New Zealand southward along the east coast, across Cook Strait, to the northern tip of the South Island (Chapter 3). To further investigate population structure and connectivity between populations, highly variable molecular markers are needed. In general, microsatellites are several orders of magnitude faster evolving than other DNA sequences (Gilbert *et al.* 1990). Therefore microsatellites are a powerful tool for detecting recent evolutionary changes within and between populations. Few microsatellites have been developed in Laminariales (Billot *et al.* 1998, Whitmer 2002, Kusumo *et al.* 2004, Daguin *et al.* 2005, Li *et al.* 2008, Faugeron *et al.* 2009, Collens 2009, Dolman & Coleman 2009, Liu *et al.* 2010). These have been used in parental analyses (Asensi *et al.* 2001, Shan & Pang 2009) but mostly for population structure and spatial analysis where huge differences in population structure and connectivity became obvious between non-floating (Billot *et al.* 2003, Kusumo *et al.* 2006, Coleman *et al.* 2009) and floating Laminariales (Macaya 2010 (Thesis) pp. 90-124). However, as microsatellites normally occur in noncoding regions where mutation rates are higher, they almost always have to be species specific (Wan *et al.* 2004, Martinez *et al.* 2005). Here I

describe the isolation and characterisation of species specific microsatellites for *L. variegata*/W. Cross-amplification to other *L. variegata* lineages was also tested.

4.3 Material and Methods

A microsatellite library for *L. variegata* was prepared following a modified protocol after Gardner *et al.* (1999). Briefly, after DNA extraction (following a modified CTAB protocol in Zuccarello & Lokhorst 2005), approximately 30µg of DNA was digested with *Sau3A* restriction enzyme (New England BioLabs, Ipswich, MA, USA). The *Sau3A* cut DNA was ligated to previously phosphorylated adapters. Adapter ligated DNA was precipitated and size fractionated in 1% agarose gel to select fragments between 200 and 1000bp in length. Bands were purified from the agarose gel using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The DNA was hybridised in a reaction containing 200pmoles of each of three 5'biotin-labelled oligonucleotides [(AT)₁₂, (CA)₁₂, and (AAAT)₆]. In order to limit the formation of concatemers, terminal priming sites were blocked by the addition of S61 primers (Gardner *et al.* 1999) to the reaction mix. Enrichment was performed while selectively capturing hybridised microsatellite repeats that bound to streptavidin magnetic beads (Promega, Madison, WI, USA). Enriched DNA was purified using High Pure PCR Product Purification Kit (Roche Applied Science, Mannheim, Germany). The purified DNA was PCR amplified (using S61 primers), cleaned with Ultraclean PCR clean-up (MoBio Laboratories, Inc., Carlsbad, CA), and ligated into pGEM-T Easy Vectors (Promega) for transformation into high-efficiency competent cells (Promega). Recombinant clones were detected using blue/white screening of colonies (Sambrook *et al.* 1989). The first 248 white colonies were selected for PCR amplification using non-biotin-labelled CA₁₂, AT₁₂, AAAT₆ and the forward and

reverse M13 primers in one reaction. Colonies tested positive for microsatellites (96 of the 248 tested colonies showed two or more bands with at least one band >236bp) were again PCR amplified with only M13 primers for sequencing. Sequences showing microsatellite motifs and sufficient flanking regions were selected and primer pairs were designed with PRIMER3 (Rozen & Skaletsky 2000).

Primers were constructed for 10 loci and dye-labelled (Table 4.1). Loci were tested for amplification in individuals from all four *L. variegata* lineages and variability was tested for *L. variegata*/W using 10 to 50 samples per locus from each of three populations that were sampled at the south-eastern part of the species range (Table 4.2).

PCR amplification was performed on a Biometra TGradient PCR machine in 15µl volumes containing 1x PCR buffer (New England BioLabs), 250µM dNTPs, 2.5mM MgCl₂, 0.84% BSA, 5pmol of each primer, 0.5U Taq polymerase (New England BioLabs) and 1µl of template DNA. PCR conditions are as indicated in Table 4.1 and involve an initial denaturation step at 94°C for 5 min (or 10 min for 33D) followed by cycles of denature at 94°C, annealing at a designated temperature (Table 4.1), extension at 72°C, and a final extension time of 10min at 72°C. The ramping speed used was 1°C sec⁻¹. Loci were pooled for genotyping on an ABI 3730 Genetic Analyser with GS 500 LIZ size standard. The results have been scored with PEAK SCANNER version 1.0

(<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=603624>), binned using MSAT-ALLELE ver. 1.01 (Alberto 2009) and double checked with MICRO-CHECKER ver. 2.2.3 (Van Oosterhout *et al.* 2004) for scoring errors and null alleles. ARLEQUIN ver. 3.5 (Excoffier *et al.* 2005) was used

to calculate deviations from Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium, as well as the amount of observed (H_O) and expected heterozygosities (H_E) (Table 4.2). Test probabilities were corrected according to Bonferroni procedures (Rice 1989).

4.4 Results and Discussion

All primers constructed for the ten loci amplified in *Lessonia variegata*/W (Table 4.2). However scoring was impossible for locus 14D, showing multiple peaks as a result of stuttering and/or multiple copies of the target sequence. Optimisation of this locus is essential before further use. Two loci were monomorphic in *L. variegata*/W (19A and 26D) but are included here as they might be polymorphic in other *L. variegata* lineages (as it is shown for 26D, Table 4.3). Another two loci (20A and 28C) showed only minor variation and were monomorphic in some populations. The remaining five loci were polymorphic in all populations with 3 to 24 alleles per locus (Table 4.2).

No significant deviation from HWE was found except for the locus 57B within the Cape Palliser population. If significant deviations from HWE result from local inbreeding or admixture then deviations should be found at all loci. As in this population only one marker (57B) departed significantly from HWE this might indicate the presence of null alleles rather than the result of local breeding structure or admixture (Van Oosterhout *et al.* 2006). Linkage disequilibrium was also only significant in the Cape Palliser population between 57B and 09B. This, however, disappeared when 57B was corrected for null alleles (using Brookfield 1 method (Brookfield 1996) implemented in MICRO-CHECKER) and linkage disequilibrium

was not evident in any other of the three populations or in any other combination of loci.

Eight loci were successfully tested for amplification in the other three lineages of the *L. variegata* species complex, showing variation within and between lineages (Table 4.3).

4.5 Tables

Table 4.1 Primer sequences, and characteristics of ten microsatellite loci from *L. variegata*/W. The values for the PCR profiles are the times in seconds, spent denaturing at 94°C/annealing at a designated temperature/extension at 72°C. Each PCR profile started with an initial denaturation step of 5min (10min for 33D) at 94°C and ended with an extension period of 10min at 72°C. One of each paired primers was dyed with FAM, VIC, PET, HEX, or NED for genotyping.

Locus	Repeat motif	Repeat unit	Primer sequence (5'-3')	PCR profile
02A	(GTA) ₇	3	FAM -GTGGTGGTCGCATTAGGAGT TCACGAGCAGTCAAGATTCG	30/30/45 at 48°C for 32 cycles
09B	(T) ₁₃	1	VIC -CACCTGTAGCTTTTCGCTCAAACG GGGAGAGAGAAAGAAGGACGAGGAT	30/30/45 at 55°C for 32 cycles
14D	(TGT) ₁₃	3	VIC -CCTAACGCTGTGCAGTTGAA AGAGAGGACGGCCCGATG	30/30/45 at 55°C for 32 cycles
19A	(ATC) ₃ ACC(ATC) ₅	3	PET -CAATCAGCATGGTCACCACCAA CAGGATGAGTATGGTCGTGGGAAT	30/30/45 at 55°C for 32 cycles
20A	(TTG) ₃ (TTA) ₂ (TTG) ₃ CTG(TTG) ₂ TCG,TAG	3	PET -TGACAGTACGCAGTACGTGTAGGT CGCTGCCACATACATGTCCTGTTA	30/30/45 at 55°C for 32 cycles
25C	(TTGTAG) ₂ TTG(TTGTAG) ₂ (TTG) ₄ (TCA) ₈ N ₃₀ (ACC) ₅ N ₆ (ATC) ₁₇	3	FAM -CATCGTCGATAACAGCATCA TACGCCGTATGTCTGTGTGC	30/30/45 at 55°C for 32 cycles
26D	TGTGGGG,TGTGGGA,(TGTGGGG) ₄ N ₁₅ (GCT) ₃ GCC(GCT) ₅	7 & 3	FAM -GCCGAACATCAGTATGTGTGA CGGATGGACTGTCTGTGAAA	30/30/45 at 55°C for 32 cycles

Table 4.1 continued

Locus	Repeat motif	Repeat unit	Primer sequence (5'-3')	PCR profile
28C	GGTTT,(GGGTT,AGGTT) ₃ ,GGGTT, (GGGTT,AGGTT),(GGGTT) ₂ ,GTGTT	5	HEX -GCACTTAGACAGTTGGGGTGT	30/30/45 at 55°C
33D	(GTT) ₉₀	3	TTTGTGCTCGAAGACACCAT NED -GATTTCACTCACTGGCCAAC	for 32 cycles 2/2/8 at 50°C
57B	(AAC) ₂₂ AGC(AAC) ₅ N ₇₅ (AAC) ₁₀ AGC,AAC,AGC,(AAC) ₂	3	ACGTGAGAACGGCAGCAG PET -TCGCATCAACAACAAAATC ACTCTTTACCCCTAACCCA	for 35 cycles 30/30/45 at 48°C for 32 cycles

Table 4.2 Number and size range of alleles per locus combined over three populations ($N_{A/L}$), populations, sample size (N), number of alleles per population (N_A), observed (H_O) and expected (H_E) heterozygosity. * significant deviation from HWE at $\alpha=0.05$; nv = not variable; N/A marker needs optimisation.

Locus	$N_{A/L}$	Size range (bp)	Population	N	N_A	H_O	H_E
33D	24	349-481	Moa Point	47	11	0.809	0.794
			Turakirae Head	49	10	0.735	0.790
			Cape Palliser	48	16	0.813	0.749
57B	10	293-317	Moa Point	50	7	0.520	0.492
			Turakirae Head	50	8	0.580	0.612
			Cape Palliser	50	8	0.600*	0.714
25C	7	331-358	Moa Point	50	4	0.220	0.236
			Turakirae Head	49	4	0.286	0.306
			Cape Palliser	50	5	0.720	0.701
02A	5	217-241	Moa Point	50	2	0.020	0.020
			Turakirae Head	50	3	0.120	0.115
			Cape Palliser	50	3	0.140	0.133
09B	3	224-231	Moa Point	50	2	0.440	0.465
			Turakirae Head	50	2	0.360	0.453
			Cape Palliser	50	3	0.240	0.251
20A	2	228-237	Moa Point	10	1	nv	nv
			Turakirae Head	10	1	nv	nv
			Cape Palliser	10	2	0.200	0.189
28C	2	166-181	Moa Point	10	2	0.200	0.189
			Turakirae Head	10	1	nv	nv
			Cape Palliser	10	1	nv	nv
19A	1	224	Moa Point	10	1	nv	nv
			Turakirae Head	10	1	nv	nv
			Cape Palliser	10	1	nv	nv
26D	1	158	Moa Point	10	1	nv	nv
			Turakirae Head	10	1	nv	nv
			Cape Palliser	10	1	nv	nv
14D	N/A	N/A	needs optimisation	-	N/A	N/A	N/A

Table 4.3 Amplification success and allele region for three additional *Lessonia variegata* lineages (sample size one) in comparison with *L. variegata*/W (sample size as in Table 4.2). Lineage abbreviations as in Chapter 2, K Kaikoura lineage, N northern lineage, S southern lineage, W Wellington lineage.

Locus	Lessonia sp.	Size range (bp)	Locus	Lessonia sp.	Size range (bp)
57B	L. variegata/W	293-317	19A	L. variegata/W	224
	L. variegata/N	330		L. variegata/N	224
	L. variegata/K	330		L. variegata/K	224
	L. variegata/S	232-238		L. variegata/S	221
25C	L. variegata/W	331-358	20A	L. variegata/W	228-237
	L. variegata/N	375		L. variegata/N	240-243
	L. variegata/K	391		L. variegata/K	240
	L. variegata/S	355-372		L. variegata/S	239
02A	L. variegata/W	217-241	28C	L. variegata/W	166-181
	L. variegata/N	254-257		L. variegata/N	196
	L. variegata/K	237		L. variegata/K	170
	L. variegata/S	248		L. variegata/S	170
09B	L. variegata/W	224-231	26D	L. variegata/W	158
	L. variegata/N	235		L. variegata/N	158
	L. variegata/K	223		L. variegata/K	149-164
	L. variegata/S	225		L. variegata/S	150-167

4.6 References

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Chapter 5

Connectivity between populations of *Lessonia variegata* (Phaeophyceae, Laminariales) across Cook Strait, New Zealand

5.1 Abstract

Cook Strait is a waterway geographically separating the North Island and the South Island of New Zealand. However, in many phylogeographic and biogeographic surveys genetic breaks of North Island lineages are found south of Cook Strait implying that the breaks are not generated by the strait. In this chapter I want to evaluate the influence that Cook Strait has on population connectivity and the reason why this geographic feature does not correlate with genetic breaks. I used the recently discovered Wellington lineage of the *Lessonia variegata* species complex as model system. Six populations have been sampled according to a sample design that allowed me to compare genetic variation on both sides of Cook Strait to genetic variation found within and between populations along a continuous coastline. Genetic variation of five recently constructed, species specific microsatellite markers were detected and evaluated with three different methods, structure analysis, principle component analysis and F-statistics. The results indicate that genetic differentiation found between populations separated by Cook Strait was weak and no differences have been found between three connected populations at the south coast of Wellington over a distance of 32 km. A strong break was detected between two populations separated by Palliser Bay, where unsuitable coastline created a gap

similar to Cook Strait in width. The differences in the strength of the genetic break between the two gaps suggested that the stronger currents within Cook Strait might be responsible for enhanced connectivity. By critically evaluating the potential of possible dispersal mechanisms I concluded that rafting might be important as a means to connect populations across Cook Strait.

Abbreviations: K, number of clusters; K^* , real number of clusters within a dataset; $L(K)$, posterior probability of K; $L'(K)$ and $L''(K)$, first and second transformation of $L(K)$ after Evanno *et al.* (2005); ΔK , third transformation of $L(K)$ modified after Evanno *et al.* (2005); CSC, Cook Strait Cluster; WC, Wairarapa Cluster; GWG, Greater Wellington Group; Fight or [Fi], Fighting Bay; Tera or [Te], Cape Terawhiti; Moa or [Mo], Moa Point; Tura or [Tu], Turakirae Head; Mata or [Ma], Mataikona; PCA, principle component analysis; R_{ST} , a measure of population divergence that assumes a stepwise mutation model of *microsatellite* evolution; F_{GT} , variation among different groupings of locations; F_{LG} , variation among locations within groups or clusters; F_{IS} , inbreeding coefficient that measures the amount of heterozygosity in subpopulations.

5.2 Introduction

To classify the coastal marine environments of New Zealand, phylogeographic (Apte & Gardner 2002, Ayers & Waters 2005, Goldstien *et al.* 2006) and/or biogeographic (Moore 1949, Knox 1975, Nelson 1994, Walls 1995, Francis 1996, Walls 2006, Shears *et al.* 2008, Ministry of Fisheries & Department of Conservation 2008; hereafter called MPA 2008) approaches have been used. In these surveys common genetic breaks have been found (reviewed in Ross *et al.* 2009), e.g., a north-south differentiation centred on Cook Strait (Moore 1949, Francis 1996, Apte & Gardner 2002, Star *et al.* 2003, Waters & Roy 2004, Ayers & Waters 2005, Goldstien *et al.* 2006, Shears *et al.* 2008, MPA 2008). Cook Strait separates the North and South Islands of New Zealand (Lewis *et al.* 1994) by at least 22km, and is known to be turbulent with sometimes strong tidal currents (Bowman *et al.* 1983). Most surveys, however, suggested the exact location of the break south of Cook Strait but not directly in congruence with the strait. A break south of Cook Strait was also found in the *Lessonia variegata* species complex (Chapter 3). In this chapter I want to evaluate the influence of this waterway on the connectivity of *Lessonia variegata* populations in comparison to populations on a continuous coastline.

The distribution and connectivity of populations in the marine realm is likely linked to the species dispersal ability (Ross *et al.* 2009). Natural dispersal in brown algae (kelp) of the order Laminariales is possible in several ways that enable kelp to disperse over a variety of spatial scales. The two most studied dispersal mechanisms are dispersal of zoospores and sporophytes. Zoospores tend to disperse over short distances (tens of meters (Dayton 1973, Reed *et al.* 1988, Norton 1992, Kusumo *et al.* 2006)), but have been recorded to travel up to 3.5 km (Reed *et al.* 2004).

However, successful recruitment in Laminariales was mostly found to take place in the vicinity of the parent (<10m) (Santelices 1990, Norton 1992, Forrest *et al.* 2000, Faugeron *et al.* 2005, Kusumo *et al.* 2006) possibly based on the complex life cycle of the Laminariales with microscopic dioecious gametophytes. As an adaptation to exposed environments where some kelps, including *Lessonia*, are found, the dispersal distance might be even more limited as quick settlement enables zoospores to remain within this habitat (Taylor & Schiel 2003). Long distance dispersal is considered to be mainly due to detached drifting sporophytes (Coyer *et al.* 2004, Thiel & Gutow 2005a, Macaya *et al.* 2005, Macaya & Zuccarello 2010). Some Laminariales (e.g., *Macrocystis pyrifera*) are positively buoyant due to gas-filled bladders called pneumatocysts, which enables detached algae to float and disperse by currents (Macaya *et al.* 2005, Macaya & Zuccarello 2010). Whereas all Laminariales have dispersive zoospores, long distance dispersal via floating is confined to the few species with gas filled structures. *Lessonia* does not float and sinks to the sea floor if detached (Martinez *et al.* 2003), which would indicate poor dispersal abilities and therefore poor connectivity between populations. Martinez *et al.* (2003), who surveyed the recovery of *Lessonia nigrescens* on a disturbed coastline, calculated an average advance of populations of less than 3km per year, and Faugeron *et al.* (2005) showed that *Lessonia nigrescens* had its highest genetic variation concentrated within plots of quadrants that are 20m apart, suggesting restricted dispersal. However, kelp gametophytes (Lane & Saunders 2005) and sporophytes (Fig. 1.10) have been found attached to other algae with the ability to float or raft, thus population connectivity is possible even for non-floating algae by this means (Thiel & Gutow 2005b). Dispersal of fertile fronds or detached individuals, even if they do not have the ability to float, has been reported for some Laminariales (Dayton 1973,

Forrest *et al.* 2000, Sliwa *et al.* 2006), including *Lessonia* (Marsden 1991a, Thiel & Gutow 2005a), and might be underestimated as a means of dispersal (Faugeron *et al.* 2005).

Lessonia variegata J.Agardh was divided into four genetic species with distinct distribution ranges based on sequences of the mitochondrial, chloroplast and nuclear genome (Chapter 2 & 3). The distribution of these genetic species with clear biogeographic breaks (Chapter 3) and their inability to float suggests that *L. variegata* is a poor disperser. A genetic break between two species of *L. variegata* was found south of Cook Strait, thus one species (*L. variegata*/Wellington lineage (W)) was confined to the central eastern region of New Zealand while spanning Cook Strait. In this study, I will use newly designed microsatellite markers (Chapter 4) to address questions of population structure and differentiation in one genetic species of *L. variegata* (*L. variegata*/W) around Cook Strait.

5.3 Material and Methods

Lessonia variegata was collected from five locations around Cook Strait and one location on the southeast coast of the North Island (Fig. 5.1). Within each location, I sampled different sites following a sampling design with increasing distances from 0 to 1000m (Fig. 5.1, inserts). Five sites were sampled at Cape Terawhiti, Moa Point, Turakirae Head, and Cape Palliser, three sites at Fighting Bay, and two sites at Mataikona. On average, ten samples (4 to 17) were collected at each site within 10-15m², dried in silica gel and returned to the laboratory. Fighting Bay, on the South Island, is separated from the other populations by Cook Strait. The closest population on the opposite side of Cook Strait is Cape Terawhiti, which is located

36km to the east, at the southwest corner of the North Island. Moa Point is 18km further to the west of Cape Terawhiti. Another 14km to the southwest is Turakirae Head, which is separated from Moa Point by the Wellington harbour. Cape Palliser is the southernmost point of the North Island and 37km (53km along the coast) southeast of Turakirae Head. A large bay, Palliser Bay, and a long beach (20km) separates Turakirae Head and Cape Palliser. Mataikona on the east coast is 127km to the northeast of Cape Palliser along a continuous rocky coastline.

DNA was extracted following a modified CTAB protocol (Zuccarello & Lokhorst 2005). PCR amplification was performed as described in Chapter 4 for five microsatellite markers, which have been developed chiefly for this species (02A, 09B, 25C, 33D, and 57B (for primer information see Chapter 4, Table 4.1)). PCR products were pooled and subsequently genotyped on an ABI 3730 Genetic Analyser with an internal GS 500 LIZ size standard (Allan Wilson Centre, Palmerston North).

Variations in size have been scored in PEAK SCANNER version 1.0 (<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catalogID=603624>) and binned using MSAT-ALLELE ver. 1.01 (Alberto 2009). Prior to statistical analysis, the binned scores were double checked for scoring errors and null alleles with MICRO-CHECKER ver. 2.2.3 (Van Oosterhout *et al.* 2004). Null alleles were corrected using Brookfield 1 method (Brookfield 1996) implemented in MICRO-CHECKER. Brookfield 1 method was selected from a range of other correction methods as all samples used were successfully amplified. Linkage disequilibrium was tested against 1,000 permutations using ARLEQUIN ver. 3.5.1.2 (Excoffier *et al.* 2005).

CONVERT ver. 1.31 (Glaubitz 2004) was used to create input files for analytical softwares and to transfer data matrixes between softwares.

ARLEQUIN ver. 3.5 (Excoffier *et al.* 2005) was used to calculate deviations from Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium, as well as the amount of observed (H_O) and expected heterozygosities (H_E) (Table 5.1). Test probabilities were corrected according to Bonferroni procedures (Rice 1989).

Genetically structured populations were defined using STRUCTURE ver. 2.3.3 (Pritchard *et al.* 2000). Using a Bayesian approach this program assigns posterior probabilities ($L(K)$) after sorting individuals based on their genotypes at multiple loci into a predefined number of clusters (K). To compute the probability of the data, I set the number of Markov chain Monte Carlo repeats to 5 million after a burnin period of 10,000 generations. The allele frequency prior, lambda (λ), was calculated for each dataset in an extra run with K set to 1 and was used in further runs as a fixed value. The admixture model was used to account for possible migration between clusters, for the same reason allele frequencies were assumed to be correlated between subpopulations. The best fitting K value, averaged over 10 independent runs, was selected with an algorithm designed to detect the uppermost level of structure, transforming $L(K)$ over three steps into ΔK (Evanno *et al.* (2005). However, instead of using the average of the absolute values of $L''(K)$ (Evanno *et al.* 2005), I defined the numerator contributing to ΔK as the absolute value taken from the average of all $L''(K)$'s. The original formula would not calculate an average value of $L''(K)$ if both, positive and negative values are found for $L''(K)$ in different runs (e.g., if $L(K+1)$ shows high variation between parallels) whereas the average is calculated if all $L''(K)$ values are either positive or negative. Thus the real K value

would not only depend on the change of differentiation between the posterior probabilities of successive K s but also on the standard deviation of $L(K+1)$. However, this is in contrast to the underlying principle of ΔK as formulated elsewhere in the article by Evanno *et al.* (2005), thus I assume the formula in the original article might have been wrongly phrased. This differentiation has proven to be critical for better detection of the number of clusters, comparable to principle component analysis (PCA) and/or results from AMOVA and the true K (K^*) was not found with the former but with the later formulation of ΔK . Outputs of multiple STRUCTURE runs were combined in CLUMPP ver. 1.1.2 (Jakobsson & Rosenberg 2007), and graphically optimised with DESTSTRUCT ver. 1.1 (<http://rosenberglab.bioinformatics.med.umich.edu/distruct.html>). CLUMPP input files were generated using STRUCTURE HARVESTER ver. 0.56.4 (http://taylor0.biology.ucla.edu/struct_harvest/).

To evaluate the degree of structure among the samples, I additionally performed a principle component analysis coding each locus as an independent variable combining the three-number code that described the length of each allele per locus into a six-number code for each individual. Thus I created a matrix with five columns (the number of microsatellite markers used). Through the variable reduction procedure of the principle component analysis (PCA), an artificial matrix consisting of only two columns was created while accounting for most of the variation within the data. Due to the sample size I only plotted the centre of gravity for each site to make the figure easier to read.

Hierarchical AMOVA was calculated in ARLEQUIN ver. 3.5.1.2 (Excoffier *et al.* 2005) and used to conduct three different series of tests according to different

spatial levels. In all tests I performed R_{ST} -like analysis. To add information about the relatedness of alleles for R_{ST} -like analysis, the allele-code had to be converted from the length of the marker sequence into the number of microsatellite repeats. Thus I had to use a dataset that was not corrected for null-alleles as for null-alleles the length or count is unknown. The first series of analyses was performed to evaluate the genetic structure of *L. variegata* populations in Cook Strait compared with the STRUCTURE output. Therefore I calculated the percentage of variation among different groupings of locations (F_{GT}). Highest F_{GT} combined with lowest F_{LG} (variation among locations within groups) would indicate maximised differentiation between groups together with minimized differentiation within groups, and thus the best combination of groups of locations. A crude index ($F_{GT/LG}$) was created to make the best combination salient, therefore I compared the absolute values of F_{GT} divided by F_{LG} (i.e., $F_{GT/LG}=|F_{GT}/F_{LG}|$) between all combinations tested. The next series of tests was performed to find potential substructure within clusters and groups defined with STRUCTURE (i.e., within the Cook Strait cluster (Fighting Bay, Cape Terawhiti, Moa Point and Turakirae Head), the Greater Wellington group (Cape Terawhiti, Moa Point and Turakirae Head), and the Wairarapa cluster (Cape Palliser and Mataikona)). In the third series of tests, small scale variation within locations and sites was evaluated. Pair-wise R_{ST} was performed for all sample sites in ARLEQUIN to additionally evaluate population genetic structure between sites within locations and among locations.

The three different methods (STRUCTURE, PCA, and hierarchical AMOVA) were used to evaluate connectivity, and breaks between and within the locations sampled. As one method (the detection of K^* (the real number of clusters

within a dataset)) was slightly modified, the other methods were also used to confirm the results that were affected by this modification. STRUCTURE was used hierarchically similar to AMOVA to further investigate clusters for groups and subgroups. Breaks found with STRUCTURE at the first hierarchical level (using an undivided dataset) were used to define “clusters”. Subsequently the dataset was divided according to these clusters and reanalysed. Breaks found at the second hierarchical level were used to define “groups”, so that the terminology gives an indication to the hierarchical level of the structure found. With the additional use of ΔK^* , (which is proposed to measure the signal strength of a break (Evanno *et al.* 2005)), STRUCTURE results of pairwise tests were interpreted as a measure of connectivity between groups. This is similar to the use of a pairwise R_{ST} matrix in combination with AMOVA results, where the mean pairwise R_{ST} value between groups of interest gives an indication of the degree of differentiation. The significance of the degree of differentiation was tested with a 2-tailed t-test comparing pairwise R_{ST} within groups with pairwise R_{ST} between groups. In turn, three series of subsequent hierarchical AMOVA were used to verify and confirm STRUCTURE results, however the third series allowed additional interpretations (e.g., the evaluation of inbreeding) and the evaluation of structure at a finer scale (e.g., among individuals within locations). PCA was used to evaluate the variation within a dataset without using information about genetic inheritance and dependence of the data.

5.4 Results

No significant deviation from HWE was found except for the locus 57B within the Mataikona population (Table 5.1). If significant deviations from HWE result from

local inbreeding or admixture then deviations should be found at all loci. As in this population only one marker (57B) departed significantly from HWE this might indicate the presence of null alleles rather than the result of local breeding structure or admixture (Van Oosterhout *et al.* 2006).

Among the four possible scoring errors that can be detected using MICRO-CHECKER (typographical errors, stuttering, large allele dropout, and null alleles) only null alleles were apparent in the data set. A total of 11 corrections have been suggested (Table S 5.1), one for the locus 57B for a sample from Mataikona, and ten for the locus 09B (affecting six alleles from Turakirae Head and four from Fighting Bay). However no evidence for null alleles was found within the Cook Strait Cluster or Greater Wellington Group. Thus, as Turakirae Head and Fighting Bay were within these groups of connected locations (see below), no corrections were made for locus 09B. Linkage disequilibrium (Table S 5.2) was found in Cape Terawhiti between loci 02A and 25C; however, as linkage was not found in any other population or any other pair of loci it might be an artefact of common evolutionary history rather than an artefact of linked loci.

Allele variability differed between loci from 6 to 31 alleles (at locus 09B and 33D, respectively). Private alleles were detected (Table S 5.3) with a maximum of 9 private alleles found for the locus 33D within samples from Cape Palliser.

Structure and substructure was found using hierarchical STRUCTURE analysis and a modified algorithm based on Evanno *et al.* (2005) (Fig. 5.2-5.4). In addition, ΔK^* (defining K^* as the real number of clusters within a dataset) of the modified Evanno transformation could be used as an indicator of the strength of the signal detected by STRUCTURE (Evanno *et al.* 2005) (Fig. 5.5 & 5.6). At the

uppermost hierarchical level, a separation was indicated between a cluster comprising Cape Palliser and Mataikona (hereafter called the Wairarapa Cluster, WC in Fig. 5.2) and locations sampled in inner Cook Strait (Fighting Bay, Cape Terawhiti, Moa Point, and Turakirae Head; hereafter called the Cook Strait Cluster, CSC in Fig. 5.2). This was the strongest break found during hierarchical testing with $\Delta K^*=100$. Within the Wairarapa Cluster the locations Mataikona and Cape Palliser formed separate groups at the second hierarchical level ($\Delta K^*=83$, Fig. 5.3). Fighting Bay was separated from the remaining locations of the Cook Strait Cluster at the second hierarchical level ($\Delta K^*=64$, Fig. 5.3). The remaining locations (Cape Terawhiti, Moa Point, and Turakirae Head) were not further structured (Fig. 5.4) and are hereafter called Greater Wellington Group. STRUCTURE and ΔK^* were also used to estimate the strength of breaks between locations or group of locations. A pairwise test of locations within the Cook Strait Cluster was conducted to evaluate the connectivity of the Greater Wellington Group locations to Fighting Bay (Fig. 5.5). The highest ΔK^* found was between Fighting Bay and Turakirae Head ($\Delta K^*=75$). Five times smaller was the value between Fighting Bay and Cape Terawhiti ($\Delta K^*=14$) and no differences were found between the other comparisons ($K^*=K1$) including the comparison between Fighting Bay and Moa Point. I also tested the connectivity of locations or groups of locations to Cape Palliser (Fig. 5.6). The lowest ΔK^* was found between Cape Palliser and Fighting Bay ($\Delta K^*=28$). These two locations are 98km apart with the Greater Wellington Group between them. In comparison the signal between Cape Palliser and Turakirae Head, its geographically closest neighbour, was 10 times stronger ($\Delta K^*=290$). The strongest signal, however, was found between Cape Palliser and the Greater Wellington Group ($\Delta K^*=888$).

Principle component analyses revealed three different clusters explaining 54.80% of the variation in the dataset (Fig. 5.7; showing the centre of gravity of sample sites). The first component (explaining 29.71% of the total variance) clearly separated the samples from Mataikona, Cape Palliser, and the remaining Cook Strait samples into three different groups. Whereas the second axis (explaining 25.09%) separated the Cook Strait Cluster from the Wairarapa Cluster. Thus, both axes supported a differentiation between the Wairarapa Cluster and the Cook Strait Cluster, which are geographically separated by Palliser Bay. The separation between Cape Palliser and Mataikona was mainly supported by the first and less by the second factorial axis; the two locations are geographically 127km apart, and connected continuous rocky coastline.

The first series of AMOVA tests was designed to evaluate the amount of structure within the dataset. Therefore the percentage of variation was calculated for different groupings of locations (Table 5.2). High F_{GT} (differentiation among groups) would be an indicator for a good grouping regime and low F_{LG} (differentiation within groups) would indicate little substructure. Thus I explored the dataset for a combination of maximised F_{GT} and minimized F_{LG} values. The highest F_{GT} value was found for the grouping [Fi/Te/Mo/Tu][Pa][Ma] (test No. 4), which equals the grouping found with the first two components of PCA. However, the AMOVA also resulted in a low but significant F_{LG} value, indicating further substructure among populations within groups, in this case within the Cook Strait Cluster ([Fi/Te/Mo/Tu]). The only non-significant F_{LG} value (indicating no further substructure within the defined groups) combined with the second highest F_{GT} (0.5257; $p < 0.0001$) was found for the grouping [Fi][Te/Mo/Tu][Pa][Ma] (test No. 6).

Test No. 6 had also the highest $F_{GT/LG}$ value and this conformed to the clusters and groups found with hierarchical STRUCTURE analysis. Thus the first test series of AMOVA supported the clusters and groups found with STRUCTURE und PCA.

The second series of AMOVA tests was performed to test for substructure within clusters and groups defined from the STRUCTURE results. Different combinations of groupings were tested between locations of the Cook Strait Cluster but only one combination was found with a significant F_{GT} value (Table S 5.4A). No significant variation was found among locations of the Greater Wellington Group (Table S 5.4B) and a high F_{LT} denoted significant variation between locations of the Wairarapa Cluster (Table S 5.4C). Thus no additional substructure was found with the second test series that was not already found in the first series of tests.

The third series of AMOVA tests was designed to evaluate the differentiation among sites within locations (Table S 5.5). Significant differentiation among sites (F_{ST}) would indicate isolation into small populations within hundreds of meters, whereas high variation within sites (F_{IS}) might indicate structure even within 10 to 15m². However, no significant variation was found within any location at any spatial level.

Pairwise R_{ST} estimates showed a moderate number of significant differences (Table 5.3). Genetic differentiation was strong between clusters (121 out of 126 comparisons have been significant) and strong to moderate within clusters between groups (between Cape Palliser and Matakona 8 out of 10 comparisons were significant and between Fighting Bay and Greater Wellington Group 8 out of 45), however, no significant differences were found between sites within locations and between sites within groups. A two-sided t-test was used to evaluate the strength and

significance of the breaks found. The t-test was used to compare the variation within groups to the variation between groups (Table 5.3 insert). The highest value was found in the comparison between clusters (0.441; $p < 0.0001$) indicating the strongest break. Within clusters between groups the t-test resulted a value of 0.409 ($p < 0.0001$) and 0.104 ($p < 0.0001$), for the comparison between Cape Palliser and Mataikona and between Fighting Bay and GWG, respectively. The latter results are an indication for a stronger break between the Eastcoast populations than between the CSC populations. Thus the pairwise R_{ST} estimates together with the 2-sided t-tests of pairwise R_{ST} values (Table 5.3 insert) confirmed the results found with the three hierarchical levels of STRUCTURE analysis.

5.5 Discussion

Although it was assumed that *Lessonia* is a poor disperser (Faugeron *et al.* 2005) I found no genetic differentiation within sites (within 10m²) nor between sites within locations (i.e., sites that are separated by 100 to 1,000m). However, small scale genetic variation cannot be ruled out and might be detected with a larger number of microsatellite markers. The small scale genetic variations as detected in other *Lessonia* species, such as *L. nigrescens* (Faugeron *et al.* 2005 [within 20m]), or in other Laminariales, such as *Postelsia palmaeformis* (Dayton 1973 [$< 3m$], Kusumo *et al.* 2006 [$< 5m$]), reflect supposedly the dispersal by zoospores and sperms (propagules). Propagule dispersal followed by successful recruitment of juveniles is governed by restrictive mechanisms in Laminariales, e.g., their complex life cycle requires male and female gametophytes to be in close vicinity for successful fertilisation (Reed 1990). Some factors that restrict dispersal of propagules are summarized in Santelices (1990). However, other forms of dispersal e.g., drifting or

floating of detached algae or fertile fragments, rafting attached to other floating devices as well as dispersal by saltation are reported for Laminariales (Dayton 1973, Schiel and Foster 1986, van den Hoek 1987, Santelices 1990, Reed *et al.* 1992, Faugeron *et al.* 2005, Lane & Saunders 2005, Macaya *et al.* 2005, Kusumo *et al.* 2006, Sliwa *et al.* 2006). Dispersal of free-floating spores should not be underestimated, as spores can develop into planktonic gametophytes that can become fertile and retain the ability to produce sporophytes after fertilization (Reed *et al.* 1992). These other forms of dispersal might be responsible for the lack of genetic structure found in this survey. For example, I detected only weak genetic differentiation between populations on either side of Cook Strait separated by a >36km broad waterway and no significant differences between populations within a 32km long, more or less continuous coast line on the south of the North Island (i.e., within the Greater Wellington Group). Strong tidal currents, eddies, and rips (Heath 1971, Bowman *et al.* 1980) but also wind stress (Bowman *et al.* 1983) within Cook Strait might assist good connectivity between these populations. The connectivity found between Cook Strait locations is of the same magnitude but slightly smaller than the differentiation found in *Ecklonia radiata* on the South coast of Australia (Coleman *et al.* 2009). Coleman *et al.* (2009) used six microsatellite markers to detect small scale [within 10s of km] genetic variation in *Ecklonia radiata*. These two related algae might have similar dispersal abilities as they possess no floating devices, are of comparable size, and share partially overlapping habitats. In contrast, large brown algae with floating devices, like pneumatocysts in *Macrocystis pyrifera* or gas-filled honeycomb structures in the blades of *Durvillaea antarctica*, have shown extreme connectivity throughout the Southern Hemisphere with little to no

differentiation between populations within the Antarctic Circumpolar Current (Macaya & Zuccarello 2010, Fraser *et al.* 2009).

The strongest break found was between Turakirae Head and Cape Palliser. These two populations are separated by 37km (53km along the coast) across Palliser Bay. There is no obvious evidence for inhibited connectivity through surface currents (pers. comm. with Craig Stevens and Mark Hadfield, Marine Physicists from New Zealand National Institute for Water and Atmospheric Research) but the populations are separated by a 15 to 20 km long beach of unsuitable substrate consisting of mudstone and/or sandstone in combination with a river delta in the middle of the bay (http://sci.waikato.ac.nz/evolution/images/geology_era.pdf). The width of Palliser Bay is comparable to the width of Cook Strait however the genetic differences found are significantly stronger (4 to 14 times stronger, dependent on the method used, i.e., mean $R_{ST}=0.1042$ between [Fi][Te/Mo/Tu] and 0.4067 between [Te/Mo/Tu][Pa] showing significant differences at $p<0.0001$; $\Delta K_2=64$ between [Fi][Te/Mo/Tu] and 888 between [Te/Mo/Tu][Pa]). This is in contrast to suggestions made by Coleman *et al.* (2009) who found that differences in gene flow were comparable between vast tracts of unsuitable coastline and where oceanic expanses existed. One explanation could be that stronger currents and turbulences within Cook Strait increase connectivity, explaining both the low genetic differentiation across Cook Strait and the insignificant differences among the Greater Wellington populations along the Cook Strait coast. Another explanation could be that steep variations in bathymetry (i.e., the Wairarapa canyon on the west side of Palliser Bay) might influence near-shore flows through the vertical flows they generate (Craig Stevens, pers. comm.). Even though there are no obvious anomalies in the surface

currents within Palliser Bay that would explain a constant barrier to gene flow, the Wairarapa canyon would prevent some of the many possible dispersal mechanisms. The genetic differentiation on these two breaks is probably a combination of many unknown causes and influencing factors (Santelices 1990). Cook Strait and the Wairarapa canyon in Palliser Bay could prevent dispersal via saltation as proposed for intermediate dispersal over moderate distances (10-100m per year) in *Undaria pinnatifida* (Sliwa *et al.* 2006). Both Cook Strait and the Wairarapa canyon also prevent dispersal of drifting algae, which tumble over the seafloor, as they are too heavy to float. This form of dispersal is limited to shallow water (Thiel & Gutow 2005a). Thus dispersal across Cook Strait and Palliser Bay might be limited to rafting, and via floating spores or spore aggregates. Floating spores have been reported to travel at least 4km (Reed *et al.* 1988,), which is only a fraction of the 36km needed. However, the maximum range of spore dispersal in nature is still unknown based on the difficulties in detecting spores in the water column (Reed *et al.* 2004). But successful settlement and fertilisation success decreases with time and distance based on several affecting factors like planktonic grazing, the dilution effect, and loss of stickiness (Santelices 1990). Thus I might speculate that a likely form of dispersal is rafting (i.e., the attachment of microscopic gametophytes or macroscopic sporophyte to other floating devices), which might be favoured by the stronger currents and turbulences within the Cook Strait narrows and thus explain the differences in genetic differentiation found between the two barriers, Cook Strait and Palliser Bay.

5.6 Conclusion

The aim of this study was to investigate the connectivity across Cook Strait in comparison to the connectivity of populations along a continuous coastline.

Therefore I sampled six locations, one on the South Island and five along the south-east coast of the North Island (Fig. 5.1). To explore the genetic diversity I used five newly developed microsatellite markers (Chapter 4). Cook Strait was found to be only a weak barrier to gene flow enabling connectivity between Fighting Bay and the Greater Wellington Group. In general, connectivity was high within the Cook Strait Cluster and no differentiation was found within the Greater Wellington Group, which consists of three sample locations distributed along a 32km long coast line. However, a strong break was found at Palliser Bay between the Cook Strait Cluster and the Wairarapa Cluster. Strong currents within Cook Strait might be the reason for increased connectivity across Cook Strait compared to connectivity across Palliser Bay where currents are weaker.

5.7 Figures & Tables

Fig. 5.1 Sample locations (stars) and different sample designs (A-C). Dark grey outlined is the distribution of *Lessonia variegata*/W in the area. Sampling design A was applied to Fighting Bay; sampling design B with increasing distances between sites (quadrats) was applied to the locations Cape Terawhiti, Moa Point, Turakirae Head, and Cape Palliser; sampling design C was used at Matakona.

Fig. 5.2 Bar plot and graphs of the genetic population differentiation at the 1st hierarchical level calculated in STRUCTURE. Bar plots show the mean of all replicates of K^* (here $K=2$). Each population is represented by a colour and each sample is represented by a single bar showing its membership coefficient to any population (the height of each bar plot equals 100%). Left graph shows the mean $L(K)$ value, right graph shows ΔK values using a modified transformation based on Evanno *et al.* (2005), which calculates the best value of K (K^*). The height of the ΔK^* value indicates the strength of the signal detected by STRUCTURE. CSC Cook Strait cluster, WC Wairarapa cluster.

Fig. 5.3 Bar plots and graphs of the genetic population differentiation at the 2nd hierarchical level calculated in STRUCTURE. See Fig. 5.2 for more details. GWG Greater Wellington group.

Fig. 5.4 Bar plots and graphs of the genetic population differentiation at the 2nd hierarchical level calculated in STRUCTURE. See Fig. 5.2 for more details. ΔK values are not shown as $K^*=1$ (i.e., no substructure was found). GWG Greater Wellington group.

testing connectivity within Cook Strait cluster

Fig. 5.5 Bar plots and graphs of STRUCTURE results, testing connectivity within Cook Strait cluster. See Fig. 5.2 for more detail. ΔK values are only shown if $K^* > 1$ (i.e., when substructure was found).

Fig. 5.6 Bar plots and graphs of STRUCTURE results, testing connectivity to Cape Palliser. See Fig. 5.2 for more detail.

Fig. 5.7 Principle component analysis (PCA). Each point represents the centre of gravity of all samples per site. Total variance is given in parentheses. Fight, Fighting Bay; Tera, Cape Terawhiti; Moa, Moa Point; Tura, Turakirae Head; Pall, Cape Palliser; Mata, Mataikona.

Table 5.1 Sample size (N), number of alleles per locus and population (N_A), observed (H_O) and expected (H_E) heterozygosity. * significant deviation from HWE at $\alpha=0.05$ (after sequential Bonferroni correction); nv = not variable.

Population	N	Locus	N_A	H_O	H_E
Fighting Bay	39	02A	1	nv	nv
	39	09B	2	0.20513	0.29837
	39	25C	5	0.15385	0.14785
	35	33D	7	0.57143	0.58219
	39	57B	4	0.61538	0.51449
Cape Terawhiti	49	02A	3	0.04082	0.04061
	50	09B	3	0.52000	0.48343
	49	25C	2	0.10204	0.13402
	49	33D	10	0.81633	0.78834
	50	57B	7	0.58000	0.55616
Moa Point	50	02A	2	0.02000	0.02000
	50	09B	2	0.44000	0.46545
	50	25C	4	0.22000	0.23636
	47	33D	11	0.80851	0.79387
	50	57B	7	0.52000	0.49232
Turakirae Head	50	02A	3	0.12000	0.11495
	50	09B	2	0.36000	0.45333
	49	25C	4	0.28571	0.30570
	49	33D	10	0.73469	0.78961
	50	57B	8	0.58000	0.61232
Cape Palliser	50	02A	3	0.14000	0.13273
	50	09B	3	0.24000	0.25131
	50	25C	5	0.72000	0.70081
	48	33D	16	0.81250	0.74934
	50	57B	8	0.60000	0.71394
Mataikona	19	02A	3	0.31579	0.35846
	21	09B	2	0.28571	0.25087
	20	25C	4	0.65000	0.69103
	18	33D	15	1.00000	0.93810
	20	57B	7	0.60000*	0.82564

Table 5.2 First series of AMOVA tests exploring variation within dataset. Groupings of locations are given in brackets. Highest $F_{CT/SC}$ was found in Test No. 6. Fi, Fighting Bay; Te, Cape Terawhiti; Mo, Moa Point; Tu, Turakirae Head; Pa, Cape Palliser; Ma, Mataikona; SS, Sum of squares; *, $p < 0.05$; **, $P < 0.001$; ***, $p < 0.0001$; ^{ns}, not significant.

	SS	% variation	Fixation indices
Test No. 1: [Fi/Te/Mo/Tu/Pa/Ma]			
Among locations	11297.2	44.09344	$F_{LT}=0.44093^{***}$
Among individuals within locations	8667	1.35765	$F_{IL}=0.02428^{ns}$
Within individuals	8454.5	54.54892	$F_{IT}=0.45451^{***}$
Total	28418.7		
Test No. 2: [Fi/Te/Mo/Tu/Pa][Ma] $F_{GT/LG}=1.1$			
Among groups	3581.4	41.19609	$F_{GT}=0.41196^{ns}$
Among locations within groups	7715.8	22.04104	$F_{LG}=0.37482^{***}$
Among individuals within locations	8667	0.89276	$F_{IL}=0.02428^{ns}$
Within individuals	8454.5	35.87011	$F_{IT}=0.6413^{***}$
Total	28418.7		$F_{ST}=0.63237^{***}$
Test No. 3: [Fi/Te/Mo/Tu][Pa/Ma] $F_{GT/LG}=0.5$			
Among groups	4342.8	18.55871	$F_{GT}=0.18559^*$
Among locations within groups	6954.4	30.91662	$F_{LG}=0.37962^{***}$
Among individuals within locations	8667	1.22695	$F_{IL}=0.02428^{ns}$
Within individuals	8454.5	49.29772	$F_{IT}=0.50702^{***}$
Total	28418.7		$F_{ST}=0.49475^{***}$
Test No. 4: [Fi/Te/Mo/Tu][Pa][Ma] $F_{GT/LG}=37.5$			
Among groups	11033.5	59.63854	$F_{GT}=0.59639^{***}$
Among locations within groups	263.6	0.64181	$F_{LG}=0.0159^{***}$
Among individuals within locations	8667	0.96456	$F_{IL}=0.02428^{ns}$
Within individuals	8454.5	38.75509	$F_{IT}=0.61245^{***}$
Total	28418.7		$F_{ST}=0.6028^{***}$
Test No. 5: [Fi][Te/Mo/Tu][Pa/Ma] $F_{GT/LG}=0.0$			
Among groups	4574.8	0.69617	$F_{GT}=0.00696^{ns}$
Among locations within groups	6722.3	43.46737	$F_{LG}=0.43772^{***}$
Among individuals within locations	8667	1.35594	$F_{IL}=0.02428^{ns}$
Within individuals	8454.5	54.48051	$F_{IT}=0.45519^{***}$
Total	28418.7		$F_{ST}=0.44164^{***}$
Test No. 6: [Fi][Te/Mo/Tu][Pa][Ma] $F_{GT/LG}=90.6$			
Among groups	11265.6	52.57038	$F_{GT}=0.5257^{***}$
Among locations within groups	31.6	-0.27524	$F_{LG}=-0.0058^{ns}$
Among individuals within locations	8667	1.15847	$F_{IL}=0.02428^{ns}$
Within individuals	8454.5	46.54638	$F_{IT}=0.53454^{***}$
Total	28418.7		$F_{ST}=0.52295^{***}$

Table 5.3 Pairwise R_{ST} . Names with numbers indicate the specific sample sites. Red font: significant values after sequential Bonferroni correction ($p < 0.05$). Insert: 2-sited t-test evaluating the strength and significance of the breaks found. The t-test was used to compare the within groups variation to the between groups variation; groups are coded as square brackets. ***, $p < 0.0001$. Fight, Fighting Bay; Tera, Cape Terawhiti; Moa, Moa Point; Tura, Turakirae Head; Pall, Cape Palliser; Mata, Mataikona.

[illegible]

Table S 5.1 Estimated null allele frequencies for all loci in a population using Brookfield 1 and Bonferroni corrections. Null alleles are evident in Fighting Bay, Turakirae Head, and Mataikona, however, no evidence for null alleles was found in GWG or CSC. Bottom row shows the number (in brackets) of homozygotes to be corrected (H_C). *, indicates significant null allele frequencies. Fight, Fighting Bay, Tera, Cape Terawhiti, Moa, Moa Point, Tura, Turakirae Head, Pall, Cape Palliser, Mata, Mataikona, GWG, Greater Wellington Group (Cape Terawhiti, Moa Point, and Turakirae Head), CSC, Cook Strait Cluster (Fighting Bay and Greater Wellington Group).

Locus	Fight	Tera	Moa	Tura	Pall	Mata	GWG	CSC
02A	0.0000	-0.0006	-0.0002	-0.0056	-0.0076	0.0246	-0.0013	-0.0008
09B	0.0691*	-0.0280	0.0142	0.0613*	0.0070	-0.0328	0.0158	0.0329
25C	-0.0069	0.0270	0.0113	0.0129	-0.0155	0.0142	0.0188	0.0148
33D	0.0016	-0.0202	-0.0129	0.0263	-0.0407	-0.0460	-0.0008	0.0263
57B	-0.0713	-0.0190	-0.0219	0.0163	0.0626	0.1136*	-0.0012	-0.0013
H_C	231 (4)			225 (2) 231 (4)		335 (1)		

Table S 5.2 Linkage disequilibrium test conducted against 1,000 permutations in ARLEQUIN. *, p<0.05 (after sequential Bonferroni correction).

Locus	02A	09B	25C	33D	57B
Fighting Bay					
02A					
09B	1.000				
25C	1.000	0.082			
33D	1.000	0.807	0.383		
57B	1.000	0.464	0.728	0.521	
Cape Terawhiti					
02A					
09B	0.042				
25C	0.000*	0.037			
33D	0.005	0.023	0.002		
57B	0.680	0.566	0.377	0.097	
Moa Point					
02A					
09B	0.140				
25C	0.096	0.702			
33D	0.801	0.142	0.104		
57B	0.441	0.344	0.774	0.909	
Turakirae Head					
02A					
09B	0.225				
25C	0.106	0.367			
33D	0.598	0.454	0.924		
57B	1.000	0.820	0.061	0.327	
Cape Palliser					
02A					
09B	0.026				
25C	0.587	0.416			
33D	0.718	0.558	0.612		
57B	0.945	0.003	0.346	0.049	
Mataikona					
02A					
09B	0.574				
25C	0.023	0.548			
33D	0.346	0.033	0.154		
57B	0.388	0.864	0.072	0.446	

Table S 5.3 Allele frequencies and private alleles (red) for all populations by locus. N_A , number of alleles; N_{PA} , number of private alleles; Fight, Fighting Bay; Tera, Cape Terawhiti, Moa, Moa Point; Tura, Turakirae Head; Pall, Cape Palliser; Mata, Mataikona.

	N_A	68	23	27	28	32	36	34
	N_{PA}	30	2	0	2	3	11	12
Locus		Alleles	Fight	Tera	Moa	Tura	Pall	Mata
02A	N_A	7	1	3	2	3	3	3
	N_{PA}	3	0	0	0	1	1	1
		217					6.00	
		229			1.00	5.00		
		235				1.00		
		238	100.00	97.96	99.00	94.00	93.00	15.79
		241		1.02			1.00	
		250		1.02				78.95
		256						5.26
09B	N_A	6	4	3	2	5	3	2
	N_{PA}	2	1	0	0	1	0	0
		223	2.56			2.00		
		224		1.00		2.00	4.00	14.29
		225	17.95	37.00	36.00	32.00	86.00	85.71
		230				2.00		
		231	76.92	62.00	64.00	62.00	10.00	
		232	2.56					
25C	N_A	9	5	2	4	4	5	4
	N_{PA}	3	1	0	1	0	1	0
		331	3.85		3.00	6.12		
		340					2.00	
		343			1.00			
		346	92.31	92.86	87.00	82.65	6.00	
		349	1.28	7.14	9.00	10.20	30.00	37.50
		352				1.02	21.00	35.00
		358					41.00	25.00
		379	1.28					
		397	1.28					2.50

Table S 5.3 Continue

Locus		Alleles	Fight	Tera	Moa	Tura	Pall	Mata
33D	N _A	31	7	10	11	10	16	15
	N _{PA}	17	0	0	0	1	9	7
		349				1.02		
		352						2.78
		355						13.89
		358						11.11
		361						13.89
		364						5.56
		367						2.78
		370						5.56
		388		3.06	1.06	2.04		
		391	40.00	25.51	28.72	28.57	5.21	
		394	2.86		4.26	8.16		
		397		2.04	1.06	1.02		8.33
		400		2.04	2.13			2.78
		403		14.29	13.83	11.22	2.08	
		406	1.43	34.69	30.85	32.65		
		409		5.10	1.06	3.06		5.56
		412	51.43	9.18	11.70	9.18		2.78
		415	1.43	3.06	3.19	3.06	2.08	8.33
		418	1.43	1.02	2.13		3.13	8.33
		421					3.13	
		424					44.79	
		427					2.08	2.78
		430	1.43				2.08	
		433					2.08	
		436					21.88	5.56
		439					4.17	
		442					1.04	
		463					1.04	
		475					1.04	
		478					3.13	
		481					1.04	
57B	N _A	13	4	7	7	8	7	8
	N _{PA}	5	0	0	1	0	0	4
		281						10.00
		293				1.00	1.00	7.50
		296	30.77	3.00	2.00	4.00	43.00	20.00
		299	5.13	4.00	4.00	3.00	8.00	
		302	62.82	64.00	69.00	53.00	22.00	
		305	1.28	2.00		2.00		
		308		6.00	4.00	3.00	2.00	12.50
		311		18.00	18.00	33.00	23.00	5.00
		314		3.00	1.00	1.00	1.00	
		317			2.00			
		332						12.50
		335						30.00
		338						2.50

Table S 5.4 Second series of AMOVA tests exploring variation within clusters and groups.

Groupings are given in brackets. A) The best grouping within the Cook Strait Cluster was found with [Fi][Te/Mo/Tu] (test No. 5; $F_{CT/SC} = 24.7$). B) No significant variation was found within Grater Wellington group. C) F_{ST} between Cape Palliser and Mataikona is significant but F_{IS} is not indicating differentiation between Cape Palliser and Mataikona with no subsequent structure in either of these groups. Fi, Fighting Bay; Te, Cape Terawhiti; Mo, Moa Point; Tu, Turakirae Head; SS, Sum of squares; *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$; ^{ns}, not significant.

	SS	% variation	Fixation indices
A) within Cook Strait Cluster			
Test No. 1: [Fi/Te/Mo/Tu]			
Among locations	263.6	3.20427	$F_{LT} = 0.03204$ **
Among individuals	4004.8	4.25371	$F_{IL} = 0.04395$ ^{ns}
within locations			
Within individuals	3741.5	92.54201	$F_{IT} = 0.07458$ ^{ns}
Total	8009.9		
Test No. 2: [Fi/Te][Mo/Tu]			
		$F_{GT/LG} = 0.1$	
Among groups	93.2	0.19881	$F_{GT} = 0.00199$ ^{ns}
Among locations within	170.4	3.06932	$F_{LG} = 0.03075$ *
groups			
Among individuals	4004.8	4.25091	$F_{IL} = 0.04395$ ^{ns}
within locations			
Within individuals	3741.5	92.48096	$F_{IT} = 0.07519$ *
Total	8009.9		$F_{ST} = 0.03268$ **
Test No. 3: [Fi/Tu][Te/Mo]			
		$F_{GT/LG} = 0.3$	
Among groups	53.8	-1.29989	$F_{GT} = -0.01300$ ^{ns}
Among locations within	209.8	4.13101	$F_{LG} = 0.04078$ ***
groups			
Within locations	7746.3	97.16888	
Total	8009.9		$F_{ST} = 0.02831$ ***
Test No. 4: [Fi/Mo][Te/Tu]			
		$F_{GT/LG} = 0.0$	
Among groups	89.5	0.04245	$F_{GT} = 0.00042$ ^{ns}
Among locations within	174.1	3.17557	$F_{LG} = 0.03177$ *
groups			
Among individuals	4004.8	4.25311	$F_{IL} = 0.04395$ ^{ns}
within locations			
Within individuals	3741.5	92.52887	$F_{IT} = 0.07471$ ^{ns}
Total	8009.9		$F_{ST} = 0.03218$ **
Test No. 5: [Fi][Te/Mo/Tu]			
		$F_{GT/LG} = 24.7$	
Among groups	232.1	7.6603	$F_{GT} = 0.07660$ *
Among locations within	31.6	-0.27795	$F_{LG} = -0.00310$ ^{ns}
groups			
Among individuals	4004.8	4.07011	$F_{IL} = 0.04395$ ^{ns}
within locations			
Within individuals	3741.5	88.54754	$F_{IT} = 0.11452$ ^{ns}
Total	8009.9		$F_{ST} = 0.07382$ ***

Table S 5.4 Continue

	SS	% variation	Fixation indices
Test No. 6: [Mo][Fi/Te/Tu]		$F_{GT/LG} = 0.8$	
Among groups	12.3	-3.85211	$F_{GT} = -0.03852^{ns}$
Among locations within groups	251.3	5.26677	$F_{LG} = 0.05071^{***}$
Among individuals within locations	4004.8	4.33236	$F_{IL} = 0.04395^{ns}$
Within individuals	3741.5	94.25298	$F_{IT} = 0.05747^{ns}$
Total	8009.9		$F_{ST} = 0.01415^{***}$
Test No. 7: [Tu][Fi/Te/Mo]		$F_{GT/LG} = 0.3$	
Among groups	69.5	-0.99277	$F_{GT} = -0.00993^{ns}$
Among locations within groups	194.1	3.72883	$F_{LG} = 0.03692^{***}$
Among individuals within locations	4004.8	4.27429	$F_{IL} = 0.04395^{ns}$
Within individuals	3741.5	92.98965	$F_{IT} = 0.07010^{ns}$
Total	8009.9		$F_{ST} = 0.02736^{***}$
Test No. 8: [Te][Fi/Mo/Tu]		$F_{GT/LG} = 0.7$	
Among groups	26.1	-3.16586	$F_{GT} = -0.03166^{ns}$
Among locations within groups	237.5	4.9083	$F_{LG} = 0.04758^{**}$
Among individuals within locations	4004.8	4.31796	$F_{IL} = 0.04395^{ns}$
Within individuals	3741.5	93.93961	$F_{IT} = 0.06060^{*}$
Total	8009.9		$F_{ST} = 0.01742^{***}$
Test No. 9: [Fi/Mo][Te][Tu]		$F_{GT/LG} = 0.6$	
Among groups	117.7	-3.85164	$F_{GT} = -0.03852^{ns}$
Among locations within groups	145.9	6.51089	$F_{LG} = 0.06269^{**}$
Among individuals within locations	4004.8	4.27767	$F_{IL} = 0.04395^{ns}$
Within individuals	3741.5	93.06308	$F_{IT} = 0.06937^{ns}$
Total	8009.9		$F_{ST} = 0.02659^{***}$
B) within Greater Wellington Group			
Among locations	31.6	-0.24654	$F_{LT} = -0.00247^{ns}$
Among individuals within locations	3006.2	3.41366	$F_{IL} = 0.03405^{ns}$
Within individuals	2863.5	96.83288	$F_{IT} = 0.03167^{ns}$
Total	5901.3		
C) within Wairarapa Cluster			
Among locations	6690.8	63.94669	$F_{LT} = 0.63947^{***}$
Among individuals within locations	4662.3	0.38164	$F_{IL} = 0.01059^{ns}$
Within individuals	4713	35.67166	$F_{IT} = 0.64328^{***}$
Total	16066		

Table S 5.5 Third series of AMOVA tests exploring variation among and within sites. No significant values were found. SS, Sum of squares; ^{ns}, not significant.

	SS	% variation	Fixation indices
Fighting Bay			
Among sites	1.8	0.59841	$F_{ST}=0.00598^{ns}$
Among individuals within sites	26.3	-0.59415	$F_{IS}=-0.00598^{ns}$
Within individuals	29	99.99574	$F_{IT}=0.00004^{ns}$
Total	57.1		
Cape Terawhiti			
Among sites	36.5	-2.02083	$F_{ST}=-0.02021^{ns}$
Among individuals within sites	730.2	-10.55157	$F_{IS}=-0.10343^{ns}$
Within individuals	998	112.5724	$F_{IT}=-0.12572^{ns}$
Total	1764.7		
Moa Point			
Among sites	98.3	1.27307	$F_{ST}=0.01273^{ns}$
Among individuals within sites	894.2	7.67872	$F_{IS}=0.07778^{ns}$
Within individuals	849.5	91.04821	$F_{IT}=0.08952^{ns}$
Total	1842		
Turakirae Head			
Among sites	67.4	-2.13079	$F_{ST}=-0.02131^{ns}$
Among individuals within sites	1179.7	12.97205	$F_{IS}=0.12701^{ns}$
Within individuals	1016	89.15874	$F_{IT}=0.10841^{ns}$
Total	2263.1		
Cape Palliser			
Among sites	314.2	4.11525	$F_{ST}=0.04115^{ns}$
Among individuals within sites	1945.3	8.3317	$F_{IS}=0.08689^{ns}$
Within individuals	1827	87.55305	$F_{IT}=0.12447^{ns}$
Total	4086.5		
Matakona			
Among sites	93.3	-3.48327	$F_{ST}=-0.03483^{ns}$
Among individuals within sites	2309.5	-5.66694	$F_{IS}=-0.05476^{ns}$
Within individuals	2886	109.15021	$F_{IT}=-0.09150^{ns}$
Total	5288.8		

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Chapter 6

Taxonomic confusion in *Lessonia* from the Falkland Islands

6.1 Abstract:

Since the first descriptions by Bory de Saint-Vincent between 1825 and 1828, the taxonomy and affiliation of Falkland Island *Lessonia* has confused phycologists. To date seven *Lessonia* species have been described or assigned to these islands in the centuries following Bory de Saint-Vincent, even though only two to three Falkland Island species have been distinguished by authorities at any time. The reason for this is the altered interpretation of Bory de Saint-Vincent's descriptions of algal material that apparently included samples from two *Lessonia* species found on Falkland Island. Bory de Saint-Vincent described his material as one species, but twice in successive years using different epithets. Here I clarify the entangled typification and nomenclature of the two *Lessonia* species found on Falkland Island.

Keywords: *Lessonia flavicans*, *Lessonia fuscescens*, *Lessonia frutescens*, *Lessonia ovata*, *Lessonia vadosa*, Falkland, typification, nomenclature, *Lessonia searlesiana*.

Protologue [ICBN Appendix VII]. Everything associated with a name at its valid publication, i.e., description or diagnosis, illustrations, references, synonymy, geographical data, citation of specimens, discussion, and comments (McNeill et al. 2006).

Original material [ICBN Art.9.2 Note 2]. For the purposes of this Code, the original material comprises: (a) those specimens and illustrations (both unpublished and published either prior to or together with the protologue) upon which it can be shown that the description or diagnosis validating the name was based; (b) the holotype and those specimens, which, even if not seen by the author of the description or diagnosis validating the name, were indicated as types (syntypes or paratypes) of the name at its valid publication; and (c) the isotypes or isosyntypes of the name irrespective of whether such specimens were seen by either the author of the validating description or diagnosis, or the author of the name (but see also Art. 7.7, second sentence, and 7.8) (McNeill et al. 2006).

6.2 Introduction

Since the first descriptions by Bory de Saint-Vincent between 1825 and 1828 the taxonomy and affiliation of Falkland Island *Lessonia* has confused phycologists (Tab. 6.1). Five authorities have subsequently described *Lessonia* species from the Falkland Islands (Bory de Saint-Vincent 1825 & 1828, Harvey & Hooker 1847, Skottsberg 1907 & 1921, Searles 1978, Asensi & de Reviers 2009). The circumstances that have led to the description of new species and which species they initially recognised will be introduced under the headline of the authority.

6.2.1 Bory de Saint-Vincent:

During the French voyage of the ‘Coquille’ under Duperrey (during 1822-25), considerable attention to Falkland Island plants was paid by Durmont d’Urville, the second in command and botanist. The main purpose of the journey was more scientific than exploratory, thus a second naturalist, Lesson, was also on board (anonymous 1859). After the voyage, the collections of the cryptogams were given to Bory de Saint-Vincent who determined and described the samples. D’Urville, probably working on his career dream to become a commander (Dunmore 2007), urged publication, thus already in 1825 the *Flore des Iles Malouines* (d’Urville 1825) was separately published in advance of the paper in the *Memoires of the Linnean Society of Paris* (d’Urville 1826). In the ‘*Flore des Iles Malouines*’, d’Urville described *Lessonia flavicans* in the general narrative part about the voyage but he referred to Bory de Saint-Vincent as the Authority (d’Urville 1825). Only a very brief description of *Lessonia flavicans* was given (Box A) in the attached species list, for which Bory de Saint-Vincent was responsible (Bory de Saint-Vincent 1825). The note was a pre-digest of the later published volumes about the botany of the

circumglobal voyage of the Coquille (Bory de Saint-Vincent 1828). No type was specified and no drawings were made. As only a single species was described and as the genus *Lessonia* was not described, the species description validates the description of the genus (Art. 42 ICBN, as recognised by Searles 1978, McNeill et al. 2006).

Bory de Saint-Vincent (1826) described the genus *Lessonia* in the ninth volume of the Dictionnaire Classique d'Histoire Naturelle together with three new *Lessonia* species; *L. fuscescens* (Box A), *L. nigrescens* (reported, by Bory de Saint-Vincent, from Cape Horn but not from the Falklands), and *L. quercifolia* (now known as *Myriodesma quercifolium* (Bory) J. Agardh). No types were specified and no drawings made, but the species was valid published.

Between 1827-1829 the volumes about the botany of the circumglobal voyage of the Coquille were released together with an atlas of 38 plates drawn by Bory de Saint-Vincent. In great detail Bory de Saint-Vincent described the three *Lessonia* species *L. flavicans*, *L. nigrescens*, and *L. quercifolia* (Bory de Saint-Vincent 1828). The entry of *L. flavicans* starts:

“Lessonie brunissante, *Lessonia fuscescens* (pl. 2, fig. 2, et pl. 3), N. Dict. class. d'hist. nat., tom. IX, p. 322; *Lessonia (flavicans)*, [...]”

followed by the same Latin description Bory de Saint-Vincent used for *L. flavicans* in 1825 and an account in French that started with a direct quote of the complete narrative part made by d'Urville (1825) in Flore des Iles Malouines, where *L. flavicans* was originally described. Note, however, that Bory de Saint-Vincent put *flavicans* in brackets and also listed *L. fuscescens* as a synonym.

6.2.2 Joseph Dalton Hooker & William Henry Harvey

Two decades later in 1847 another species was described from the Falkland Island as a result of the voyage of H. M. Discovery Ships “Erebus” and “Terror” in the years 1839 and 1843 under the command of Captain Sir James Clark Ross. The botanist and the youngest of the 128 man crew on the ship was J. D. Hooker, who, together with W. H. Harvey, described *L. ovata* as a new species in Hooker’s book, known as ‘Flora Antarctica’ (Hooker 1847). From the Falklands, Hooker and Harvey recognised three *Lessonia* species; *L. fuscescens* (putting *L. flavicans* in a synonymous status), *L. nigrescens* [however, no *L. nigrescens* is found on the Falklands (Searles 1978) and they do not have air-cavities as described and drawn by Hooker (fig. 167-168. C)], and a new species *L. ovata* (Harvey & Hooker 1847) (Box A). In 1855 the Atlas of the Flora was printed where Hooker dedicated three plates to *Lessonia*, illustrating fronds and whole individuals of *L. fuscescens* (fig. 167-168. A & fig. 171. D) and *L. ovata* (fig. 167-168. B & fig. 171. C) in relationship to *Macrocystis*. About *L. fuscescens* they wrote that it is found always far beyond low-water mark and that it has lacunae (air-cells) (fig. 167-168. A1). They did not explain why they accepted *L. fuscescens* instead of *L. flavicans*.

6.2.3 Carl Skottsberg

For his PhD, Skottsberg worked on the Antarctic and Sub-Antarctic marine algae with a focus on the ‘Phaeophyta’. He made extensive collections during the Swedish Antarctic expedition between 1901 and 1903 on Grahams Land (Antarctic Peninsula), South Georgia, Terra del Fuego, and the Falkland Islands. On the Falklands, he recognized *L. nigrescens*, *L. flavicans* (while using *L. fuscescens* and *L. ovata* as synonyms), and a new species *L. frutescens* (Box A). However, no *L.*

nigrescens is found on the Falklands (Searles 1978); furthermore, *L. nigrescens* does not have lacunae (Schleimraeme) as described and illustrated by Skottsberg (1907) but it is possible that he followed the description made by Hooker (1847), who also recognised *L. nigrescens* on the Falkland Islands and who depicted them with lacunae (air-cavities) (Scottsberg 1921). Skottsberg's (1907) detailed description of *L. nigrescens* is identical to the later described *L. vadosa*, indicating a missidentification. About *L. flavicans* he stated that Bory de Saint-Vincent (1828) changed the name into *L. fuscescens* while putting *L. flavicans* in brackets, however, he recognised *L. flavicans* as the older name. Skottsberg referred to Hooker & Harvey (1847 p.459) when he described *L. ovata* as a species that is smaller (but treelike) than *L. flavicans* with laminae that are broader at the base with a round stipe. However, he found round stipes together with laminae that are narrow at the base and laminae with a wide base that merge into very flat stipes. His material indicated to him that a roundish, blunt base is characteristic of young individuals, whereas in older algae the base of the laminae becomes progressively narrow and the whole blade becomes longer and narrower. As this supported Hooker and Harvey's speculation that *L. ovata* might be the younger stage of *L. fuscescens*, Skottsberg treated *L. ovata* as a synonym of *L. flavicans* (*L. fuscescens*).

Skottsberg (1907) noted that *L. frutescens* differs from *L. ovata* as it is shorter and has more stipes. *L. frutescens* differs from *L. flavicans* as in *L. flavicans*, even in young algae, the stipes are already longer than in *L. fuscescens* and the blades are narrower and dentate. The holdfast is weaker than in *L. flavicans*. *L. frutescens* is found in the upper sublittoral and is often fully exposed during ebb tide.

Numerous mucilage cavities can be found in the blade, however, none were found in haptera or young parts of the stipe.

Skottsberg admitted in his 1921 paper: “Still, it is possible that *L. frutescens* is nothing but a local form of *L. nigrescens*”. In the same publication he described two new algae, *L. nigrescens* f. *montagnei* and *L. nigrescens* f. *lacunifera*. He explains: “No ‘true’ *L. nigrescens* has been found in the Falkland Islands. Still, from reasons given under *L. flavicans*, I retain the Falkland plant under the old name, adding ‘f. *lacunifera*’ for convenience sake.” And to the ‘true’ *L. nigrescens* he added ‘f. *montagnei*’ (Skottsberg 1921).

6.2.4 Richard B. Searles

Searles (1978) made an extensive survey of the *Lessonia* species in Southern Chile and Argentina. Whereas some scientists (e.g., Agardh 1877) already assumed that lacunae might be species specific, Searles (1978) was the first who used this character to distinguish between the two species recognized by him on the Falkland Islands. The species are: *L. flavicans* (with *L. fuscescens*, *L. ovata*, and *L. frutescens* as synonyms) and the new described *Lessonia vadosa*. He also excluded any records of *L. nigrescens* from the Falkland Islands (see Hooker & Harvey and Skottsberg).

As no holotype was assigned to *L. flavicans* he assigned a lectotype from Bory de Saint-Vincent’s herbarium, which comprises samples made by d’Urville and Lesson, choosing a specimen collected by Lesson on the Falkland Islands (Searles 1978). The specimen had a richly branched holdfast, a single stipe and entire blades that lack lacunae. Why this specimen was chosen is not clear, however it fitted the description of *L. ovata*, which was placed in a synonymous status. As Skottsberg (1907) had not assigned a holotype to his *L. frutescens*, Searles also assigned a

lectotype to this species, recognising the algal specimen depicted in the original description in Skottsberg's herbarium. When he found that the lectotype bears lacunae in the blade, in contrast to Skottsberg's description, he decided to assign *L. frutescens* as a synonymy of *L. flavicans* and described a new species, *L. vadosa* (Searles 1978) (Box A).

Searles (1978) adds to the description that "Plants of this species (fig. 9) are differentiated from *L. flavicans* by their narrow blades (fig. 3), wider blade base angle relative to the blade width (fig. 4), lacunate cortex and a shallow, subtidal habitat" whereas *L. flavicans* is found in deeper water.

6.2.5 Aldo Asensi & Bruno de Reviers

The latest attempt to clarify the situation was undertaken by Asensi & deReviers (2009), who found that Searles has chosen the wrong lectotype for *L. flavicans*, from material that was actually not eligible. The reason is that Searles (1978) has chosen an individual collected by Lesson, however, Lesson was not mentioned in the first description of *Lessonia flavicans* (d'Urville 1825), and thus only material collected by d'Urville, who wrote the narrative to the original description, should be treated as original material, from which types can be selected (Asensi & deReviers 2009). Bory de Saint-Vincent (1825) has not indicated any type location however d'Urville mentioned in the protolog that *Lessonia* is often found at the coasts of l'île aux Pingouins [Long Island, Berkeley Sound, East Falkland Island] (d'Urville 1825). Accordingly, Asensi & de Reviers (2009) chose a new lectotype of *L. flavicans* collected by d'Urville at l'île aux Pingouins on the Falkland Island. Among the original material they were able to choose the very same individual that was depicted by Bory de Saint-Vincent in the atlas of the 'voyage autour du monde' (Bory de

Saint-Vincent 1828). Their microscopic analyses of the blade anatomy revealed lacunae in the new lectotype of *L. flavicans*, and consequently *L. vadosa* was regarded as a taxonomic synonym to the new *L. flavicans sensu* Asensi & de Reviers. Thus not only the lectotype has changed but also the designation of the species and the species *L. flavicans sensu* Searles was now nameless. Asensi & de Reviers (2009) designated an individual among Searles' original material of *L. flavicans sensu* Searles to describe the new species *L. searlesiana*. What was known as *L. flavicans sensu* Searles is now *L. searlesiana* and what was known as *L. vadosa* is now *L. flavicans*. No taxonomic synonyms have been listed for either of the new designated species except for *L. vadosa* as a synonym for *L. flavicans*.

6.3 Discussion

6.3.1 Species one: *L. flavicans*

Bory de Saint-Vincent's (1825) description of *L. flavicans* is short and from today's perspective both Falkland Islands *Lessonia* species can be assigned to this description, thus the designation of a type specimen is essential. *L. flavicans* was validly published (ICBN Art. 32.1, McNeill *et al.* 2006) as shown by Chamberlain (1965) on the example of *Iridaea undulosa*. The protologue to the species comprises the narrative part made by d'Urville and the species description made by Bory de Saint-Vincent (ICBN Appendix VII, McNeill *et al.* 2006). As no holotype was specified and no drawings have been made, the protologue is used to gather information in order to assign a species to the name or the authority to an autonym (e.g., in the narrative d'Urville validated the autonym *L. flavicans* and assigned it to Bory de Saint-Vincent when he finished with the sentence "C'est cette plante que *M. Bory de Saint-Vincent* a nommée *Lessonia flavicans*") (McNeill *et al.* 2006). The

additional information given by d'Urville (1825) includes information about the possible type locality (“[...] l’île aux Pingouins”) where *Lessonia* is very common; but also about the stipe, the blades and the holdfast when he writes, “Its trunk, which is cylindrical, somewhat wooden and often as thick as a thigh, divides into multiple-dichotomous and compressed branches that always end with spear-like, undulating, smooth and fine dentate fronds. It is seldom longer than 8-10m (quatre ou cinq toises), and I never saw the fronds reaching out of the water. They usually reach only until a few inches (pouces) to the sea surface, where they form relatively dense foliations. The stem sticks to stones or other solid bodies with a fleshy, thick and somewhat thick-bordered disk, which is sometimes divided into three or four parts at its base. Without penetrating the material on which it grows it adheres so strongly that one would break the trunk instead of detaching it...” [Translated from the French original by S. Bourguignon].

In Bory de Saint-Vincent’s herbarium both species are found under the name *L. fuscescens* but the samples given to Bory de Saint-Vincent by Lesson show *L. flavicans sensu* Searles whereas the samples given to Bory de Saint-Vincent by d’Urville show *L. flavicans sensu* Asensi & de Reviers (the samples I have seen are PC0062744, PC0062747, PC0062748, PC0062749, PC0062750; preserved at PC). D’Urville’s (1825) description in the protologue, especially the characters of the algal height, thickness of the stipe and the morphology of the holdfast, best describes the alga shown in Fig. 1.7 D&F (Chapter 1) and fig. 167-168 A & fig. 171 D in Harvey & Hooker (1847). As this is most likely the same species as shown on the herbarium vouchers made by d’Urville, I will follow the re-typification as proposed by Asensi & deReviers (2009). Asensi & deReviers (2009) also argue that Bory de

Saint-Vincent (1828) might have preferred *L. fuscescens* as the species epithet as he put *flavicans* in brackets when he combined the two species into one. However, brackets were used in this series to emphasise species names not the other way around, thus the plea to conserve the name *L. fuscescens* against *L. flavicans* (Asensi & deReviers 2009) is not justified.

An emended species description could be the one used by Searles (1978) for *L. vadosa* (see diagnosis of *L. vadosa* in Box A). Note that the alga might become bigger as indicated in Searle's description (see d'Urville 1825 and Fig. 1.7 C&D). In the original paper referring to *L. vadosa* Searles (1978) indicated different growth forms: "Plants of the sublittoral fringe appear to be limited in height by the water depth and are short and bushy. These small bushy plants might also occasionally initiate an adventitious erect stipe from the haptera of the holdfast; the basal segment of the stipe might be short and thick. Larger plants from slightly deeper water have a long unbranched lower stipe and a bushy crown (fig. 11). The dichotomies of the branch system in this species are often asymmetric; the larger of a pair of blades divides more frequently and leaves the smaller blade behind on the stipe in a pattern reminiscent of *Macrocystis*, but not as pronounced. The smaller blade and branch might subsequently break off and the scar is smoothed over by growth of the meristoderm to produce a long, unbranched lower stipe."

A few species have been described for the Falkland Islands and some must be considered as synonyms to this typification, whereas others most likely refer to a second species. The ones to be considered as synonyms to *Lessonia flavicans* Bory, 1825 are:

Lessonia vadosa Searles, 1978 (as recognised by Asensi & deReviers (2009)); and *Lessonia nigrescens* f. *lacunifera* Skottsberg, 1921.

Misapplied names: *L. nigrescens sensu* Hook. *fil.* & Harv. 1847; *L. nigrescens sensu* Skottsberg, 1907.

Descriptions that might refer to a second species are *Lessonia fuscescens* Bory, 1826; *Lessonia ovata* J.D. Hooker & Harvey, 1847; *Lessonia frutescens* Skottsberg 1907 (partim. lectotype without lacunae (Searles 1978)); *Lessonia searlesiana* Asensi & de Reviers, 2009.

6.3.2 Species two: *L. fuscescens* [?], *L. ovata*, (*L. frutescens*, (*L. searlesiana*))

6.3.2.1 *L. searlesiana*

L. searlesiana was described in 2009 by Asensi & de Reviers to account for the problem that *L. flavicans sensu* Searles would be without a name after the new lectotypification of *L. flavicans* (Asensi & deReviers 2009). Yet, as not only the lectotype changed but also the concept of *L. flavicans* every species that was in synonymy with the old meaning would have been available.

6.3.2.2 *L. frutescens*

No type specimen was assigned to *L. frutescens* by its authority Skottsberg (1907). On March 15 in 1978 Searles lectotypified *L. frutescens* using the only specimen available in Skottsbergs collection, and which is identical to the picture (taf.8) in Skottsberg (1907). For the description, Skottsberg (1907) could not use a specimen from his own collection as only pieces of the specimen he had sampled at Berkeley Sound were available and did not yield useful information. This lack was because the ‘Antarctic’, the ship utilized for the Swedish South Polar expedition, foundered on

February 12, 1903 and with it its valuable scientific cargo. Skottsberg could only save a packet from the sinking ship that included some algae on herbarium sheets. The biggest part of the Falkland collection, however, was packed and brought to land earlier (Skottsberg 1907 p.1). Thus he used a specimen collected by S. Birger at Port Stanley a year later (Skottsberg 1907 p.78 footnote). Parts of the diagnosis (especially habitat descriptions) do not correspond to the lectotype, which is based on the figure (taf. 8) of the parologue. In plant nomenclature, figures, drawings, and types have priority to diagnoses and consequently Searles had to move *L. frutescens* into synonymy with *L. flavicans sensu* Searles when he found, contrary to the description, no lacunae in the cortex of the blade. The lectotype of *L. frutescens* corresponds to the concept of *L. flavicans sensu* Searles (wide blade base angle, width of blade, holdfast structure, and with lacunae) and would have had priority over *L. searlesiana*. However, the alga Skottsberg wanted to describe was a different one that grows in the intertidal, has lacunae in the blade, and is only found on Falkland (e.g., Fig. 1.7 A).

6.3.2.3 *L. ovata*

L. ovata was discovered during the English voyage of the ships Erebus and Terror to the Antarctic in the years 1839-1843 and described by J.D. Hooker (who was assistant surgeon and botanist to the expedition) and Harvey in 1847 (Hooker 1847). No type was assigned but Hooker made some nice drawings in an atlas that was released in 1855 (Hooker 1855). In the 1847 publication he referred to the drawings within the atlas in the diagnosis to *L. ovata* (Tab. CLXVII.-CLXVIII. B; et Tab. CLXXI. C.). Searles (1978) writes that the *L. ovata* plates CLXVII.-CLXVIII. B and B1 correspond to *L. flavicans* and that Plate CLXXI could represent either *L.*

flavicans or *L. vadosa*. He also wrote that he could not find any type specimen in the herbaria of Kew, the British Museum or Trinity College corresponding to Hooker & Harvey's description. At Trinity College there are no *L. ovata* specimens (pers. comm. with Professor John Parnell, Head of the School of Natural Sciences, Trinity College Dublin, Dublin). Kew has given their algae collection on permanent loan to the British Museum (pers. comm. Prof. David J. Mabberley, Keeper of the Herbarium, Library, Art & Archives, Royal Botanic Gardens, Kew) but their specimens of *L. ovata* are preserved; two bearing the handwriting of J.D. Hooker stating "*Lessonia ovata* Fl. Ant. Antarctic Sea J.D.H." (K in BM without number). Both sheets are very similar to the drawing in Hooker's Atlas (Hooker 1855) and are indicated with "Fl. Ant." for Flora Antarctica, the short name of Hooker's book from 1847.

As no types are assigned to *L. ovata* it is necessary to designate a lectotype (ICBN, Art. 9.9; McNeill *et al.* 2006). I suggest this should be one of the two herbarium specimens found in BM and the other should be an isolectotype. Both specimens have the appearance of *L. searlesiana* and thus might have priority as being the older name although additional anatomic study might be required. I discovered unpublished drawings attached to a herbarium sheet of a alga determined to be *L. ovata* and collected by Moseley at Heard Island (BM000937207). These drawings, which are made of *L. ovata* by Hooker (with unknown handwriting stating "drawings of type"), show sections of blade and stipe. Within the blade and stipe, no lacunae are drawn but there is a large area of ruptured cells in the blade section.

6.3.2.4 *L. fuscescens*

After Bory de Saint-Vincent described *L. flavicans* in 1825 he described another species *L. fuscescens* a year later (Bory de Saint-Vincent 1826) with a different diagnosis. As noted above, both species are found in Bory de Saint-Vincent's herbarium under the epithet *L. fuscescens*, however, the ones collected by d'Urville (PC0062749, PC0062750) are different to the ones collected by Lesson (PC0062744, PC0062747, PC0062748). In the diagnosis to the new species, Bory de Saint-Vincent referred to Lesson as the collector and 'Conception du Chili' as the type locality. Thus Bory de Saint-Vincent used a different sample from a different collection (Lesson's collection) when he made the diagnosis to *L. fuscescens* (as a reminder: d'Urville's collection is believed to be the source for the description of *L. flavicans* and based on the protologue in d'Urville (1825) 'l'île aux Pingouines' is considered to be the type locality). As far as I know, there is only one sample in Bory de Saint-Vincent's collection labelled as being from Concepción, Chile (PC0062747). At the bottom of the sheet, Bory de Saint-Vincent noted that it was given to him by Lesson. For this reason it is not mandatory to assume that for his description of *L. fuscescens* Bory (1826) 'definitely included the type' of *L. flavicans*, and therefore this name is neither superfluous nor can it be treated as *nomen illegitimum* (ICBN Art. 52.1, McNeill *et al.* 2006) as suggested by Searles (1978) and Asensi & deReviers (2009). That two years later Bory de Saint-Vincent (1828) merged the two species into one with the name *L. flavicans* is for taxonomic decisions irrelevant as only information known at the time of publication are essential (ICBN Art. 52.1, McNeill *et al.* 2006). Bory de Saint-Vincent (1826) also writes that d'Urville reported that 'it' [*L. fuscescens*] is found on the Falkland Islands, where it grows in great quantities at some distance to the shore (see

introduction to this Chapter for the original text). As *L. flavicans* (the one d'Urville sampled) is found close to the shore and the second species is found in some distance from the coast (Searles 1978; remember that Searles used the opposite meaning of the epithet) this might indicate that d'Urville was aware of two species at Falkland.

A type has not been assigned so far, thus I suggest to lectotypify (ICBN, Art. 9.9; McNeill *et al.* 2006) the sample, which is preserved under the number PC0062747 at the 'Museum National d'Histoire Naturelle' in Paris (PC) for reasons given above. However, there are possibly two different species mounted; one with slightly narrower blades on the left and another with broader blades on the right. It has to be carefully examined to determine whether they belong to one or two species. As the collection site of Concepción is out of the species range reported for the *Lessonia* species also found on the Falklands, it is possible that one or both morphotypes are identical to *L. trabeculata*. If one or both morphotypes, however, are found to have no lacunae in the cortex it would indicate that the species range of *L. ovata* has to be extended to Concepción. Montagne (1852) reported *L. ovata* from Concepción; he also found that *L. fuscescens* and *L. ovata* have been mixed by Bory de Saint-Vincent in d'Urville's collection, but he might have confused Lesson's collection with d'Urville's collection as in d'Urville's collection no specimen from Concepción was found. Synonym status for *L. ovata*, *L. frutescens* and *L. searlesiana* species to *L. fuscescens* might be possible but has to be further investigated.

6.4 Conclusions

Nobody has shown so far that the occurrence of lacunae is species specific i.e., correlated with genetic evidence. However, based on morphology two different species were separated by Searles (1978). Blade width and the depth where the alga was collected have been correlated to lacunae (Searles 1978), thus individuals found in greater depth, with broad blades (> 8cm in width), and no lacunae in the blade were referred to *L. flavicans sensu* Searles. For the reasons explained above they were newly described as *L. searlesiana* (Asensi & deReviers 2009). But the Latin diagnosis (see Skottsberg (1907), p.78; and introduction above) and the lectotype (S: A1388; assigned by Searles 19 May 1979) of *L. frutescens* describe the same species; therefore *L. frutescens* has priority over *L. searlesiana*. However, the even earlier described *L. ovata* (Harvey & Hooker 1847) has, based on Latin and English description and morphology (blade width), priority over both epithets. A type has to be assigned to *L. ovata* and I suggest one of the two samples determined as *L. ovata* and signed by Hooker, which are found in BM.

Even though *L. flavicans* and *L. fuscescens* have often been used as synonyms there is no need to believe that they are based on the same type material (as suggested by Searles 1978 and Asensi & deReviers 2009). Whereas *L. flavicans* is based on a sample from “Île aux Pingouins” in d’Urville’s collection (Asensi & deReviers 2009), *L. fuscescens* is, according to the protologue (Bory de Saint-Vincent 1826), based on an individual sampled by Lesson in Concepción, Chile. As no type is assigned so far, I suggested PC0062747 (PC), as it is the only sample known to me that was collected by Lesson at “Concepción au Chili”. Based on the

sample site it is most likely a *L. trabeculata*-like individual but additional anatomic study is required.

6.5 Tables

Box A: Original diagnosis of species found on the Falkland Islands and their English translations (translations of *L. flavicans*, *L. fuscescens*, *L. ovata*, and *L. frutescens* made by P. Martin; the other translations are given by the authorities):

***Lessonia flavicans*.** *Caule sub-arboreo cylindrico, ramis compressis, foliis ovato-linearibus, subdenticulatis, flavicantibus.* (Bory de Saint-Vincent 1825, n° 29)

Lessonia flavicans. Stipe somewhat treelike cylindrical, branches compressed, blades oval-linear, somewhat denticulated, yellowish.

***Lessonia fuscescens*, N.,** *a tige arborescente, inferieurement simple, se divisant a son extremite en rameaux nombreux, cylindriques, qui a leur tour se fourchent en ramules entrelacees, fort comprimees, noiratres, supportant des frondes lineaires ou ovaes-allongees, acuminees inferieurement et superieurement, a bords legerement ou fort obscurement dentes quand ces bords ne sont pas d'une integrite parfaite. Cette espece nous fut d'abord communiquee par Lesson qui l'avait recueillie a la Concepción du Chili et par Durville qui l'a rapportee des iles Malouines, ou elle croit en grande quantite a quelque distance du rivage. Elle sera figuree dans la relation du voyage de la Coquille.* (Bory de Saint-Vincent 1828)

Lessonia fuscescens, N., stipe treelike, at the bottom simple, divides at the top into numerous, cylindrical branches, those further divide in to smaller, entangled, very

compressed, blackish branches, they support linear or oval-elongated fronds that acuminate at the bottom and top, the edges are fine or course dentated if not entirely smooth. This species was first communicated to us by Lesson, who collected the species at Concepción, Chile and by d'Urville, who reported that it is found on the Falkland Islands, where it grows in great quantities at some distance to the shore. It is correlated to the voyage of the Coquille.

Lessonia ovata, Hook. fil. et Harv.; stipite brevi vage dichotome ramoso, ramis brevibus divaricatis, frondis laciniis breviter petiolatis, petiolo in laminam ovatam lineari-ovatamve olivaceo-fuscescentem submembranaceam dilatato. (Tab. CLXVII.-CLXVIII. B; et Tab. CLXXI. C.)

Hab. Hermite Island, Cape Horn, and the Falkland Islands, very abundant.

Radix e fibris perplurimis crassis intricatis massam 1-2ped. latam efficientibus. Stipites e radice plurimi (ut in Macrocyti) 4-6 unc. longi, torti v. flexuosi, erassitie pollicis humanae, dichotome fissi, demum solitarii, incrassati, subarborescentes. Laminae pedales, colore et substantia L. fuscescentes, juniores basi obscure sinuato-dentatae; adultae integerrimae. (Harvey & Hooker 1847)

Lessonia ovata, Hook. fil. et Harv.; short stipes, here and there dichotomously branched, branches short and split at a wide angle, fronds shortly after the petiole cleaved, the petiole broadens into an oval or linear-oval, olive-darkish, thin (somewhat membranous) lamina. (Tab. CLXVII.-CLXVIII. B; et Tab. CLXXI. C.)

Hab. Hermite Island, Cape Horn, and the Falkland Islands, very abundant.

The root is formed from many thick fibres that form an entangled mass 30-60cm

broad. Stipes emerge numerous from the root (like in *Macrocystis*) they are 4-6 unc. [?] long, twisted or flexuous, *erassitie* a human thumb, split dichotomously till completely separated, thick, somewhat like a tree. The bases of the blades are of the colour and substance as *L. fuscescens*, the base in young individuals is weakly sinuated to dentated, but entire in adult algae.

L. frutescens Skottsberg *n. sp.* – Tab. 8. *Inde a basi dichotoma, stipite primario fere nullo; rami breves, crassi, 1,5-4cm longi, ± complanati. Folia ovato-lanceolata, basi rotundato-angustata, adulta 50-60 cm longa, 10-17 lata, margine integerrima, elastica. Hapterae numerosae, crassae, massam diam. circ. 15 cm formantes. Color fuscus vel olivacea-fuscus. Cetera ignota.* (Skottsberg 1907)

L. frutescens [becoming shrubby] *n. sp.* Splits dichotomous right at the base, primary stipe almost lacking; branches short, thick, 1.5-4cm long, ± flattened. Blades ovate-lanceolate, at the base narrow rounded, adult blades are 50-60cm long, 10-17cm broad, margins entire, elastic. Haptera numerous, thick, forming a mass of circa 15cm in diameter. Colour grey-brown or at least olivaceous-grey-brown. The rest is unknown.

Lessonia vadosa Searles *sp.nov.* *Plantae ad 3 m altae; una stipes dichotome vel subdichotome ramata, ex systemate hapterorum separatim ramatorum et distinctorum exorta. Rami minores ex furcis veterioribus in plantis magnis deperditi, quarum postea ima stipes inramata videtur. Laminae angustae, 2-6(9) cm latae, (8)12-68(89) cm longae; margines integri vel subdentati. Summa lamina uniformiter*

pigmentifera; cortex laminae et stipitis lacunas sphaericas vel ellipticas habet.

Lessonia vadosa. Plants to 3 m tall; one dichotomously to subdichotomously branched stipe arising from a system of freely branched, distinct haptera. Lesser branches of older dichotomies lost by large plants, the lower stipe then appearing unbranched. Blades narrow, 2-6(9) cm wide, (8)12-68(89) cm long, margins entire or with small teeth. Surface of blade uniformly pigmented, cortex of blade and stipe with spherical to ellipsoidal lacunae. (Searles 1978)

Lessonia searlesiana Asensi et Reviere, sp. nov.

Diagnosis: Species Lessonia, e meridiei regione quae ultra Oceanum spectat, denso cortice parenchymato instructa, in lamina tamquam in stipite, qua re a Lessonia berteriana differt, cujus cortex filis instructus est, differtque a Lessonia flavicanti lacunis absentibus, quibus lacunis illius speciei propriis, differtque tandem a Lessonia nigrescenti in systemate hapterorum quod stipitem unum gignit pro mole solida, quae nonnullos gignit; lamina angulusque ab ima laminae parte latius patent quam in Lessonia flavicanti et species altius quam Lessonia flavicans crescit.

South-American *Lessonia* species with a solid parenchymatous cortex of blades and stipe, not filamentous like *L. berteriana* and lacking the lacunae typical of *L. flavicans*; with only one stem originating from ramified haptera, not numerous ones from a massive holdfast like in *L. nigrescens*. Wider blades and wider blade base angle, compared to *L. flavicans*; vertical distribution extending deeper in subtidal than in *L. flavicans*.

Misapplied name: *L. flavicans sensu* Searles (1978) British Phycological Journal 13:

361-381.

Holotype: PC0124194 (Figure 13) (Duke University Algal Herbarium 16175 given by R.B. Searles to PC)

Type locality: “Just west of Punta Conway on Isla de los Estados, Argentina”

Collectors: R.B. Searles, G.L. Leister and J.F. Brauner on 4 May 1973.

Etymology: the epithet is in honour of R.B. Searles who first depicted the species.

(Asensi & deReviers 2009)

Table 6.1 Affiliation of the South American species (*L. flavicans*, *L. ovata*, *L. nigrescens*, and *L. trabeculata*) to selected authorities and their understanding of the species. *L. flavicans* has been confused with *L. fuscescens*, *L. nigrescens*, *L. frutescens*, *L. nigrescens* f. *lacunifera*, and *L. vadosa*; whereas, *L. ovata* has been confused with *L. fuscescens*, *L. flavicans*, *L. frutescens*, and *L. searlesiana*. N, number of species recognised on the Falkland Islands (species recognised on the Falkland Islands have a grey background); the first three letters of the name have been used for species and genera (if not *Lessonia*) abbreviation; na, not applicable; nm, not mentioned.

Reference	N	<i>L. flavicans</i> lacunae	<i>L. ovata</i> w/o lacunae	<i>L. nigrescens</i>	<i>L. trabeculata</i>	other	Remarks
Bory de Saint-Vincent 1825	1	<i>L. flavicans</i>					based on d'Urvills collection Type locality: l'île aux Pingouins, Falkland Lectotype: PC0062750
Bory de Saint-Vincent 1826	1	nm	<i>L. fuscescens</i> [?]	<i>L. nigrescens</i>	<i>L. fuscescens</i> [?]	<i>L. quercifolia</i>	<i>L.fus.</i> : based on Lesson's collection; Type locality: Concepción de Chili; might be identical to <i>L. trabeculata</i> (collection site), <i>L. searlesiana</i> (width of blade on right), or <i>L. flavicans</i> (not likely). No type has been assigned; thumbnail shows proposed type (PC0062747). <i>L.nig.</i> : based on Lamouroux collection [he called it <i>Laminaria ramosissima</i> (Bory 1826) or <i>ramosa</i> (nom.herb. PC0062752)]; Type locality: Cape Horn; [The name <i>Laminaria ramosissima</i> is not mentioned in Lamouroux (1813) despite it was claimed by Etcheverry (1951)]. Isolectotype (PC0062752) shown. <i>L.que.</i> : [currently <i>Myrodesma quercilolium</i>]

Table 6.1 continued

Bory de Saint-Vincent 1828	1	<i>L. flavicans</i>	<i>L. nigrescens</i>	<i>L. quercifolia</i>	Combined <i>L.fla</i> and <i>L.fus</i> into one species, which he called <i>L.fla</i> (Pl.3 in Bory de Saint-Vincent 1828). <i>L.nig</i> : (Pl.5 in Bory de Saint-Vincent 1828)
Suhr 1839	na	<i>Chordaria spicata</i>			Type locality: Valparaíso, fig. 41 [?], collector Lucas
Postels & Ruprecht 1840	?	<div> <i>L. fuscescens</i> (<i>L. flavicans</i>) </div> <div> <i>L. nig</i> <i>L. laminariaeoides</i> <i>L. ciliata</i> </div>			no reference to the collection site; the transverse sections of the blade of <i>L.fus</i> (Tab. XXXIX Fig. 15&18) show lacunae with trabeculae
Suhr 1841	na	<i>Laminaria scissa</i>			Type locality: Valparaíso
Agardh 1841	na	<i>L. suhrii</i>			<i>L.suh</i> : Changed the name <i>Chordaria spicata</i> into <i>Lessonia suhrii</i> [nom. illeg.; the comb. nov. <i>Lessonia spicata</i> should have been made] <i>Lam.sci</i> : <i>Laminaria scissa</i> can scarcely be distinguished from <i>L.nig</i>

Table 6.1 continued

Montagne 1842	na			<i>L. berteroana</i>		Type locality: Coquimbo; Collector: Bertero et cel. Gaudichaud; Montagne: "Could possibly be a synonym to <i>Cordaria spicata</i> Suhr"
Kuetzig 1843	?	<i>L. fuscescens</i>				p.88 the gigantic thick caulom of <i>L.fus</i> has also the biggest vasa mucifera; p.348 states that <i>L.fus</i> and <i>L.nig</i> have the same distribution
Gaudichaud 1844-6	na			<i>L. berteroana</i>	<i>L. fuscescens</i>	<i>L.ber</i> : Type collected by Gaudichaud and reported here in more detail; <i>L.fus</i> : might be <i>L. trabeculata</i> (<i>Ad oras Peruviae circa Callao</i>)
Harvey & Hooker 1847	3	<i>L. fuscescens</i> (<i>L. flavicans</i>) with lacunae <i>L. nigrescens</i> with lacunae [!]	<i>L. ovata</i>			Both, <i>L.fus sensu</i> H&H and <i>L.nig sensu</i> H&H are depicted with lacunae; However, <i>L.nig</i> is not found on the Falklands (Searles 1978) <i>L.ova</i> : Type locality: Hermit Island (Cape Horn); has most likely priority to <i>L. searlesiana</i> . No type has been assigned; thumbnail shows proposed type (unnumbered herbarium sheet in BM).
Agardh 1848	2	<i>L. fuscescens</i>	<i>L. ovata</i>	<i>L. suhrii</i> <i>L. nigrescens</i>	<i>Species inquirendae: L. ciliata, L. laminariaeoides, L. sinclairi</i>	<i>L.suh</i> : syn of <i>Chordaria spicata</i> 1839 and <i>L.ber</i> (<i>Himanthalia durvillaei</i> (Bory de Saint-Vincent 1828. p. 135) ad Areschougia ad hanc trahitur) <i>L.nig</i> : syn <i>Lam. scissa</i> <i>L.fus</i> : <i>sensu</i> Hooker

Table 6.1 continued

Kuetzig 1849	3	<i>L. fuscescens</i> (<i>L. flavicans</i>)	<i>L. ovata</i>	<i>L. nigrescens</i> <i>L. berteroana</i>	<i>L. laminariaeoides</i>	<i>L.fus</i> (<i>L.fla</i>): Hab: <i>ad oras Americae, insula Falkland, Kerguelensland</i> : in Terra Louis-Philippe <i>L.ova</i> : Hab: with former <i>ad</i> Cap Horn, ins. Falkland, Hermite Isl. <i>L.nig</i> : Hab: with <i>L.fus</i> [indicating that it is found on Falkland]; syn of <i>Lam.ram.</i> , <i>Lam.sci.</i> , <i>Lessonia binderi</i> Sonder. [<i>nom.herbariorum</i> ? A&R09] <i>L.ber</i> : Hab: <i>ad oras Chilenses</i> ; syn of <i>L.suh.</i> , <i>Cho.spi</i> .
Montagne 1852	?	<i>L. fuscescens</i>	<i>L. ovata</i> [?]	<i>L. berteroana</i> <i>L. nigrescens</i>	<i>L. fuscescens</i> [?] <i>L. ovata</i> [?]	<i>L.ber</i> : syn <i>L.suh</i> 1841 and <i>Chordaria spicata</i> 1839; Coquimbo; (<i>L.ber</i> has nothing in common with <i>Himanthalia durvella</i> as reported by Areschoug) <i>L.nig</i> : syn <i>Laminaria scissa</i> ; Concepción, Valparaiso, etc.[Montagne recognised <i>L.ber</i> and <i>L.nig</i>] <i>L.fus</i> : often confused with <i>L.nig</i> and <i>L.ova</i> it was collected at Concepción by Lesson and Valparaiso by Bertero <i>L.ova</i> : it was collected at Concepción shores [Montagne found both species mixed up in Bory de Saint-Vincent's collection][<i>L.fus</i> --> might been confused with <i>L. trabeculata</i>];

Table 6.1 continued

J.Agardh 1877	?		<i>L. fuscescens</i> (<i>partim?</i>) <i>L. ovata</i>	<i>L. suhrrii</i> <i>L. nigrescens</i>	<i>L. variegata</i>	Agardh makes the point that there are two sections of <i>Lessonia</i> . The first section includes those without lacunae as <i>L.suh.</i> , <i>L.nig.</i> , <i>L.fus</i> (<i>partim?</i>), <i>L.ova</i> (= <i>L.fus</i> <i>fide spec.</i>) and the second includes those in Chile and New Zealand with lacunae. Those with lacunae he called <i>L. variegata</i> (including those with lacunae found in Chile). <i>L.nig</i> : syn <i>Lam.sci</i>
Dickie 1877a&b	?	<i>L. fuscescens</i>	<i>L. ovata</i>	<i>L. nigrescens</i>		Algae from Kerguelen (<i>L.fus</i>) and Heard Island (<i>L.nig</i> , <i>L.ova</i>); collected by Moseley during the 'Challenger' expedition
Hariot 1889	3	<i>L. fuscescens</i> <i>L. nigrescens</i> [!]	<i>L. flavicans</i>	<i>L. suhrrii</i>		<i>L.fus</i> (after drawings by Bory de Saint-Vincent 1828): Hab: Terre de Feu, Malouines, one of the more common magellanian seaweeds. She is very abundant in all the canals of Terra del Fuego, especially in the South, and on the Falklands. <i>L.flav</i> : syn <i>L.fus</i> (excl. drawings), <i>L.ova</i> ; Hab: Terre de Feu, Malouines; refers to Rostafinski (in herb. Musei Parisiensis) as the one who re-established the true meaning of <i>L.flav</i> & <i>L.fus</i> ; Hariot states that he found an alga in d'Urville's collection annotated as <i>L.flavicans</i> in Bory de Saint-Vincent's handwriting, which might be the original <i>L.flav</i> and nearest to <i>L.ova</i> . <i>L.nig</i> : Hab: Terre de Feu, Malouines; Hariot referred to Hooker's (1847) drawings of <i>L.fus</i> [!] <i>L.suh</i> : synonymous with <i>Himanthalia durvillaei</i> (following Areschoug (1884) despite the opposite opinion of Montagne (1852))

Table 6.1 continued

J.Agardh 1894	?	<i>L. fuscescens</i>	<i>L. ovata</i>	<i>L. Suhrii</i> <i>L. nigrescens</i>	<i>L. brevifolia</i> <i>L. variegata</i>	<p><i>L.fus</i>: two forms with lanceolate-linear blades, young ones with lacunae, adult ones with some small lacunae but rare, however teeth on the edge of the blades are conspicuouse with nobbs at pointed elongated teeth.</p> <p><i>L.ova</i>: ovalo-lanceolate blades, "lacunae slightly conspicuous, bigger, more or less equalling several disrupted cells, I have seen this only here."</p>
Skottsberg 1907	3	<p><i>L. nigrescens</i> <i>L. frutescens</i> [partim. (habitat description)]</p>	<p><i>L. flavicans</i> <i>L. frutescens</i> [partim. (anatomy and picture)]</p>			<p><i>L.nig</i>: following Hooker (1847) he recognised and lengthily described a <i>L.vadosa</i>-like alga as <i>L.nig</i> [he called it later in 1921 <i>L.nig f. lacunifera</i>].</p> <p><i>L.fla</i>: his understanding is nearest to <i>L.ova</i> (following Hariot).</p> <p><i>L.fru</i>: he described an intertidal alga that is nearest to his understanding of <i>L.nig</i> [i.e., <i>L.nig f. lacunifera</i> or <i>L.vadosa</i>-like but intertidal; this form is only found on Falkland; left picture], however, the alga he got from S.Birger that he used for anatomic studies and pictured in Taf.8 is a <i>L.fla</i>-like individual [right picture; lectotype of <i>L.fru</i> (A1388 in S)]</p>
Cotton 1915	3	<p><i>L. nigrescens</i> <i>L. frutescens</i> [partim. (habitat description)]</p>	<p><i>L. flavicans</i> <i>L. frutescens</i> [partim. (anatomy and picture)]</p>			<p>following Skottsberg</p> <p><i>L.nig</i>: besides Skottsberg (Port William) and Hooker who gave accounts on <i>L.nig</i> at E.Falkland he also cited Vallentin for <i>L.nig</i> at W.Falkland (Roy Cove, in rock-pools)</p>

Table 6.1 continued

Skottsberg 1921	?	<i>L. nigrescens</i> f. <i>lacunifera</i> <i>L. frutescens</i>	<i>L. flavicans</i>	<i>L. nigrescens</i> f. <i>montagnei</i>		<p><i>L.fla</i>: enormous stem, which resembles the trunk of a small tree; some have lacunae some do not. "Are there two species? I hardly think so: the use of this character for the separation of species, otherwise quite identical, does not appear very promising."</p> <p><i>L.nig</i>: admits that he has not seen a real <i>L.nig</i> with unbranched haptera on Falkland and thus divides <i>L.nig</i> in two forms, <i>f.montagne</i> the 'real' <i>L.nig</i> and <i>f.lacunifera</i> [a <i>L.vad</i>-like alga].</p> <p><i>L.fru</i>: "Still, it is possible that <i>L.fru</i> is nothing but a local form of <i>L.nig</i> [<i>f.lacunifera</i>!]"</p>
Searles 1978	2	<i>L. vadosa</i>	<i>L. flavicans</i>	<i>L. nigrescens</i>	undescribed	<p><i>L.vad</i>: Type loc: Isla de los Estados; syn <i>L.nig f.lacunifera</i>; Hab: from Chiloe south through Beagle Channel and along the Argentinean coast till Puerto Deseado; Falkland; shallow subtidal;</p> <p><i>L.fla</i>: syn <i>L.fus</i>, <i>L.ova</i>, <i>L.fru</i>; Hab: as <i>L.vad</i> including Cape Horn; subtidal (3)7-38m deep; assigned Lectotype to <i>L.fla</i></p> <p><i>L.nig</i>: syn <i>Cho.spi</i>, <i>Lam.sci</i>, <i>L.Suh</i>, <i>L.ber</i>, <i>L.nig f.montagnei</i>; Hab: Chile & Peru; lower intertidal and sublittoral fringe; assigned Lectotype to <i>L.nig</i></p>
Villouta & Santelices 1986	?	<i>L. vadosa</i>	<i>L. flavicans</i>	<i>L. nigrescens</i>	<i>L. trabeculata</i>	<p>same concept as Searles</p> <p><i>L.tra</i>: Type loc: Coquimbo (MNH SGO 102908); selected by J.Vasquez; Hab: Chile between 20°-40°S; subtidal</p>

Table 6.1 continued

Hay 1987	2	<i>L. vadosa</i>	<i>L. flavicans</i>	<i>L. nigrescens</i>	<i>L. trabeculata</i>	<i>L. adamsiae</i> <i>L. brevifolia</i> <i>L. corrugata</i> <i>L. variegata</i>	same concept as Searles
Asensi & de Revers 2009	?	<i>L. flavicans</i>	<i>L. searlesiana</i>	<i>L. nigrescens</i>	<i>L. trabeculata</i>	all described species mentioned	<i>L.fla</i> : re-lectotypification of <i>L.fla</i> ; found lacunae in new Lectotype thus changed Searles concept of <i>L.fla</i> putting his <i>L.vad</i> in a synonymous status. <i>L.sea</i> : [is certainly syn of <i>L.ova</i> & <i>L.fru</i> (possibly also a syn of <i>L.fus</i>)]

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Chapter 7

Summary and directions for future research

General patterns of phylogeographic research of coastal benthic invertebrates and plants have revealed a significant negative correlation between larval duration and genetic differentiation (Ross *et al.* 2009). Larval duration is not the only factor responsible for the dispersal potential of a species, as studies on *Macrocystis* and *Durvillaea antarctica* have shown (Macaya & Zuccarello 2010, Fraser *et al.* 2009). Due to the ability of these species to float, they are able to connect populations throughout the Southern Hemisphere and single widespread haplotypes have been observed in both species (Macaya & Zuccarello 2010, Fraser *et al.* 2009). My aim was to study a widespread non-floating taxon with limited dispersal abilities but with the possibility of being attached to rafts that enables long distance dispersal. I surveyed *Lessonia* at different taxonomic and geographic levels while examining the global phylogeny at the generic level (Chapter 2), the phylogeography of *Lessonia* species in Australasia (Chapter 3), and the population genetics across Cook Strait (Chapter 5).

Data collected in the course of this study revealed the taxonomic relationship and the time of speciation events in *Lessonia* (Chapter 2), although more work is needed to test hypotheses about the origin of *Lessonia*. Some concrete hypotheses were formulated to describe the dispersal history of *Lessonia* in New Zealand (Chapter 3). I identified features and processes that create and maintain genetic breaks, emphasizing the importance of rafting as a means to connect meta-

populations (Chapter 3 & 5). The known biodiversity has been increased by three species, which remain to be formally described (Chapter 2 & 3). New microsatellite markers have been developed to study genetic differences within and between populations (Chapter 4). I also clarified the nomenclatural confusion among South American *Lessonia* (Chapter 6) based on an extensive literature research. This was necessary as the confusion led to wrongly assigned species in publications and in GenBank. Alignments of sequences assigned to the Falkland Islands in GenBank support the occurrence of two different species. However, the affiliation to the species is misleading (Table 7.1).

Table 7.1 *Lessonia* haplotypes from GenBank. Sp., species affiliation according to this Chapter; det., determined (species affiliation in GenBank according to the Author). *L.flu*, *L. flavicans*; *L.ovu*, *L. ovata*; *L.vad*, *L. vadosa*; atp8, spacer region between atp-dehydrogenase subunit 8 and t-RNA serine; trnW-sp, the spacer between the t-RNA's for tryptophan and isoleucine; nd6, a partial sequence of the gene coding for NADH dehydrogenase subunit 6; rbc, spacer between the large and short subunit of the ribulose-1;5-bisphosphate carboxylase/oxygenase gene; ITS, nuclear marker covering the internal transcribed spacers (1 and 2) between the large and small-ribosomal RNA subunits; and the 5.8S ribosomal RNA; gb, GenBank accession number; code, haplotype in the network.

Sp.	det.	marker	gb	sample site	code
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593743.1	Patagonia	1.a
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593742.1	Patagonia	1.a
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593741.1	Patagonia	1.a
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593740.1	Patagonia	1.b
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593739.1	Patagonia	1.b
<i>L.flu</i>	<i>L.flu</i>	atp8	FJ410130.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593738.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593737.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	atp8	GU593736.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	atp8	<i>Seal001</i> (-10m)	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	atp8	<i>Xmas001</i> (-17m)	Falkland	2.a
<i>L.flu</i>	<i>L.vad</i>	atp8	<i>BEAU2</i> (-15m)	Falkland	2.a
<i>L.ovu</i>	<i>L.vad</i>	atp8	EU652986.1	Patagonia	3.a
<i>L.ovu</i>	<i>L.flu</i>	atp8	<i>GREEN1</i> (-20m)	Falkland	3
<i>L.ovu</i>	<i>L.flu</i>	atp8	<i>BEAU4</i> (-20m)	Falkland	3

Table 7.1 continued

Sp.	det.	marker	gb	sample site	code
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593943.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593942.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593941.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593940.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593939.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593938.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593937.1	Falkland	2.1
<i>L.flu</i>	<i>L.vad</i>	trnW	GU593936.1	Falkland	2
<i>L.ova</i>	<i>L.flu</i>	trnW	EU652961.1	Patagonia	3.1
<i>L.ova</i>	<i>L.vad</i>	trnW	EU652962.1	Patagonia	3

Table 7.1 continued

Sp.	det.	marker	gb	sample site	code
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593843.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593842.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593841.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593840.1	Patagonia	1.1
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593839.1	Patagonia	1.1
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593838.1	Falkland	2.1
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593837.1	Falkland	2.2
<i>L.flu</i>	<i>L.vad</i>	nad6	GU593836.1	Falkland	2
<i>L.flu</i>	<i>L.flu</i>	nad6	AY857931.1	Falkland	2

Table 7.1 continued

Sp.	det.	marker	gb	sample site	code
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593893.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593892.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593891.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593890.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593889.1	Patagonia	1
<i>L.flu</i>	<i>L.flu</i>	rbc	FJ410101.1	Patagonia	1
<i>L.flu</i>	<i>L.flu</i>	rbc	AF318992.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593888.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593887.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	rbc	GU593886.1	Falkland	2
<i>L.flu</i>	<i>L.flu</i>	rbc	AY851543.1	Falkland	2
<i>L.ova</i>	<i>L.vad</i>	rbc	FJ410102.1	Patagonia	3
<i>L.ova</i>	<i>L.vad</i>	rbc	AF318993.1	Chubut, AR	3

Table 7.1 continued

Sp.	det.	marker	gb	sample site	code
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593793.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593792.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593791.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593790.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593789.1	Patagonia	1
<i>L.flu</i>	<i>L.flu</i>	ITS	AF319031.1	Patagonia	1
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593788.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593787.1	Falkland	2
<i>L.flu</i>	<i>L.vad</i>	ITS	GU593786.1	Falkland	2
<i>L.flu</i>	<i>L.flu</i>	ITS	AY857900.1	Falkland	2
<i>L.ova</i>	<i>L.vad</i>	ITS	FJ410083.1	Patagonia	3
<i>L.ova</i>	<i>L.vad</i>	ITS	FJ410059.1	Patagonia	3
<i>L.ova</i>	<i>L.vad</i>	ITS	AF319032.1	Chubut, AR	3.1

7.1 What processes maintain genetic breaks in the distribution of

Lessonia in New Zealand?

Genetic (e.g., Apte & Gardner 2002, Waters & Roy 2004, Ayers & Waters 2005, reviewed in Ross *et al.* 2009) and biogeographic (Shears *et al.* 2008, Ministry of Fisheries & Department of Conservation 2008; hereafter called MPA 2008) breaks have been studied in the New Zealand marine environment to aid in conservation planning, to understand recruitment pathways, or to identify processes structuring marine ecosystems. Using mitochondrial and chloroplast markers I tested whether genetic breaks in *Lessonia* correspond to biogeographic and phylogeographic breaks found earlier. The haplotype distribution was mapped in order to find genetic breaks and to show the distribution range and boundaries of the Australasian species based on more extensive sampling (Chapter 3). Within the distribution area of *Lessonia* in New Zealand, the genetic breaks have been found to correspond well to existing biogeographic classifications (Fig. 3.1 and 3.6).

Newly developed microsatellite markers (Chapter 4) were used to investigate the influence of geographic breaks on the connectivity of populations. The Cook Strait area was selected as a study site to compare the genetic differences found between two geographic breaks of similar width, Cook Strait (a seaway separating the North and South Island of New Zealand) and Palliser Bay (a gap created by unsuitable coastline), and the genetic variation within and between populations along a continuous coastline (Chapter 5). The results, based on the frequencies of five microsatellite markers, indicated only a weak genetic barrier between populations separated by Cook Strait, and no differences have been found between three connected populations at the east side of Cook Strait over a distance of 32 km, but a strong break was detected between two populations separated by Palliser Bay. The differences in the strength of genetic breaks between the two gaps suggested that stronger currents within Cook Strait might be responsible for enhanced connectivity (Chapter 5). Longer stretches of unsuitable habitat (e.g., 40km separating *L. variegata*/W and *L. variegata*/K) have been shown to act as barriers to gene flow and maintain differentiation between species (Chapter 3). Thus the strength of a genetic break is correlated to the width of unsuitable habitat and to the strength of connecting currents. The *atp8*-sp haplotype distribution (Fig. 3.6) indicates that breaks between *Lessonia* species are often linked to either eddies (e.g., the break at East Cape between *L. variegata*/N and *L. variegata*/W) or stretches of unusable habitat (e.g., the break east of the Marlborough Sounds between *L. variegata*/W and *L. variegata*/K or the break spanning Pegasus and Canterbury Bay between *L. variegata*/K and *L. variegata*/S). Upwelling north of Kaikoura was sometimes used to explain genetic breaks between northern and southern haplotypes (Apte & Gardner 2002, Waters & Roy 2004, Ayers & Waters 2005). This oceanographic

process can be dismissed as the reason for the genetic differentiation between *L. variegata*/W and *L. variegata*/K as the distribution of the Kaikoura lineage extended to the north across the upwelling zone. Also, this upwelling region is of postglacial origin and might be too young to fully explain genetic differentiation at the species level (Goldstien *et al.* 2006). However, within the Kaikoura lineage, the populations north of the upwelling zone have a unique haplotype different from their southern populations indicating that in *Lessonia* upwelling might play a role in meta-population structure. Additional research on *L. variegata*/K with fine scale markers might help to understand the structuring process of this upwelling zone.

7.2 How important is rafting as a means of dispersal in *Lessonia*?

The different dispersal mechanisms have been summarized in the introduction (Chapter 1.3; Fig. 1.10). For the case study of connectivity within Cook Strait I systematically excluding some possible dispersal mechanisms (i.e., first, the maximum distance measured for dispersal of propagules in other Laminariales was only a fraction of the 20+km needed to cross Cook Strait or Palliser Bay; 4 km for *Pterygophora californica* propagules (Reed 1988), 3.5 km for *Macrocystis pyrifera* propagules (Reed 2004); second, the sea floor topography within Cook Strait and Palliser Bay prevents dispersal of detached algae or dispersal by saltation). As a consequence I concluded that rafting (i.e., as an associate to a float) was important as a means to connect populations across Cook Strait and Palliser Bay (Chapter 5). The weak genetic break detected between populations on both sides of Cook Strait might further suggest that rafting is not rare. As mentioned above, the strength of a genetic break is correlated to the width of unsuitable habitat and/or to the strength of connecting currents. As a consequence, I assume rafting to be very important for

dispersal in *Lessonia* and in some cases it might be the only means to connect populations further than 20km apart. However little is known about rafting in *Lessonia*, neither whether it is the gametophyte or the sporophyte that is most successful nor what floating material might be used. Further research is suggested to find out the relative abundance of *Lessonia* sporophytes that grow on, or are overgrown by *Durvillaea antarctica* or *Macrocystis pyrifera*, the two most common rafting species in the Cook Strait area. Fox & Swanson (2007) used nested PCR to detect gametophytes of Laminariales on rocks; the same method might be modified to detect *Lessonia* gametophytes on attached and floating specimens of *Durvillaea* and *Macrocystis*. During my study I detected haplotypes of New Zealand lineages in samples from Chile (data not shown) together with the expected Chilean haplotype, indicating that dispersal from New Zealand to Chile is possible, ongoing, and not rare (given the relatively small number of South American specimens surveyed).

7.3 How many *Lessonia* species are there?

Currently nine *Lessonia* species are recognised (see Introduction) but with the use of molecular methods, cryptic species were detected. Recently *L. nigrescens* has been split into two species (Tellier *et al.* 2009) though the whole species range of *L. nigrescens* was not investigated. In the current study *L. variegata* was found to be polyphyletic and four cryptic species were detected (Chapter 2). Each of these species was confined to a distinct area (Chapter 3). Genetic differentiation was also found in *L. trabeculata* but the surveyed populations were located very far apart, thus it was interpreted as a result of isolation by distance rather than speciation. I interpreted the genetic differentiation within *L. flavicans* in the same way (Chapter 2). *Lessonia flavicans*, *L. ovata*, and *L. nigrescens* (Dickie 1876 a & b and 1879,

Delepine 1963)) are reported from the remote islands, Kerguelen and Heard. As connectivity with South America or Australasia might be very low there is a good chance that these are in fact undescribed species. At present thirteen *Lessonia* species can be genetically recognised.

Additional research is needed to survey the status of *Lessonia* on Kerguelen and Heard Island (i.e., whether they are known or new species). The inclusion of these species into an overall phylogeny (Chapter 2) might improve the interpretation of the Southern Hemisphere phylogeography and dispersal history of this genus. Further research is also needed to investigate the morphology of the cryptic species found in *L. nigrescens* and *L. variegata* (Chapter 2&3) in order to be formally described.

Four traits, which were previously used to describe and differentiate known species, were preliminary studied (Fig. 7.1-7.4, Table 7.2). These traits were colour and colour pattern on the lamina, the appearance of the holdfast, the number of stipes emerging from the holdfast, and the occurrence of lacunae within the blade.

With few exceptions, only individuals of the Wellington lineage (*L. variegata*/W) possess variegations on the blades (Fig. 7.1), whereas the blades of the other *L. variegata* lineages are uni-coloured (Fig. 7.3). The exceptions are some northern-lineage (*L. variegata*/N) populations on the Coromandel Peninsula (Sailors Grave) where variegated blades were found (Fig. 7.2). However, as the variegations appear longer and rather parallel to each other and to the edge of the blade they differ from those of the Wellington lineage, which are short and scattered (Fig. 7.1). All features used to describe *L. variegata* fit the Wellington lineage and as Wellington

(Lyll Bay) is also the type locality of *L. variegata*, I consider this lineage as the true *L. variegata* (Chapter 2).

The holdfast morphology is another feature, which was often used to differentiate *Lessonia* species and some species (*L. tholiformis*, *L. trabeculata*, or individuals belonging to the *L. nigrescens* species complex) can be recognised on holdfast morphology alone. Also in *L. variegata*, the holdfast differs between the lineages (Fig. 7.1-7.3). In *L. variegata*/W the holdfast is a massive woody structure that forms a rib-like buttress with numerous primary stipes (Fig. 7.1). No buttresses are formed in the northern lineage and the holdfast forms an appressed network on the substrate (Fig. 7.2). Young stipes at early stages might grow along the bottom surface and produce additional haptera thus forming a flat, central hapteron. Many long primary branches emerge from the flat holdfast. Stipes and branches are often flat, too. The holdfast of the Kaikoura (*L. variegata*/K) and Southern lineage (*L. variegata*/S) are very much alike and hard to distinguish (Fig. 7.3). They do not form a buttress (in contrast to *L. variegata*/W) but are not appressed to the ground either (in contrast to *L. variegata*/N); instead they form a doom-like structure but, in contrast to *L. tholiformis*, with very distinctive haptera. In *L. variegata*/K numerous stipes emerge from this holdfast whereas in *L. variegata*/S the stipes are few (Fig. 7.3). In both lineages, but more so in *L. variegata*/S, the stipes and branches are short.

A fourth feature used is the occurrence of lacunae in the blades, which is used as a distinguishing characteristic in other *Lessonia* species. All *L. variegata* lineages have lacunae (Fig. 7.4), however, they are numerous and large in *L. variegata*/W and numerous but small in *L. variegata*/N. They are less frequent (2-

3/mm in a transverse section) in *L. variegata*/S and they are rare in *L. variegata*/K (average of 1/mm in a transverse section).

The four morphological and anatomical characteristics appear promising in their ability to differentiate between the four lineages of the *L. variegata* species complex. However, the small sample size used does not guarantee that any of these characters is species-specific as the within species variability is unknown and only the Wellington lineage has been proven to be easily distinguished by sight (pers. observation).

Fig. 7.1 *L. variegata*/W displaying morphological features of the ‘real’ *L. variegata*; sampled at Lyall Bay in Wellington (Type locality); left: close up on blades showing variegation; middle: close up on buttress; right whole alga.

Fig. 7.2 *L. variegata*/N multiple stipes arose from a flattened holdfast; blades, which are normally uni-coloured show long and parallel variegations in some populations (left).

Fig. 7.3 Multiple stipes emerge from the holdfast in *L. variegata*/K (left), but only a few from *L. variegata*/S (right; showing two algae).

Fig. 7.4 Transverse sections showing possible differences between the four *L. variegata* lineages as specified in Table 7.1.

Table 7.2 Morphological characteristics and habitat of *L. variegata* lineages (tentative).

Feature	<i>L. variegata</i> /W	<i>L. variegata</i> /N	<i>L. variegata</i> /K	<i>L. variegata</i> /S
Lamina colour	light brown to yellow; variegated	light brown to green often brighter at the edge	brown	
Lacunae	numerous, big	numerous, small	few (2-3/mm)	rare (in average 1/mm)
Holdfast	massive, woody; dichotomously branched haptera	Flat appressed on the substrate; at least in young algae flattened stipes with the ability to produce haptera	Massive, building half of a sphere; haptera very distinct not moulded	
Buttress	rib-like	no		
Stipes emerging from holdfast	up to 20 and more			few

7.4 Where did *Lessonia* originate?

The main results of the global phylogeny have been that the Australasian species form a clade nested within a paraphyletic group of South American species. The South American species form a pectinate or unbalanced tree base with *L. flavicans* in a sister relationship to the Australasian clade (Chapter 2). Although the supermatrix approach (multiple matrices, i.e., atp8-sp, trnW-sp, nd6-gene, rbc-sp, and ITS datasets, were combined into one supermatrix) gave good support to the phylogeny of *Lessonia*, no phylogeographic conclusions were made as to the place of the origin of *Lessonia*.

In the following I will describe two different hypothetical dispersal scenarios (Chapter 2) and introduce a third scenario that combines our present day knowledge of the relationship and distribution of *Lessonia* haplotypes.

According to the standard hypothesis as used in DIVA or TREEFITTER (Sanmartin *et al.* 2007, fig. 3) and given the phylogeny as shown in Fig. 2.2, *Lessonia* would have originated in South America and a single dispersal event would have predated the radiation in Australasia, as the Australasian clade is nested within a paraphyletic group of lineages from South America. This phylogeographic interpretation of the phylogeny has not been contested for many years but, for dispersive species where unidirectional currents are the main dispersal agents, the same phylogeny can be interpreted differently (Crisp & Cook 2005). Following the ‘nested ancestral area’ hypothesis (Cook & Crisp 2005) Australasia would be origin of *Lessonia* and source of multiple dispersal events, which would have led to multiple colonisations into the downstream area (i.e., South America). The rarer

those dispersal events are the more like they will lead to allopatric speciation (Thiel & Haye 2006). These two hypotheses are the extremes of more, equally parsimonious, hypotheses that follow the ‘nested ancestral area’ hypothesis but with a different starting point (e.g., a South American origin of *Lessonia* followed by dispersal to Australasia, which becomes the nested ancestral area for the remaining South American species). These intermediate hypotheses are special cases when unidirectional agents form ring systems like the WWD in the southern Oceans but are not possible, for example, in river systems, where unidirectional dispersal might also occur. In any case, all these phylogeographic hypotheses are interpretations of the same phylogeny, thus in systems with unidirectional dispersal no conclusions regarding the ancestral area can be drawn based on the phylogeny alone (Cook & Crisp 2005, Crisp & Cook 2005).

Currently DIVA (Ronquist 1997) is the most used program to infer ancestral areas, even though it is only able to reconstruct simple evolutionary histories and fails if dispersal patterns are more complex (Kodandaramaiah 2010). In the past, programs to infer complex evolutionary histories have not been available but currently many programs are in development to address this problem. Kodandaramaiah (2010) concludes that model-based methods are promising in determining ancient dispersal patterns. But also simulation tools like IMa2 (Isolation with migration; Hey 2010a & b) might be altered in a way to test different dispersal hypotheses in species rather than populations. The key to answer the question about the origin of *Lessonia* might lay in South America, since, if the ‘nested ancestral area’ hypothesis is valid, each South American species must have gone through a bottleneck as the result of the founder effect, which should have followed the long

distance dispersal with colonisation events (Thiel & Haye 2006, Wilson *et al.* 2009). Different programs, e.g., IMA2, are able to detect bottlenecks, but more sampling in South America would be required.

Additional extensive sampling might be required to discriminate between the different dispersal hypotheses, thus research would benefit from a method that might allow making inferences based on the dataset used to acquire the phylogeny. I propose that gene networks would reflect the differences of the alternative phylogeographic hypotheses, i.e., the networks would be starlike if reflecting the ancestral area hypothesis and not starlike if reflecting the standard hypothesis. If true, this would be an easy method to test phylogeographic hypotheses; however, it might also be an oversimplification. Different evolutionary histories might generate starlike species networks and starlike networks might be an artefact of markers evolving too slowly to detect the true pattern of speciation. In the case of *Lessonia*, starlike networks were generated with the rapidly evolving mitochondrial *atp8*-sp and *trnW*-sp, but non-starlike networks resulted from the chloroplast *rbc*-sp, and the nuclear ITS1, and ITS2 dataset (networks not shown). Thus the mitochondrial spacer regions might support a ‘nested ancestral area’ hypothesis whereas the chloroplast and nuclear spacer regions might not.

More sampling and more data in addition to a better understanding of organelle inheritance and speciation processes (e.g., speciation through hybridisation) are needed to fully resolve questions about the origin and dispersal history of *Lessonia*. However, it is worth considering a different hypothesis can be determined based on the contrary information gained by the single marker networks (mitochondrial-DNA vs. chloroplast- and nuclear-DNA). This hypothesis would

invoke South America as the origin of *Lessonia*. Long distance dispersal of individuals following the WWD would have led to the colonisation of Australasia. Australasia would become the nested ancestral area of all remaining *Lessonia* (as in the intermediate hypothesis mentioned above). However, dispersal back to South America would not create new species due to allopatric speciation, but due to hybridisation. This interpretation would combine the conflicting hypotheses inferred from single marker networks of the mitochondrial, chloroplast, and nuclear-DNA, without being in conflict with the phylogenetic calculations of the combined dataset.

The validity of any of these dispersal hypothesis remains to be tested. As algae do not fossilize well, clues have to be found elsewhere e.g., in geological and climatic history and processes, or in the comparison with other live forms that share the same habitat, or the same distribution, or the same dispersal abilities. The use of different markers and the comparison of gene trees might be helpful, as well as the use of different methods or models like trees, splittrees, supertrees (a tree derived from the combination of different gene trees), networks, and methods to infer coalescence and demography, but also methods to simulate different scenarios where hypotheses can be tested on artificial datasets to compare the output with the original data.

While the global phylogeography remains speculative, but might be narrowed down to fewer possibilities with developing tools and increasing knowledge, more could be inferred about the phylogeography of *Lessonia* in Australasia. In Chapter 3, the relationship and distribution of haplotypes was combined with knowledge of the recent and past geography of the area to infer phylogeographic hypotheses and to reconstruct the dispersal history of *Lessonia* in Australasia. Thus I formulated the

hypotheses about the post glacial dispersal history of *L. corrugata*, the dispersal history of *L. variegata*/W, and the possibility of hybrid speciation of *L. adamsiae* from a cross between *L. variegata*/S and *L. corrugata*. As pointed out in Chapter 3, the hypothesis of the post glacial dispersal history of *L. corrugata* can be tested by sequencing samples from the west coast of Tasmania to search for the proposed intermediate haplotypes. The finding of these haplotypes would strengthen the hypotheses of a proposed north-western refugium followed by postglacial dispersal along both sides of Tasmania (Fig. 3.5).

7.5 References

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