# The phylogeny, biology and biogeography of the Southern African kelps *Ecklonia maxima* and *Laminaria pallida*.

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Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biological Sciences

UNIVERSITY OF CAPE TOWN

February 2015

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ABSTRACT

Brown algae of the order Laminariales, commonly referred to as kelps, are the largest and most productive primary producers in the coastal inshore environment. Three genera of kelps are present on the southern African coast: *Ecklonia*, *Laminaria* and *Macrocystis*, of which the first two are ecologically and economically important and the focus of this study.

The taxonomy of the genus *Ecklonia* is investigated. The genus *Ecklonia* (Phaeophyceae, Lessoniaceae) consists of seven species with four species in the Northern Hemisphere and three in the southern Hemisphere. *Ecklonia* was recently transferred to the family Lessoniaceae based on phylogenetic analyses of nuclear and chloroplastic markers, though the type of the genus was not included, and its relationship to the allied genera *Eckloniopsis* and *Eisenia* remained unresolved. The present study is the first to produce a phylogeny focussed on the genus *Ecklonia*. It included sequences from nuclear, mitochondrial and chloroplastic DNA, for most of the distribution range of the three current Southern Hemisphere species (*E. radiata*, *E. maxima* and a sample of a putative *E. brevipes* specimen), sequences for East Asiatic species (*E. cava*, *E. kurome* and *E. stolonifera*), as well as the closely related genera *Eckloniopsis* and *Eisenia*. Results confirmed *E. radiata* and *E. maxima* as two distinct species in South Africa, *E. radiata* as a single species throughout the Southern Hemisphere (in South Africa, Australia and New Zealand) and East Asiatic species as a distinct lineage from the Southern Hemisphere clade. Results further indicated a close sister relationship between *Eckloniopsis radicosa* and two *Eisenia* species (including the type species: *Eisenia arborea*), and the genus *Ecklonia,* suggesting that the genera *Eckloniopsis* and *Eisenia* are superfluous.

A molecular study of *Laminaria pallida* was undertaken. Along the Southern African west coast, *L. pallida* is morphologically variable, at the extreme presenting two forms: i) a solid-

stiped form with the stipe widest near the holdfast and ii) a hollow-stiped form which has a longer, hollow stipe that is wider in the middle, narrowing to the distal and proximal ends (at one stage regarded as a separate species, *L. schinzii*). Using sequences from nuclear, mitochondrial and chloroplastic DNA, from Southern African as well as *Laminaria* species from other regions, a phylogeny of *Laminaria* was constructed. Results confirmed that *L. pallida*, in Southern Africa, irrespective of form, is all a single species with the northern Atlantic species, *L. ochroleuca*, as its sister. The *rbc*L/ITS analysis showed that there is a separation between the species of *Laminaria* from the Eastern pacific, Western Pacific and the Atlantic while the atp8/trnWI analysis, focused on the Atlantic species of *Laminaria*, resolved two subclades: a *L. digitata/hyperborea* subclade, and a *L. ochroleuca/pallida* subclade.

*Ecklonia maxima* and *Laminaria pallida* form large and extensive kelp beds along the west coast of Southern Africa. While they co-dominate these beds, their vertical and geographical patterns of dominance change, and the next section of this study describes and attempts to explain these patterns.

On the southern part of the west coast, *E. maxima* is the dominant inshore kelp, generally forming a surface canopy in shallow water (0- 5 m or more), with *L. pallida*  growing underneath it but extending beyond the depth limit of *E. maxima*, down to 20 m or more. However, northwards along the west coast, this pattern gradually changes, as *L. pallida* progressively replaces *E. maxima* as the dominant kelp in shallow water, until only *L. pallida* remains, north of Lüderitz in Namibia.

The morphology of *E. maxima* and *L. pallida* sporophytes in relation to the changeover in dominance between these two species on the Southern African west coast was investigated. Seven sites in South Africa and one site in Namibia were sampled. Sporophytes (> 50 cm

stipe length), from shallow water (< 5 m), were collected from 1 x 1 m quadrats, along transects, running perpendicular to the shore, and extending from the outer edge of the surface-reaching (visible) kelp bed, to the shore. Various morphological characters were measured: stipe length, stipe weight, stipe outer diameter, stipe inner diameter, length of hollow section in the stipe, and frond weight. In addition environmental data (seawater temperature, seawater turbidity, cloud/fog data, day length, and wave and wind data) were collected from various sources and analysed.

*E. maxima* showed no geographical gradient in stipe length or hollowness, but as *L. pallida* sporophytes became more dominant, their stipes became longer and increasingly hollow. For the first time the changeover in dominance between the two species, as well as the gradual increase in *L. pallida* stipe hollowness was quantified.

Among the environmental variables investigated, turbidity, wind speed and wave hight showed significant differences along the coast. However, only turbidity showed a steady trend, increasing northward in terms of all indicators obtained from NASA satellite data (Chlorophyl a, Particulate Inorganic Carbon, Particulate Organic Carbon) while wind speed and wave hight showed a general decreasing trend. We hypothesize that *L. pallida* sporophytes may progressively outcompete *E. maxima* northward, perhaps because they are more low-light tolerant, and that by developing a hollow stipe the sporophytes may grow faster, potentially increasing their competitive advantage in the shallow water where they would compete with sporophytes of *E. maxima*.

If increasingly turbid water (and therefore lower light levels) were responsible for the change in dominance of the two kelps along the west coast, the differences could lie in the responses of various stages in their life histories. A study was therefore done to compare *E. maxima* with *L. pallida* in terms of early growth stages under various light levels, as these stages are

amenable to laboratory study. In addition some circumstantial evidence of differences in *in situ* growth rates of young sporophytes is presented and discussed.

Gametophytes and the young sporophytes which they produced, were grown in the laboratory at 15 °C at different irradiance levels (dark, 10, 30, 80 and 120 µmol photons $m^{-2}$ $s^{-1}$). In the dark, gametophytes of both species died after 3 days. In both species, development of the gametophyte stage was so rapid (10 - 15 days from spore settlement to the appearance of spophytes) that we concluded that at this stage in their development, any competitive advantage is unlikely. Young sporophytes were grown at the same irradiance levels as above. Under low light (30 – 80 µmol photons $m^{-2}$ $s^{-1}$) young sporophytes of *L. pallida* increased in length significantly faster than those of *E. maxima*. An *in situ* rope culture study at Oudekraal, South Africa, showed that *L. pallida* grew significantly faster than *E. maxima* at 6 – 9 m depth.

The interaction between the two dominant kelp species on the Southern African west coast is complex and directions for future studies are discussed in the light of the results presented here.

DEDICATION

I dedicate this work to my late father Leslie Brian Rothman who first sparked my interest in Biology and a love for nature. He was an amazing teacher, had a brilliant mind and a keen sense of humour. Daddy, you will not be forgotten.

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CHAPTER 1

GENERAL INTRODUCTION

This is a study of the Southern African species of two genera from the Laminariales, *Ecklonia* and *Laminaria*. It first uses molecular methods to examine the systematics and phylogenetic status of the Southern African species on relation to their close relatives. The approach here is to firstly elucidate the phylogenetic position of the two most abundant and economically valued kelps to serve as a basis for the following chapters. The study then uses various measures to examin the ecological and geographic variences between the two dominant west coast species (*E. maxima* and *L. pallida*), and concludes with a culture study of these two species under different light conditions.

Kelps are large, brown seaweeds of the order Laminariales (Bolton 2010), and although there are not many species in the order (Steneck et al. 2002) with 112 species in 33 genera (Bolton 2010), they are distributed worldwide on sub- and intertidal rocky substrata in warm to cold temperate waters (Steneck and Johnson 2013), as well as in Arctic regions (Chapman and Lindley 1980, Sjøtun and Gunnarsonn 1995, Bolton 2010, Lüning 1990, Kain 1979, Steneck and Johnson 2013). Extensive beds have also been found in the geographical tropics where there are cool-water micro-habitats that creates suitable conditions (Graham et al. 2007).

*Kelp productivity*. Kelp beds form an integral part of inshore marine communities along most temperate rocky shores globally (Bolton 2010) and because of their ability to modify the environment (Velmirov and Griffith 1979, Dayton et al. 1984, Wernberg et al. 2005), they have been referred to as ecosystem engineers (Steneck et al. 2002). The communities they support are unique, variable and ecologically important in that they are very productive systems (Newell et al. 1982, Witman 1998, Foster and Vanblaricom 2001, Pereira et al. 2011). For example, Dayton (1985) compared the productivity of some kelp populations and

reported ranges of 120 – 1900 g C m$^{-2}$yr$^{-1}$ for *Laminaria*, 600 – 1300 g C m$^{-2}$yr$^{-1}$ for *Macrocystis* and 350 – 1500 g C m$^{-2}$yr$^{-1}$ for *Ecklonia* populations, the upper limits in most cases equalling or exceeding production levels in most terrestrial systems (Lüning 1990).

*General taxonomy*. In this order, eight families are recognised on Algaebase (Guiry and Guiry 2014): Agaraceae Postels & Ruprecht, Akkesiphycaceae Kawai & Sasaki, Alariaceae Setchel & Gardner (Silva 1980), Aureophycaceae Kawai & Ridgway, Chordaceae Dumortier (Silva 1980), Pseudochordaceae Kawai and Kurogi (Kawai and Kurogi 1985), Laminariaceae Bory (Silva 1980), and Lessoniaceae Setchel & Gardner (Womersley 1987). Lane et al. (2006) does not recognise the family Agaraceae and places the five constituent genera (combining Dictyoneuropsis and Dictyoneurum) in the family Costariaceae. Guiry and Guiry (2014) also list an additional family: Laminariaceae *familia incertae sedis* (an uncertain family containing 4 genera from Russia).

Sporophytes of a typical kelp-like appearance are considered more advanced and are from the families Alariaceae, Laminariaceae and Lessoniaceae (referred to as the ALL families by Lane et al. 2006) and contain most of the kelp species. The genus with the most species is *Laminaria* (24 species, Bolton 2010), with all but two species found in the Northern Hemisphere. The recently resurrected genus, *Saccharina* Stackhouse (20 species, Bolton 2010), has the next most species currently attributed to it, followed by *Lessonia* Bory de Saint-Vincent (10 species, Guiry and Guiry 2014) and *Ecklonia* (seven species, Bolton 2010).

It is suggested that members of the Laminariales had their origin in the Northern Hemisphere (Estes and Steinberg 1988) and crossed the tropics (Lüning 1990, Bolton and Anderson 1994), to evolve into species that are better adapted to new environments in the Southern Hemisphere. The present study is mainly focussed on the kelps of the Southern Hemisphere where many inshore rocky environments are dominated by large kelps. *Ecklonia* dominates

many kelp forests of Australia, New Zealand and South Africa/Namibia (Lüning 1990, Stegenga et al. 1997, Bolton 2010), while the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh forms dense forests in south eastern Australia, New Zealand, many southern Ocean Islands and forms sporadic patches in isolated areas of southwestern South Africa (Druehl 1978, Graham et al. 2007, Macaya and Zuccarello 2010, Guiry and Guiry 2014).

*Eisenia* Areschoug occurs along the South American east coast in Peru, Chile and the Galápagos Islands (Parada et al. 2012, Guiry and Guiry 2014). *Laminaria abyssalis* A.B. Joly & E.C. Oliveira (found in subtidal upwelling regions along the Brazilian coast) and *L. pallida* Greville (along the Southern African west coast) are the two *Laminaria* species confined to the Southern Hemisphere (Marins et al. 2012, Stegenga 1997). Species of *Lessonia* are largely found along the South American west coast in Chile and Peru, and also in Argentina, the Falkland Islands, Australia, New Zealand and surrounding islands (Martin 2011, Martin and Zuccarello 2012, Guiry and Guiry 2014).

*Environmental factors affecting kelp*. There are many different factors, environmental or biological, that shape or influence kelp bed communities in general. While temperature is the main factor controlling geographical distribution of marine organisms (Lüning 1990), the ecology of kelp beds communities is controlled by various other factors: wave action (Field and Griffiths 1991, Wing et al. 2007, Wernberg & Goldberg 2008, Bekkby et al. 2009), nutrients, photoperiod, salinity, tides, availability and topography of the substrata for attachment (Fowler-Walker and Connell 2007, Bekkby et al. 2009), disturbance (Odum 1963, Dayton 1994), patch dynamics, dispersal, grazing (Dayton 1985) and depth (Bekkby et al. 2009). However, to determine the role that each of these factors play is difficult as they usually act in combination (Dayton 1985, Rothman et al. 2006).

*Temperature*. Genetically determined temperature tolerances are the most important factor controlling the large-scale geographical distribution of marine plant and animal species (Breeman 1988, Lüning 1990), and sometimes even local and regional patterns of distribution (Henkel and Hofmann 2008). On the South African coast, Bolton (1986), Bolton and Anderson (1987), Bolton et al. (2004) and Anderson et al. (2007) have shown a strong relationship between seawater temperature and the geographical distribution of seaweed species. Southern Africa (South Africa and Namibia) has five temperature regions (Bolton 1986, also see Smit et al. 2013, Stephenson 1947): East Coast region (with annual means above 20°C; a south coast-east coast transition zone (annual mean of 19.3; a south coast region; a south caost region (annual means 17.2 – 18.2°C); a south coast-east coast transition zone (annual means 15.3 – 15.6°C); and the west coast region which stretches into Namibia. These regions are driven by two major currents: the cold, nutrient-rich Benguela Current (characterised by intense inshore upwelling) flows northward along the west coast (Andrews and Hutchings 1980, Shannon 1985, Shannon and Pillar 1986) while on the east coast the warm tropical Agulhas Current flows southwards (Beal et al. 2011, Rouault et al. 2010, Schumann 1988, De Clerck et al. 2005). The interactions between these two currents are complex and have profound effects on the Southern African coast (Partridge et al. 2004, Schmitz 1995, Shillington et al. 2006, Walker 1990).

*Nutrients*. In the Benguela Marine Province of the South African/Namibian west coast, seawater temperature can serve as a proxy for nutrients, with a generally inverse relationship between the two within certain limits (Dieckmann 1980, Waldron and Probyn 1992). Because seaweed distribution patterns are also linked to temperature, one can infer a relationship also with nutrients. *Laminaria digitata* (Hudson) J.V. Lamouroux for example, has the ability to store nutrients for periods of low nutrient conditions (Young et al. 2007). Similarily *L. hyperborea* (Gunnerus) Foslie stores nutrients in summer for use during autumn and winter

(Lüning 1990). The Laminariales' ability to translocate stored nutrients has been investigated and proved essential for survival in conditions where light availability is unpredictable (Lüning 1990). Kelps such as *Macrocystis pyrifera* are able to translocate nutrients from parts of the plants, which are bathed in higher nutrients, to parts which are nutrient-limited (Jackson, 1977). North et al. (1993) noted that the largest *Macrocystis* forests develop during periods of low temperatures and high nutrients. *Saccharina latissima* (Miyabe) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (as *Laminaria longicruris* Bachelot de la Pylaie), is able to store nutrient for up to two months which allows it to increase its growth rate during the summer (Chapman and Craigie 1977). Similarly, *L. abyssalis* stores nutrients in the spring for increased growth during summer (Marins et al. 2014). The clonal kelp *Laminaria sinclairii* (Harvey ex J.D. Hooker & Harvey) Farlow, Anderson & Eaton can store carbon in its rhizomes possibly for expansion of the thallus via rhizomatous growth (Demes and Graham 2011). *Saccharina diabolica* (Miyabe) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (as *Laminaria diabolica* Miyabe), is the most widely distributed *Saccharina* species, because of its nutrient storage abilities (Li et al. 2009). Interestingly, both *L. pallida* and *E. maxima* can store nutrients but they do not store it seasonally (Dieckmann 1980, Probyn and McQuaid 1985).

*Depth.* Depth is sometimes cited as important in controlling the vertical limits of distribution of kelps insofar as it is directly related to light attenuation (e.g. Lüning 1990 and Bekkby et al. 2009). However, there are instances where kelp grows at depths greater than expected. Graham et al. (2007) modelled probable suitable habitats for kelps and discovered new *Eisenia* kelp beds in deep cooler water (> 60 m), in Galapagos Islands, where kelps received adequate irradiance.

*Kelp canopy and light*. Because kelps often form a distinct and well developed canopy, there have been many studies investigating the effects of canopy removal, usually but not always in relation to the effects of kelp harvesting. These effects include an increase in turf algae due to an increase in the bottom irradiance (Graham 1997, Edwards 1998, Rothman 2006, Schmidt and Scheibling 2007), the bleaching of coralline algae as a result of light damage (Irving et al. 2004), and in *Laminaria hyperborea* beds in Norway, the rapid formation of a new canopy by understorey kelp responding to increased light levels (Christie et al. 1998). Similarly, Toohey and Kendrick (2007) in an *Ecklonia radiata* bed in Australia, found that canopy removal formed a new environment for the understorey and that just over 50% of the juvenile kelp survived in the 'new environment'. In Tasmania, Edgar et al. (2004) found that large invertebrates prefer turf algae rather than the shelter of a kelp canopy and that the removal of the canopy had little effect on the algal community. In Norway, shading by the kelp canopy and sea urchin grazing were considered largely responsible for the mortality of small kelp sporophytes (Sjøtun et al. 2006).

Wiencke et al. (2000, 2004) showed that depth distribution of kelp and kelp-like brown algae is determined by the susceptibility of zoospores to ultraviolet radiation due to the inhibition of germination (Wiencke et al. 2007). It is well documented that *Laminaria* species are well adapted to growing in low light (Lüning 1990) so it is not surprising that the Arctic *Laminaria solidungula* J. Agardh can grow its blades during the long dark winter season so that the photosynthetic material is available for the arrival of the supply of light. In South Africa a study by Levitt et al. (2002) showed that the understorey communities did not change when the *E. maxima* kelp canopy was removed. A similar study, on the same species, by Rothman et al. (2006) also showed that different degrees of canopy removal had no effect on the growth rate of juvenile recruits because those particular kelp beds were shallow (3 – 4 m) and light was probably not limiting.

*Kelp morphology and the environment*. Studies show that morphological characters of seaweeds in general can be a function of environmental conditions, among which wave action is an important variable (Dayton et al. 1984, Jackelman and Bolton 1990, Hurd and Pildith 2011). The larger the organism, the bigger the drag forces that are exerted on it, and organisms often adapt to reduce the effects of these forces (Friedland and Denny 1995). For example, these authors measured water velocities of up to $10 - 20$ m/s$^{-1}$ with accelerations of $400$ m/s$^{-1}$ acting on thalli of the kelp *Egregia menziesii* (Turner) Areschoug. Kelps need to be flexible and give way as waves sweep over them and to have strong, flexible thalli (Norton et al. 1982). *Nereocystis luetkeana* Postels & Ruprecht stipes are able to bend up to 60° to the stipe axis due to cellulose fibrils in their cortical cell walls, and usually break because of urchin grazing rather than hydrostatic bending forces (Koehl and Wainwright 1977). Furthermore, sporophytes are often ripped from the substratum by becoming entangled, which increases the drag force on them (Koehl and Wainwright 1977, Dayton et al. 1984).

Wernberg and Thomsen (2005) showed that, in *E. radiata* kelps in Australia, plant size, blade morphology, holdfast size, and stipe thickness are weakly correlated to wave exposure. Their results showed that lateral width was the only character that was consistently influenced by wave action and that the fronds of *E. radiata* were narrower in wave exposed environments. They conclude that in their system the effects on morphology of wave exposure may be confounded by other variables (e.g. grazing, sediment load, reef geology and geomorphology). Friedland and Denny (1995) demonstrated that *Egregia menziesii* (Turner) Areschoug has a strap-like shape in the intertidal to reduce the drag in high wave-exposed conditions. Norton et al. (1982) showed that *Saccharina longicruris* (as *Laminaria longicruris*) growing in sheltered areas has up to three times the surface/mass ratio of populations growing in exposed areas. In general kelp sporophytes growing in exposed areas are tougher, sturdier, and more strongly attached compared to those in sheltered areas,

because they have to withstand high water velocities (Friedland and Denny 1995). Other seaweeds also show water-motion induced morphological variability, for example the dictyotalean brown algae *Dictyopteris undulata* Holmes and *Zonaria farlowii* Setchell & N.L. Gardner (Stewart and Carpenter 2003). However, Li et al. (2009) showed that differences in morphology could also be a result of how nutrients are stored within the sporophytes of some *Laminaria* species, indicating that factors other than water motion may influence the morphology of kelps.

The life history of kelps has an alternation of heteromorphic generations, with microscopic haploid gametophytes and macroscopic diploid sporophytes (Dayton 1985). The sporophytes produce motile zoospores which are released into the water and settle on the bottom or any other substrata. There they develop into male and female gametophytes. The male produces sperm which are released to fertilise the egg produced by the female. The result is a diploid zygote that develops into the sporophyte, which attaches to any solid substratum, even the stipes and primary blade of other sporophytes (M. Rothman, personal observation).

*Kelp recruitment.* There are many processes involved in recruitment of kelps. Firstly it involves the release of spores by the previous generation of adult sporophytes (Joska and Bolton 1987, Reed 1990), then the dispersal of these spores (Amsler and Neushul 1989, Cie and Edwards 2011), followed by their settlement on suitable substrata and their subsequent germination, then the growth, maturation and fertility of the gametophytes, culminating in fertilisation and germination of a new generation of sporophytes (Reed 1990) which may or may not survive to become adults.

Environmental conditions can affect any stage in the recruitment cycle, but stable kelp bed densities are often ensured even by low survival rates of recruits (Sjøtun et al. 2006). Among the factors affecting recruitment are storms (Dayton et al. 1984, Kennelly 1987), grazing (Fricke 1979, Harrold and Reed 1985, Anderson et al. 1997), irradiance (Cie and Edwards

2008), kelp harvesting (Sjøtun et al. 2006), and temperature (tom Dieck 1993). However, the two most important factors influencing the early stages of development of kelps are usually temperature and irradiance levels (Lüning 1980, Dysher and Dean 1986).

Generally, large numbers of spores are released by kelp sporophytes: between $10^8 – 10^9$ spores per hour for a single *Alaria marginata* Postels and Ruprecht sporophyte (McConnico and Foster 2005), 3.15 X $10^{12}$ spores per annum by a *Macrocystis pyrifera* sporophyte (Neushul 1981), and 3.07 X $10^{10}$ spores per annum by an *Ecklonia maxima* sporophyte (Joska and Bolton 1987). However, there may be significant seasonal differences in spore production and spore release (Joska and Bolton 1987), and consequently on recruitment of gametophytes or of sporophytes. Also, the distance that spores are dispersed can be affected by many factors, including the size of the spore source, kelp densities and individuals' fecundity (Gaylord et al. 2004). Furthermore, propagule dispersal directly affects the genetic composition of kelp communities (Graham 2003).

Heteromorphic life histories enable organisms to survive in different habitats and environments because different phases can have different environmental requirements (Edwards 2000). Kain and Jones (1964) and Lüning (1980) showed that gametophytes of *Laminaria digitata* (Hudson) J.V. Lamouroux, *L. hyperborea*, and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (as *L. saccharina* (Linnaeus) J.V. Lamouroux) can survive in darkness for up to six months, and then, under light produce sporophytes in two weeks. Gametophytes of *Ecklonia maxima* have a broader temperature range for growth and survival than the sporophytes (Bolton and Levitt 1985, Bolton and Lüning 1982) which is also true in *Undaria pinnatifida* (Harvey) Suringar (Henkel & Hofmann, 2008). Garbary et al. (1999) showed that, in the north-eastern Pacific Ocean, some

kelp gametophytes live as endophytes in the cell walls of red algae, presumably to facilitate succession of the sporophyte generation.

Dayton et al. (1984) observed that under a *Macrocystis pyrifera* canopy there are always juvenile sporophytes present to take over and form part of the canopy once surface-reaching sporophytes are removed. Rothman et al. (2006) showed that once *E. maxima* juveniles reach a stipe length of 25 mm, they have a 75% chance of becoming an adult and forming part of the canopy, probably because they are by then far less accessible to benthic grazers such as sea urchins. It is therefore a reasonable generalisation that once sporophytes are established and have reached a certain size, mortality is reduced, and these young sporophytes have a good chance of maturing to become adult plants that contribute to the kelp canopy.

In South Africa, four species of kelps are currently recognised: *Macrocystis pyrifera*, *Ecklonia maxima*, *E. radiata* and *Laminaria pallida*. *Macrocystis pyrifera* is found only in a few sheltered sites on the southwest coast, and is not dealt with in this study. *Ecklonia maxima* and *Laminaria pallida* form extensive beds and dominate inshore waters on the west coast (see below), and *Ecklonia radiata* grows along the south coast right up the east coast but is usually limited to small stands in the sublittoral fringe (see below). Of particular interest are certain ecological and systematic relationships between some of these species, and these are investigated in this study.

*Molecules vs morphology*. Until relatively recently, identifications of kelps were based on morphological and anatomical characters. These included external characters, e.g. colour, size, blade shape, frond shape, branching, and internal microscopic characters, eg. cell size, cortical cell depth and width, size of paraphyses, etc. While morphological characters are

important, their use is limited when they might be environmentally plastic and therefore inconsistent. In recent years there has been a shift from morphological identification techniques to molecular techniques, often combined with morphological studies (e.g. Leliaert et al. 2014), and it is now almost commonplace to collect tissue, and to analyse the DNA.

In 2006 Lane et al. published the first molecular phylogeny of the ALL families. Their molecular data suggested that the genus *Laminaria* be split into two genera: *Laminaria* (with most of the sporophytes having split blades) and the resurrected genus *Saccharina* (with most of the sporophytes having non-split blades). There is a growing number of examples of morphologically similar species of seaweeds which, on the basis of molecular phylogeny, are clearly different, often known as cryptic species (eg. De Clerck et al. 2005a, Fraser et al. 2009, Soehner et al. 2012, Francis et al. 2015). Two cryptic species of *Lessonia nigrescens* are a particularly good example (Tellier et al. 2011). Morphologically similar sporophytes, thought to be a single species, but separated by a barrier, were molecularly (using mitochondrial and microsatellite markers) shown to be two cryptic species.

The current organisation of the genus *Ecklonia* is largely based on morphology because little molecular work has been done on this genus. This has caused much confusion especially with the Asiatic species of *Ecklonia* and Southern Hemisphere *E. radiata* (Bolton and Anderson 1984, Lane et al. 2006). *Ecklonia radiata* has the widest distribution of the genus and based on morphological characters, *E. fastigiata*, *E. richardiana* and *E. biruncinata* were considered to be synonymous with *E. radiata* (Bolton and Anderson, 1994). *Ecklonia radiata*, due to its morpological variability, has caused some taxonomic confusion, especially in Australia.

*Ecklonia maxima* is very distinctive and is found on the Southern African west coast from Koppie Allen at De Hoop Nature Reserve, South Africa to just north of Lüderitz in Namibia.

The adult has a long hollow stipe that terminates in a spear-shaped primary blade from which the secondary laterals grow bilaterally, often with tertiary branching in adult sporophytes. Until recently the distributions of *Ecklonia maxima* and *E. radiata* did not overlap, but in the last decade *E. maxima* spread ca. 70 km eastwards to De Hoop, where populations of these two species now co-occur (Bolton et al. 2012) and where the distinction between some individuals of the two species is not always clear.

The other Southern Hemisphere *Ecklonia*, *E. brevipes*, is interesting because it can propagate using adventitious attachment organs produced from the blades. Similar holdfast-like protrusions have only previously been observed for *E. maxima* (Fig. 1), however there is no evidence that these can reattach to substrata. The sporophytes of *E. brevipes* have blades that are flabellate or erratically arranged lobes and occurs in shallow water on loose substrata.

Apart from *E. stolonifera* (which can propagate *via* rhizoid-like haptera that extend laterally) the other Northern Hemisphere species of *Ecklonia* can be difficult to distinguish morphologically (Bolton and Anderson 1994). Few sequences have been published for *Ecklonia*, and a proper phylogenetic study is lacking.

The genus *Laminaria*, on the other hand, has received much attention (Yoon et al. 2001, Oudot-Le Secq et al. 2002, Lane et al. 2006, Zhang et al. 2011, 2013, Chi et al. 2014). However, species from the Southern Hemisphere have received much less attention. The only molecular study on a Southern Hemisphere *Laminaria* is by Marins et al. (2012) who used nuclear, mitochondrial and chloroplastic markers and found that *L. abyssalis* (entire blade) and *L. bicyclis* (split blade), previously described for Brazil, comprise a single species.

Figure 1. Haptera-like protruberences on an *Ecklonia maxima* frond.

In Southern Africa two *Laminaria* species were at one stage recorded for the region: *L. pallida* and *L. schinzii*. The differences between the two were based on stipe morphology and size, with *L. pallida* having a solid stipe that is wider near the holdfast, narrowing towards the distal end, while *L. schinzii* was larger and described as having longer, hollow stipes that are widest in the middle narrowing towards the ends (Molloy and Bolton 1996). Stegenga et al. (1997), considered that, as the populations graded into one another and also that these forms were apparently interfertile (tom Dieck and Oliveira 1993), the morphological differences between the two forms were insufficient to recognise separate species. Stegenga et al. (1997) thus regarded *L. schinzii* as a form of *L. pallida*. It was been reported that *L. pallida* in its southern distribution is solid with only the occasional sporophytes being hollow, while on the northern part of the west coast the plants are longer with a greater proportion of hollow-stipe individuals (Stegenga et al. 1997).

The fourth kelp species present in South Africa is *Macrocystis pyrifera* (Linnaeus) C. Agardh. It was previously known as *M. angustifolia* Bory, but recent molecular evidence showed that there is only one species worldwide (Macaya and Zuccarello 2010). In South Africa it is only found in isolated patches on the southern part of the west coast, and it is not dealt with here.

Along the Southern African west coast, *L. pallida* and *E. maxima* co-exist as the dominant kelp species. In the south of this region *E. maxima* dominates the inshore, forming near homogeneous stands, with *L. pallida* forming a subcanopy at deeper depths from about 6 m down to 30 m (Molloy and Bolton 1996). Northwards, *L. pallida* becomes progressively more dominant until it completely replaces *E. maxima* just north of Lüderitz, Namibia. The

ecological relationship between these two species was poorly understood and is the subject of part of the present study.

The first part of this study is a molecular investigation of the systematic and evolutionary relationships of Southern African *Ecklonia* species and their relationship with *Ecklonia* species from other parts of the world. The questions I attempt to answer are:  1. are *E. maxima* and *E. radiata* two distinct species in South Africa? 2. Is the entity known as *E. radiata* in South Africa conspecific with other Southern Hemisphere entities of the same name? 3. What is the relationship between the Southern Hemisphere and Northern Hemisphere species of *Ecklonia* and the other genera of the Lessoniaceae?

Chapter 3 is a molecular study of Southern Hemisphere species of *Laminaria*. The questions I investigate are 1. Are all Southern African morphotypes of *Laminaria,* including the former *L. schinzii*, in fact one species? 2. What is the phylogenetic relationship between the Southern Hemisphere species of *Laminaria* and those from the Northern hemisphere?

Chapter 4 examines the ecological changeover in dominance in shallow inshore waters, between *Ecklonia maxima* on the southern part of the west coast and *Laminaria pallida* on the northern parts of the west coast and into Namibia.   Here I investigate the physical environment on this coast, using satellite and other available data, and how this may influence the distribution, morphology and biomass of the two dominant kelps in order to try to explain the changeover.

Chapter 5 is a culture study investigating the growth and development of the juvenile life stages of the sporophytes of *E. maxima* and *L. pallida,* from the northern west coast of South Africa (Port Nolloth) and the southern west coast (Oudekraal on the Cape Peninsula) in an

attempt to discover differences in their response to temperature and light that might explain the ecological changeover between these species that was quantified in Chapter 4.

Chapter 6 is a general discussion and recommends topics for future research.

CHAPTER 2

A MOLECULAR INVESTIGATION OF THE GENUS *ECKLONIA* (PHAEOPHYCEAE, LAMINARIALES) WITH SPECIAL FOCUS ON THE SOUTHERN HEMISPHERE[1]

INTRODUCTION

*Ecklonia* Hornemann is a genus of brown seaweed in the family Lessoniaceae (previously Alariaceae) of the Laminariales (Lane et al. 2006). *Ecklonia* is distributed in both Hemispheres (Steneck et al. 2002, Steneck and Johnson 2013, Guiry and Guiry 2014) but largely limited to east Asia in the Northern Hemisphere, with only small populations recorded in cooler deep water habitats in Oman and off the north-west coast of Africa (Sheppard et al. 1992, Guiry and Guiry 2014). In the Southern Hemisphere, it is found in South Africa, Australia, and New Zealand (Lüning 1990, Stegenga et al. 1997, Bolton 2010, Guiry and Guiry 2014). According to Guiry and Guiry (2014), out of a total 23 species and infraspecific names available for the genus, only ten *Ecklonia* species (and three forms) are currently accepted taxonomically. However, on the basis of morphology, Bolton and Anderson (1994), considered *E. fastigiata* (Endlicher & Diesing) Papenfuss, and *E. richardiana* J. Agardh to be synonymous with *E. radiata* (C. Agardh) J. Agardh. They further included *E. biruncinata* (Bory de Saint-Vincent) Papenfuss in *E. radiata,* following the treatments of Womersley (1967) and Novaczek (1980), thus leading to a final count of seven current species. Four of those species occur exclusively in the Northern Hemisphere: *E. muratii* Feldmann in Mauritania (Northeastern Atlantic), *E. cava* Kjellman, *E. stolonifera* Okamura and *E. kurome* Okamura in Japan and Korea, with *E. kurome* also occurring in China (Bolton 2010, Guiry and Guiry 2014). Two species occur exclusively in the Southern Hemisphere: *E. maxima* (Osbeck) Papenfuss (Fig. 1 a) and *E. brevipes* J. Agardh. *Ecklonia brevipes* was described from northern New Zealand (Lindauer 1961, Adams 1994) but was also tentatively recorded

from Hamelin Bay, Western Australia (Huisman 2000), while *Ecklonia maxima* forms large kelp forests along the west coast of Southern Africa (Stegenga et al. 1997, Guiry and Guiry 2014). The latter has also been reported from several islands in the south Atlantic, Indian and Pacific Oceans: St Helena, Tristan da Cunha, Falkland Island, St Paul Island, and Auckland Island (Guiry and Guiry 2014). However, except for St Paul Island, which has a number of other seaweed species in common with South Africa, Papenfuss (1942) considered these reports doubtful, and they have not subsequently been substantiated.

*Ecklonia radiata* (Fig 1 b) is the most widely distributed species, occurring in the Southern Hemisphere in South Africa, Australia and New Zealand but also reported from Madagascar, and in the Northern Hemisphere from Oman and the central-eastern Atlantic Ocean (Mauritania, Senegal, the Canary and Cape Verde Islands) (Stegenga et al. 1997, John et al. 2004, Wing et al. 2007).

The characters separating species of *Ecklonia* are almost entirely based on external morphology, particularly stipe and holdfast characteristics. As Bolton and Anderson (1994) pointed out, the morphological distinction between some species is unclear, apart from the very different modes of growth of *E. stolonifera* (spreading stolon-like holdfast) and *E. brevipes* (forming new holdfasts from the tips of blades), and the very long, hollow stipe of *E. maxima*. *Ecklonia radiata* is particularly polymorphic (Fig 1 j-m) (Wernberg et al. 2003). In Australia, Wernberg and Vanderklift (2010) described variations in rugosity, spinosity, stipe length, frond thickness and frond densities which they linked to wave exposure. In New Zealand, Wing et al. (2007) also measured morphological variations of *E. radiata* (frond length, width, thickness and number, as well as stipe length and diameter) and found some of these to depend on light levels related to wave exposure. Morphological variations in South African populations of *E. radiata* have also been observed (Fig 1 b-e), including a range of

Fig. 1. (a) An adult *E. maxima*, (b-e) Different morphologies of *E. radiata* at De Hoop, South Africa; (f-h) indicate different frond morphologies of E. radiata at De Hoop, South Africa, (i) an *E. radiata* with haptera-like protrutions from Hamelin Bay, Australia, (j-m) Different morphologies of *Ecklonia radiata* at different sites in Australia; (j) Ballina, New South Wales, Australia, (k) Abrolhos Island, Western Australia, (l) Albany, Western Australia (m) Jervis Bay, New south Wales, Australia.

frond morphologies (spiny to smooth), frond colours (striped to plain) and marginal serration (Fig. 1 f-g). Differences such as these have contributed to taxonomic confusion in the past, and the description of a number of different species and subspecies, now considered synonymous (see Bolton and Anderson 1994).

Bolton and Anderson (1994) noted that *E. radiata*, *E. cava*, *E. kurome*, and *E. muratii* are difficult to tell apart based on morphological descriptions. They also noted that *E. muratii*, as described by Feldmann (1973), was similar to plants from Oman under the name *E. radiata* and reassigned to an "*E. radiata* complex" including the four species mentioned above. Moreover, the main morphological character used to separate *Ecklonia maxima* from *E. radiata* is the morphology of the stipe, which in the former is long (sometimes up to 10 m) and hollow, and in the latter is shorter and solid. However, because intermediate morphologies have been observed, the distinction between these species also is not clear in some South African populations (M. Rothman, personal observation).

Consequently, the taxonomic confusion surrounding the genus *Ecklonia* has been evident for a long time (Bolton and Anderson 1994). While a few sequences have been published, no study has yet assessed *Ecklonia* species molecularly or examined their phylogenetic relationships. Using small subunit (SSU) rDNA sequences, Boo et al. (1999) confirmed the placement of *Ecklonia cava* in the order Laminariales. Subsequently, Boo and Yoon (2000), using ITS and *rbc*L sequence data sets, constructed a scheme of the Laminariales which grouped *Ecklonia*, *Eckloniopsis* (Kjellman) Okamura and *Eisenia* Areschoug in a clade that was later upheld by Lane et al. (2006). Based on phylogenetic analyses of the large subunit (LSU) rDNA, ITS, the RuBisCO operon, and the NADH dehydrogenase subunit 6 (nad6) regions, Lane et al. (2006) further proposed the transfer of *Ecklonia, Eckloniopsis* and *Eisenia* from the family Alariaceae to the Lessoniaceae. The authors stressed the need to include a sequence of *E. maxima*, the type species of the genus *Ecklonia*, to elucidate the

status of *Eckloniopsis* and *Eisenia*. Finally, using ITS, Wing et al. (2007), confirmed that polymorphic populations of *E. radiata,* from 14 different fjords in New Zealand, formed a single species.

The aim of the present study was to produce the first phylogeny of *Ecklonia*, in order to answer three questions: (i) are *E. radiata* and *E. maxima* two distinct species? (ii) is *E. radiata* from South Africa and Australia/New Zealand a single species?; (iii) what are the relationships between Northern and Southern Hemisphere species of *Ecklonia* and other genera of the Lessoniaceae?

MATERIALS AND METHODS

*Sample collection and identification. Ecklonia maxima and E. radiata* samples were collected from Southern Africa and Australia along their distribution ranges from a total of 13 and 6 localities, respectively. *Ecklonia cava*, *E. kurome*, and *E. stolonifera* were collected from 6 localities around Japan. For a detailed sampling list see Table 1. Voucher specimens are lodged at the Seaweed Unit, Department of Agriculture, Forestry and Fisheries, South Africa. Samples from mature sporophytes were collected to represent observed morphological variability within populations at the various sites either using SCUBA, snorkelling or walking at low tide. Whenever possible, photographs of the specimens were taken. Tissue samples (3-5 cm$^2$) of each specimen were collected from the youngest part of the frond of the first secondary blade and stored in silica gel for later DNA extraction (Chase and Hills 1991). Specimens were identified based on characters as described in Okamura (1927), Lindauer (1961), Womersley (1987), Adams (1994), Stegenga et al. (1997), and Yoshida (1998).

*DNA extraction, amplification and sequencing*. DNA extraction and PCR amplification were done in the Systematics laboratory of the Department of Biological Sciences of the University of Cape Town (South Africa). Silica gel-dried material (0.2 - 0.3 g) was ground in

a Retsch mixer mill MM 4000 (Retsch GmbH, Haan, Germany). Two extraction protocols were used to extract the DNA. The first method used a Qiagen plant DNA extraction kit following the manufacturer's protocol, but success was limited, probably due to high levels of polysaccharides and phenols in the samples. A higher success rate was obtained using a combination of CTAB and SDS method as described by Maeda et al. (2013). Genomic DNA was then purified using the GENECLEAN® III Kit (MP Biomedical, LLC; France) following the manufacturer's protocol.

The purified DNA was used for the PCR amplification in 25 μL volumes in a 2700 GeneAmp PCR System (Applied Biosystems, Foster City, CA, USA). The reaction mix contained 1 μL DNA, 20.25 μL ultra-pure water, 2.5 μL KAPA *Taq* buffer (Kapa Biosystems, Cape Town, South Africa), 0.5 μL KAPA dNTP Mix, 0.25 μL of each primer and 0.25 μL KAPA *Taq* DNA Polymerase Ready Mix or SuperTherm*Taq* DNA Polymerase (Roche, Mannheim, Germany).

Four genetic markers were amplified: (i) two mitochondrial intergenic spacer regions: spacer between the t-RNAs for tryptophan and isoleucine genes (trnWI) and between the atp8 and t-RNA serine genes (hearafter mentioned as atp8) using primers from Voisin et al. (2005); (ii) one nuclear marker covering the ITS1, 5.8S gene, was amplified with the primer pair LB1 and LB2 from Yoon et al. (2001); (iii) and one chloroplastic marker: the large subunit of the RuBisCO (*rbc*L) using primers KL2 and KL8 from Lane et al. (2006). These markers have been chosen to cover all three algal genomes (mitochondrial, chloroplastic, and nuclear) and to beable to include sequences from previous studies (e.g. Martin 2011).

The thermal profile for PCR amplification of *rbc*L was as follows: an initial denaturation cycle of 95 °C for 1 min, followed by 35 cycles of 95 °C for 30 sec, 45 °C for 30 sec, and 72 °C for 1 min. A final annealing step at 72 °C was extended for 10 min. The thermal profile for PCR amplification for ITS were as follows: an initial denaturation cycle of 94 °C for 5

min, followed by 35 cycles of 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min. A final

annealing step at 72 °C was extended for 4 min. For both trnW-I and atp8-trnS, the thermal

profile for PCR amplification were as follows: an initial denaturation cycle of 95 °C for 2

min, followed by 32 cycles of 95 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 30 sec. A

final annealing step at 72 °C was extended for 5 min. All PCR products were purified using

the NucleoFast membrane (Machery-Nagel, Düren, Germany) on a Freedom Evo robot

(Tecan Group Ltd., Männedorf, Switzerland) and sequenced with PCR primers using the

BigDye Terminator v3.1 sequencing kit (Life Technologies, Johannesburg, South Africa).

Purifying and sequencing was done by the Sequencing Unit at the University of Stellenbosch

(South Africa).

Sequences were edited, manually aligned and concatenated in Bioedit (Hall 1999). Sequences

representing as many species of *Ecklonia, Eisenia* and *Eckloniopsis* as possible from as many

localities as possible, were downloaded from Genbank and added to the alignments. Although

many sequences were available for all four markers for *Ecklonia*, no atp8 and trnWI

sequences were available for *Eisenia* and *Eckloniopsis.* As a consequence, two different

datasets were analysed: a concatenated atp8/trnWI dataset for studying the relationships of

species within the genus *Ecklonia*, and a concatenated *rbc*L/ITS dataset for studying the

relationships of taxa within the family Lessoniaceae.

The best-fit models were estimated for each individual alignment with FindModel (available

online at http://hiv.lanl.gov) and were all found to be GTR plus gamma. A Bayesian

inference was applied to a concatenated data sets consisting of atp8 + trnWI and *rbc*L + ITS

using BEAST v.1.7.5 (Drummond et al. 2012) through the online CIPRES Science Gateway

Platform (Miller et al. 2010). The analysis used estimated base frequencies, 4 T categories to

model among-site rate heterogeneity, a relaxed log-normal molecular clock, a coalescent tree

prior with a randomly generated starting tree, and run for 50 million generations. A tenth of

the tree was discarded as burn-in. Convergence of the Markov chains was checked using Tracer v.1.4 (Rambaut and Drummond 2007). The Maximum Likelihood analysis was performed using RAxML-HPC2 on XSEDE using a GTR-MIX evolutionary model (Stamatakis 2006). A multi-parametric bootstrap resampling with 1000 iterations provided bootstrap supports. The concatenated atp8/trnWI tree was rooted with sequences of *Lessonia* Bory de Saint-Vincent species (downloaded from GenBank), with the aim of looking into relationships between species of *Ecklonia*, because *Lessonia* is closely related to *Ecklonia* (Lane et al. 2006). The concatenated *rbc*L/ITS tree was rooted with *Laminaria* J.V. Lamouroux species (one sequence from this study and the rest downloaded form GeneBank), a species from the closely related family, Laminariaceae (Lane et al. 2006), with the aim of looking into relationships within the Lessoniaceae familly.

RESULTS

A total of 44 samples were successfully sequenced (Table 1), including 24 from South Africa (14 *E. maxima*, 10 *E. radiata*), two from Namibia (both *E. maxima*), eight from Australia (seven *E. radiata* and one *E. brevipes*) and ten from Japan (three *E. cava*, five *E. kurome* and two *E. stolonifera*). Sequences previously published and available on GenBank were used for: *Ecklonia cava* (Japan), *Ecklonia kurome* (Japan), *Ecklonia radiata* (New Zealand and Australia), *Ecklonia stolonifera* (Japan), *Eckloniopsis radicosa* (Kjellman) Okamura (Japan), *Eisenia arborea* Areschoug (Canada), and *Eisenia bicyclis* (Kjellman) Setchell (Korea). For the Outgroup of the concatenated atp8/trnWI tree: *Lessonia adamsiae* C.H. Hay (New Zealand), *L. brevifolia* J. Agardh (Chile), *L. nigrescens* Bory de Saint-Vincent (Chile), *L. tholiformis* C.H. Hay (New Zealand), *L. trabeculata* Villouta & Santelices (Chile) and *L. vadosa* Searles (Falkland Islands) sequences were downloaded from GenBank. For the outgroup of the concatenated *rbc*L/ITS analysis: *Laminaria digitata* (Hudson) J.V. Lamouroux (United Kingdom and Canada), and *L. hyperborea* (Gunnerus) Foslie (France)

sequences were downloaded from GenBank while one sequence was obtained during the present study for *L. pallida* Greville (South Africa). Final alignments included 28 sequences for the *rbc*L/ITS and 48 for atp8/trnWI and were 1367 and 538 base pairs long including gaps, respectively.

The concatenated atp8/trnWI analysis (Fig. 2) recovered two clades: a Southern Hemisphere clade (posterior probabilities (PP) = 0.99; bootstrap (B) < 60 %) and an Asiatic one (PP = 1; B = 81 %). The Southern Hemisphere clade was further subdivided into two subclades representing *Ecklonia radiata* (PP = 1, B = 78 %, sequence divergence < 1 %) and *Ecklonia maxima* (PP = 1; B = < 65 %, sequence divergence < 1 %). The *E. radiata* subclade included sequences from South Africa, Australia and New Zealand while the *E. maxima* subclade was represented by South African and Namibian sequences only (with an interspecific sequence divergence of 1.2 – 2.7 %). One specimen, from Hamelin Bay, Western Australia, which had been tentatively identified as *Ecklonia brevipes* (specimen D1741) was recovered in the '*E. radiata* clade'. The Northern Hemisphere clade was further subdivided in two subclades, one containing the available sequences for *E. cava* (with an intraspecific sequence divergence < 1 %), and the rest of the Asiatic species (with a sequence divergence of 0.2 – 1.4 %). Only the second clade was significantly supported (PP = 1, B = 65%).

The *rbc*L/ITS analysis (Fig. 3) produced two fully supported clades, one representing the genus *Lessonia* (PP = 1; B = 99-100 %) and the other one representing mainly the genus *Ecklonia* but also containing the available sequences for *Eckloniopsis* and *Eisenia* (PP = 1; B = 99 %). The *Ecklonia* clade was further subdivided into two subclades, one representing the Southern Hemisphere species of *Ecklonia* as well as a sequence for *Eisenia arborea* (PP = 0.82; B = 62 %)*,* and one containing the Asiatic species of *Ecklonia* as well as sequences for *Eckloniopsis radicosa* and *Eisenia bicyclis* (PP = 0.93; B = 99 %). The Southern Hemisphere clade was subdivided into two groups representing *Ecklonia maxima* (PP = 0.99, B = 70 %,

intraspecific sequence divergence < 1 %) and *Ecklonia radiata* (PP = 0.94; B < 65 %, intraspecific sequence divergence < 1 %), with the sequence for *Eisenia arborea* standing
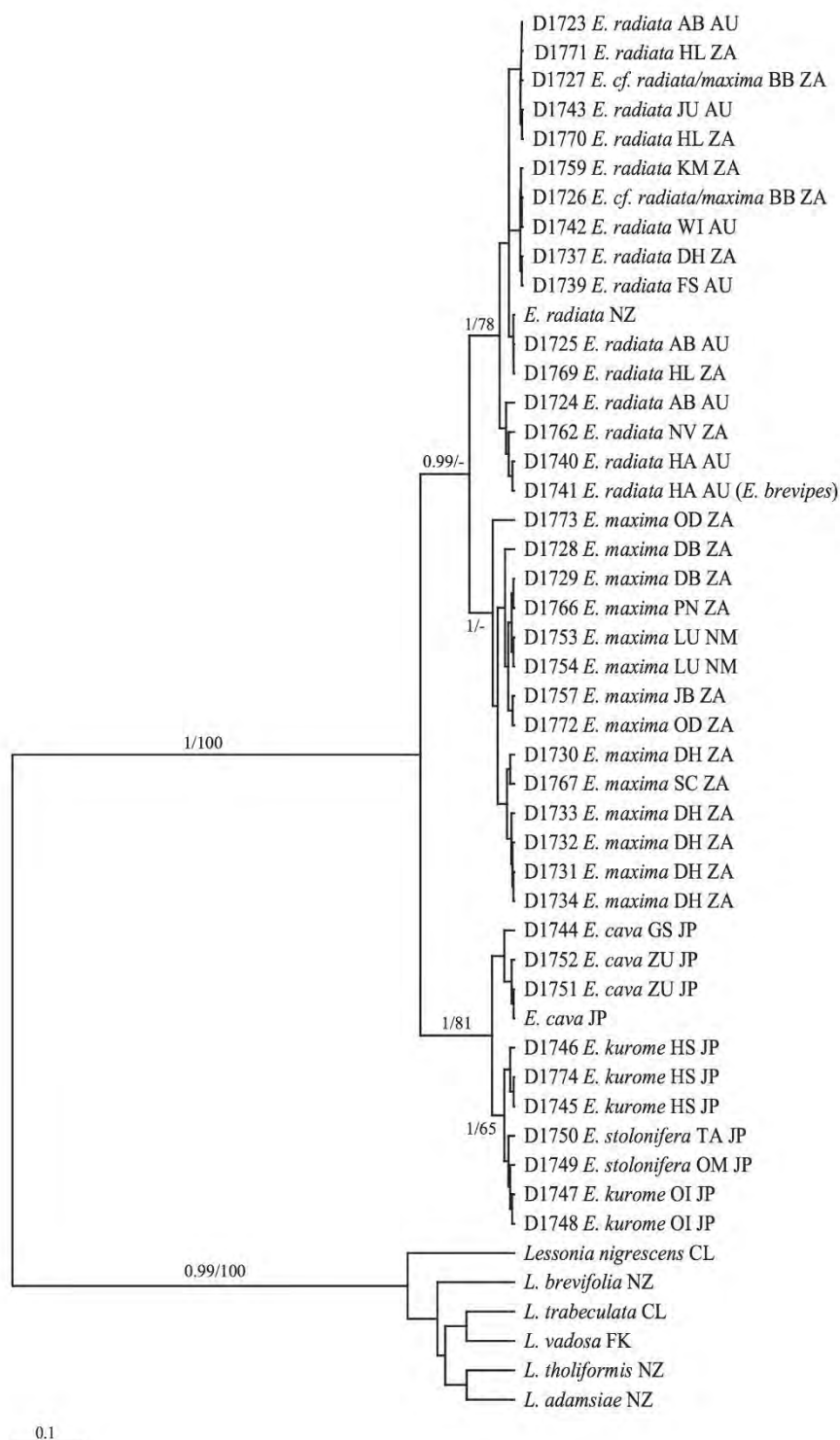


Fig. 2. Bayesian phylogeny based on the concatenated atp8/trnWI alignment. Posterior probabilities below 0.8 and bootstrap less than 60% represented by "-". Branch numbers indicate Bayesian analysis; Maximum likelihood.
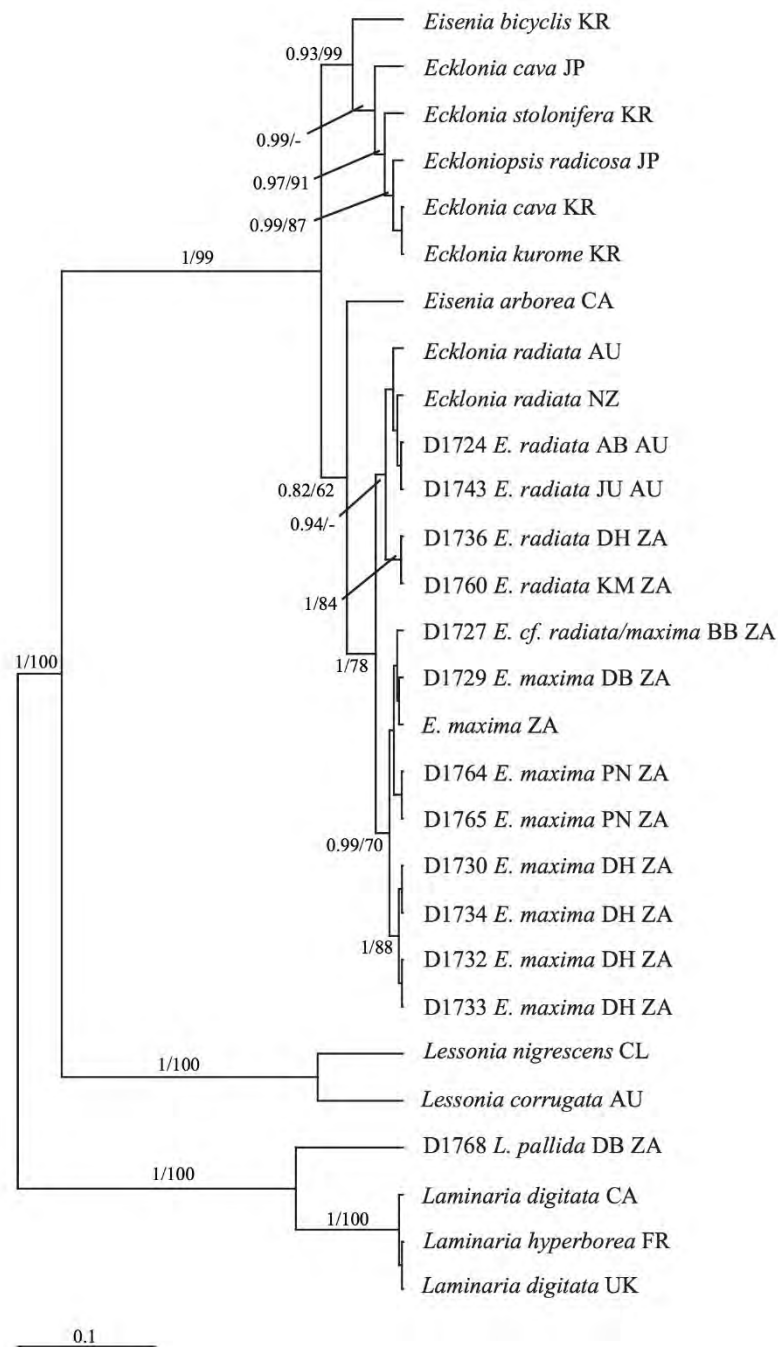
Fig. 3. Bayesian phylogeny based on the concatenated *rbc*L/ITS alignment. Posterior probabilities below 0.8 and bootstrap less than 60% represented by "-". Branch numbers indicate Bayesian analysis; Maximum likelihood.

intraspecific sequence divergence < 1 %), with the sequence for *Eisenia arborea* standing alone. Interspecific divergence between *E. radiata* and *E. maxima* ranged from 3 to 7 %. The *Ecklonia radiata* group included sequences from South Africa, Australia and New Zealand while the *E. maxima* group contained only sequences from Southern Africa (South Africa and Namibia). The Northern Hemisphere clade did not produce any well supported subclades and sequence divergence ranged from 0 to 1.4 %. Interestingly, one *Ecklonia* specimen (D1727) from Bordjiesrif, (near Cape Point, South Africa) produced atp8 and trnWI sequences matching with the *E. radiata* clade (Fig. 2) while *rbc*L and ITS sequences matched the *E. maxima* clade (Fig. 3). The sample was re-extracted and sequenced again but results remained the same.

DISCUSSION

Our phylogeny of *Ecklonia* included sequences for most of the distribution range of the three current Southern Hemisphere species (*E. radiata*, *E. maxima* and a specimen tentatively identified as *E. brevipes*), sequences for East Asiatic species (*E. cava*, *E. kurome* and *E. stolonifera*) as well as closely related genera *Eckloniopsis* and *Eisenia*. Our results confirmed *E. radiata* and *E. maxima* as two distinct species in South Africa, *E. radiata* as a single species throughout the Southern Hemisphere (in South Africa, Australia and New Zealand) and East Asiatic species as a lineage distinct to the Southern Hemisphere clade. We further showed the close sister relationship between *Eckloniopsis radicosa*, two *Eisenia* species (including the type species *Eisenia arborea*) and the genus *Ecklonia*, suggesting the two former genera are superfluous and should be subsumed in *Ecklonia*.

*Diversity and distribution of* Ecklonia *in the Southern Hemisphere.* Both the concatenated *rbc*L/ITS and the concatenated atp8/trnWI analyses recovered *E. maxima* and *E. radiata* as two distinct species with comparable intraspecific divergence (< 1 %).

 Sporophytes of *Ecklonia maxima* can grow a stipe of up to 10 m long and usually form extensive kelp forests along the South African west coast. *Ecklonia maxima* is distributed from Koppie Alleen in De Hoop Nature Reserve, South Africa (34°28'42.55"S 20°30'37.23" E) (Bolton et al. 2012) westwards to north of Lüderitz, Namibia (26°37'52.56"S 15°09'06.31"E) (Stegenga et al. 1997). In the south it dominates shallower inshore waters, forming near homogeneous stands of floating kelp forests, from the subtidal fringe down to 5-10 m deep which are gradually replaced northward by another kelp, *Laminaria pallida.*

*Ecklonia radiata* sporophytes are, in South Africa, generally less than 1 m long (Stegenga et al. 1997) but in some sites in Australasia they can reach 2 m long (see Fig. 1 j and Wernberg et al. 2003), consisting of a primary blade with secondary lateral fronds, a stipe and holdfast. Unlike *E. maxima*, *E. radiata* sporophytes have solid stipes, fronds that are sometimes spiny, rugose or smooth, or varying combinations on a single sporophyte (Wernberg et al. 2003). In South Africa, *Ecklonia radiata* occurs inshore on the south and east coasts, from Koppie Alleen in De Hoop Nature Reserve eastwards to Port Edward (31°3'24"S 30°13'24"S). It is far less abundant than *E. maxima* and mostly occurs in the sublittoral fringe or very shallow subtidal. However, there are also records from further east than Port Edward, but all from deeper subtidal habitats, to at least 40m deep (De Clerck et al. 2005b). The recording of subtidal *E. radiata* at Bordjiesrif (South Africa) by Stegenga et al. (1997), extends the species distribution about 200 km further west than its generally accepted distribution limit at Koppie Alleen. However, no sporophytes have been found in the region between Koppie Alleen and Bordjiesrif, and results of our molecular analyses could not confirm the presence of this species at Bordjiesrif. Specimens collected from Bordjiesrif had a morphology intermediate

between *E. radiata* and *E. maxima* (general *E. maxima*–like appearance with a solid stipe and broad, rugose fronds with serrated edges) while molecular analyses also provided mixed results. Hence, the specimens sequenced clustered within the *E. maxima* clade in *rbc*L/ITS analysis (D1727) and within the *E. radiata* clade in the atp8/trnWI analysis (D1726 & D1727) (Figs. 2 & 3). The presence of possible hybrids between the two species at Bordjiesrif requires further studies.

In Australia, *Ecklonia radiata* is distributed around the western and southern coasts from Kalbarri and the Abrolhos Island on the west coast around southern Australia and Tasmania to Caloundra in Queensland (Womersley 1987, Huisman 2000, Wernberg et al. 2003). Upon studying the description of *E. radiata* (Lindauer 1961, Hommersand 1986, Adams 1994) it is clear that, apart from the Australasian *E. radiata* sometimes having a slightly longer stipe, *E. radiata* in the Southern Hemisphere has many overlapping morphological characters (Wernberg et al. 2003). *Ecklonia radiata* is further distributed throughout New Zealand and on many New Zealand Islands: Three Kings Island/Manawatawhi, Stewart Island/Rakiura, Snares Island/Tini Heke (Guiry and Guiry 2014).

The third Southern Hemisphere *Ecklonia* species, *E. brevipes*, was described by J. Agardh (1877) from the Bay of Islands in New Zealand. Since then it has been recorded from this locality, Stewart Island (Lindauer 1961, Adams 1994), Bay of Islands (North I.), Fiordland (South I.) (Adams 1994), and "tentatively" from Hamelin Bay in Western Australia (Huisman 2000). *Ecklonia brevipes* was described as having blades that are flabellate or erratically arranged lobes angled in any direction without order, and with marginal teeth that can develop into adventitious attachment organs. Its habitat is sublittoral, in shallowish water, on somewhat loose substratum of sand and corallines (J. Agardh 1877, Lindauer 1961). Adams (1994) also describes its habitat as subtidal on a muddy substratum in sheltered, turbid areas and mentioned that colonies of *E. brevipes* can develop from detached, sunken plants and that

they can increase by fragmentation. During the present study, one specimen collected from Hamelin Bay was tentatively identified as *E. brevipes* (Fig. 1 i) following the morphological description and illustration provided by Huisman (2000). It was similar to *E. radiata* except for the presence of haptera-like protrusions at the frond tips that appeared to be reattachment organs, but was different from the descriptions of specimens of *E. brevipes* from the type locality (J. Agardh 1877, Lindauer 1961, Adams 1994). Results of our molecular analysis further indicated that this specimen was molecularly similar to *E. radiata* (Fig. 3) thus not supporting the presence of *E. brevipes* as a distinct species in Australia. Similar reattachment organs to those observed on specimens from Hamelin Bay, although not as pronounced, have been observed from the margins of the secondary fronds in *E. maxima* sporophytes from Southern Africa (Bolton and Anderson 1994; aslo see Ch 1, Fig. 1). We believe that *E. brevipes* does not occur in Australia, and material from the type locality needs to be assessed molecularly to establish whether this species is indeed separate from *E. radiata*. The specimen from Hamelin Bay might be an *E. radiata* exhibiting an ecotype or abnormality that is amplified under specific environmental conditions conducive to vegetative propagation.

*The East Asiatic clade*. Phylogenies produced during the present study supported the East Asiatic sequences as a lineage independent from the Southern Hemisphere clade. In the concatenated atp8/trnWI analysis, *Ecklonia cava* formed a clade (which was however not supported) separate from *E. kurome* and *E. stolonifera*. Okamura (1927) pointed out the difficulty in distinguishing *E. cava* from *E. kurome* but stated that the central rachis of *E. kurome* varies while that of *E. cava* does not, and that the former is palatable while the latter is not. Results of the atp8/trnWI analysis indicate that these species could be distinct, but the corresponding clade was not significantly supported nor did it appear in the *rbc*L/ITS analyses, indicating that more sequences from these species are required to fully resolve their statuses. Similarly, previous studies by Yoon et al. (2001) and Lane et al. (2006) could not

differentiate between *E. cava* and *E. stolonifera,* raising questions about whether they are distinct taxa.

The results further showed that the available sequences for *Eckloniopsis radicosa* and *Eisenia bicyclis* are part of the East Asiatic *Ecklonia* clade. *Eckloniopsis radicosa* was first described by Kjellman et al. (1885) as *Laminaria radicosa* Kjellman but was later transferred to *Ecklonia* as *Ecklonia radicosa (*Kjellman) Okamura (Okamura 1892). Okamura (1927) re-examined the species and decided it was sufficiently different from *Ecklonia* to belong to a distinct and new monospecific genus which he named *Eckloniopsis.* The two major differences (as compared to *Ecklonia*), on which Okamura based the description of his new genus, were its frond morphology and the absence of secondary blades arising from the meristem. There are however examples of *Ecklonia* species showing similar characters, for example *E. stolonifera* lacks secondary fronds and substantial ecotypic frond plasticity has been observed in *Ecklonia radiata* (Wernberg and Thomsen 2005, Fowler-Walker et al. 2006, Wernberg and Vanderklift 2010). The results of the present study resolved the available sequences for *Eckloniopsis radicosa* within the *Ecklonia* clade and highlighted its sister relationship to Japanese species (Fig. 3). Based on this result, we consider *Eckloniopsis* as superfluous and propose to transfer the species "*radicosa"* back to the genus *Ecklonia* and therefore consider *Eckloniopsis radicosa* (Kjellman) Okamura (Okamura 1927) as an homotypic synonym of *Ecklonia radicosa* (Kjellman) Okamura (Okamura 1892). More sequences are needed to clarify its relationship to the other Asiatic species of *Ecklonia*. The situation of *Eisenia bicyclis* is discussed below.

Ecklonia *and* Eisenia. The genus *Eisenia* was described based on *Eisenia arborea* (type from San Francisco, USA) by Areschoug (1876). *Eisenia bicyclis* (Kjellman) Setchell was first described as *Ecklonia bicyclis* by Kjellman et al. (1885), but was transferred to *Eisenia* by Setchell (1905), because of the splitting of the meristem at the top of the stipe which is

characteristic of the genus. Yendo (1911) later proposed *Eisenia bicyclis* as a form of *E. arborea* but the name is not currently accepted (Guiry and Guiry 2014). Apart from the split in the meristem at the top of the stipe, there is little morphological difference between *Ecklonia* and *Eisenia*.

Based on *rbc*L and ITS Boo and Yoon (2000) produced a systematic scheme of the Laminariales, placing *Ecklonia*, *Eckloniopsis* and *Eisenia* in an *Ecklonia* group. This group was later confirmed by Yoon et al. (2001) who placed *Ecklonia*, *Eckloniopsis* and *Eisenia* in a robust group with a 100 % bootstrap. Furthermore, similar results were obtained by Lane et al. (2006) who also doubted that *Eisenia*, based on their molecular analysis, is a distinct genus. However, because their analysis did not include the type of *Ecklonia* (*E. maxima*), they were reluctant to make this modification.

Results of the concatenated *rbc*l/ITS phylogeny presented in our study, including sequences for *E. maxima*, did not support *Eckloniopsis* (discussed above) nor *Eisenia* as distinct from *Ecklonia*. The sequences for *Eisenia bicyclis* from Korea grouped in a well-supported clade with Asiatic *Ecklonia* species, while the sequence for the type species of *Eisenia* (*E. arborea*) collected from Canada clustered with the Southern Hemisphere *Ecklonia* species (Fig. 3). Based on our results and the discussions of previous authors we believe that *Eisenia* is superfluous and propose to transfer the species *Eisenia arborea* and *E. bicyclis* to *Ecklonia*. We therefore consider *Eisenia arborea* Areschoug and *Eisenia bicyclis* (Kjellman) Setchell to be homotypic synonyms of *Ecklonia arborea* (Areschoug) Rothman, Mattio & Bolton *comb. nov.* (see below) and *Ecklonia bicyclis* Kjellman respectively. Okamura (1927) believed that the other *Eisenia* species might one day be proved to be one and same species, or at least varieties of a single species. We find it likely that remaining species of *Eisenia* also belong to *Ecklonia*, but the status of these species needs to be assessed using molecular data.

The present study shows that *Ecklonia* is divided into two independently evolving lineages, both including species with split (formely *Eisenia*) and non-split meristems. One of the lineages appeared centred in the East Asiatic region (Japan, Korea and China) whereas the other lineage appeared more widespread with species occurring in the Pacific, Atlantic and Indian Oceans, but mostly in Southern Africa, Australia and New Zealand and along the west coast of the Americas. Where and when the genus originated is difficult to determine, but our results suggest that split or non-split meristems have evolved twice independently. We believe that the analysis of sequences for other species of *Eisenia* and a calibrated phylogeny are necessary to further discuss this hypothesis.

*Taxonomic conclusions.* From the data presented here we conclude that two distinct *Ecklonia* species occur in Southern Africa (*E. maxima* and *E. radiata*), and confirm *E. radiata* as a single species throughout the Southern Hemisphere in South Africa and Australasia. Based on our chloroplastic and nuclear analysis we resurrect *Ecklonia radicosa* (Kjellman) Okamura and *Ecklonia bicyclis* Kjellman, and consider *Eisenia arborea* synonymous with the new combination *Ecklonia arborea* (Areschoug) Rothman, Mattio & Bolton *comb. nov*.

*Ecklonia arborea* (Areschoug) Rothman, Mattio & Bolton *comb. nov. Basionym: Eisenia arborea* Areschoug *in De tribus Laminarieis* (*Egregia aresch*., *Eisenia aresch*., *Nereocystis*) *et de Stephanocystide osmundacea(turn.). trevis. observationes praecursorias offert*. *Botaniska Notiser* 1876:69.

Table 1. Collection details[a] or references, sample and GenBank accessions for species used in this study.

| Species | Location, Collector | Sample no | rbcL | ITS | atp8 | trnWI |
|---|---|---|---|---|---|---|
| Ecklonia cava | Goza-Shirahama, JP, K. Kogame | D1744 | | | KM575758 | KM575814 |
| Ecklonia cava | Goza-Shirahama, JP, K. Kogame | D1744 | | | KM575758 | KM575814 |
| Ecklonia cava | Zushi, JP, S. Uwai | D1751 | | | KM575765 | KM575820 |
| Ecklonia cava | Zushi, JP, S. Uwai | D1752 | | | KM575751 | KM575809 |
| Ecklonia cava | Martin, 2011, JP | na | GU593873 | GU593773 | GU593723 | GU593923 |
| Ecklonia cava | Yoon et al., 2001, KR | na | AF318967 | AF319009 | | |
| Ecklonia kurome | Hatozu, Saeki, JP, S. Uwai | D1745 | | | KM575746 | KM575806 |
| Ecklonia kurome | Hatozu, Saeki, JP, S. Uwai | D1746 | | | KM575762 | KM575812 |
| Ecklonia kurome | Ohmi, Itoi-gawa, JP, M. Okaji | D1747 | | | KM575763 | KM575824 |
| Ecklonia kurome | Ohmi, Itoi-gawa, JP, M. Okaji | D1748 | | | KM575749 | KM575808 |
| Ecklonia kurome | Hatozu, Saeki, JP, S. Uwai | D1774 | | | KM575760 | KM575823 |
| Ecklonia kurome | Feng et al. Unpubl, CN | na | EF407572 | EF407574 | | |
| Ecklonia maxima | Doring Bay, ZA, M. Rothman | D1728 | | | KM575766 | KM575798 |

| Species | Location | ID | | | | |
|---|---|---|---|---|---|---|
| *Ecklonia maxima* | Doring Bay, ZA, M. Rothman | D1729 | KM575789 | KM575775 | KM575756 | KM575800 |
| *Ecklonia maxima* | De Hoop, ZA, R. Anderson | D1730 | KM575782 | KM575769 | KM575757 | KM575829 |
| *Ecklonia maxima* | De Hoop, ZA, R. Anderson | D1731 | | | KM575729 | KM575825 |
| *Ecklonia maxima* | De Hoop, ZA, R. Anderson | D1732 | KM575792 | KM575778 | KM575733 | KM575826 |
| *Ecklonia maxima* | De Hoop, ZA, R. Anderson | D1733 | KM575783 | KM575770 | KM575734 | KM575827 |
| *Ecklonia maxima* | De Hoop, ZA, R. Anderson | D1734 | KM575793 | KM575779 | KM575735 | KM575828 |
| *Ecklonia maxima* | Lüderitz, NM, A. Plos | D1753 | | | KM575748 | KM575807 |
| *Ecklonia maxima* | Lüderitz, NM, A. Plos | D1754 | | | KM575754 | KM575799 |
| *Ecklonia maxima* | Jacobs Bay, ZA, M. Rothman | D1757 | | | KM575745 | ---- |
| *Ecklonia maxima* | Port Nolloth, ZA, M. Rothman | D1764 | KM575788 | KM575774 | | |
| *Ecklonia maxima* | Port Nolloth, ZA, M. Rothman | D1765 | KM575786 | KM575772 | | |
| *Ecklonia maxima* | Port Nolloth, ZA, M. Rothman | D1766 | | | KM575752 | KM575797 |
| *Ecklonia maxima* | Stanford's Cove, ZA, M. Rothman | D1767 | | | KM575761 | KM575822 |
| *Ecklonia maxima* | Oudekraal, ZA, M. Rothman | D1772 | | | KM575755 | KM575795 |
| *Ecklonia maxima* | Oudekraal, ZA, M. Rothman | D1773 | | | KM575767 | KM575810 |
| *Ecklonia maxima* | Feng et al. Unpubl, ZA | na | EF407573 | EF407575 | | |

| Species | Location | Sample | | | | |
|---|---|---|---|---|---|---|
| *Ecklonia radiata* | Hamlin Bay, AU, M. Mohring | D1723 | | | KM575740 | KM575796 |
| *Ecklonia radiata* | Abrolhos, AU, M. Mohring | D1724 | KM575784 | KM575781 | KM575741 | KM575821 |
| *Ecklonia radiata* | Abrolhos, AU, M. Mohring | D1725 | | | KM575743 | KM575817 |
| *Ecklonia cf. radiata/maxima* | Buffels Bay, ZA, D. Kemp | D1726 | | | KM575736 | KM575830 |
| *Ecklonia cf. radiata/maxima* | Buffels Bay, ZA, R. Anderson | D1727 | KM575785 | KM575771 | KM575753 | KM575805 |
| *Ecklonia radiata* | De Hoop, ZA, C. Boothroyd | D1736 | KM575790 | KM575776 | | |
| *Ecklonia radiata* | De Hoop, ZA, C. Boothroyd | D1737 | | | KM575738 | KM575801 |
| *Ecklonia radiata* | Hamlin Bay, AU, M. Mohring | D1739 | | | KM575730 | ---- |
| *Ecklonia radiata* | Hamlin Bay, AU, M. Mohring | D1740 | | | KM575737 | KM575815 |
| *Ecklonia radiata* | Hamlin Bay, AU, M. Mohring | D1741[b] | | | KM575739 | KM575802 |
| *Ecklonia radiata* | Hamlin Bay, AU, M. Mohring | D1742 | | | KM575731 | ---- |
| *Ecklonia radiata* | Jurien Bay, AU, M. Mohring | D1743 | KM575787 | KM575773 | KM575747 | KM575803 |
| *Ecklonia radiata* | Kei Mouth, ZA, D. Kemp | D1759 | | | KM575732 | KM575831 |
| *Ecklonia radiata* | Kei Mouth, ZA, D. Kemp | D1760 | KM575791 | KM575777 | | |

| Species | Location/Source | Code | | | | |
|---|---|---|---|---|---|---|
| *Ecklonia radiata* | Kei Mouth, ZA, D. Kemp | D1762 | | | KM575768 | KM575804 |
| *Ecklonia radiata* | Hluhluwe, ZA, C. Boothroyd | D1769 | | | KM575742 | KM575813 |
| *Ecklonia radiata* | Hluhluwe, ZA, C. Boothroyd | D1770 | | | KM575744 | KM575818 |
| *Ecklonia radiata* | Hluhluwe, ZA, C. Boothroyd | D1771 | | | KM575759 | KM575816 |
| *Ecklonia radiata* | Lane et al. 2006, AU | na | AY851552 | AY857898 | | |
| *Ecklonia radiata* | Martin, 2011, NZ | na | GU593874 | GU593774 | GU593724 | GU593924 |
| *Ecklonia stolonifera* | Oma, JP, S. Uwai | D1749 | | | KM575764 | KM575819 |
| *Ecklonia stolonifera* | Oma, JP, S. Uwai | D1750 | | | KM575750 | KM575811 |
| *Eckloniopsis radicosa* | Yoon et al. 2001, JP | na | AF318969 | AF319011 | | |
| *Eisenia arborea* | Lane et al. 2006, CA | na | AY851550 | AY857899 | | |
| *Eisenia bicyclis* | Yoon et al. 2001, KR | na | AF318963 | AF319012 | | |
| *Laminaria digitata* | Lane et al. 2006, CA | na | AY857886 | AY851559 | | |
| *Laminaria digitata* | Yoon et al. 2001, UK | na | AF318971 | AF319014 | | |
| *Laminaria hyperborea* | Yoon et al. 2002 , FR | na | AF318972 | AF319015 | | |
| *Laminaria pallida* | Doring Bay, ZA, M. Rothman | D1768 | KM575794 | KM575780 | | |
| *Lessonia adamsiae* | Martin, 2011, NZ | na | | | GU593749 | GU593949 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Lessonia brevifolia* | Martin, 2011, NZ | na | | | GU593753 | GU593953 |
| *Lessonia corrugate* | Lane et al. 2006, AU | na | AY851545 | AY857902 | | |
| *Lessonia corrugate* | Martin, 2011, AU | na | | | GU593744 | GU593944 |
| *Lessonia nigrescens* | Lane et al. 2006, CL | na | AY851544 | AY857901 | | |
| *Lessonia nigrescens* | Martin, 2011, CL | na | | | GU593925 | GU593725 |
| *Lessonia tholiformis* | Martin, 2011, NZ | na | | | GU593746 | GU593946 |
| *Lessonia trabeculata* | Martin, 2011, CL | na | | | GU593733 | GU593933 |
| *Lessonia vadosa* | Martin, 2011, FK | na | | | GU593736 | GU593936 |

[a]Country abbreviations: AU = Australia, CA = Canada, CL = Chile, CN = China, FK = Falkland Islands, FR = France, JP = Japan, KR = Republic of Korea, UK = United Kingdom, ZA = South Africa

[b]Specimen originally identified as *E. brevipes*

CHAPTER 3

A MOLECULAR SYSTEMATIC INVESTIGATION OF SOUTHERN AFRICAN
*LAMINARIA* (LAMINARIALES, PHAEOPHYCEAE)

The genus *Laminaria* J.V. Lamouroux is one of 33 genera in the family Laminariales. The genus was described in 1813 (Lamouroux 1813) and since then more than 260 species, including varieties and different forms, have been attributed to this genus (Kain 1979, Bartsch et al. 2008) but most of these have been synonymised, with only 24 currently accepted species (Bolton 2010). *Laminaria* inhabits coastal waters of warm- to cold-temperate regions and the Arctic mainly in the northern hemisphere: from the Mediterranean Sea, along the European coast, Scandinavia, the Baltic Sea, into the Arctic and including both the North east and west Pacific (Bolton 2010, Steneck and Johnson 2013), making it ecologically important and economically valuable in many regions (Bartsch et al. 2008).

In Norway, United Kingdom and France, *L. hyperborea* (Gunnerus) Foslie and *L. digitata* (Hudson) J.V. Lamouroux are dredged by boat for the alginate industry (Critchley and Ohno 1998). In South Africa, beachcast *L. pallida* Greville is collected, dried and milled to be exported, mainly for the extraction of alginate (Levitt et al. 1992, Anderson et al. 2003) which is used in the pharmaceutical, cosmetic and food industries.

Species of the genus are morphologically plastic (Kain 1979, Molloy and Bolton 1996, Selivanova et al. 2007, Klochkova et al. 2010, Marins et al. 2012) which has led to taxonomic confusion, and it was only in the last quarter century, with the application of molecular techniques (Bhattacharya and Druehl 1990; Druehl 1990), that species names could be confirmed and assigned with more certainty.

There are several studies generating entire genomes for certain *Laminaria* species. Oudot-Le Secq et al. (2002) reported the complete sequence of the mitochondrial genome of *L. digitata*, and Zhang et al. (2011) produced the complete sequence of the mitochondrial genome of *L. hyperborea*. Recent revisions to *Laminaria*, based on molecular analyses, have had a major taxonomic impact on the genus. Yoon et al. (2001), using RuBisCo spacer and ITS, showed that *Laminaria* was polyphyletic. The most recent study aimed at elucidating the phylogeny of the Laminariaceae is that of Chi et al. (2014) who used mitochondrial (Cox1), nuclear (ITS) and chloroplastic (*rbc*L) markers. Although their phylogeny is the most comprehensive of the order Laminariales, their analysis only confirms the findings of Lane et al. (2006) who did a substantial re-organisation of the genus *Laminaria*, by resurrecting the genus *Saccharina* Stackhouse using plastid (ITS) and mitochondrial (nad6) and chloroplastic (RUBISCO) gene markers. *Laminaria* now consists of the split-bladed (formerly Digitatae section of *Laminaria*) species, and *Saccharina* the simple (formerly Simplices section of *Laminaria*). However, there are exceptions. *Laminaria ephemera* Setchell and *L. solidungula* J. Agardh are two species of *Lamiaria* with a non-split blades, while *Saccharina dentigera* (Kjellman) C.E. Lane, C. Mayes, Druekl & G.W. Saunders has a split-blade. Such revisions emphasise the importance of combining morphology with molecular analysis for meaningful taxonomic studies.

A recent example studied *L. brasiliensis* A.B. Joly & E.C. Oliveira (split blade) and *L. abyssalis* A.B. Joly & E.C. Oliveira (non-split blade), occurring along the Brazilian east coast at depths of between 45 – 120 m, which were considered separate species due to their different morphologies. Marins et al. (2012), using molecular analyses (nuclear (ITS), mitochondrial (CoxI) and chloroplastic (*rbc*L) markers), showed that *L. brasiliensis* and *L. abyssalis* are conspecific. They ascribed the morphological differences, between these populations, to the unstable rhodolith substrate that shifts during storms causing a splitting of

the *L. abyssalis* blades. Another example of a species that has individuals with split and non-split blades, but molecularly shown to be the same species, is *Saccharina groenlandica* (McDevit and Saunders 2010). Some species of *Laminaria* can exist as entire blades (without splits) in very sheltered water; an example is *L. hyperborea* the entire bladed form first described as *L. cloustonii* Edmondston (Kain 1979). However, this sheltered form of *L. hyperborea*, is known as *L. hyperborea f. cucullata* P.Svensden & J.M.Kain and develops where there is little or no water movement (Kain 1971) and intermediate forms are often found in intermediate wave exposure conditions. Also *L. pallida* occurs in a non-split form in the Two Oceans Aquarium in Cape Town (JJ Bolton, personal communication), where water movement is minimal, but this has not been observed in nature (this study).

In the Southern Hemisphere, *L. pallida* was, because of its morphological plasticity, previously considered to represent two separate species, *L. pallida* and *L. schinzii* Foslie. The "*L. pallida*-form" has a solid stipe which is wider at the base than at the transition zone between the blade and stipe, while the "*L. schinzii*-form" has a longer, hollow stipe which can grow up to 5 m long and is wider in the middle than at either ends (Molloy and Bolton 1996). Stegenga et al. (1997) did not distinguish between the two species and considered *L. schinzii* to be a synonym of *L. pallida*. In the south eastern Atlantic, *L. pallida* is the only species of *Laminaria* currently recognised, and it forms large kelp forests between Lüderitz and Rocky Point, close to the Angola border in northern Namibia (Molloy and Bolton 1996).

Apart from *L. pallida*, only a few other taxa of the Laminariales have hollow and solid stiped forms. *Ecklonia arborea* (Areschoug) Rothman, Mattio & Bolton (previously *Eisenia arborea* Areschoug, Rothman et al. 2015) (in the Eastern Pacific) has hollow and solid stiped forms and it has been shown that this hollowness varies along a latitudinal gradient (Matson and Edwards 2006), and that hollowness increases in exposed compared to protected sites (Parada et al. 2012). Others are *Saccharina latissima* (Miyabe) C.E. Lane, C. Mayes, Druehl

& G.W. Saunders, a hollow-stiped form now considered conspecific with the solid stiped *Saccharina longissima* (Miyabe) C.E. Lane, C. Mayes, Druehl et G.W. Saunders (Sjøtun and Gunnarsson 1995, McDevitt and Saunders 2010). *Ecklonia maxima* (Osbeck) Papenfuss, which is speculated to have originated from *E. radiata* (C. Agardh) J. Agardh (Bolton and Anderson 1994, also Rothman et al. 2015) is always hollow when mature, while the latter is always solid (see Ch 4).

This study had two aims: firstly to use molecular methods to determine if Stegenga et al. (1997) were correct in considering the hollow and solid forms of "*L. pallida*-form" and the "*L. schinzii*-form" conspecific; and secondly, to assess where *L. pallida/schinzii* fits in the general phylogeny of the genus *Laminaria*.

MATERIALS AND METHODS

*Sample collection and identification.* Samples of *Laminaria pallida*/*L. schinzii* were collected from seven localities along its distribution range in Southern Africa (Table 1). A sample of *L. ochroleuca* Bachelot de la Pylaie was obtained from J. Franco (Amorosa; 41°38'33.04" N 08°49'29.48" W) in Portugal. Voucher specimens and/or photographs of specimens are lodged at the Seaweed Unit, Department of Agriculture Forestry and Fisheries, South Africa. Samples from mature sporophytes were collected to represent observed morphological variability within populations at the various sites either using SCUBA, snorkelling or wading at low tide. Whenever possible, photographs of the entire sporophytes were taken. Tissue samples ($3 - 5$ cm$^2$) of each specimen were collected from the youngest part of the frond of the first secondary blade and stored in silica gel for later DNA extraction (Chase and Hills, 1991).

*DNA extraction, amplification and sequencing.* DNA extraction and PCR amplification were done in the Systematics laboratory of the Department of Biological Sciences of the

University of Cape Town (South Africa). Silica gel-dried material (0.2-0.3 g) was ground in a Retsch mixer mill MM 4000 (Retsch GmbH, Haan, Germany). Two extraction protocols were used to extract the DNA. The first method used a Qiagen plant DNA extraction kit following the manufacturer's protocol, but success was limited, probably due to high levels of polysaccharides, tannins and phenols in the samples. A higher success rate was obtained using a combination of CTAB and SDS method as described by Maeda et al. (2013). Genomic DNA was then purified using the GENECLEAN® III Kit (MP Biomedical, LLC; France) following the manufacturer's protocol.

The purified DNA was used for the polymerase chain reaction (PCR) amplification in 25 µL volumes in a 2700 GeneAmp PCR System (Applied Biosystems, Foster City, CA, USA). The reaction mix contained 1 µL DNA, 20.25 µL ultra-pure water, 2.5 µL KAPA *Taq* buffer (Kapa Biosystems, Cape Town, South Africa), 0.5 µL 0.8 mM KAPA dNTP Mix, 0.25 µL of each primer and 0.25 µL 1 U KAPA *Taq* DNA Polymerase Ready Mix or SuperTherm*Taq* DNA Polymerase (Roche, Mannheim, Germany).

Four genetic markers were amplified:
(i) two mitochondrial intergenic spacer regions: one spacer between the t-RNAs for tryptophan and isoleucine genes (trnWI), and one between the atp8 and t-RNA serine genes (hearafter mentioned as atp8) using primers from Voisin et al. (2005); (ii) one nuclear marker covering the ITS1 and 5.8S region, was amplified with the primer pair LB1 and LB2 from Yoon et al. (2001); and (iii) one chloroplastic marker: the large subunit of the RuBisCO (*rbc*L) using primers KL2 and KL8 from Lane et al. (2006). These markers have been chosen to cover all three algal genomes (mitochondrial, chloroplastic, and nuclear) and to be able to include sequences from previous studies (e.g. Martin 2011).

The thermal profile for PCR amplification of *rbc*L was as follows: an initial denaturation cycle of 95 °C for 1 min, followed by 35 cycles of 95°C for 30 sec, 45°C for 30 sec, and 72°C for 1 min. A final annealing step at 72°C was extended for 10 min. The thermal profile for PCR amplification for ITS were as follows: an initial denaturation cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min. A final annealing step at 72°C was extended for 4 min. For both trnW-I and atp8-trnS, the thermal profile for PCR amplification were as follows: an initial denaturation cycle of 95°C for 2 min, followed by 32 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec. A final annealing step at 72°C was extended for 5 min. All PCR products were purified using the NucleoFast membrane (Machery-Nagel, Düren, Germany) on a Freedom Evo robot (Tecan Group Ltd., Männedorf, Switzerland) and sequenced with PCR primers using the BigDye Terminator v3.1 sequencing kit (Life Technologies, Johannesburg, South Africa). Purifying and sequencing was done by the Sequencing Unit at the University of Stellenbosch (South Africa).

Sequences were edited, manually aligned and concatenated in Bioedit (Hall, 1999). Sequences representing as many species of *Laminaria* from as many localities as possible, were downloaded from Genbank and added to the alignments. Although many sequences were available for all four markers for *Laminaria*, there were very few instances in which the sequences for all four markers came from the same specimen. As a consequence, in order to study relationships within the Laminariaceae, three different datasets were analysed: a concatenated atp8/trnWI dataset, an *rbc*L/ITS dataset as well as a data set only consisting of ITS sequenses. The combination of these data-sets allowed us to concatenate the maximum number of sequences generated in this study and those downloaded from GenBank. The ITS data contained most of the available sequences for species of *Laminaria* and was used to study relationships mainly between the Southern Hemisphere species.

The best-fit models were estimated for each individual alignment with FindModel (available online at http://hiv.lanl.gov) and were all found to be GTR plus gamma. A Bayesian inference was applied to a concatenated data sets consisting of atp8 + trnWI and *rbc*L + ITS using BEAST v.1.7.5 (Drummond et al. 2012) through the online CIPRES Science Gateway Platform (Miller et al. 2010). The analysis used estimated base frequencies, 4 T categories to model among-site rate heterogeneity, a relaxed log-normal molecular clock, a coalescent tree prior with a randomly generated starting tree, and run for 50 million generations. A tenth of the tree was discarded as burn-in. Convergence of the Markov chains was checked using Tracer v.1.4 (Rambaut and Drummond 2007). The Maximum Likelihood analysis was performed using RAxML-HPC2 on XSEDE using a GTR-MIX evolutionary model (Stamatakis, 2006). A multi-parametric bootstrap resampling with 1000 iterations provided bootstrap supports. The concatenated atp8/trnWI and *rbc*L/ITS trees were rooted with sequences of the closely related *Macrocystis* (Linnaeus) C. Agardh (downloaded from GenBank).

RESULTS

A total of 16 samples were successfully sequenced (Table 1), including 13 from South Africa (*L. pallida*), two from Namibia (*L. pallida*) and one from Portugal (*L. ochroleuca*). Sequences previously published and available on GenBank were selected to represent as many taxa as possible in the genus *Laminaria* but with additional sequences for *Saccharina* (see Table 1). Final alignments included 19 sequences for the *rbc*L/ITS and 30 for atp8/trnWI, which were 1592 and 529 base pairs long, including gaps, respectively.

The concatenated atp8/trnWI analysis (Fig. 1) recovered two clades: a *Saccharina* clade (posterior probabilities (PP) = 1; bootstrap (B) 100 %) and a *Laminaria* clade (PP = 0.96; B = 78 %). The *Laminaria* clade was further subdivided into two subclades representing a poorly

supported *L. digitata/hyperborea* (PP = 0.51; B 50 %) subclade and the other representing a fully supported *L. ochroleuca/pallida* (PP = 1; B 100 %) subclade. The *L. ochroleuca/pallida* subclade resolved the two species well (PP = 0.94; B = 84 %, intraspecific sequence divergence (SD) = 0 – 0.5% for *L. ochroleuca* and PP = 1; B = 99 %, intraspecific SD = 0 – 0.3% for *L. pallida*, with an interspecific SD = 1.9 – 3 %). The *L. pallida* group included sequences from Namibia and South Africa, with both hollow and solid forms. Although the L. *digitata/hyperborea* subclade was poorly supported (PP = 0.51/-) it resolved well into a *L. digitata* group (PP = 1; B = 100 %) and a *L. hyperborea* group (PP = 1; B = 84 %).

The *rbc*L/ITS (Fig. 2) similarly produced two fully supported clades, one representing the genus *Saccharina* (PP = 1; B = 100 %) and the other representing the genus *Laminaria* (PP = 1; B = 99 %). *Laminaria pallida* (which included hollow and solid stiped individuals) forms a well supported clade, with *L. ochroleuca* as a sister species. These two groups were in a larger "Atlantic clade" (containing *L. digitata* and *L. hyperborea*) which was also well supported (PP = 1; B = 98 %; interspecific SD = 3.6 – 3.9%). This Atlantic clade is nested in the larger *Laminaria* clade containing the rest of the sequences available for species present in the north eastern Pacific.

The ITS analysis (Fig. 3) also produced the two fully supported clades consisting of a *Saccharina* and a *Laminaria* clade. The *Laminaria* clade was further subdivided into a North-east Pacific clade (containing *L. sinclarii*, *L. setchellii* and *L. farlowii*) and a subclade containing Atlantic species and the species with a discoid holdfast (*L. ephemera*, *L. solidungula* and *L. yezoensis*). This latter subclade also resolved a Southern Hemisphere clade including *L. ochroleuca*, a Northern Atlantic clade (containing *L. digitata* and *L. hyperborea*), as well as the clade containing the species with discoid holdfasts.

Fig. 1. Bayesian phylogeny based on the concatenated atp8/trnWI alignment. Posterior probabilities below 0.8 and bootstrap less than 60% represented by "-". Branch numbers indicate Bayesian analysis; Maximum likelihood.
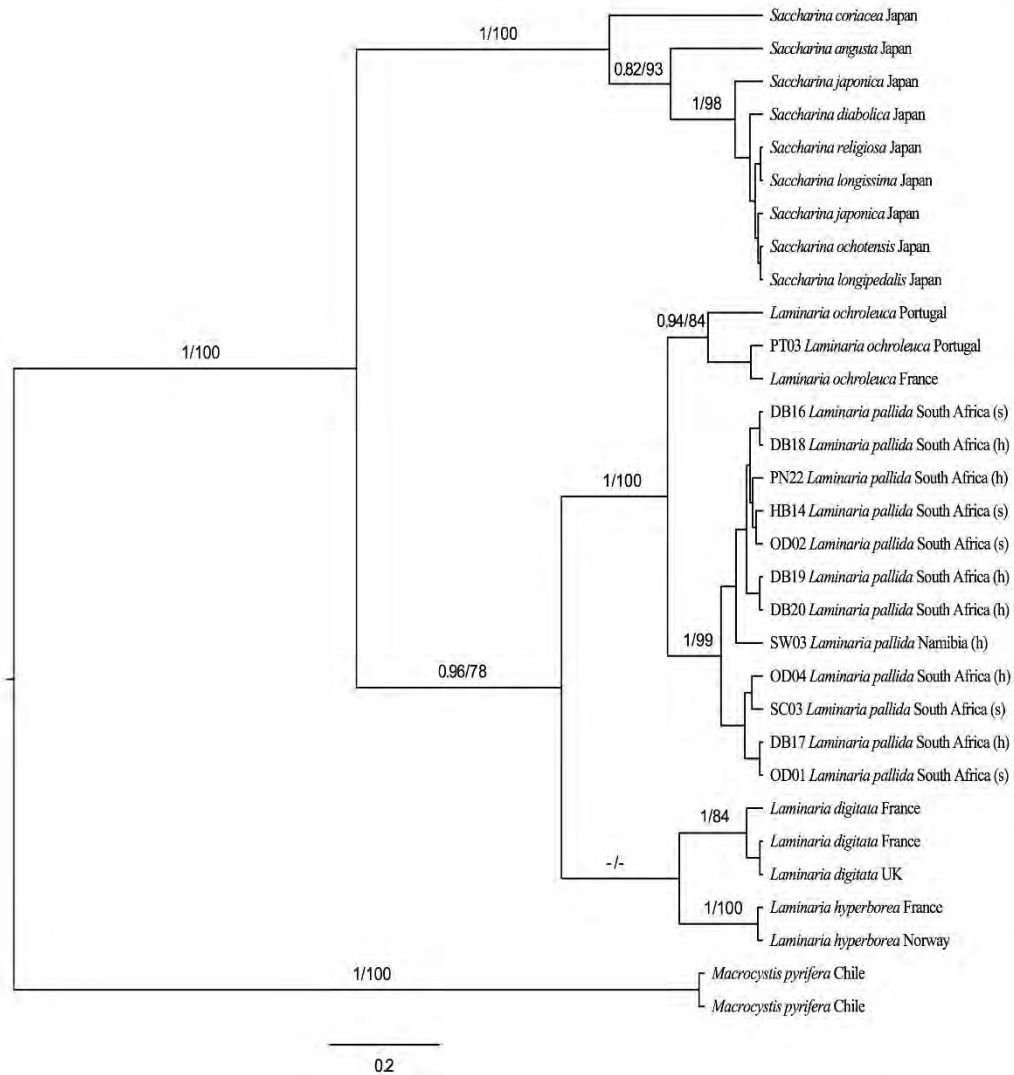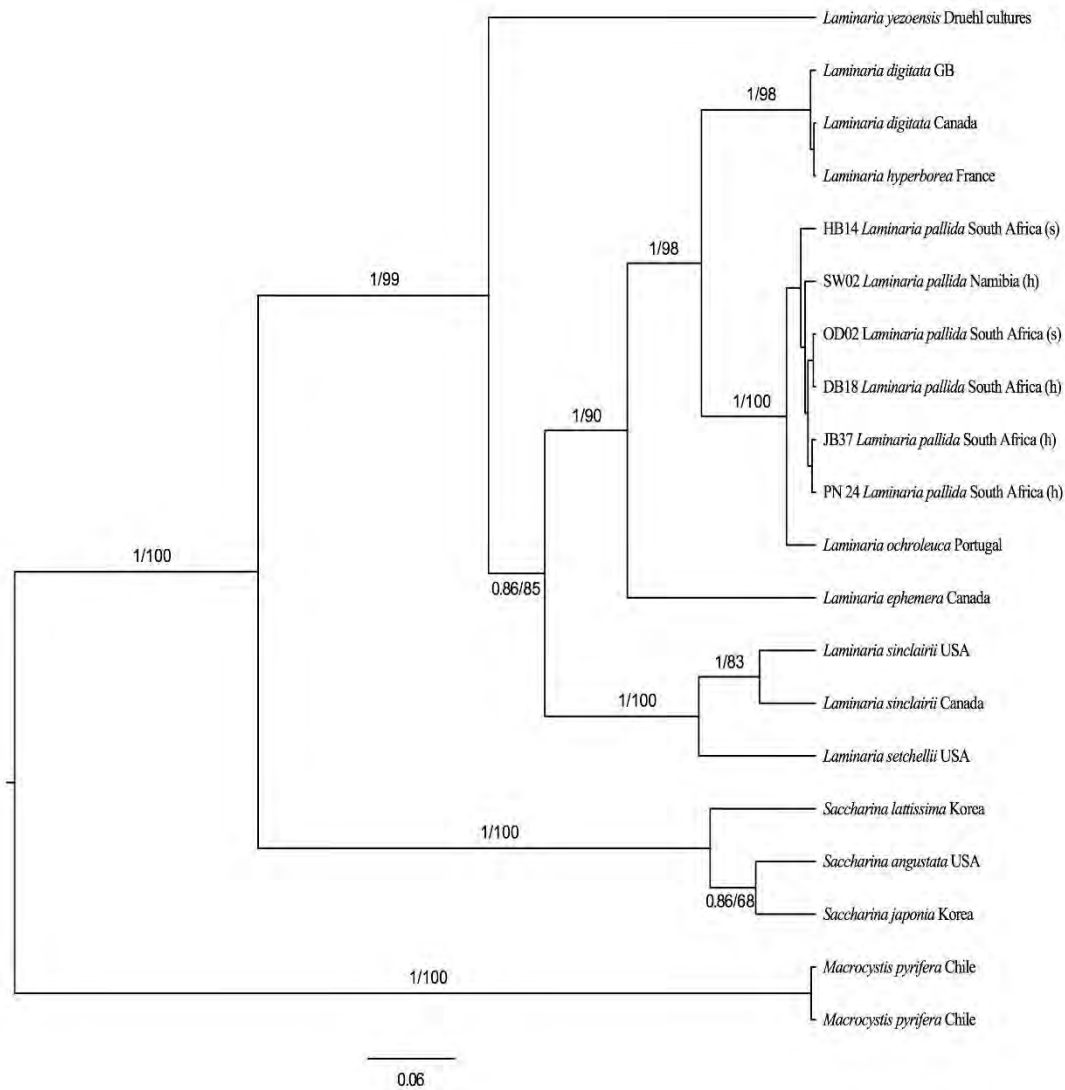
Fig. 2. Bayesian phylogeny based on the concatenated *rbc*L/ITS alignment. Posterior

probabilities below 0.8 and bootstrap less than 60% represented by "-". Branch numbers

indicate Bayesian analysis; Maximum likelihood.
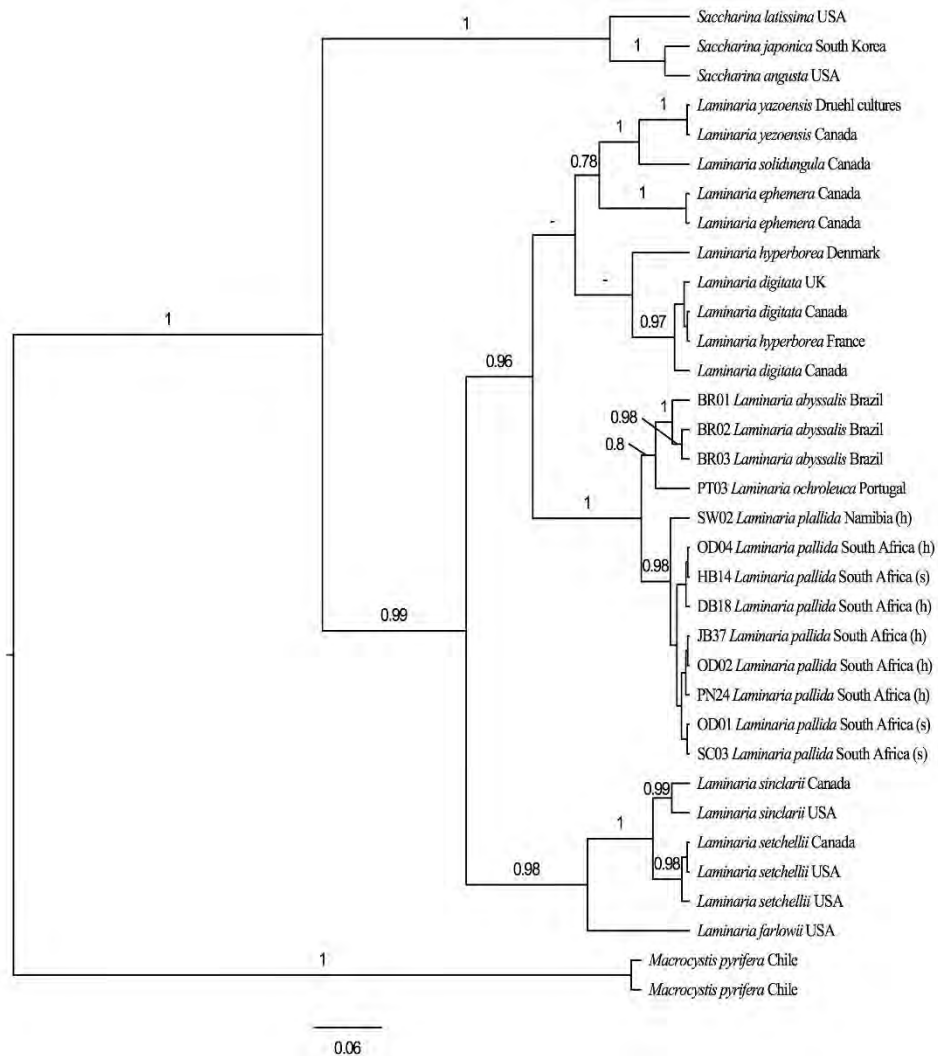
Fig. 3. Bayesian phylogeny based on the ITS alignment. Posterior probabilities below 0.8 is represented by "-".

DISCUSSION

The phylogeny of *Laminaria* includes sequences of the most abundant and widespread species from both hemispheres. Results confirmed that *L. pallida* must be considered as a single species as proposed by Stegenga et al. (1997) with *L. ochroleuca* as its sister. The *rbc*L/ITS analysis showed that there is a separation between the species of *Laminaria* from the Eastern pacific, Western Pacific and the Atlantic, while the atp8/trnWI analysis, focused on the Atlantic species of *Laminaria*, resolved two subclades: a *L. digitata/hyperborea* subclade, and a *L. ochroleuca/pallida* subclade.

*Southern African* Laminaria. Both concatenated *rbc*L/ITS and the atp/trnWI analyses indicated a single species, *L. pallida*, in Southern Africa. *Laminaria pallida* sporophytes can grow a stipe of up to 5 m long (solid or hollow) with a single smooth broad blade that splits into many regular longitudinal, belt-like fronds (Stegenga et al. 1997). Despite previous reports (Molloy 1990, Molloy and Bolton 1996) indicating that *L. schinzii* has a longer, hollow stipe, tapering towards the base, Stegenga et al. (1997) considered *L. pallida* and *L. schinzii* conspecific because of their general morphological similarity, and the absence of an abrupt biogeographical or ecological change in morphology. A very important non-molecular precursor to this study is the hybridization study of the *L. pallida*-form and *L. schinzii*-form by tom Dieck and de Oliveira (1993). Their study showed that crosses between these forms resulted in morphologically normal $F_1$ sporophytes suggesting that a single biological species may be present. Furthermore, their study also showed that no there was no full fertility barrier between Northern Atlantic and Southern Atlantic species because a cross between *L. digitata* and *L. pallida* was successful, resulting in healthy $F_1$ sporophytes. This is interesting as these two species are in different clades in the trees presented here. There is no evidence as yet that either of these crosses could produce fertile offspring. The current study molecularly

confirms the conspecificity of *L. pallida* and *L. schinzii,* with all analyses producing well supported clades including both the hollow and solid forms.

This provides evidence that these particular morphological characters, previously used to distinguish the two forms, are likely controlled environmentally (see Ch 4). Lane et al. (2006) also remarked how unreliable stipe size and blade morphology are to distinguish species of L*aminaria*.

*Laminaria pallida* is found from Danger Point (34°37'45.16"S 19°17'33.59"E), on the South African south coast, to Rocky Point (18°59'37.73"S 12°28'30.23"E) in northern Namibia. Along its distribution range *L. pallida* can occur as deep as 30 m (Dieckmann 1980). In the south (e.g. around the Cape Peninsula) and in shallow water (between 5 - 8 m deep), *Laminaria pallida* forms a subcanopy under *Ecklonia maxima* (Osbeck) Papenfuss, the fronds of which form a dense canopy up to the surface. North of Cape Columbine (32°49'29.46"S 17°50'38.15"E) (ca 120 km north west of Cape Town), *L. pallida* becomes progressively dominant in shallower water, so that many kelp beds in the Northern Cape are mixed beds, until it completely replaces *E. maxima* north of Lüderitz in Namibia (26°37'52.56"S 15°09'06.31"E) (Molloy and Bolton 1980, Stegenga et al. 1997). Northern *L. pallida* sporophytes tend to be hollow while in the south only occasional plants are hollow (see Ch 4). Other studies have shown morphological variability in *Laminaria* species (Kain 1979; Bartsch et al. 2008), Marins et al. (2012) in particular, have observed variation in blade morphology within *L. abyssalis* from Brazil.

*Diversity and distribution of* Laminaria. *Laminaria* species are primarily distributed in the northern Hemisphere, only a few are recorded in the Southern Hemisphere and they are are restricted to the southern Atlantic (*L. abyssalis* and *L. pallida*). Lane et al. (2006), using the large subunit (LSU) rDNA, ITS, the RuBisCO operon, and the NADH dehydrogenase

subunit 6 (nad6), made major revisions to the Family Laminariaceae. Their results indicated two *Laminaria* clades, which led them to resurrect the genus *Saccharina* for the *Laminaria* clade that did not include the type for *Laminaria*, *L. digitata*. This modification drastically changed the known distribution of *Laminaria*, especially in the North West Pacific where of the 22 *Laminaria* species previously recorded, now only 6 remain: *L. appressirhiza* J.E. Petrov & V.B. Vozzhinskaya*, L. digitata, L. inclinatorhiza* J.E. Petrov & V.B. Vozzhinskaya*, L. philippensis* J.E. Petrov & M.V. Suchovejeva, *L. platymeris* Bachelot de la Pylaie and *L. yezoensis*. To explain the occurrence of sister taxa of *Laminaria* in both the North Pacific and North Atlantic, Stam et al. (1988) suggested that the opening of the Bering Strait would have allowed seaweeds to migrate via the coast of the ice-free Arctic Ocean. Lüning (1990) placed the opening of the Bering Stait at 3 Ma, but more recent evidence showed that the Bering Strait opened much earlier than previously thought, between 5.4 and 5.5 Ma (Gladenkov et al. 2002), meaning that this seaweed migration could have happened much earlier. *Laminaria yezoensis* is the only *Laminaria* that occurs in both the North West and North East Pacific, while *L. ephemera* occurs in the North East Pacific and *L. solidungula* occurs in the Arctic, Alaska, Greenland, Spitsbergen and Northwest Atlantic (Guiry and Guiry 2014). Interestingly those three species are the only *Laminaria* species to have discoid holdfasts, the other *Laminaria* species having rhizoidal holdfasts. A discoid holdfast was described in *Aureophycus aleuticus* H. Kawai, T. Hanyuda, Lindeberg and S.S. Lindstrom, which according to Kawai et al. (2013) is considered ancestral to the rhizoidal holdfasts of more derived kelps. However, Kawai et al. (2013) consider the discoidal holdfasts of *L. ephemera*, *L. solidungula* and *L. yezoensis* to have evolved secondarily from rhizoidal holdfasts because their sister taxa have rhizoidal holdfast and because they form rhizoidal structures in their early developmental stages. The ITS analyses of Chi et al. (2014) and the present study resolved a clade (PP = 0.78) consisting of these three species of *Laminaria*. The close

relationship between those three northern Atlantic species of *Laminaria* with discoid holdfasts supports the hypothesis of Stam et al. (1988), echoed by Sasaki et al. (2001), and Bolton (2010) that the Laminariales originated in the Northern Pacific, and then migrated via the Bering Strait to the Arctic and Northern Atlantic.

Another important relationship is between the Southern Hemisphere species of *Laminaria*, *L. pallida* and *L. abyssalis,* occurring in the south east and south west Atlantic respectively, and the north east Atlantic species, *L. ochroleuca*. A hypothesis would be that a north eastern Atlantic ancestor (closely related to *L. ochroleuca*) crossed the equator, *via* a single or two distribution events, during a glaciation event to colonize the southern part of the Atlantic (see van den Hoek 1982). The results of our Bayesian analysis of the ITS region (Fig. 3) supports this hypothesis with the grouping of *L. ochroleuca* and the two southern Atlantic species (*L. pallida* and *L. abyssalis*) within the same clade, and the sister relationship of this clade to the rest of the northern Atlantic species. Further supporting evidence for this sister relationship can be seen in the study by tom Dieck (1992), who crossed *L. pallida* and *L. ochroleuca*. The authors found that although initial development of the hybrids was normal, the sporophytes later became deformed and further development was retarded. The timing and origin of *L. pallida* and *L. abyssalis* remains to be explored in more detail but from our results we believe that they share a common northern Atlantic ancestor with *L. ochroleuca*.

The trees also revealed a close relationship between the Northern Atlantic *L. digitata*/*L. hyperborea* and the *L. abbysalis*/*L. pallida*/*L. ochroleuca* clades. Although the resolution here does not allow us to expand on the relationship, it is however, interesting to note that the former clade are more cold temperate and restricted to higher latitude in the Atlantic while the latter are warm temperate and occurs at lower latitudes in the Atlantic. The ITS analysis here has provided impetus for further studies into the relationship between the Southern Hemisphere and Northern Hemisphere species.

Based on the results and discussion of this study, a likely hypothesis on the evolution of the genus is an origin in the northern Pacific, a migration through the Bering Strait to colonize northern Atlantic and further migration south in the Atlantic, with South Africa and Brazil being the most recently colonized regions. A time-calibrated phylogeny and sequences for more species and specimens in both the Atlantic and the Pacific would provide evidence for the timing of these events.

Table 1. Collection details[a] or references, sample and Genbank accession numbers for species used in this study.

| Species | Location, Collector | Sample no | rbcL | ITS | atp8 | atp8/trnWI |
|---|---|---|---|---|---|---|
| *L. abyssalis* (BR01) | Marins et al. (2012), Brazil | | | JN645269 | | |
| *L. abyssalis* (BR02) | Marins et al. (2012), Brazil | | | JN654270 | | |
| *L. abyssalis* (BR03) | Marins et al. (2012), Brazil | | | JN654267 | | |
| *L. digitata* | Lane et al. (2006), NB, Canada | | AY851559 | AY857886 | | |
| *L. digitata* | McDevit and Saunders (2009), NB, Canada | | | FJ042764 | | |
| *L. digitata* | Engel et al. Unpubl., France | | | | DQ841608 | DQ841670 |
| *L. digitata* | Oudot-Le Secq et al. (2002), France | | | | AJ344328 | AJ344328 |
| *L. digitata* | Yoon et al. (2001), Great Britain | | AF318971 | AF319014 | | |
| *L. digitata* | Engel et al. Unpubl., United Kingdom | | | | DQ841607 | DQ841669 |
| *L. ephemera* | Lane et al. (2006), BC, Canada | | AY851557 | AY857887 | | |
| *L. ephemera* | McDevit and Saunders (2009), BC, Canada | | | FJ042733 | | |
| *L. hyperborea* | Erting et al. (2004), Denmark | | | AY441772 | | |
| *L. hyperborea* | Yoon et al. (2001), France | | AF318972 | AF319015 | | |

| Species | Source | Code | | |
|---|---|---|---|---|
| *L. hyperborea* | Engel et al. Unpubl., France | | DQ841609 | DQ841671 |
| *L. hyperborea* | Engel et al. Unpubl., Norway | | DQ841610 | DQ841672 |
| *L. ochroleuca* | Engel et al. Unpubl., France | | DQ841612 | DQ841674 |
| *L. ochroleuca* | Amorosa, Portugal, T, J. Franco | PT03 | | |
| *L. ochroleuca* | Engel et al. Unpubl., Portugal | | DQ841611 | DQ841673 |
| *L. pallida* (s) | Doring Bay, South Africa , C. Boothroyd | DB16 | | |
| *L. pallida* (h) | Doring Bay, South Africa, C. Boothroyd | DB17 | | |
| *L. pallida* (h) | Doring Bay, South Africa, C. Boothroyd | DB18 | KM575794 | KM575780 |
| *L. pallida* (h) | Doring Bay, South Africa, C. Boothroyd | DB19 | | |
| *L. pallida* (h) | Doring Bay, South Africa, C. Boothroyd | DB20 | | |
| *L. pallida* (s) | Hondeklip Bay, South Africa, R. Anderson | HB14 | | |
| *L. pallida* (h) | Hondeklip Bay, South Africa, R. Anderson | JB37 | | |
| *L. pallida* (s) | Oudekraal, South Africa, C. Boothroyd | OD01 | | |
| *L. pallida* (h) | Oudekraal, South Africa, C. Boothroyd | OD02 | | |
| *L. pallida* (h) | Oudekraal, South Africa, C. Boothroyd | OD04 | | |
| *L. pallida* (s) | Port Nolloth, South Africa, M. Rothman | PN22 | | |

| Species | Location / Reference | Code | | |
|---|---|---|---|---|
| *L. pallida* (h) | Port Nolloth, South Africa, M. Rothman | PN24 | | |
| *L. pallida* (s) | Stanford's Cove, South Africa, S. John | SC03 | | |
| *L. pallida* (h) | Swakopmund, Namibia, B. Rothman | SW02 | | |
| *L. pallida* (h) | Swakopmund, Namibia, B. Rothman | SW03 | | |
| *L. sinclairii* | Yoon et al. (2001), United States | | AF318974 | AF319017 |
| *L. sinclairii* | Lane et al. (2006), BC, Canada | | AY851558 | AY857889 |
| *L. setchellii* | Lane et al. (2006), BC, Canada | | | AY857890 |
| *L. setchellii* | Yoon et al. (2001), Oregon, United States | | AF318973 | AF319016 |
| *L. solidungula* | McDevit and Saunders (2009), BC, Canada | | | FJ042756 |
| *L. yezoensis* | Druehl cultures, BC, Canada | | AY851555 | AY857885 |
| *L. yezoensis* | McDevit and Saunders (2009), BC, Canada | | | FJ042749 |
| *Macrocystis pyrifera* | Yoon et al. (2001), United States | | AF318997 | AF319036 |
| *Macrocystis pyrifera* | Yoon et al. (2001), United States | | AF318998 | AF319037 |
| *Macrocystis pyrifera* | Engel et al. Unpubl., Chile | | DQ841620 | DQ841684 |
| *Macrocystis pyrifera* | Engel et al. Unpubl., Chile | | DQ841619 | DQ841683 |
| *Saccharina angusta* | Yotsukura et al. (2010), Japan | | AP011498 | AP011498 |

| | | | | | |
|---|---|---|---|---|---|
| *Saccharina angusta* | Lane et al. (2006), United States | AY851554 | AY857891 | | |
| *S. coriacea* | Yotsukura et al. (2010), Japan | | | AP011499 | AP011499 |
| *S. diabolica* | Yotsukura et al. (2010), Japan | | | AP011496 | AP011496 |
| *S. japonica* | Yoon et al. (2001), Korea | AF318976 | AF319018 | | |
| *S. japonica* | Yotsukura et al. (2010), Japan | | | AP011493 | AP011493 |
| *S. japonica* | Zhang et al. (2011), Japan | | | JF937591 | JF937591 |
| *S. latissima* | Yoon et al. (2001), USA | AF318980 | AF319019 | | |
| *S. longipedalis* | Yotsukura et al. (2010), Japan | | | AP011497 | AP011497 |
| *S. longissima* | Zhang et al. (2013), Japan | | | JN099684 | JN099684 |
| *S. ochotensis* | Yotsukura et al. (2010), Japan | | | AP011495 | AP011495 |
| *S. religiosa* | Yotsukura et al. (2010), Japan | | | AP011494 | AP011494 |

[a]*L. pallida* key: (h) = hollow stipe, (s) = solid stipe

CHAPTER 4

GEOGRAPHICAL VARIATION IN MORPHOLOGY OF THE TWO DOMINANT KELP SPECIES, *ECKLONIA MAXIMA* AND *LAMINARIA PALLIDA* (PHAEOPHYCEAE, LAMINARIALES), ON THE WEST COAST OF SOUTHERN AFRICA.

The Southern African kelp flora consists of four species: *Ecklonia maxima* (Osbeck) Papenfuss, *Ecklonia radiata* (C. Agardh) J. Agardh, *Laminaria pallida* Greville and *Macrocystis pyrifera* (Linnaeus) C. Agardh (previously known in South Africa as *Macrocystis angustifolia* Bory de Saint-Vincent). *Ecklonia maxima* and *L. pallida* have by far the highest biomass in the inshore, rocky environment and are the subject of this study. *Macrocystis pyrifera* is only found in isolated patches, generally inside inshore beds of *Ecklonia maxima* on the southern part of the west coast of South Africa (Kommetjie, Oudekraal, Robben Island (10 km offshore from Cape Town), Melkbosstrand (50 km south of Yzerfontein), Dassen Island (10 km offshore from Yzerfontein), Jacobs Bay) (Stegenga et al. 1997) (see Fig. 1). *Ecklonia radiata* is limited almost entirely to populations in rock pools and the sublittoral fringe, between De Hoop Nature Reserve (70 km east of Cape Agulhas) and Port Edward in southern KZN, and to a few records from deep reefs (to 50 m and more) off the south and east coasts, with one rather anomalous population reported at depths of 6 - 25 m in False Bay (Rothman et al. 2015). In Southern Africa, subtidal kelp forests in the accepted sense only occur from De Hoop Nature Reserve in the south to Rocky Point in northern Namibia (Stegenga et al. 1997). These comprise either *E. maxima* or *L. pallida* or a mixture of both (Molloy 1990).
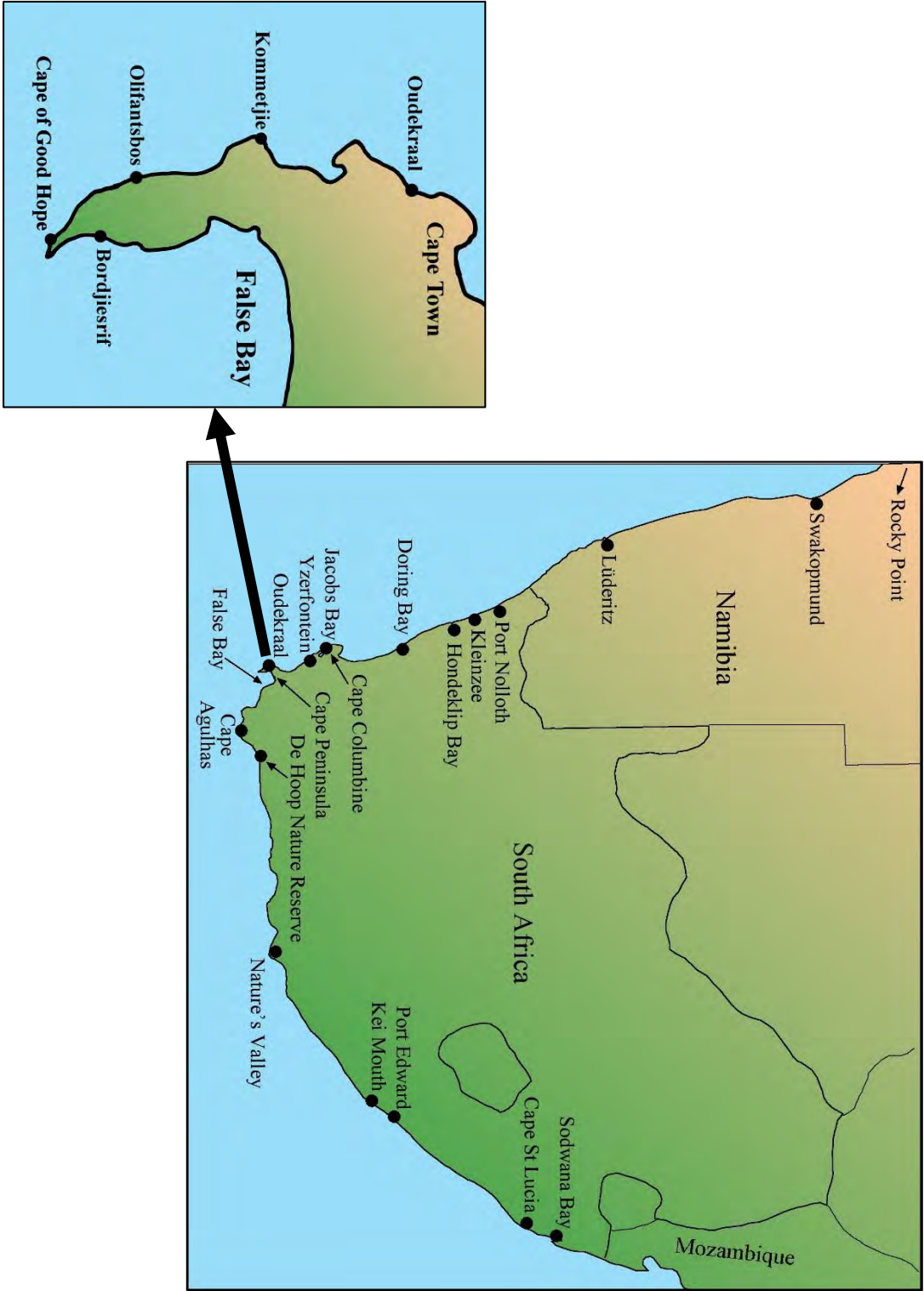
Fig. 1. Coastline of South Africa and Namibia showing sites mentioned in the text. Insert shows the Cape Peninsula enlarged.

Anderson et al. (2007) mapped the inshore beds (those detectable by infra red aerial photographs) of *E. maxima* and *L. pallida* on the west coast of South Africa and estimated the total standing crop of surface-reaching sporophytes to be close to 600 000 t fresh weight. Jarman and Carter (1981) estimated the standing crop of *E. maxima* at 336 370 t fresh weight, but their estimate was based on published data from only a few quadrats, and may be less reliable. There are no biomass or density data available for the south and east coasts of South Africa (east of Cape Agulhas), where only relatively narrow patches of *Ecklonia* are found.

*Ecklonia maxima* is one of the largest members of the Laminariales (Anderson et al. 1997). Extensive beds of this species are found from just west of Cape Agulhas (although small beds appeared recently 70 km east of Agulhas, at De Hoop Nature Reserve, Bolton et al. 2012), to Hottentotspunt, 60 km north of Lüderitz in Namibia (Molloy 1990, Stegenga et al. 1997). This species forms extensive forests down to a depth of about 10 m (Simons and Jarman 1981, Bolton and Anderson 1997, Anderson et al. 2007) and can comprise up to 70% of the total plant biomass in the inshore zone (Velimirov and Griffiths 1979, Field et al. 1980, Rothman 2006).

*Laminaria pallida*, the other dominant kelp in South Africa and the dominant species in Namibia, is found from Danger Point (about 100 km east of Cape Town) in South Africa to Rocky Point, south of the Kunene River in northern Namibia (Molloy 1990, Stegenga et al. 1997). The distribution of *L. pallida* therefore extends about 5° into the tropics which is related to the northward penetration of cold upwelled water near the coast. While Graham et al. (2007) used oceanographic and ecological models to predict that deep-water refuges may exist for kelp off much of the West African coast, there is as yet no evidence of such populations, although *Ecklonia muratii* Feldmann occurs in deeper upwelling regions of the coast of Mauretania and Senegal in western North Africa (Price et al. 1978, John et al. 2004).

Jarman and Carter (1981) estimated the standing stock of *L. pallida* between Cape Point and Cape Columbine to be almost 218 000 tons fresh weight (no data are available for the rest of the distribution area). Along the southern west coast, *L. pallida* forms extensive subtidal, understorey forests below and beyond the canopy of *E. maxima* and can extend to depths of 20 m or more (Field et al. 1980, Molloy and Bolton 1996, Anderson et al. 2007). Northward, *L. pallida* has been reported to become more dominant inshore, gradually replacing *E. maxima* (Velimirov et al. 1977, Stegenga et al. 1997), but still with extensive deeper subtidal beds, where solid substrata are available because much of the coast of Namibia has little rocky substratum, particularly the last stretch of Namib desert between the Lüderitz region in the south and Swakopmund/Walvis Bay in the north (Bird et al. 2010).

In its southern area of distribution, *Laminaria pallida* was reported to have a solid stipe that is widest near the holdfast, while in the north it was reported to have a longer, hollow stipe that is widest in the mid-region and narrows towards the distal and proximal ends (Molloy and Bolton 1996). Previously these two forms were considered to be separate species, with the name *L. pallida* applied to the former (southern) entity and the name *L. schinzii* Foslie applied to the latter. However, Stegenga et al. (1997) considered the latter to be a form of *L. pallida,* an opinion that has now been molecularly confirmed (see Ch 3). In the south only occasional sporophytes are hollow-stiped (M. Rothman personal observation), but this form becomes more common north of St Helena Bay on the South African west coast (Stegenga et al. 1997).  Molloy and Bolton (1996) showed that the external morphology of *L. pallida* is greatly affected by depth (between 0.25 – 2.25 m), but they observed that the greatest interaction was observed between water motion and blade thickness with thin blades being observed in wave sheltered areas.

The general pattern of the distribution and biomass of the Southern African kelps seems to be related to sea temperatures. Biogeographically, the temperate Southern African region is

subdivided into the Benguela Marine Province (BMP) on the west coast of Southern Africa and the Agulhas Marine Province (AMP) on the south and southeast (Spalding et al. 2007). Spalding's study gave a global overview of marine ecoregions, while Anderson et al. (2009) provide a map showing our current understanding of the temperate Southern African region with respect to seaweed biogeography. The BMP is dominated by cool, nutrient rich upwelled water and is divided by Bolton (1986) into three sub-regions (one of which, Saldanha Bay, is anomalous due to much higher than normal average temperatures and therefore omitted here): i) Cape of Good Hope to Port Nolloth (with minimum and maximum monthly mean temperatures of between 11.5 – 14.0 °C); ii) north of Port Nolloth into Namibia (with minimum and maximum monthly mean temperatures of between 14.1 -18.0 °C) (Bolton 1986).  Along the south coast of South Africa, from Cape Agulhas to East London in the Eastern Cape, the AMP has minimum and maximum monthly mean temperatures of between 14.1 – 21.5 °C (Bolton 1986). The Agulhas Current, which carries tropical water, originates near Madagascar and cools gradually as it moves southward (Anderson et al. 2005). Near Cape St Lucia in northern KwaZulu-Natal the continental shelf widens forcing the South Indian Subtropical Water onto the shelf, with an attendant slight drop in water temperature causing a transition from tropical to warm temperate inshore conditions (Bolton et al. 2004, De Clerck et al. 2005b).

Between the Benguela and Agulhas Marine Provinces is a biogeographic transition zone stretching from Cape Agulhas westwards to Cape Point (Anderson et al. 2009). Within this transition zone False Bay is interesting in that it is largely sheltered from the prevailing south-easterly wind that causes the upwelling of cold nutrient-rich water on the west coast (Atkins 1970 a, 1970 b). This bay forms the western boundary of the overlap region between the Benguela and Agulhas Marine Provinces and here inshore monthly mean water temperatures rise suddenly from 14 to 16 °C and the mid-summer mean can reach 18°C (Smit

et al. 2013). However, there has been an increase observed in *Ecklonia maxima* abundance in False Bay, between 1966 – 2007, which could be linked to the lower recent temperatures, recorded in the bay (Griffiths et al. 2011). A second overlap zone, sometimes known as the 'subtropical east coast' (Porter et al. 2013) exists east of the Agulhas Marine Province, and extends from East London/southern Transkei area (about 60 km south of Kei Mouth) to St Lucia, giving way further north to the tropical Indo-West Pacific Marine Province (IWP) where monthly mean seawater temperatures are always above 20°C, and may exceed 27 °C during the warmest months (Smit et al. 2013).

Sea water temperature is the major factor influencing seaweed distributions (van den Hoek 1982, Bolton 1986, Breeman 1988). For example, the distribution range of *E. radiata* and *E. maxima* overlap on the south coast, but only *E. radiata* is found along the warmer east coast, while *E. maxima* occurs along the cool west coast. Similar range restrictions and overlapping distributions can be observed for many seaweed species (Bolton 1986).

Even small morphological changes can have large consequences for the success or survival of kelps (Koehl 1996). Morphological plasticity in kelps, induced by environmental conditions, is well documented. Hurd and Pilditch (2011) showed that the width and thickness of *Macrocystis pyrifera* blades varies with wave exposure, and that these adaptations reduce drag while maximizing nutrient uptake. In sheltered areas blades of *M. pyrifera* are thin and have ruffled margins, but in exposed areas they are flat and smooth (Druehl and Kemp 1982, Hurd and Pilditch, 2011). Similarly *L. pallida* sporophytes have a decreased frond surface area where water motion is greater, to reduce drag resistance (Molloy and Bolton 1996). Norton et al. (1982) found that *Saccharina longicruris* (as *Laminaria longicruris* Bachelot de la Pylaie) growing in sheltered areas has three times the surface area:mass ratio of populations growing in exposed areas, while plants growing in wave-exposed areas are sturdier, tougher, and more securely attached. Serisawa et al. (2002a) reported morphological

changes in *Ecklonia cava* from Japan in response to varying environmental conditions. They

attributed longer stipes and wrinkled primary blades to higher water temperatures. However,

subsequent transplant studies by Serisawa et al. (2002b, 2003) showed that the stipe length of

*E. cava* is genetically controlled and not influenced by the environment. Druehl (1978)

found that *Macrocystis pyrifera* had longer fronds in highly wave-exposed sites. This is

similar to the findings of Wernberg & Thomsen (2005) in *Ecklonia radiata* populations in

Australia, but there the differences were observed on a geographic scale (Wernberg et al.

2003). A transplant study conducted on Australian *E. radiata* confirmed that morphological

variations between individuals in exposed and sheltered sites are environmentally driven

(Fowler-Walker et al. 2006). Individuals transplanted from sheltered to exposed

environments underwent a rapid and extreme response in morphology. The sporophytes

developed thicker stipes, narrower laterals and larger holdfasts. The responses of *Ecklonia*

*kurome* to differing wave exposures were similar. These Japanese kelps had narrow primary

blades on exposed shores while wider blades were observed in sheltered sites (Tsutsui et al.

1996). The authors concluded that blade morphology was influenced by habitat although they

also suggested a possible role for genetic factors. Koehl et al. (2008) illustrated, using

*Nereocystis luetkeana* Postels & Ruprecht, how different water flow regimes caused changes

in blade shape, reducing the hydrodynamic drag experienced by the kelp.

Until relatively recently the identification of seaweeds relied entirely on morphological

characters, sometimes resulting in much taxonomic confusion. In Russia *Saccharina*

*gurjanovae* A.D. Zinova (as *Laminaria gurjanovae* A.D. Zinova), because of its abnormal

morphology, was misidentified as a new species, *L. multiplicata* J.E. Petrov & M.V.

Suchovejeva (Klochkova *et al*., 2010) Using ITS1 and ITS2 they recovered DNA from type

specimens as well as newly collected material and found them to be conspecific. Two

Brazilian kelps, *L. abyssalis* (non-split blade) and *L. brasiliensis* (split-blade) were

considered to be separate species based on their frond morphology. However, molecular analysis using the *rbc*L, ITS and coxI genes showed them to be conspecific (Marins et al. 2012). The authors attributed these morphological differences to the mobility of the rhodolith substrate, suggesting that plants in a more mobile environment developed split blades. Wing et al. (2007), using the ITS gene region, showed that morphologically different *E. radiata* populations in fjords around New Zealand are all one species.  Similarly, in Chapter 2 of this thesis it was shown that all Southern Hemisphere specimens of *E. radiata*, irrespective of morphology, are all one genetic species. Also in Chapter 3 it was shown, using different molecular markers, that the hollow and solid-stiped *L. pallida* in Southern Africa are morphological variants of a single species.

*Eisenia arborea* is the only other known example of a kelp sporophyte that becomes hollower along a geographical gradient (Matson and Edwards 2006). Chapter 3 places this species in the genus *Ecklonia* using molecular methods. A number of studies suggest that the flexibility of kelp stipes might be related to the wave exposure especially in shallow water (Friedland and Denny 1995, Gaylord and Denny 1997, Denny et al. 1997). These studies imply that kelp sporophytes' strategy for survival in high water motion environments is flexibility rather than strength and resistance.

Many studies have shown how environmental variables such as wave exposure, water temperature, and even substrate stability can dramatically change the morphology of a kelp sporophyte (Norton et al. 1982, Tsutsui et al. 1996, Serisawa 2002b, 2003, Fowler-Walker et al. 2006, Klochkova et al. 2010, Hurd and Pildith 2011, Marins 2012). While the above studies demonstrate the importance of environment in influencing kelp morphology, relatively few have examined changes over large gradients of latitude or longitude, and most have examined the effects of wave exposure at geographically close sites.  The exceptions are the studies of Wernberg et al. (2003) with *Ecklonia radiata* and Matson and Edwards (2006)

with *Eisenia arborea*. However, although *E. arborea* was shown to develop a hollow stipe over a latitudinal gradient, no explanation was advanced for this morphological change.

Although it has been reported that *L. pallida* gradually replaces *E. maxima* in inshore kelp beds up the west coast of Southern Africa and develops a hollow stipe (Molloy 1990, Molloy and Bolton 1996, Stegenga et al. 1997), it has never been confirmed quantitatively, nor has this transition been explained. The present study set out to measure this ecological transition by quantifying the morphological patterns (especially stipe morphology) in this species and its co-dominant, *E. maxima*, in shallow water along the coast. This study focuses on mature (plants with a stipe length of more than 50 cm), shallow-water sporophytes, in < 5 m depth, where these two species' vertical distribution ranges overlap and they may be expected to compete for space and other resources.

Attempts will be made to relate patterns in any of the obvious and measurable environmental variables that might change over the latitudinal gradient along the approximately 1500 km of Southern African west coast shore where *E. maxima* and *L. pallida* co-dominate. The environmental variables considered were: gradients in water temperature, nutrients, swell height (as a general indicator of wave exposure), light penetration/turbidity, fog/cloud cover and day length. Furthermore, the morphological variables that might be altered by these environmental variables were studied. These included various morphometric characteristics that were measured along the Southern African west coast as well as the flexibility of hollow *vs* solid-stiped *L. pallida* sporophytes; the geographical change in maximum stipe lengths of sporophytes, as well as the deepest occurrence of kelp sporophytes. We hypothesise that the distribution and morphological variation of *L. pallida* and *E. maxima* is controlled by inshore light availability.

MATERIALS AND METHODS

*Study area.* This study was conducted along the Southern African west coast from False Bay to as far north as was logistically possible in the *L. pallida* distribution (Swakopmund, Namibia). We measured a number of morphological characters in *L. pallida* and *E. maxima*, and as many environmental factors as possible, at a number of sites along the Southern African west coast.

*Morphometrics and biomass*. *Ecklonia maxima* and *Laminaria pallida* sporophytes were collected from eight sheltered to semi-exposed sites: seven sites in South Africa (Bordtjiesrif (34°18'50.58"S 18°27'49.43"E), Kommetjie (34° 8'30.24"S 18°19'8.10"E), Jacobs Bay (32°58'33.03"S 17°52'50.69"E), Doring Bay (31°48'51.74"S 18°14'6.15"E), Hondeklip Bay (30°18'26.64"S 17°16'7.52"E), Kleinzee (29°42'32.10"S 17° 3'15.80"E), Port Nolloth (29°14'23.61"S 16°51'9.11"E)) and one site in Namibia, Swakopmund (22°42'5.03"S 14°31'12.44"E)  where only *L. pallida* is found (Fig. 1.).

At all sites except Swakopmund, shallow water kelp sporophytes were collected, using SCUBA, along a transect perpendicular to the shore, starting from the outer edge of the surface-reaching kelp and working towards the shore.  Quadrats were formed by placing two 1 m-long  metal rods at right angles to demarcate a 1 m$^2$ area, and all the plants with stipes longer than 50 cm were harvested at the base of the stipe. A minimum of ten quadrats (between 3 – 5 m apart depending on the width of the bed) were sampled at each site (see later). Because of the low density of *L. pallida* in shallow water at Kommetjie, only one sporophyte was recorded in the quadrats. At Swakopmund 30 shallow-water sporophytes were haphazardly collected along a transect between 0.5 – 2.5 m depths. This was because it was not possible to reach depths exceeding 3 m because the reef is shallow and stretches 150 m offshore and SCUBA equipment was unavailable.  At all sites except Swakopmund, three

depth classes were selected: deep (3.1 – 5 m), intermediate (1.1 - 3 m), and shallow (0 - 1 m). Some of the kelp beds were in remote locations and a boat was not available. For this reason a depth range was chosen that allowed shore-based sampling. Also, kelp at depths beyond 5 m were often too sparse for this study which focused on dense, inshore kelp beds, and at some sites there were sandy areas deeper, with no kelp bed.

The following measurements were taken on each of the plants collected.

Stipe length: measured from the holdfast to the junction between the primary blade and the stipe, with a tape measure.

Stipe hollowness: the stipe was cut from both ends until the hollow section was reached, and air could easily be blown through that section of stipe. The length of the hollow section was measured with a tape measure, and expressed as a percentage of the total stipe length.

Stipe weight: the fresh weight of the entire stipe was measured with a spring balance, to the nearest 25g.

Frond weight: the entire primary and secondary frond mass, from junction of stipe and blade, was weighed with a spring balance to the nearest 25g.

All the above variables were analysed using the multivariate analysis package Primer 6 (Plymouth Routines In Multivariate Ecological Research, Clarke and Gorley 2006). Data were normalized before Principal Components Analysis (PCA) and non-metric Multi-Dimensional Scaling (MDS) were used to illustrate similarity between samples (MDS showed extremely similar patterns to PCA and thus is not shown here). In addition, regressions, box plots and ANOVA were used to analyse in more detail the major contributing factors as shown by the vectors from the PCA.

*Kelp flexibility*. Thirty *L. pallida* sporophytes (at each depth) were collected haphazardly from a very protected part of a kelp bed at Kommetjie (where sporophytes almost all have solid stipes) and Doring Bay (where sporophytes almost all have hollow stipes). Sporophytes were collected from shallow (< 4 m) and deep water (between 10 - 15 m). Shallow water kelp was collected on snorkel while the deep water sporophytes were collected on SCUBA from an inflatable. Measurements were taken on the shore. Kelp was kept fresh while measurements were being made by placing it in sheltered pools or crevices in the shallows.

To measure the flexibility, each kelp stipe was separated from the holdfast and primary blade and its basal 20 cm held in a rigid and immovable clamp (see Fig. 2). The stipes were held parallel to the ground and bent horizontally to a 45° and then a 90° angle. The angles were measured by reference to a bar that pivoted at the clamp, and were effectively the angles between clamp and the point of attachment of the pulling force, which was applied at two distances from the clamp: 50 and 70 cm. The tension required to reach each angle was measured with a spring balance to the nearest 25 g. Gravity and frictional forces were mitigated by resting the stipe on the edge of a ruler acting as a track over which the stipe could be pulled. After this the stipes were sectioned to check whether they were hollow or solid.

*Geographical variation in maximum stipe lengths*. Washed-up sporophytes of *Ecklonia maxima* and *Laminaria pallida* from eight bays along the South African west coast were sampled to obtain an estimate of the maximum stipe lengths of kelp at the various sites. One site was east of False Bay (Grotto Bay (34°24'57.00"S 19°17'20.52"E) 80 km west of Cape Agulhas) while the other sites included the area from the Cape Peninsula to close to the South Africa/Namibia border (Bordjiesrif, Cape of Good Hope (34°21'16.61"S 18°28'20.26"E),
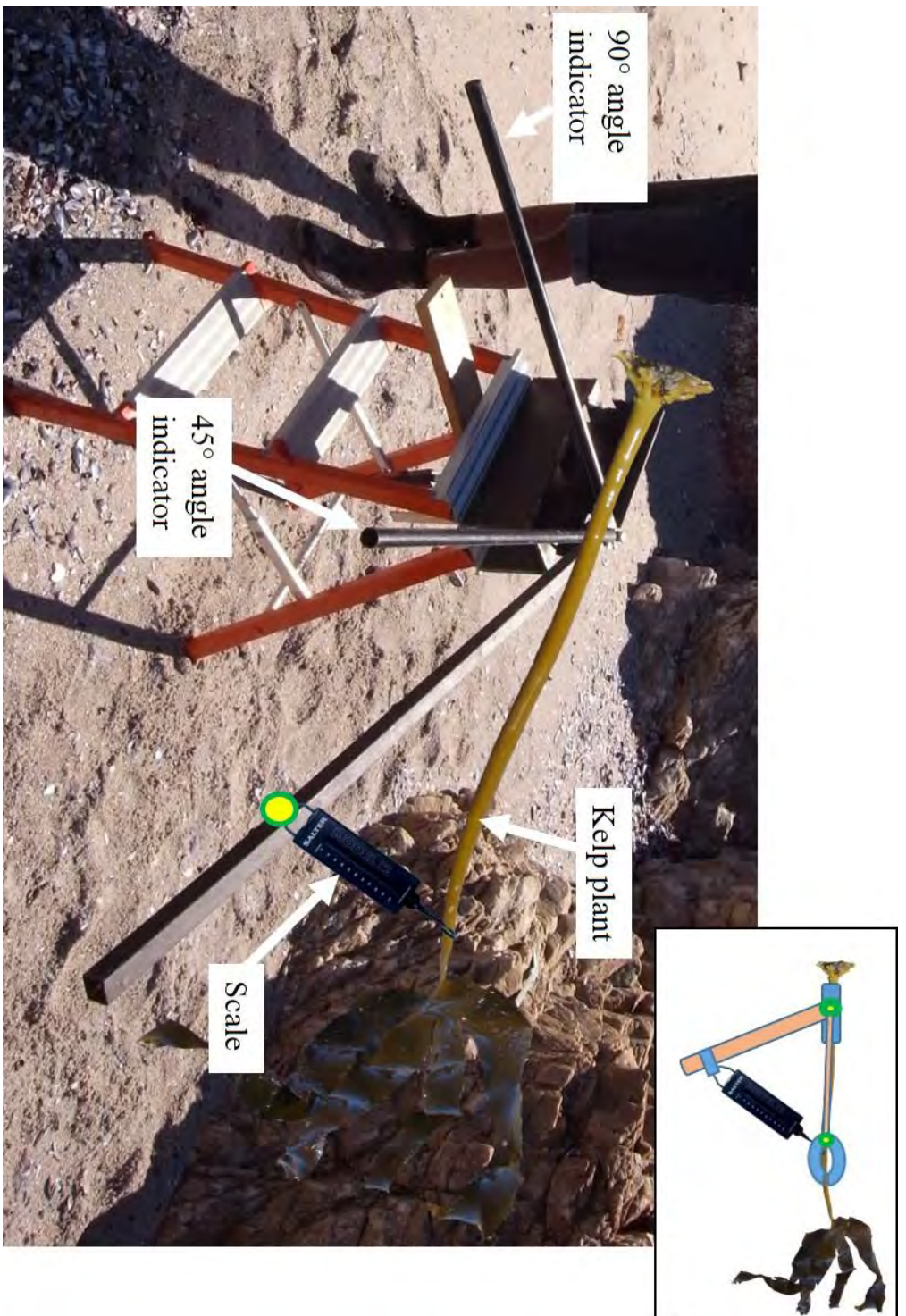
Fig. 2. Clamp is attached to a stand which holds the kelp in position (entire kelp is only for illustration purposes) to measure the flexibility of the stipe. One bar is attached to the scale to keep the distance from the clamp to the attachment point constant, while the other two bars indicate the 45° and 90° angle respectively. Insert is a schematic representation.

Yzerfontien (33°21'36.30"S 18° 9'22.40"E), Doring Bay (31°47'57.51"S 18°13'46.77"E),

Hondeklip Bay,

Brazil (29°48'45.29"S 17° 4'43.96"E – 15 km south of Kleinzee) and Port Nolloth) (Fig. 1). It

was assumed that the beach-cast sporophytes came from close to the area where they were

collected at each site. Forty of the longest washed-up sporophyte stipes were measured and

recorded, but only the 20 longest measurements were used in the analysis. If 20 washed-up

sporophytes, of a particular species could not be collected, then no measurements were taken.

A 1-way ANOVA and Tukey *post-hoc* analysis were performed to establish significance.

*Existing data on the occurrence of deepest kelp sporophytes*. Maximum depth data of *E.*
*maxima* and *L. pallida* sporophytes was extracted from unpublished data of Jeremy Cliff

(Seaweed Unit, Sea Fisheries Branch, Department of Industries) from 1979/80 for various

sites (Brand se Bay (31°18'7.48"S 17°52'55.04"E – 70 km north of Doring Bay), Donkin Bay

(31°56'1.08"S 18°15'48.22"E – 13 km south of Doring Bay), Jacobs Bay, Hondeklip Bay, and

Port Nolloth) along the South African west coast. Similar data was also extracted from Field

et al. (1980) for one site east of False Bay (Betty's Bay (34°22'18.00"S 18°53'41.15"E – 115

km west of Cape Agulhas) and two sites on the Cape Peninsula (Olifantsbos (34°15'31.41"S

18°22'47.69"E) and Kommetjie) (Fig 1).

Measurement of environmental factors

*Turbidity*. Monthly composite Aqua MODIS 4 km resolution ocean colour images were

downloaded from NASA Ocean Colour website (http:// http://modis.gsfc.nasa.gov/), for the

previous five years (2008-2013; i.e. 180 data points for each site, 60 per variable), and

manually analysed. Colours from the images were matched by eye to the key to extract

turbidity values at the eight sites where morphometric data were collected (a similar method

to that used by Smale and Wernberg (2009) to generate a temperature data set in Western

Australia). Parameters used as proxy for turbidity were: Chlorophyll a concentrations (Chl a), Particulate Organic Carbon (POC) and Particulate Inorganic Carbon (PIC). Data were analysed by ANOVA and a Tukey post-hoc test to establish significance. Images from the MODIS sensors onboard the Terra and Aqua satellites have previously been shown to reliably measure water turbidity (Chen and Muller-Karger 2007, Doxaran et al. 2009, Petus et al. 2010). A 1-way ANOVA and Tukey *post-hoc* tests were used to determine significant differences in turbidity between sites.

*Cloud/fog data*. Cloud data were visually extracted from MODIS aqua and terra 1km resolution images that were downloaded from the NASA website for 2011. Presence/absence of clouds was recorded for the same sites where morphometric data was collected, for every day during 2011. The satellite passed overhead twice daily, once in the morning and once in the afternoon. Data were analysed using a 1-way ANOVA and a Tukey *post-hoc* test to establish significance.

*Daylength*. Daylength data for Cape Town, Port Nolloth and Swakopmund (two sites at the extreme of the study area and one close to the middle) were extracted from the 2013 South African Tide Tables (Hydrographer, South African Navy). Daylengths were recorded daily and a 1-way ANOVA was used to determine significance.

*Temperature data*. Seawater temperature data were obtained from the South African Weather Service for the following sites: Kommetjie, Paternoster (32°48'20.54"S 17°53'24.59"E – 20 km north of Jacobs Bay), Lamberts Bay (32° 5'32.93"S 18°18'2.90"E – 30 km south of Doring Bay), Doring Bay, Hondeklip Bay, and Port Nolloth. The temperatures were measured daily by direct measurements at the surface using a thermometer. Temperatures were for a period of between 15 and 30 years (1981-2011). Bordjiesrif temperature data were obtained from the Starmon-Mini ® underwater temperature recorders (Star-Oddi, Iceland) of

Department of Agriculture, Forestry and Fisheries, placed at 8 m depth. The Swakopmund temperature data are from NASA Sea Surface Temperature data, corrected to reflect true coastal seawater temperatures (see Smit et al. 2013). Results were plotted to show maximum and minimum monthly means, and annual means of the entire data set at each site.

*Wave and wind data*. Windguru (http://www.windguru.cz/int/) data were downloaded for the following variables: wave speed, wave period, wave direction, wind speed, wind direction. Data were measured every three hours from February 2013 till July 2014. All the above variables were analysed using the multivariate analysis package Primer 6. Data were normalized before Principal Components Analysis (PCA) and Multi-Dimensional Scaling (MDS) were used to investigate similarity between sites. In addition regressions, box plots, and ANOVA and Post-hoc comparisons were used to closely analyse the major contributing factors that were shown by vectors for the PCA. Wind and wave directions were analysed and plotted using the freeware WRPLOT (Lakes Environmental Software: http://www.weblakes.com) to generate the plots to compare the different sites. The roses indicated direction as well as wave height (in the case of swell) and wind speed (in the case of wind). Regression analyses were performed: 1. to indicate relationship between the average wind speed and wave height versus the distance of the site from Cape Point; 2. To indicate the average maximum wave height versus the distance of the site from Cape Point.

RESULTS

Kelp biological data:

*Kelp dominance*. There was a gradual changeover in the dominance of the canopy kelps, with *L. pallida* replacing *E. maxima* from south to north (Fig. 3). In inshore shallow water, all of the sporophytes at Bordjiesrif were *E. maxima* while at Swakopmund in Namibia (about 1600

km from Bordjiesrif), all were *L. pallida*. At Hondeklip Bay (about 600 km from Bordjiesrif) there was a 42/58 split between the two species.

*Ecklonia maxima* biomass per m$^2$ (Fig. 4) increased from Bordjiesrif in False Bay to Kommetjie (26 km further) on the Atlantic side of the Cape Peninsula. The subsequent declining trend in biomass, northward to Kleinzee (700 km from Bordjiesrif), was not significant, but the lowest average biomass was observed at Kleinzee. In contrast, the highest average biomass of *L. pallida* was found at Kleinzee and only a single plant was sampled at Kommetjie and none at Bordjiesrif. Hondeklip Bay appeared to be in a transition zone for dominance between the two species, with approximately equal biomass of each species. Mean total plant weight of *E. maxima* was lowest at Bordjiesrif and highest at Kommetjie, east of which this parameter showed little variation (Fig. 5). *Laminaria pallida* sporophytes, however, had the highest average total plant weight at Kleinzee while sporophytes from Swakopmund had similar average plant weights to sporophytes from Jacobs Bay and Hondeklip Bay (Fig. 5) (there is insufficient data for Kommejie - only one sporophyte was found in the quadrats).

*Hollowness and stipe length*. *Ecklonia maxima* stipes were always hollow with no significant difference between sites (with relative hollowness between 86 - 90%; $r^2 = 0.7455$) (Fig. 6 a).

However, the linear regression (with a polynomial fit) shows that there was a clear gradient of increasing stipe hollowness in *L. pallida* from south to north, with relative hollowness ranging between solid at Kommetjie to 87% hollow at Swakopmund ($r^2 = 0.9599$: Fig. 6 a). At Kommetjie, only a single sporophyte was collected in the transects, but a separate study at the same site, that sought out only large, older *L. pallida* sporophytes, showed that 10% of them had some degree of hollowness in the stipe (n = 40). The results here, however, only use the transect data, in order to ensure methodological consistency of comparisons. In the

case of *E. maxima,* hollowness was not affected by the length of the stipe (Fig. 6 b). The stipe

length of *E. maxima* was variable, but hollowness was not (n = 366; *p* = 0.2533; *r²* = 0.6487).

By contrast, in *L. pallida* there was a positive linear correlation between stipe length and

hollowness.  (n = 153; *p* < 0.05; *r²* = 0.5291) (Fig. 6 b).
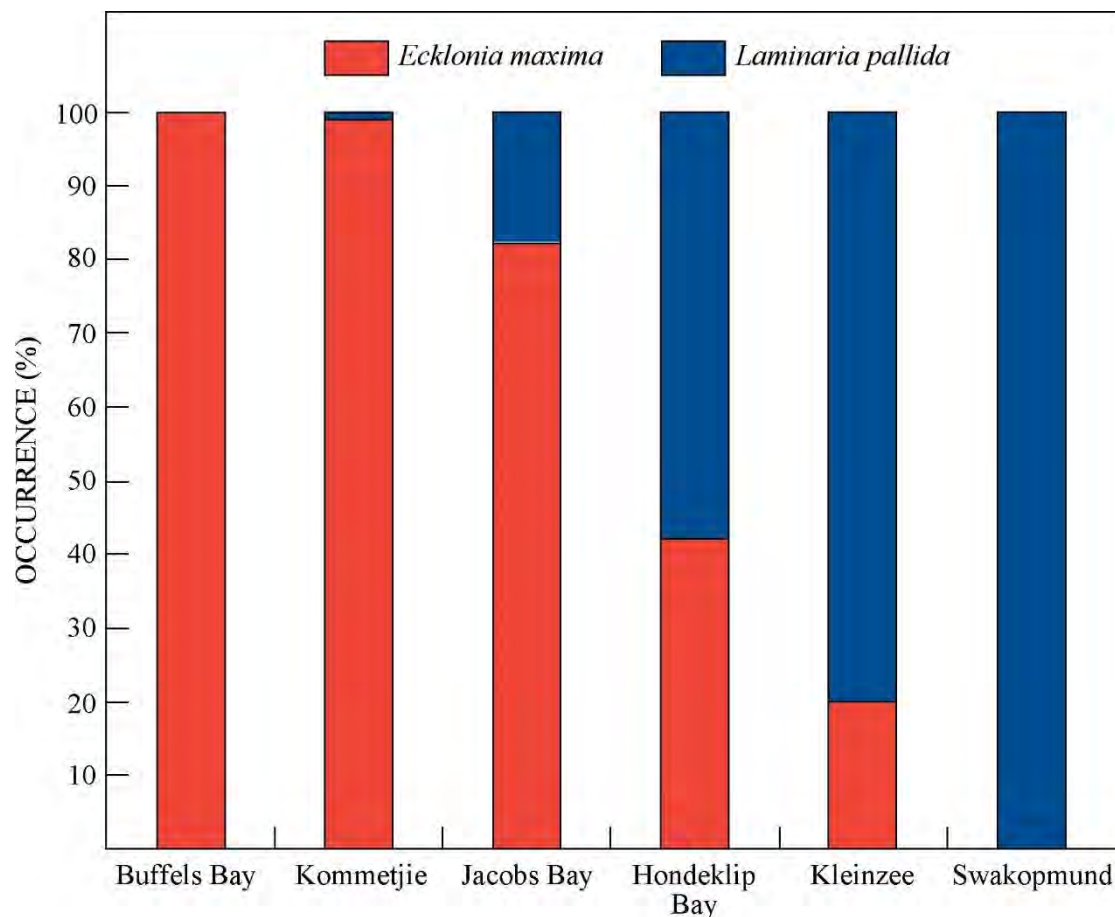


Fig. 3. Relative occurrence of *E. maxima* (red) and *L. pallida* (blue) along the Southern
African West Coast. Data was collected by benthic quadrats.

Fig. 4. Biomass of *E. maxima* (red) compared to *L. pallida* (blue) along the South African west coast. Bars indicate 95% confidence limits.

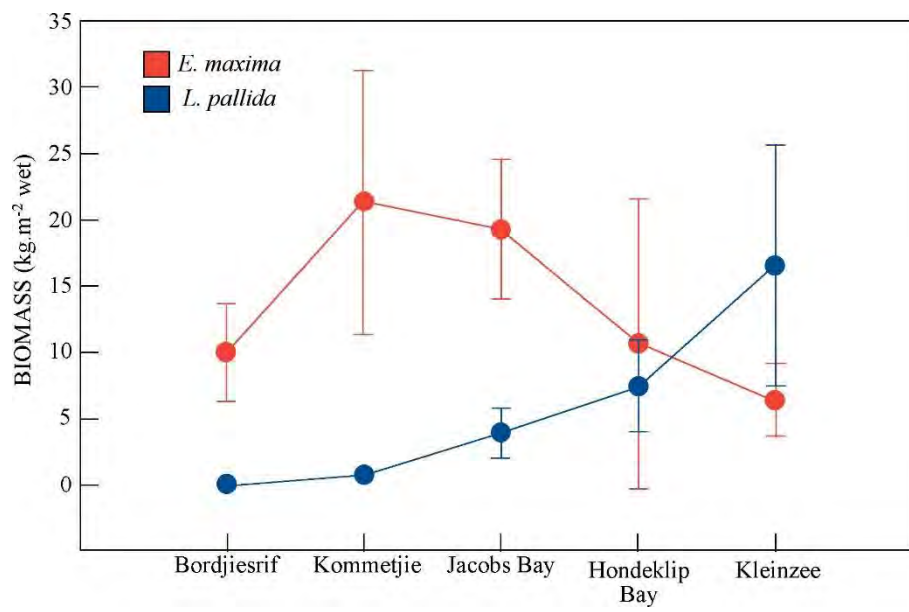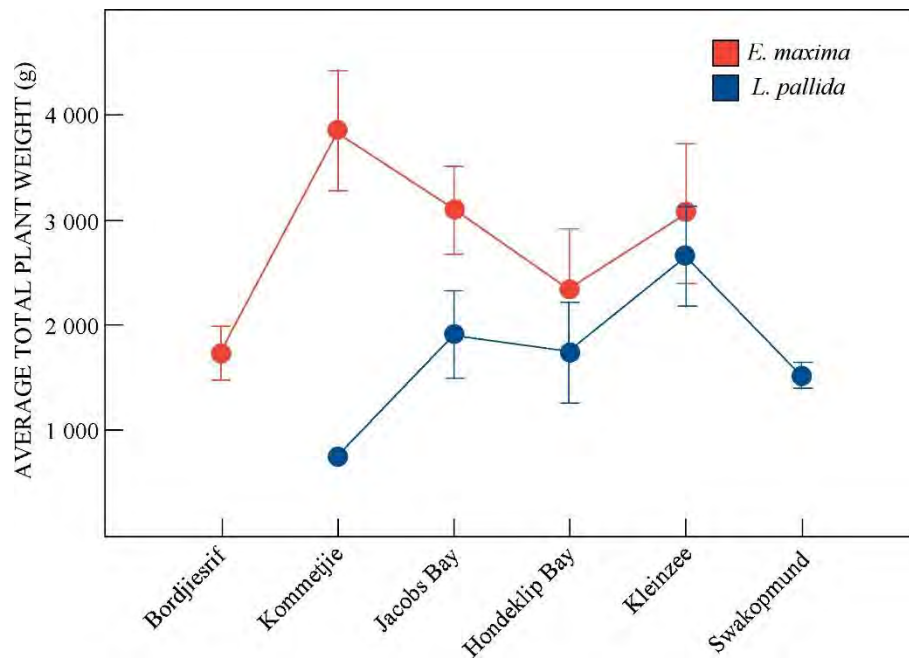Fig. 5. Average Total plant weight of shallow water *E. maxima* (red) and *L. pallida* (blue) along the Southern African west coast. Bars indicate 95% confidence intervals.

Laminaria *stipe flexibility*. Tukey *post-hoc* analysis showed that hollowness did not affect the flexibility of *L. pallida* stipes (p > 0.05) (Fig. 7 and Table 1), irrespective of whether stipes were bent to 45 or 90 degrees.

*Maximum depth and stipe length*. Maximum length of washed up *E. maxima* stipes from Cape Point (Fig 1) eastwards was much greater (average between 622 – 780 cm) than those west from Cape of Good Hope (average between 165 – 315 cm) (Fig. 8 a). Sporophytes from Cape of Good Hope had the longest stipes, while stipes from Hondeklip Bay were the shortest. The average maximum stipe length of *L. pallida* ranged between 145 cm at Hondeklip Bay to 266 cm at Port Nolloth. A decreasing trend was observed for *E. maxima* maximum stipe lengths from south to north ($r^2 = 0.6954$) (Fig. 8 b). The opposite holds for *L. pallida*, where between Hondeklip Bay and Port Nolloth a strong increasing trend was observed ($r^2 = 0.9842$) (Fig. 8 b). *Laminaria pallida* sporophytes had a deeper maximum depth at all sites compared to *E. maxima* (Fig. 8 c). Northwards along the west coast, the depth range of *E. maxima* decreased.

<u>*Multivariate analysis of morphological variables*</u>. The PCA of the *E. maxima* morphological data (Fig. 9), indicated that none of the sites (except some of the individuals at Kommetjie) separated out, indicating no effect of site on morphology (vectors from the PCA analysis indicated no significance). However, in the *L. pallida* morphological data, the PCA (Fig. 10) showed some separation of three groups of sites. The first group comprised the northernmost sites (Kleinzee and Swakopmund) with the percent hollowness (and the co-related factor of stipe length/weight: see Fig. 6 b) being the major forcing vector for this separation. The second group comprised mostly the intermediate Hondeklip Bay site, with some overlap between Jacobs Bay, Hondeklip Bay, Kleinzee, Swakopmund, and the only sporophyte from Kommetjie. The third group was from Jacobs Bay with little overlap from Hondeklip Bay (there was no *L. pallida* sampled at Bordjiesrif).
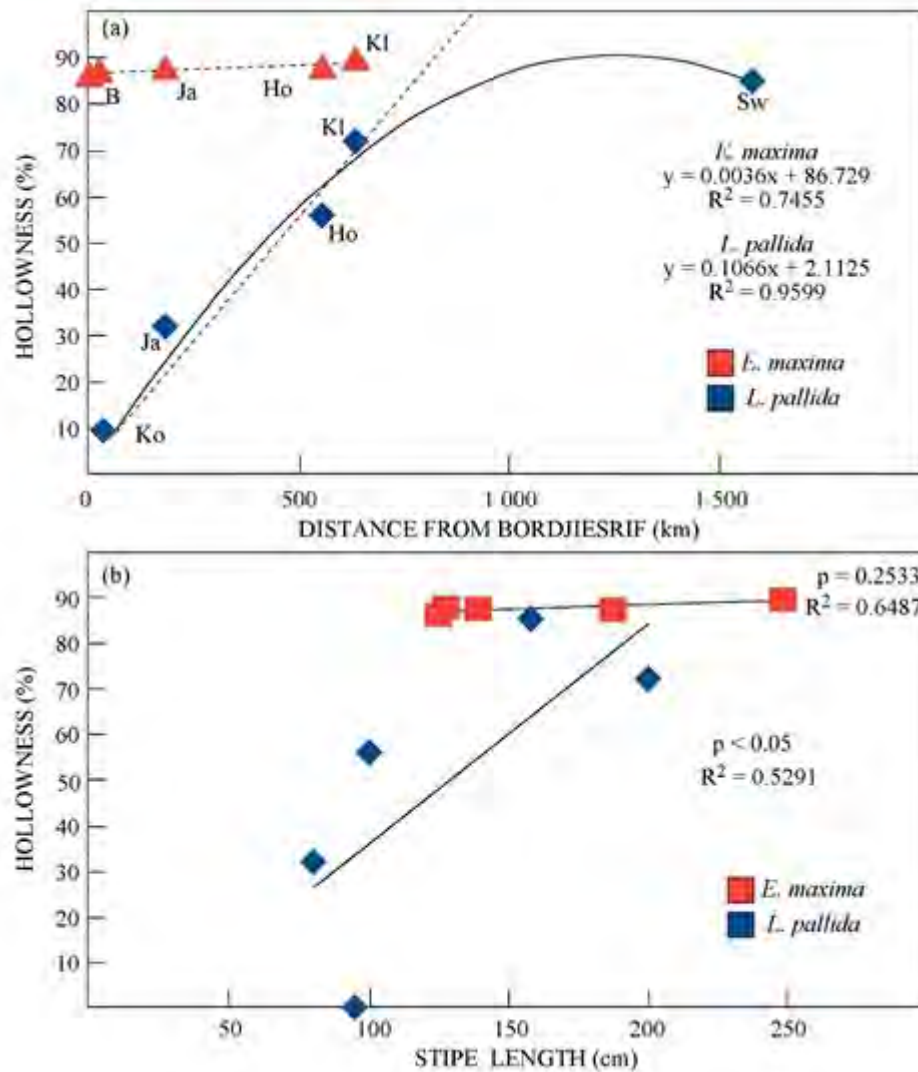
Fig. 6. (a) Relative hollowness of *E. maxima* (red) and *L. pallida* (blue) with increased distance from Bordjiesrif and (b) Relative hollowness (B) of *E. maxima* (blue) and *L. pallida* (red) with increase in stipe length.
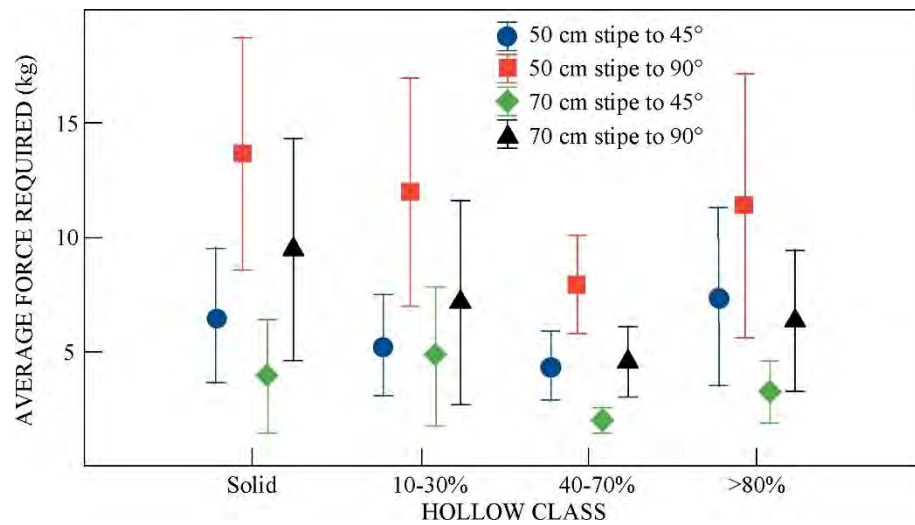
Fig. 7. The average force required to bend a L. pallida stipe of different degrees of hollowness. Blue markers indicate the force required to bend a stipe to a 45° angle when the scale is attached 50 cm from the clamp. The red markers indicate the force required to bend a stipe to a 90° angle when the scale is attached 50 cm from the clamp. The green marker indicates the force required to bend a stipe to a 45° angle when the scale is attached 70 cm from the clamp. The black markers indicate the force required to bend a stipe to a 90° angle when the scale is attached 70 cm from the clamp. Bars indicate 95% confidence limits.

Table 1. Significant values ($p$) between the different hollowness classes (ANOVA, Tukey *post-hoc* HSD test).

| Hollow Class | Solid | 10 – 30% | 40 – 70% |
|---|---|---|---|
| 10 - 30% | 0.9225 | | |
| 40 - 70% | 0.5223 | 0.9522 | |
| > 80% | 0.9771 | 0.7421 | 0.2609 |
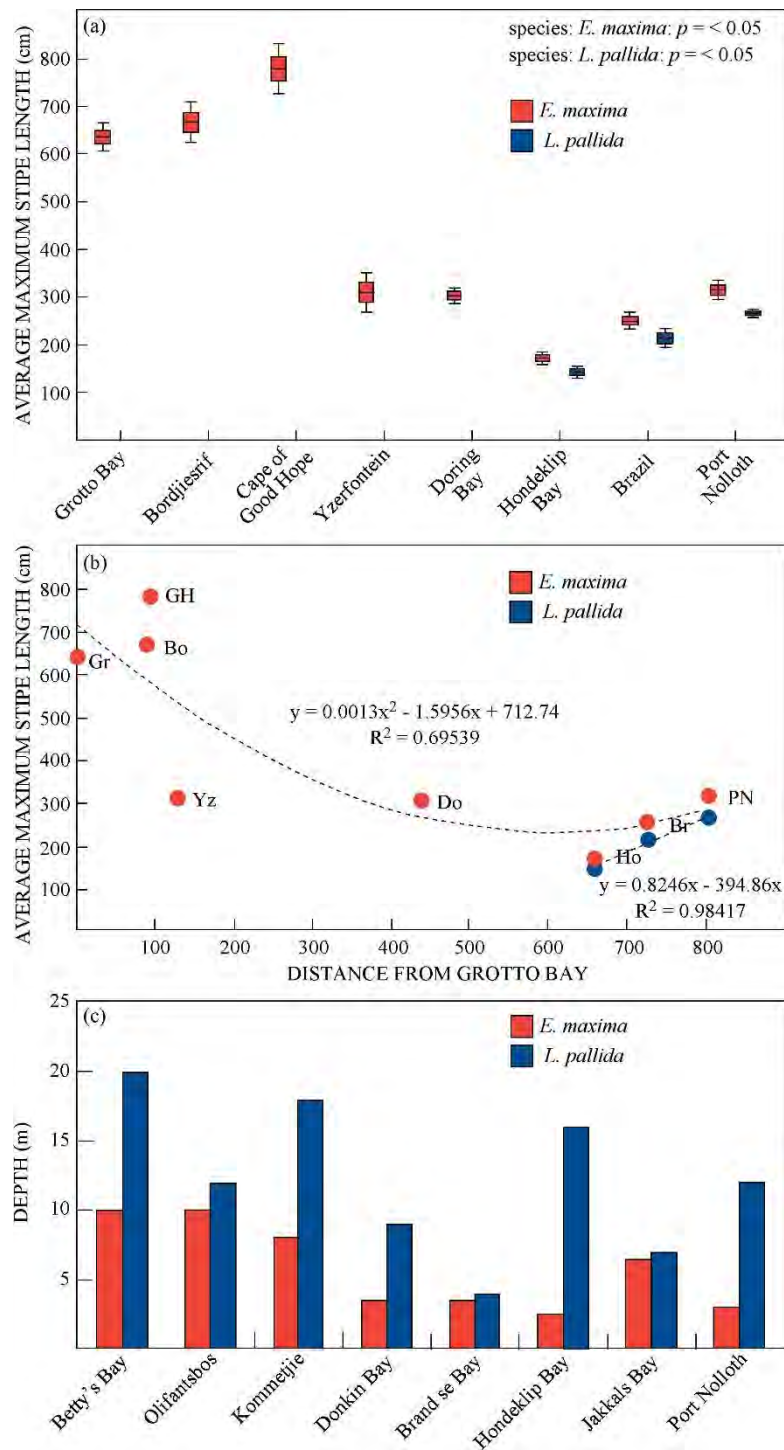
Fig. 8. (a) Average maximum stipe length of *E. maxima* (red) and *L. pallida* (blue) at different sites along the South African west coast (Bar indicate 95% confidence limits. (b) Average maximum stipe length of *E. maxima* (red) and *L. pallida* (blue) at different sites at various distances from Grotto Bay. (c) Maximum depth of *E. maxima* and *L. pallida* at different sites (data from Field et al. 1980 and Jeremy Cliff Unpubl. Sites are arranged from south to north.
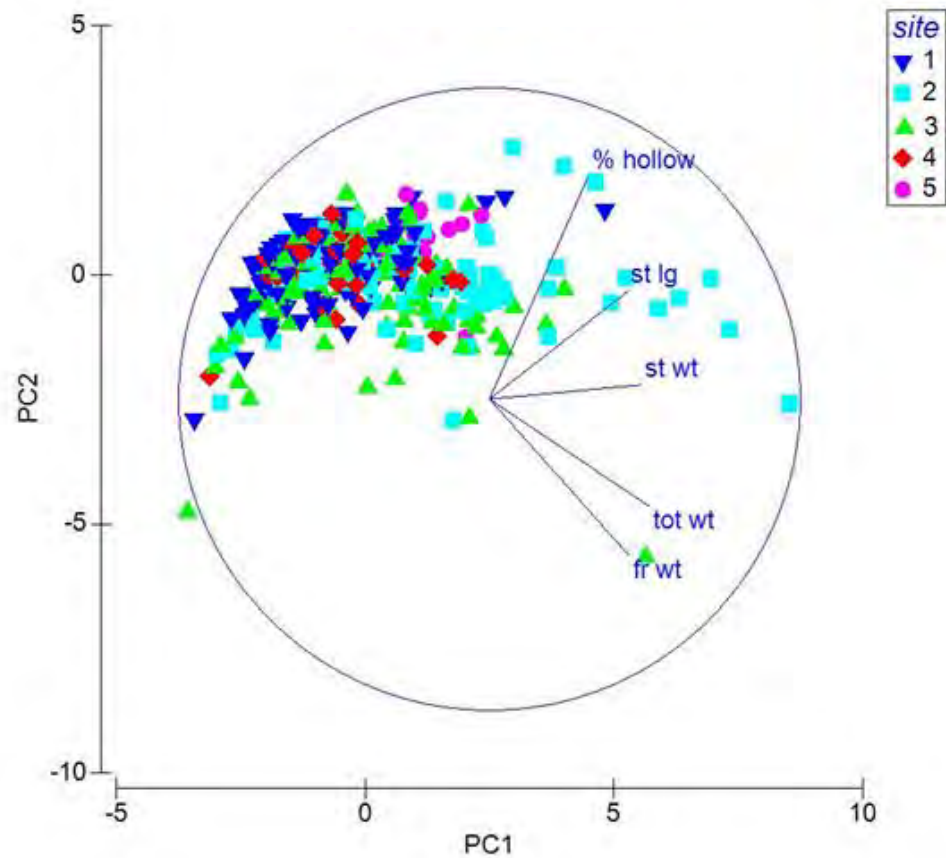
Fig. 9. Principal Components Analysis plot of *E. maxima* at different sites: 1 = Buffels Bay, 2 = Kommetjie, 3 = Jacobs Bay, 4 = Hondeklip bay, 5 = Kleinzee. Morphological characters: % hollow = portion of stipe that is hollow, st lg = stipe length, st wt = stipe weight, tot wt = total weight, fr wt = frond weight.
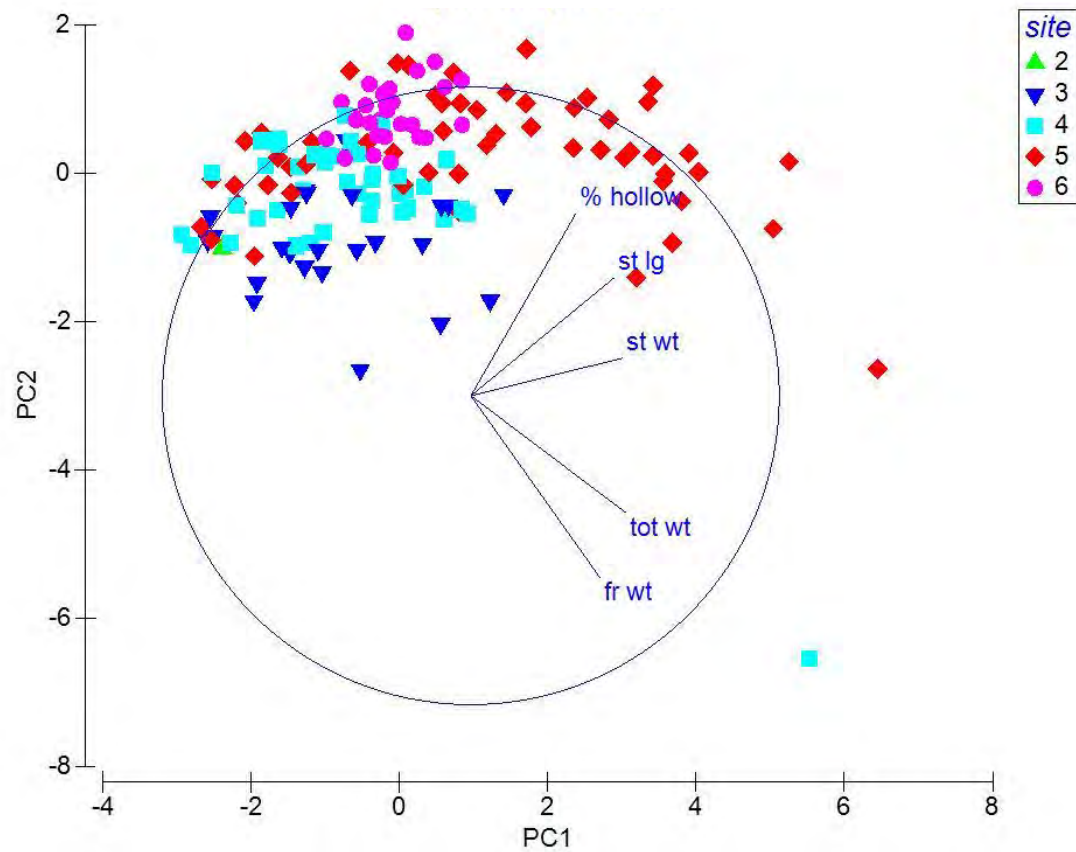
Fig. 10 Principal Components Aalysis plot of *L. pallida* at different sites: 2 = Kommetjie, 3 = Jacobs Bay, 4 = Hondeklip bay, 5 = Kleinzee, 6 = Swakopmund. Morphological characters: % hollow = portion of stipe that is hollow, st lg = stipe length, st wt = stipe weight, tot wt = total weight, fr wt = frond weight.

Environmental variables

*Turbidity*. The values of all three variables that were used as proxies for turbidity increased

from south to north (Fig. 11 a-c). Moving northwards from Bordjiesrif, all three variables

showed a progressive increase, but Jacobs Bay, Hondeklip Bay and Kleinzee all had similar

levels of Chl a, PIC and POC. The highest levels for all three variables were observed for

Swakopmund in Namibia. The linear regressions (with a polynomial fit) showed that there

were very good direct positive relationships between Chl a ($r^2 = 0.7778$; $p < 0.05$), PIC ($r^2 =$

$0.8931$; $p < 0.05$), and POC ($r^2 = 0.8854$; $p < 0.05$) the further west you move from

Bordtjiesrif.

*Fog or cloud cover*. The relative fog or cloud cover (Fig. 12; Table 2) showed no significant

variation over the South African portion of the study area for the year 2010, with values

ranging from  28.5 to 35.6 % (ANOVA, $p = 0.2818$).  However, the relative cloud/fog cover

at Swakopmund (46 %) was significantly higher than the South African sites ($p < 0.05$).

*Day length*. Summer day length in Cape Town is 18 minutes and 49 minutes longer than at

Port Nolloth and Swakopmund respectively (Fig. 13).  In winter Swakopmund (the northern

site) has winter days 29 minutes and 46 minute longer than at Port Nolloth and Cape Town

respectively.

*Temperature data*. After a sudden decrease from 15.5 – 13.3°C, between Bordjiesrif and

Kommetjie, the mean annual seawater temperature decreased slightly northwards to 12.3 °C

at Port Nolloth, after which, over the next 880 km, it rose again to 14.7 °C at Swakopmund

(Fig. 14). The extreme ends of the area under investigation, Bordjiesrif in the south and

Swakopmund in the north, showed the highest mean annual seawater temperatures of 15.5

and 14.7 °C respectively. Maximum and minimum mean monthly temperatures followed the

same general trends as the mean temperatures, varying between $\pm$ 1.8 – 4 °C. Again the sites at the extreme of the study area, Bordjiesrif and Swakopmund, had the highest minimum and maximum monthly mean temperatures. Swakopmund showed the biggest range between mean monthly maximum and minimum (8°C).

*Wind and waves*. For almost all sites, average wind direction (Fig. 15 a) was South-South Westerly between 200-210°, except for Lüderitz where it was South (177°). Average wind speed was highest at the southernmost sites (Cape Point = 7.5 knots and Kommetjie = 6.5 knots). Lüderitz had similar average wind speed as Paternoster and Shelly Bay (, which is higher than Elands Bay and Doring Bay, the, Elands Bay and Doring Bay as well as the most northerly site, Swakopmund had the lowest average wind speed (Fig. 15 b). Average and maximum wave height (Fig. 15 b) showed a similar trend with the highest values at the southernmost sites (Cape Point and Kommetjie) and lowest at the most northern site (Swakopmund). For both these variables Paternoster, Shelly Point, Elands Bay Doring Bay and Lüderitz all had similar values (ranging between 6 – 5.5 m for maximum wave hight and 2.5 – 2.6 m for average wave hight).

*Multivariate analysis*. The PCA (Fig. 16) partly separated out three groups of sites. The first group included Cape Point and Kommetjie (the most southerly sites), the second group, Lüderitz and Swakopmund (the most northerly sites) and the final group comprised the sites that lie in between (Jacobs Bay, Shelly Point, Elands Bay, and Doring Bay). The direction of the arrows revealed that the most southerly sites had high values for wind speed, and low values for those factors linked with water turbidity (Chl a, PIC, POC).
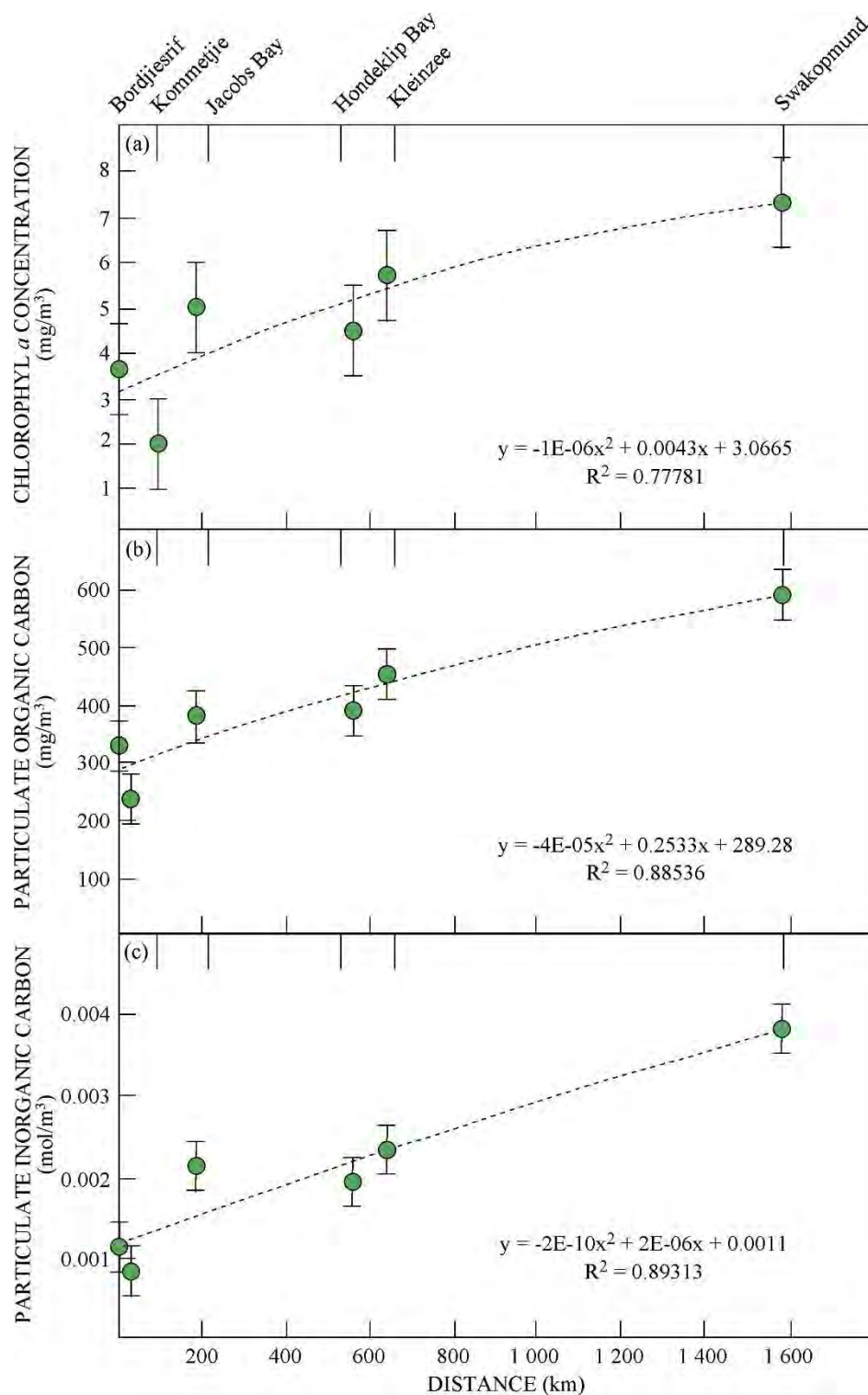
Fig. 11. Three variables indicating turbidity of seawater, at various distances from Bordjiesrif along the Southern African west coast: (a) Chlorophyl *a* concentration, (b) Particulate Organic Carbon, (c) Particulate Inorganic Carbon. Bars indicate 95% confidence intervals.
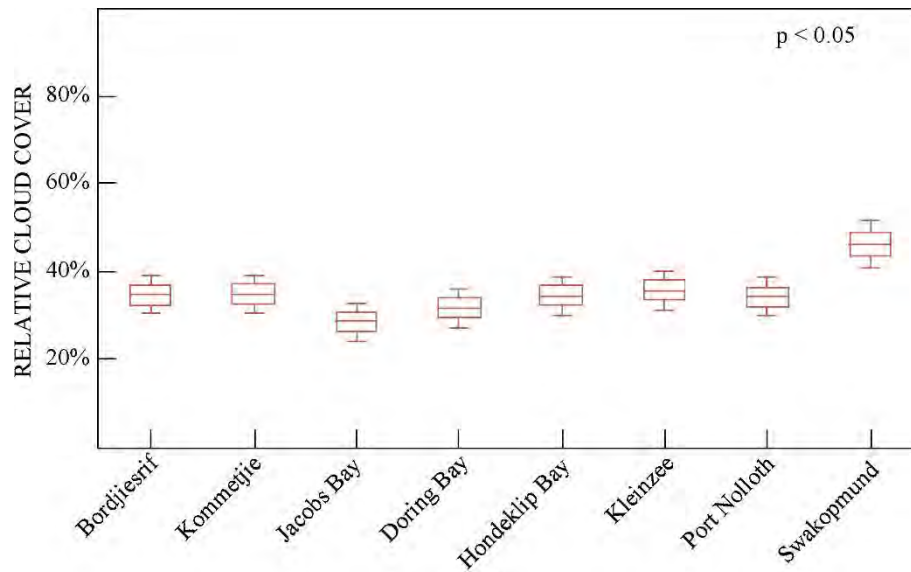
Fig. 12. Relative fog or cloud cover for Southern African west coast for 2010. Bars indicate 95% confidence intervals. Boxes indicate Standard Error; lines indicate mean values and whiskers indicate 95% confidence limits.

Table 2. Significant values ($p$) of cloud cover at different sites along the Southern African west coast (ANOVA, Tykey *post-hoc* HSD test). Values in red are significant.

| Site | Buffels Bay | Kommetjie | Jacobs Bay | Hondeklip Bay | Kleinzee |
|------|-------------|-----------|------------|---------------|----------|
| Kommetjie | 1.0000 | | | | |
| Jacobs Bay | 0.4194 | 0.3917 | | | |
| Hondeklip Bay | 1.0000 | 1.0000 | 0.4478 | | |
| Kleinzee | 0.9995 | 0.9998 | 0.2383 | 0.9991 | |
| Swakopmund | *0.0062* | *0.0072* | *0.0000* | *0.0053* | *0.0180* |
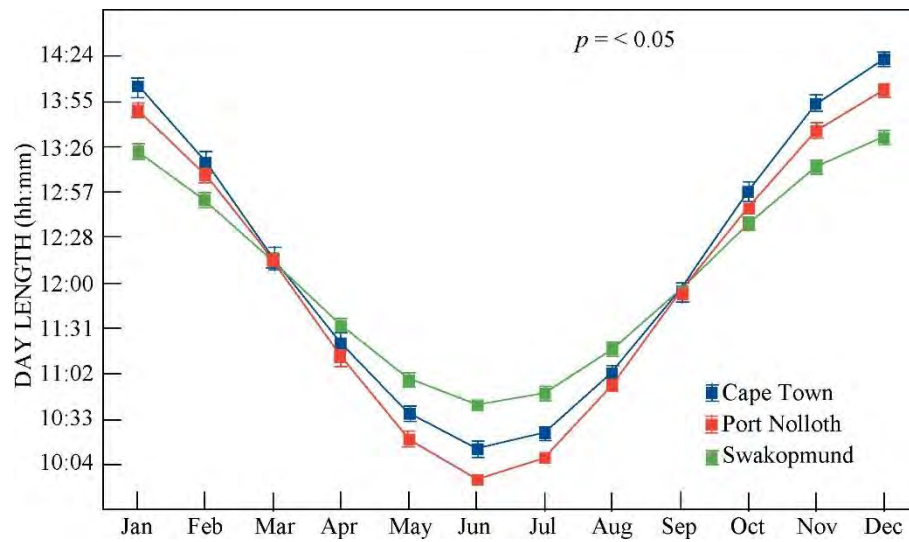
Fig. 13. Average monthly day length for Cape Town (blue), Port Nolloth (red) and Swakopmund (green) for 2013. Data from the South African Tide Tables, The hydrographer, South African Navy. (b) Bars denote 95% confidence limits
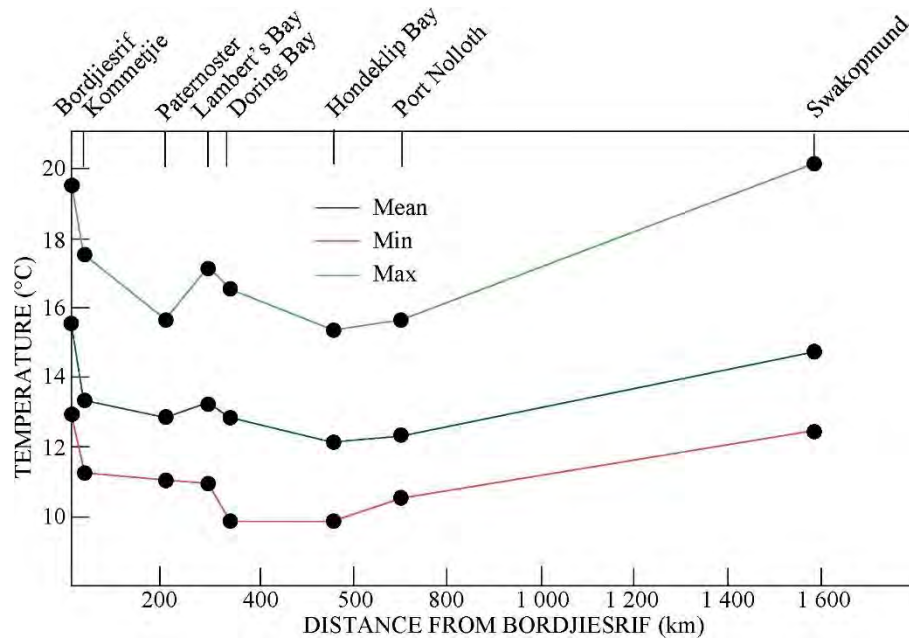
Fig. 14. Average seawater temperatures for the South African west coast for a period of about 30 years (1981-2011). The blue line indicate the mean annual temperatures while the green and brown lines indicate the maximum and minimum monthly mean temperatures respectively. Data from the South African Weather Services. Bordjiesrif data from a temperature recorder at 8 m depth from Department of Agriculture Forestry and Fisheries. Swakopmund data is from NASSA SST corrected by AJ Smit.
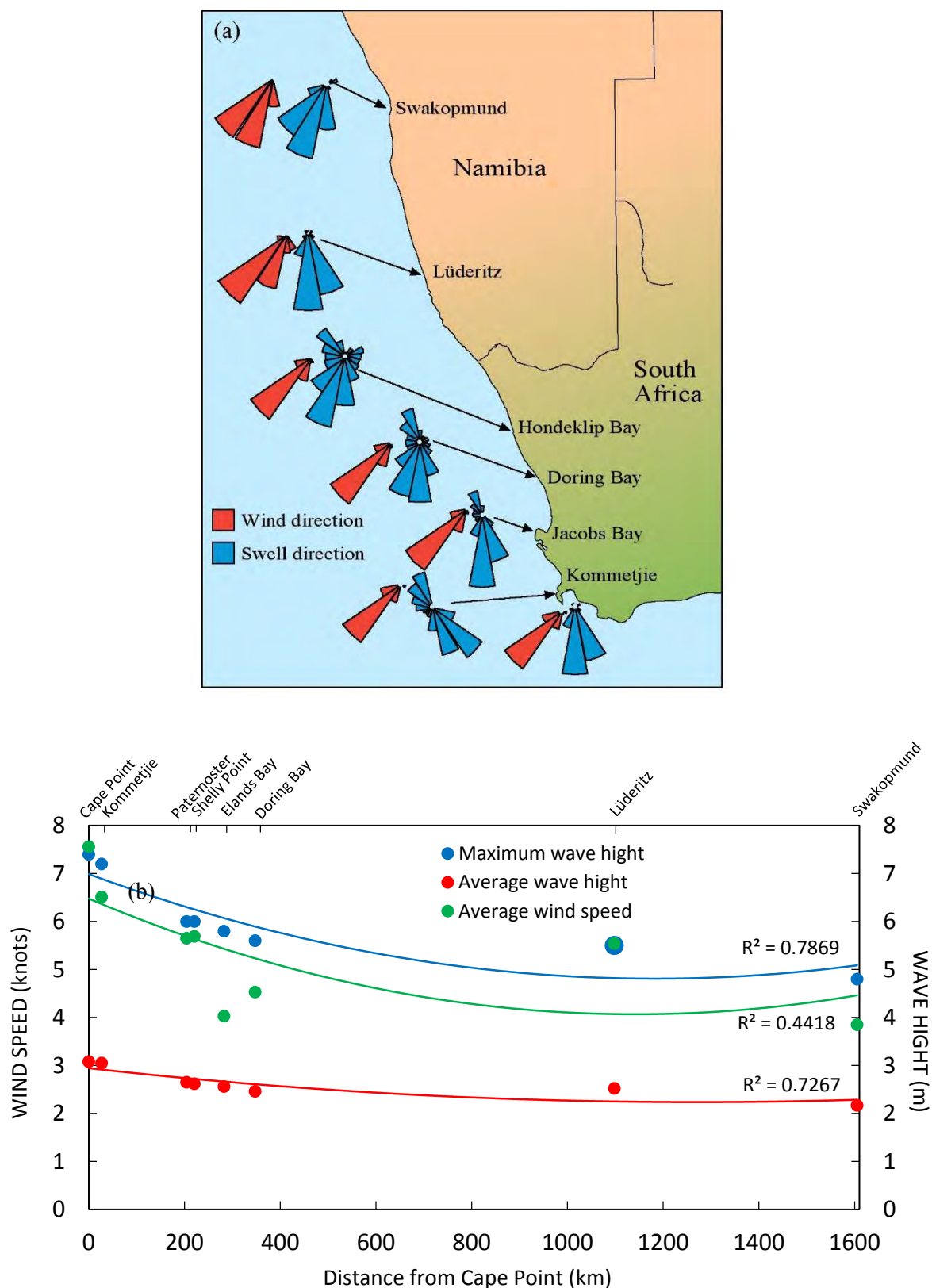
Fig. 15. (a) Wind (red rose) and wave (blue rose) directions for different sites along the Southern African west coast. (b) Red markers indicate the average wave height; the green markers indicate the average maximum wave height; the blue markers indicate the average wind speed. Sites are arranged accoding their distance from Cape Point.
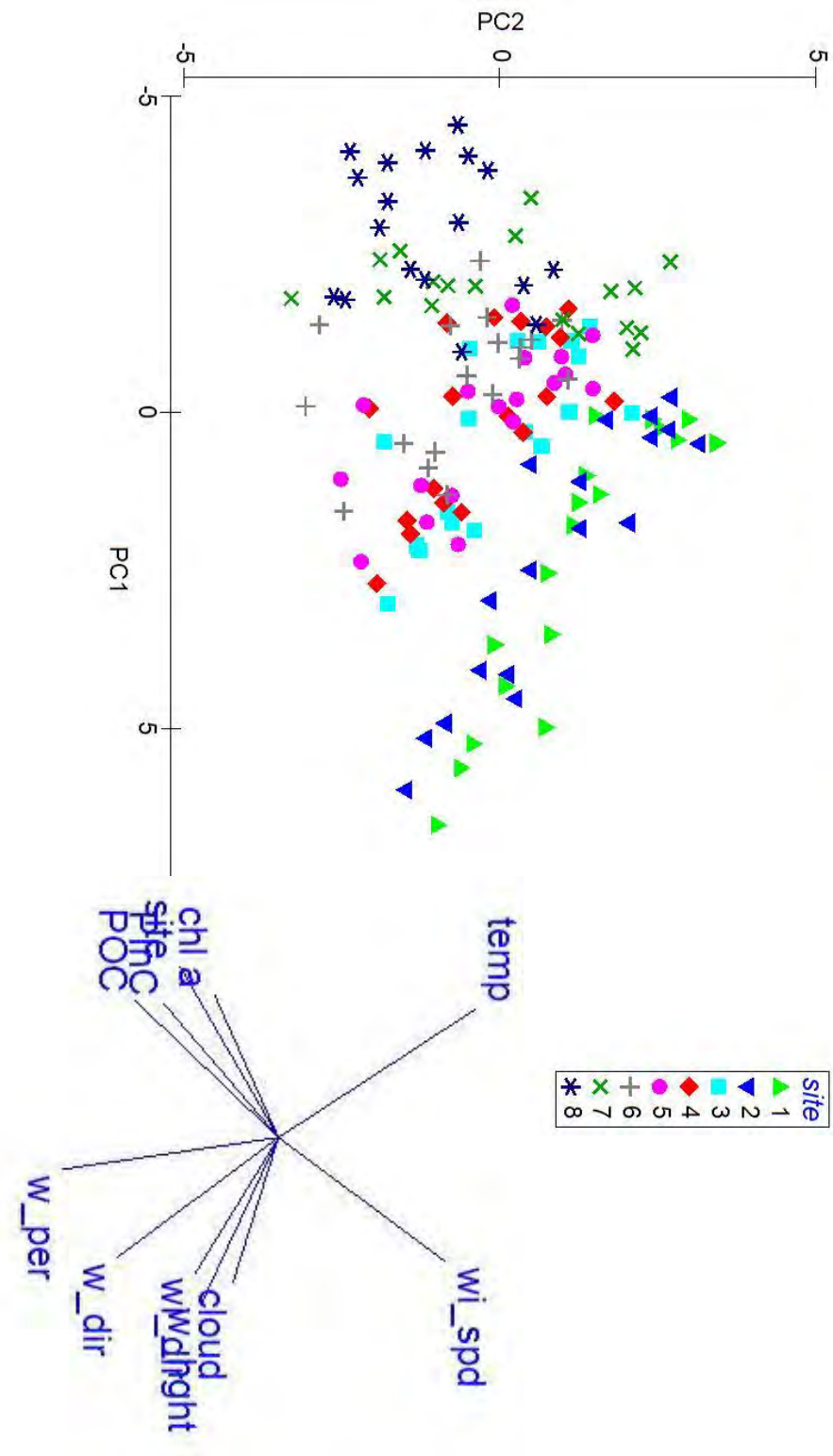
Fig. 16 PCA plot of environmental factors at different sites: 1 = Cape Point, 2 = Kommetjie, 3 = Jacobs Bay, 4 = Shelly Point, 5 = Elands Bay, 6 = Doring Bay, 7 = Luderitz, 8 = Swakopmund. Environmental factors: temp = temperature, wi spd = wind speed, wi dir = wind direction, w dir = wave direction, w hgt = wave hight, w per = wave period, cloud = relative cloud cover, chl a = chlorophyll a, PInC = particulate inorganic carbon, POC = particulate organic carbon. Red oval represents Group a; the purple oval Group b; and the green oval Group c.

DISCUSSION

This study investigated the morphology of shallow water (0 – 5 m) *E. maxima* and *L. pallida* sporophytes over most of their distribution ranges and attempted to pinpoint the environmental factors that might be responsible for any morphological patterns. It is the first study to quantify the change in dominance between the two kelps *E. maxima* and *L. pallida* along a latitudinal gradient in kelp beds on the west coast of Southern Africa, largely confirming previous anecdotal observations (e.g. Stegenga et al. 1997). Both relative occurrence (Fig. 3) and biomass data (Fig. 4) showed a clear transition from the south, where inshore beds are dominated by *E. maxima*, to the north, where beds are dominated by *L. pallida*.

Along this latitudinal gradient there was no change in the morphology of *E. maxima* sporophytes, other than some inter-site differences in mean and maximum stipe length (which may relate to turbidity - see later). However, for *L. pallida* there was a distinct gradient in relative hollowness, quantitatively confirming previous observations that northern plants have hollow stipes (e.g. Stegenga et al. 1997). Although weak, a positive correlation was also observed between stipe length and hollowness, with stipe length also showing a geographic gradient: *L. pallida* sporophytes in the north had longer stipes than those in the south. On the northern part of the South African west coast (Hondekip Bay, Brazil and Kleinzee), where washed-up stipes of both *L. pallida* and *E. maxima* were measured, there was a trend of increasing stipe length northwards between these three sites, which may partly explain the increasing hollowness northward, since there was a positive relationship between length and hollowness in shallow water sporophytes. There is also a similar pattern in mean sporophyte weight. However, this does not obscure the significant change in hollowness of *L. pallida* stipes in shallow water from south to north, over some 1000 km along the Southern African west coast.

*Saccharina latissima* (as *Laminaria agardhii* Kjellman) and *S. longicruris* (as *L. longicruris*) (now a single species) showed decreasing stipe length and hollowness as wave exposure increased at three sites in Nova Scotia (Chapman 1973). Other examples of hollow-stiped kelps are the annual *Nereocystis luetkeana* (Denny and Cowen 1997) and *Ecklonia maxima* (Stegenga et al. 1997). However, the only other comparable study is that of Matson and Edwards (2006) who showed a similar gradual change in stipe hollowness of *Eisenia arborea* northwards along the west coast of North America. However, they offered no explanation for this phenomenon.

Having established clear latitudinal patterns in relative dominance of *L. pallida* and *E. maxima*, and in relative hollowness of *L. pallida* stipes, the question arises – if the causes are environmental, what factors could be involved?

*Multivariate analyses*. Because the morphology of *E. maxima* in shallow water was unaffected by geographical location (e.g. Fig. 9, PCA) this species is morphologically clearly unaffected by whatever environmental factors affect the morphology of *L. pallida*.

In the case of *L. pallida* the pattern that emerged showed a latitudinal trend related most strongly to relative hollowness of the stipes (Fig. 10). The hollowest sporophytes grouped at the most northerly sites (Swakopmund and Kleinzee), followed by a mixture of sites with less-hollow sporophytes (Hondeklip Bay, some Jacobs Bay, and Kleinzee plants), that are more central on the coast, and finally the group with the least hollow sporophytes were southerly (most of Jacobs Bay, but with some Hondeklip Bay plants). The most southerly site, Bordjiesrif, had no *L. pallida*, and only one plant was collected in the sampling at Kommetjie, which fell in the southerly group. The PCA indicates that the major contributing factors to these groupings are percent hollowness, stipe weight and stipe length, as indicated

by the direction and length of the vectors. However, the question remains: what might cause this hollowness pattern along a geographical gradient, in shallow water?

*Temperature.* The latitudinal pattern in water temperatures does not correspond at all with the clear latitudinal patterns in *Laminaria* dominance and hollowness. While water temperatures are markedly higher at Swakopmund and Bordjiesrif than at any of the intermediate sites, the former has only *L. pallida* and the latter only *E. maxima*. At Bordjiesrif there is no *L. pallida* except at depths greater than about 5 - 6 m (M. Rothman, personal observation), but temperatures are similar to or slightly higher than at Swakopmund. Interestingly, at Hondeklip Bay, *L. pallida* and *E. maxima* each make up almost 50% of kelp numbers, and this site has the coldest average seawater temperature and is a center of upwelling (Shannon 1985). Although on a biogeographical scale temperature is the main driving force controlling seaweed species biogeographical limits (Lüning 1990, Bolton and Anderson 1987, Anderson et al. 2007), these results indicate that factors other than seawater temperature must drive the northward dominance and increasing hollowness of *L. pallida*.

*Nutrients.* Studies on this coast have shown that, in near-shore environments, there is a negative correlation between temperature and nutrients (Dieckmann 1980, Waldron and Probyn 1992). Thus as temperature does not correlate with the observed changes in dominance and *Laminaria* morphology, the influence of nutrients on the form and abundance of *L. pallida* along the Southern African west coast, can be disregarded. Perhaps more importantly, all of these sites lie within the Benguela Marine Province (except Bordjiesrif, which however is only ca 4 km from Cape Point) and all have relatively high nutrient levels that are associated with upwelling.

*Swell and wave height.* Between Cape Point and Swakopmund there was a detectable trend of decreasing wave height northwards along this coast (Fig. 15 b). However, Bordjiesrif, for

which wave height data were not obtainable, lies in an east-facing bay within the greater

False Bay, and is a relatively more sheltered site than any of the others, so that were data

available, they would certainly indicate average wave height values much lower than

Swakopmund (R. Anderson, personal communication). If dominance and hollowness of *L.*

*pallida* were associated with reduced wave exposure, this species should be prevalent in

shallow water there, and not limited to deeper water. Also, it could be argued that the

difference between the extremes of average wave height measured here (3 m at Cape Point

and 2 m at Swakopmund, 1500 km north) is not great in absolute terms. Even more so, the

difference between means at Jacobs Bay (2.6 m) and Swakopmund (2 m) is only 0.6 m, yet

the former has less than 20% *L. pallida* and sporophytes that are only 32% hollow, while at

the latter there is 100% *L. pallida* and the sporophytes are over 80% hollow. Similarly,

Hondeklip Bay, which is only 370 km north of Jacobs Bay, has similar environmental

conditions to Jacobs Bay (including temperature), yet there is 40% more *L. pallida* present at

the forms site and a higher percentage stipe hollowness (56% vs 32%).  In most of the studies

of wave exposure on kelp morphology, very sheltered sites are contrasted with very exposed

sites (e.g. Norton et al. 1982, Serisawa et al. 2002b, 2003, Hurd and Pildith 2011). However,

similar studies comparing morphology over a large latitudinal gradient are lacking.

*Wind speed and direction*. Upwelling along the Southern African west coast is wind-driven

(Shannon 1985, Monteiro and Largier 1999, Berger and Wefer 2002). Southerly longshore

winds drive surface water northward, and in conjunction with Coriolis forces cause surface

water to move offshore. This results in the upwelling of deeper, cold, clear, nutrient-rich

water. Along the Southern African west coast, the predominant wind direction is south-south

west, which, together with the high wind speeds observed at the southern sites (Fig. 15 a &

b), will result in more intense upwelling at these sites. In contrast the most northern site,

Swakopmund, had a significantly lower wind speed compared to the more southern sites and upwelling there is weak and sporadic (Nelson and Hutchings 1983).

*Light/turbidity*. Among the environmental variables measured here, those that affect underwater light showed the most convincing latitudinal gradients. Underwater light could be affected by water turbidity, cloud cover or fog, day length, and depth (Shirley 1929, Burgess 2009) or upwelling (see above).

Depth can be discounted as a latitudinal factor, since all sites were sampled in the same depth range. Daylength is unlikely to be the cause of the geographic variation in *L. pallida*, for several reasons. Firstly, while day length is known to correlate with annual growth rate in *L. pallida* (Dieckmann 1980), the distance (and therefore difference in day length) between even Swakopmund and Bordjiesrif is relatively slight (with the maximum difference being about 49 minutes; Fig. 13), which would imply that day length does not play a big part in the gradual hollowing of *L. pallida*. Also, photoperiodic day length effects on algal life histories/growth work in the form of on/off switches (Lüning and Dring 1972, Izquierdo et al. 2002, Muñoz et al. 2004, Oppliger et al. 2012) and would not explain the gradient observed in the *Laminaria* morphological changes. Secondly, the overall differences in the amount of light (total quanta incident per day) between distant sites are slight, and seasonal patterns may cancel out most or all of the latitudinal differences; i.e. Swakopmund has shorter summer days, but longer winter days. To understand this better one would have to measure the PAR *in situ* at the various sites over a year, and this was logistically not possible. Also the difference in the amount of energy received, from extra-terrestrial solar radiation, between sites along the distribution range of *E. maxima* and *L. pallida* would be negligible (P. Burgess, personal communication)

Clouds and fog cause scattering of incident light and reduce the amount of light that reaches the earth (Blinn 1982). Although anecdotal reports of increasing fog and clouds northwards up the west coast led us to investigate this, the satellite image data for 2011 showed no significant trends, except that there was significantly more cloud/fog cover at Swakopmund. This however, cannot account for differences in the *L. pallida* at the other sites, and we are left with water turbidity as the only factor that shows a convincing latitudinal gradient.

Data extracted from NASA satellite imagery showed a northward increase in turbidity for all three variables that were measured (Fig. 11), indicating a northward reduction in underwater light along the coast. On the west coast, a gradual decrease in underwater light corresponds well with the gradual increase in dominance of *L. pallida* in the near shore environment (and the increasing prevalence of hollow stipes in this species). From this it is hypothesized that *L. pallida* may be adapted to lower light compared to *E. maxima.*

This seems reasonable in view of the fact that, in the south *L. pallida* grows under the canopy of *E. maxima*, and all along the coast *L. pallida* forms extensive beds in deeper water down to 20 m and more. From this it appears that where there is good light in shallows (e.g. Cape Peninsula, down to about 5 m depth), *E. maxima* out-competes *L. pallida*. While *Ecklonia maxima* may well be depth-limited by how long its stipe can grow, (because it seldom occurs deeper than about 12 m, it might also be depth limited at more than 12 m because even when the water is clear, it is *light-limited*. Using the Jerlov (1977) system of optical water types, Lüning (1990) showed that water clarity can have an enormous effect on light penetration: the deepest *Laminaria* in Helgoland is found at 8 m depth, but in the Mediterranean at 95 m. The quantity of light at the former site is drastically reduced by turbidity. It is possible that on the Southern African west coast, the northward gradient of increasing dominance of *L. pallida* in shallow water is a result of the gradient in water turbidity and hence light: as inshore light decreases, *L. pallida* may be able to outcompete *E. maxima*.

*Maximum stipe lengths*. The maximum lengths of *E. maxima* stipes recovered from beach-casts around Cape Point were almost double those collected from Hondeklip, Brazil and Port Nolloth (Fig. 8 a), where the water is more turbid.  Stipes from Yzerfontein were also relatively short, but there are few or no deeper reefs there, whereas  the other three sites have extensive reefs running from shallow to deep water, so that substratum availability cannot limit the lengths of  *E. maxima* there. The results indicate that southern sporophytes are longer than the northern ones (Fig. 8 b).

Published data on the maximum depths at which the two kelps were recorded (Fig. 8 c) provides further support for this idea. Among the five northerly sites, only Jakkalsbaai had *E. maxima* sporophytes deeper than 5 m, while at the three southern sites, depths ranged from 8 m (Kommetjie) to 10 m at the remaining two sites.  The pattern for *L. pallida* is less clear, but it grows down to more than 15 m at three of the sites. In any case, clearly *L. pallida* grows deeper than *E. maxima*, supporting the idea that it can tolerate lower light levels.

*Hollowness*. This study demonstrated a gradual change in turbidity along the distribution area of *L. pallida*. Similarly, a gradual increase in the dominance of *L. pallida* northwards was observed, which corresponded with a gradual increase in the degree of hollowness of *L. pallida* stipes. It is interesting to note that *Ecklonia* in the Southern Hemisphere has a solid-stiped species (*E. radiata*) and a closely related hollow-stiped species (*E. maxima*) (Rothman et al. 2015). It is hypothesized that the latter evolved from the former (Bolton and Anderson 1994, Rothman et al. 2015) to adapt to the cold, nutrient rich environment of the Southern African west coast. Although the degree of hollowness in *E. maxima* does not change along the west coast the stipe length is variable and could thus indicate that stipe length in *E. maxima* is genetically determined. The degree of hollowness in *L. pallida* does vary and an investigation into a causal relationship is warranted and begs the question: why does *L.*

*pallida* have a hollow stipe in shallow water on the northern parts of the coastline? Two reasons are considered.

The first reason might be that where *L. pallida* is growing in a much more wave-stressed (shallower) environment, the hollowness may make it more flexible and better able to survive (see Friedland and Denny 1995 and Denny et al. 1997).

The flexibility of kelp stipes is an important property that allows these plants to become large under conditions of very high wave action (Denny et al. 1997). The hydrodynamic forces imposed on plants are reduced by their flexibility and their ability to 'go with the flow' (Koehl 1986, Denny *et al*. 1997). Several studies have shown the importance of flexibility of seaweeds in the intertidal (Friedland and Denny 1995, Utter and Denny 1996, Denny et al. 1997). However, other mechanical properties of the tissues also influence the survivorship of individuals. Demes et al. (2013) showed that *Egregia menziesii* had a higher survival rate if the fronds are weak because the fronds self-prune, decreasing hydrodynamic drag forces and decreasing mortality. Both *E. maxima* (e.g. Mann et al. 1979) and *L. pallida* (Dieckmann 1980) lose material from the ends of their fronds, effectively "self-pruning" and limiting the drag force exerted on the sporophytes. It appears that this, together with the overall flexibility of the sporophytes, is sufficient to allow them to survive in the extremely turbulent shallow water of the Southern African west coast. But most importantly, flexibility did not increase with hollowness in *L. pallida*, indicating that flexibility cannot explain its increase in hollowness northwards.

Secondly, a hollow stipe might mean less investment in energy compared to building a solid medulla. This would enable the sporophytes to spend more energy in becoming longer and reaching the surface quicker where more light is available. Although a solid cylinder is stronger than a hollow cylinder in absolute terms, the ratio of strength to weight is higher in a

hollow cylinder of the same diameter (for a mathematical explanation, see Bhandari 2010). By becoming hollow, a stipe will lose some strength, but lose a disproportionately greater amount of weight (i.e. medullary tissue). If a slight loss of strength is less important than a relatively large saving in energy (in the form of medullary tissue that would have been necessary to produce a solid stipe), these plants will have an advantage, because they should be able to grow faster than solid plants. *L. pallida* may be exploiting this relationship to grow faster in more turbid water, leading to a northward increase in hollowness. This hollowness may therefore simply be an energy-saving adaptation, since the present results show that the force needed to bend a *L. pallida* stipe to a predetermined position does not depend on the hollowness of the stipe (Fig. 7, Table 1). This suggests that *L. pallida* sporophytes are not hollow to mitigate the higher wave action that prevails in shallow water. Also, at Swakopmund, where *L. pallida* had the highest percentage hollowness, wave height was lowest among all the sites measured (Fig. 15 b), and all indicators of turbidity were highest (Fig. 11).

The PCA model separated out three groups and showed that the main driving vectors for this separation were wind speed and turbidity, acting in opposite directions. It is established that upwelling is wind-induced (Andrews and Hutchings 1980, Shannon 1985) and that during periods of upwelling, water is clearer and underwater irradiance is considerably higher (Anderson and Botlon 1985). This inverse relationship between wind speed (Fig. 15 b) and water turbidity (Fig 11) is evident for various sites along the west coast, and supports the idea that turbidity is an important environmental factor that must influence kelps along this coast. However, consideration should also be given to the possibility of incipient speciation (see Roberson and Coyer, 2004) that could influence the morphology of *L. pallida* at the genetic level but a study at that level of genetic detail is well out of the scope of this study.

*Conclusion*: *Laminaria pallida* sporophytes gradually replace *E. maxima* northwards in shallow water on the Southern African west coast until they completely replace *E. maxima* in central Namibia. Concurrent with this, the stipes of *L. pallida* become more hollow moving northwards. The results discount a number of environmental variables that could be responsible for the distribution pattern of *L. pallida* and the hollowness of its stipe. Seawater temperature, and by inference nutrient levels, do not correlate with these patterns, but that there is a strong geographic gradient in water turbidity that must directly affect underwater light regimes. From the data here, cloud/fog cover was shown to be potentially important in reducing inshore light levels only at the northern-most site, Swakopmund. However, we would expect that other sites in northern Namibia (for which we do not have data), where desert coast line impinges on cool upwelled water, are also likely to have high cloud/fog cover. Dust storms from deserts can also increase turbidity in the adjacent ocean, and although Southern Africa deserts are not a major source of dust (Washington et al. 2008) offshore transport of particulates from the Namibian desert may contribute to turbidity in these waters. From these results we conclude that there is strong evidence that *L. pallida* progressively outcompetes *E. maxima* northward because it is more low-light tolerant and that by developing a hollow stipe it may grow faster allowing it to reach the surface quicker.

CHAPTER 5

RESPONSE OF JUVENILE STAGES OF *ECKLONIA MAXIMA* AND *LAMINARIA*

*PALLIDA* (PHAEOPHYCEAE, LAMINARIALES) TO DIFFERENT LIGHT

CONDITIONS, AND  GROWTH OF  YOUNG SPOROPHYTES IN THE SEA.

Recruitment of kelp largely depends on the production of spores by the adult sporophytes

(Joska and Bolton 1987), the dispersal of these spores in the water and then the production of

eggs and sperm by the microscopic male and female gametophyte stage (Kain 1979) to form

sporophytes which develop into adult plants. Some kelps, for example *Macrocystis pyrifera*

(Linnaeus) C. Agardh, *Ecklonia maxima* (Osbeck) Papenfuss, and *Laminaria pallida* Greville

are fertile year-round (Buschmann et al. 2006, Joska and Bolton 1987, Dieckmann 1980,

pers. obs.), while other species reproduce only in certain seasons (Schiel and Foster 2006,

Buchholz and Lüning 1999). While recruitment is clearly very important in maintaining a

dynamic marine community (Roughgarden et al. 1988), the structure of a community is

greatly affected by the interactions between the organisms and the physical and biological

environment (Reed and Foster 1984). Marine communities are complex (Dayton 1985), but

the distribution and abundance of marine populations are largely driven by recruitment

(Deysher and Dean 1986, Anderson et al. 1997).

Deysher and Dean (1986) coined the phrase 'recruitment window', to refer to a period when

environmental conditions (e.g. temperature and irradiance levels) are favourable for large-

scale recruitment to take place in kelp populations. An understanding of how environmental

conditions may affect kelp recruitment can be improved by experimental studies on early

development stages, such as zoospore production and germination, gametophyte growth and

gametogenesis, and the early stages of sporophyte growth. For example, Carney (2011)

showed that in unfavourable sea conditions *M. pyrifera* gametophytes were able to remain in

a viable, one- to two-cell state for at least 7 months, but once conditions improved the normal life cycle continued. Chapman (1986) referred to banks of microscopic stages that wait for favourable conditions: this mechanism implies no overall loss of fecundity but is a strategy to promote recovery after extended periods of nutrient-limiting conditions.

Kelps have a heteromorphic life history (Kawai 2014) in which the macroscopic sporophyte bears the microscopic unilocular sporangia in sori. The sori are mainly borne on the vegetative blades of the adult sporophyte and appear as large, discoloured, raised patches on both surfaces of the blades. Zoospores produce microscopic male and female gametophytes. These zoospores have a motile period that ranges from a few minutes to a few hours (Papenfuss 1942) after which they settle and secrete a wall around themselves, before germinating.

The female gametophyte is large-celled, exhibits limited growth, is seldom branched, and is sometimes only a single cell. The male, on the other hand, is small - and multi-celled and usually branched. Kain (1979) showed that the recruitment of Laminarian sporophytes depends on the successful production of eggs and sperm by the gametophytes. She stated that gametogenesis is controlled by a complex suite of physiochemical factors of which, according to Lüning (1980), irradiance is the most important. He showed that in three species of *Laminaria*, the amount of irradiance needed for gametogenesis increased with temperature. Once the spore germinates, its protoplasm moves out into a germination tube, as shown in *L. pallida* (Fig. 1 a). A wall forms and the first cell of the gametophyte is formed. During the next 8-10 days a few more cells are formed (Fig. 1 b - c).

The antheridia produce the sperm and each oogonium liberates a single egg. After fertilization a zygote is formed, which remains attached to the oogonial wall, and develops into a sporophyte (Fig. 1 d-f). The juvenile sporophytes have one entire lanceolate blade.
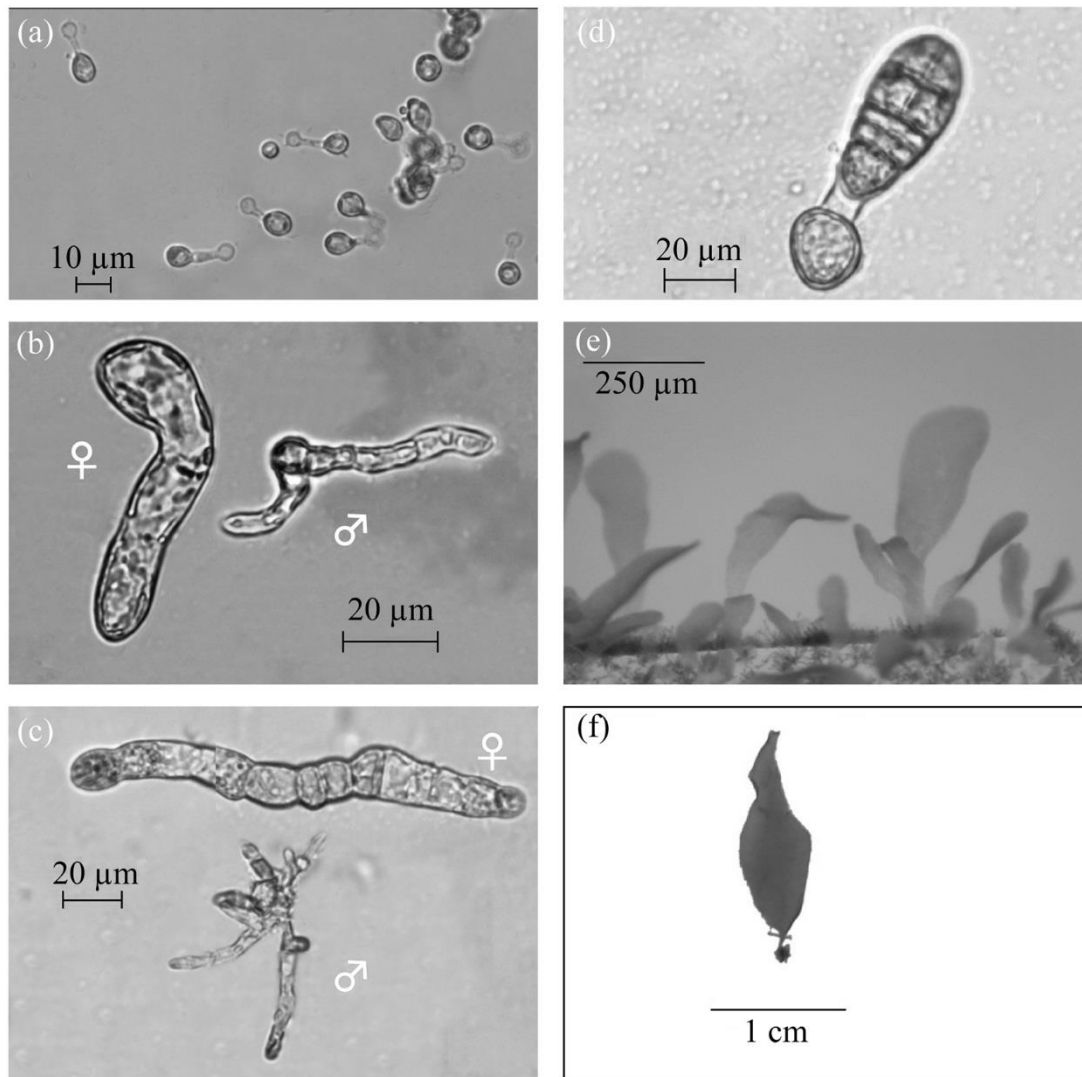
Fig. 1. *Laminaria pallida* stages of development. (a) spore tube at day 4; (b) male and female gametophytes at day 11; (c) male and female gametophytes at day 18, (d – f) first stages in development of young sporophyte.

The most rapid growth of sporophytes of South African *Laminaria pallida* occurs between ages 2-3 years (Dieckmann 1980). He has shown that these sporophytes can become more than 9 years old. *Laminaria hyperborea* (Gunnerus) Foslie has a recorded maximum age of 18 years (Lüning 1980), while *Ecklonia radiata* (C. Agardh) J. Agardh in New Zealand can attain an age of 10 years (Novaczek 1981). Some kelps (*Nereocystis* Postels & Ruprecht and *Undaria* Suringar) have annual sporophytes and survive the winter as gametophytes (Kidder 2006, Thornber et al. 2004).

Gametogenesis is a complex process because there are many factors that influence it, as shown by different studies. Using video-motion analysis Amsler and Neushul (1989) showed that spores of *Macrocystis pyrifera* and *Pterygophora californica* Ruprecht swim towards a nitrogen source. They concluded that this chemotactic behaviour enables spores to settle in microhabitats suitable for successful gametophyte growth. Generally, kelp spores do not disperse long distances from the parent plant. However, long-distance transport of the zoospores can be facilitated by unidirectional currents (Cie and Edwards 2011).

Lewis et al. (2013) showed the importance of iron on oogenesis and vegetative growth in six species of kelp. Their results indicated that in the absence of iron, oogenesis decreased in all species tested, showing that iron is essential for the early stages of kelp development. These studies indicated that a combination of factors, including the presence of iron, and presence of blue light (as shown by Lüning and Neushul 1978) are important factors influencing and maybe controlling oogenesis.

Lüning (1980) showed that irradiance plays an integral part in gametogenesis. He found that as temperature increased, so too the amount of light needed for gametogenes increases. A combination of light and temperature also affects the fertility of gametophytes in culture (Deysher and Dean 1986). Choi et al. (2005) and Mohring et al. (2013) had similar results

with *Undaria pinnatifida* (Harvey) Suringar and *Ecklonia radiata,* respectively. They found that the fastest growing gametophytes were produced from spores released early in summer, when days were longest.

A few culture studies have been done on the Southern African kelps, *Ecklonia maxima* and *Laminaria pallida* (Branch 1974, Bolton and Levitt 1985, Bolton and Anderson 1987, tom Dieck and de Oliveira 1993), but none focused on comparing growth rates of gametophytes from different parts of the coast nor directly comparing the growth rates of juvenile stages of the different species of kelp in South Africa.  Bolton and Levitt (1985) reported that *E. maxima* gametophytes were light saturated at 20 $\mu$E m$^{-2}$ s$^{-1}$, and that maximal growth occurred at 17.5 and 20 °C. They observed that rapid egg production corresponded to low cell numbers of female gametophytes at 60 $\mu$E m$^{-2}$ s$^{-1}$, and at 15 and 17.5 °C.  Their results indicated that *E. maxima* is a warm temperate organism. Bolton and Anderson (1987) showed that gametophytes of *E. radiata* (as *E. biruncinata* (Bory de Saint-Vincent) Papenfuss) could tolerate temperatures of 2 – 3 °C higher than those of *E. maxima*. Crosses between the two species resulted in juvenile sporophytes phenotypically similar to the parents with temperature optima intermediate between the parent species. The crossing experiments of tom Dieck and de Oliveira (1993), using hybridisation techniques of gametophytes in culture, showed that *L. ochroleuca* and the Southern Hemisphere species (*L. abyssalis* and *L. pallida*) form part of a warm temperate group which might point to the origin of the Southern Hemisphere species of *Laminaria* from a *L. ochroleuca*-like ancestor. This is consistent with findings in Chapter 3 which showed that, in the ITS Bayesian analysis, *L. abyssalis*, *L. ochroleuca* and *L. pallida* formed a distinct clade.

Both *Ecklonia maxima* and *Laminaria pallida* occur on the west coast of Southern Africa. However, northwards of Cape Columbine, *E. maxima* is replaced as the dominant inshore (shallow-water) species by *L. pallida*,  which then completely  replaces  *E. maxima* north of

Lüderitz, Namibia (as was shown in Chapter 3). In the southern portion of their distribution, these two species' vertical distribution ranges overlap strongly at depths of around 5m and here they would be expected to compete strongly for space. In deeper water light probably becomes a limiting factor and there one would expect that the species that is better low-light adapted would outcompete the other.

Several possible controlling factors (e.g. temperature, light, nutrients, substrate, etc.) can act on the different life history stages of kelps, although Lüning (1980) showed that light is a major factor controlling early stages of gametophyte development.

The aim of this study was to investigate the early development of sporophytes, under different light conditions, to better understand whether these conditions could influence the changes in dominance and distribution patterns of the two kelp species. Here I attempted to:

1) Compare elongation rates (vegetative growth) of young sporophytes of *L. pallida* and *E. maxima* from different sites in the north and south of the species distribution under different light conditions in culture.

2) Compare stipe and frond elongation rates of small sporophytes of *E. maxima* and *L. pallida* in an out-plant experiment at a west coast site in natural sea conditions.

MATERIALS AND METHODS

*Collection of the material.* Fertile fronds of both kelps were collected from two sites, Port Nolloth (in the north with 100% of *Laminaria pallida* sporophytes hollow) and Oudekraal (in the south where only the occasional *L. pallida* sporophyte is hollow, but only solid-stiped sporophytes were chosen). Hollowness of the adult *L. pallida* sporophyte was confirmed by cutting the stipe and checking it visually. Fertile blades of *L. pallida* and *Ecklonia maxima*

sporophytes were collected at depths of between 2 - 5 meters. Single fronds from 5 different young, healthy, epiphyte-free sporophytes of *Ecklonia maxima* and 5 of *Laminaria pallida,* each bearing mature sori, were collected and kept separate in marked bags. Pieces of frond ($\pm$ 10 cm long), bearing the mature sori, were put in distilled water for 1 minute to kill or remove any organisms on the surface of the fronds that might contaminate the cultures. The fronds were then rinsed in sterile seawater and wrapped in paper towel and black plastic and transported to the laboratory in a cooler box, to be kept at 10 °C overnight. After 24 hours each of the pieces of fertile fronds was placed separately in a crystallizing dish containing 200 ml of sterile seawater, in 80 μmol photons $m^{-2}$ $s^{-1}$, at 15 °C for 1 - 3 hours to release the spores. The 5 different spore suspensions were then combined in a 1 l beaker and placed on a magnetic stirrer to keep the spores in suspension.

*Collection and preparation of seawater.* The seawater (collected from Fisheries Branch, Department of Agriculture Forestry and Fisheries' research facility at Sea Point, Cape Town) was filtered through a 0.22 μm filter to remove sediments and particulate matter and  then stored at 10 °C in closed, dark containers until needed. Before use the seawater was again filtered using Schleicher and Schuell no. 0860 filter paper over a vacuum. Nutrients were enriched using Provasoli medium (Provasoli, 1968): this was added at a ratio of 20 ml for every litre of seawater, followed by Germanium Dioxide (0.2 mL per L) to inhibit diatom growth (Shea and Chopin 2007). The enriched seawater solution (culture medium) was then stored at 15°C until needed.

*Releasing of the spores.* Spores were settled on  single glass slides in each of thirty crystallizing dishes, containing 200 ml of culture medium, at a concentration of 100 spores per $mm^2$ (spores were counted using a haemocytometer, and final spore concentration was calculated for 200 ml solution). The dishes were covered by 10.5 X 10.5 cm clear Perspex lids to limit evaporation. Temperature, in the dishes, was monitored to ensure that the

Perspex covers did not cause heating of the water. The experiment was conducted in a constant temperature room, at 15 °C, at irradiances of (0 (dark), 10, 30, 80 and 120 µmol photons $m^{-2}$ $s^{-1}$). For each of the species three replicate dishes were subjected to each of the growth conditions. The light regime was set at 16:8 h Light:Dark. In each of the three replicate dishes, in each of the light treatments, cultures were examined every 5 days until sporophytes appeared. As soon as sporophytes were visible, the ten biggest per dish, per treatment, were measured. Measurements were taken every 5 days. Data were analysed by ANOVA and a post-hoc test using the Bonferroni correction and considering significance at p < 0.05.

Out-plant experiment.

*Seeding the ropes.* Polypropylene ropes (7 mm diameter) were suspended in glass aquarium tank (dimensions: 90 cm X 30 cm X 50 cm) containing 75 l of seawater (collected from Fisheries Branch, Department of Agriculture Forestry and Fisheries' research facility at Sea Point, Cape Town) to which Provasoli medium was added (concentration: 20 ml per litre of seawater) and $GeO_2$ (concentration: 0.2 ml of saturated solution per litre of seawater) to control diatoms (Shea and Chopin 2007). The ropes were 2.5 m long and suspended on a wooden frame with clothes pegs to hold the rope in place (see Fig. 2 a - b). Air bubbling was used to aerate and to create water motion. Fertile kelp fronds were collected from Oudekraal, Cape Town, and spores were released as above. One litre of spore solution was added to the tanks and allowed to stand (with no bubbling) overnight to allow spores to settle on the ropes. Tanks were cleaned once a week when culture medium was changed. The culture room was kept at a constant 13 °C and irradiance in the tanks was approximately 80 µmol photons $m^{-2}$ $s^{-1}$. Ropes were kept in the aquarium for 3 months, until numerous small sporophytes (0.5 - 1 cm long) could be seen on them.
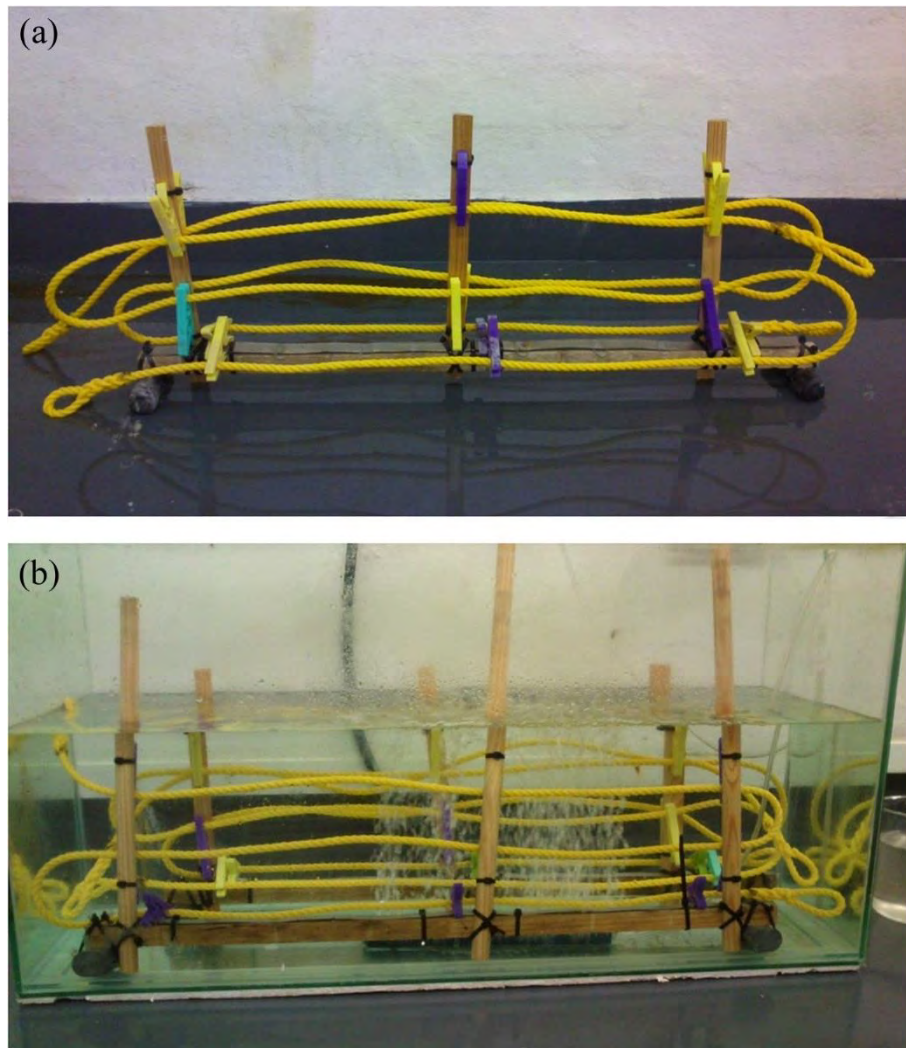
Fig. 2. (a) Ropes attached to wooden frame with clothes pegs; (b) Aquarium setup in glass tanks

*Out-plant experiment*. Six ropes, 3 seeded with *Ecklonia maxima* and three with *Laminaria pallida* sporophytes, were installed vertically (anchored at 9 m depth) in the sea at Oudekraal on the Cape Peninsula, where the substratum is of Granite, with very gentle slope. The substratum was prepared by removing all the kelp that could interfere with the vertical culture ropes. Holes were drilled into the rock with an underwater pneumatic drill, and filled with Fischer® FIS EM 390 S 2-phase Chemical Mortar (Fischerwerke GmbH & Co. KG, Germany), before a 10 mm thick eyebolt was inserted and allowed to set overnight.

The following day the ropes were attached to the eyebolts with steel shackles, and kept upright with floats at the top.  The sporophytes on the ropes were measured every 4 months, either underwater (while diving on SCUBA) or ropes were briefly lifted onto a boat, kept wet, and plants measured. The vertical ropes extended from 9 m to 6 m depth, a range that corresponds to the transition depth between the dominance of *E. maxima* and *L. pallida* in the southwest of South Africa (Field et al. 1980) and where one would expect the two species to compete for space and/or light. Measurements of the young sporophytes were taken 4 months after installing the ropes in the sea and then again after 8 months. Stipe length and frond length (longest frond from the junction between stipe and frond to the tip of the frond) was measured with a tape measure and recorded. Ten sporophytes were measured along the length of the rope, 4 from the top section, three from the middle section and three from the bottom section. Data were analysed using an ANOVA and Tukey *post-hoc* test for significance.

RESULTS

*Laboratory culture experiment*. Spores of both species died in the dark treatment after 3 days. Although they developed a germination tube, they failed to develop any further.

During the first 3 – 4 days a germination tube developed after which male and female gametophytes developed differently and at different rates. Development was quick with sporophytes appearing between 10-15 days. After 28 days, small sporophytes became visible to the naked eye, with holdfast, a stipe and the primary blade (Fig. 1 a - e)

Results from an ANOVA indicated that site and species and the interactions between site (north vs south) and species vs light, and site and species vs day were all significantly different ($p < 0.0001$) (Table 1 a).

Almost all culture conditions tested exhibited a significant difference in young sporophyte length when cultured under different light conditions (Tukey *post-hoc* test, $p < 0.0001$). The only exception was comparing irradiances of 30 and 80 µmol photons m$^{-2}$ s$^{-1}$ where no significant difference was observed ($p = 0.6632$) (Fig. 3, Table 1 B).

Results showed that overall there was a significant difference between the growth of the two species (Table 1). Under 30 and 80 µmol photons m$^{-2}$ s$^{-1}$ irradiances young *L. pallida* sporophytes increased in length faster than *E. maxima,* with *L. pallida* from Port Nolloth showing the greatest increase in length. Under all light conditions and for both species the treatments in the 10 and 120 µmol photons m$^{-2}$ s$^{-1}$ irradiances were out-performed by those grown under 30 and 80 µmol photons m$^{-2}$ s$^{-1}$ irradiances.

*Rope Culture*.  When the ropes were installed in the sea after 3 months of culture in the aquarium, the sporophytes of both species were between 0.5 - 1 cm total length (Fig. 5 a). After 4 months and 8 months in the sea (Fig. 4 also see Fig. 5 e - f), at a depth of between 6 - 9 m, the *L. pallida* sporophytes out-grew the *E. maxima* sporophytes. Average stipe length of *L. pallida* was 57% longer than *E. maxima* after 4 months (11.3 mm for *E. maxima* and 17.8 mm for *L. pallida*) and 250% longer (15.5 mm for *E. maxima* and 55.75 for *L. pallida*) after 8 months. Similar results were observed for the average frond length:   *L. pallida* was 55%

longer after 4 months (189.8 mm for *E. maxima* and 295 mm for *L. pallida*) and  98% longer after 8 months (279 mm for *E. maxima* and 552.5 for *L. pallida*) (Fig. 4).

*Laminaria pallida* sporophytes started resembling mature sporophytes after 4 months in the sea and in some plants the blades started splitting (Fig. 5 b). *Ecklonia maxima* sporophytes developed much slower, and after 4 months in the sea all sporophytes still resembled a rugose juvenile form (Fig. 5 c). After 8 months some of the sporophytes started developing secondary blades, but the long, rugose primary blades were still present (Fig. 5 g).

Table 1. (a) ANOVA results indicating the following interaction: site; species; site vs species; light; day. (b) Post-hoc test results for light and day. Bonferroni correction was used with a significant level of $p < 0.05$. Significan interactions in red.

(a)

| Number of Observations = 1465 | | | | $R^2 = 0.9231$ | |
|---|---|---|---|---|---|
| Root MSE = 0.1291 | | | | Adjusted $R^2 = 0.9226$ | |
| Source | Partial SS | df | MS | F | P-value |
| Model | 290.76209 | 9 | 32.306899 | 1941.17 | <0.0001 |
| site | 0.6800748 | 1 | 0.6800748 | 40.86 | <0.0001 |
| species | 11.122044 | 1 | 11.122044 | 668.27 | <0.0001 |
| Site vs species | 4.6057796 | 1 | 4.6057796 | 276.74 | <0.0001 |
| light | 30.188825 | 3 | 10.062942 | 604.63 | <0.0001 |
| day | 264.24917 | 3 | 88.083055 | 5292.49 | <0.0001 |
| Residual | 24.215602 | 1455 | 0.016643 | | |
| Total | 314.9777 | 1464 | 0.2151487 | | |

(b)

| Category: Light: µmol photons m$^{-2}$ s$^{-1}$ | Compared to: Light: µmol photons m$^{-2}$ s$^{-1}$ | T-stat | P-value | Ref p-value | Conclusion |
|---|---|---|---|---|---|
| | 30 | -9.7925 | <0.0001 | 0.0083 | Signif |
| | 80 | -9.0222 | <0.0001 | 0.0083 | Signif |
| 10 | 120 | -5.5939 | <0.0001 | 0.0083 | Signif |
| | 80 | 0.4356 | 0.6632 | 0.0083 | NS |
| 30 | 120 | 4.1209 | <0.0001 | 0.0083 | Signif |
| 80 | 120 | 3.5497 | 0.0004 | 0.0083 | Signif |
| Category: Day | Compared to | T-stat | P-value | Ref p-value | Conclusion |
| | 15.000 | -11.2069 | <0.0001 | 0.0083 | Signif |
| | 20.000 | -34.8845 | <0.0001 | 0.0083 | Signif |
| 10 | 25.000 | -50.1992 | <0.0001 | 0.0083 | Signif |
| | 20.000 | -43.3491 | <0.0001 | 0.0083 | Signif |
| 15 | 25.000 | -63.578 | <0.0001 | 0.0083 | Signif |
| 20 | 25.000 | -16.5206 | <0.0001 | 0.0083 | Signif |
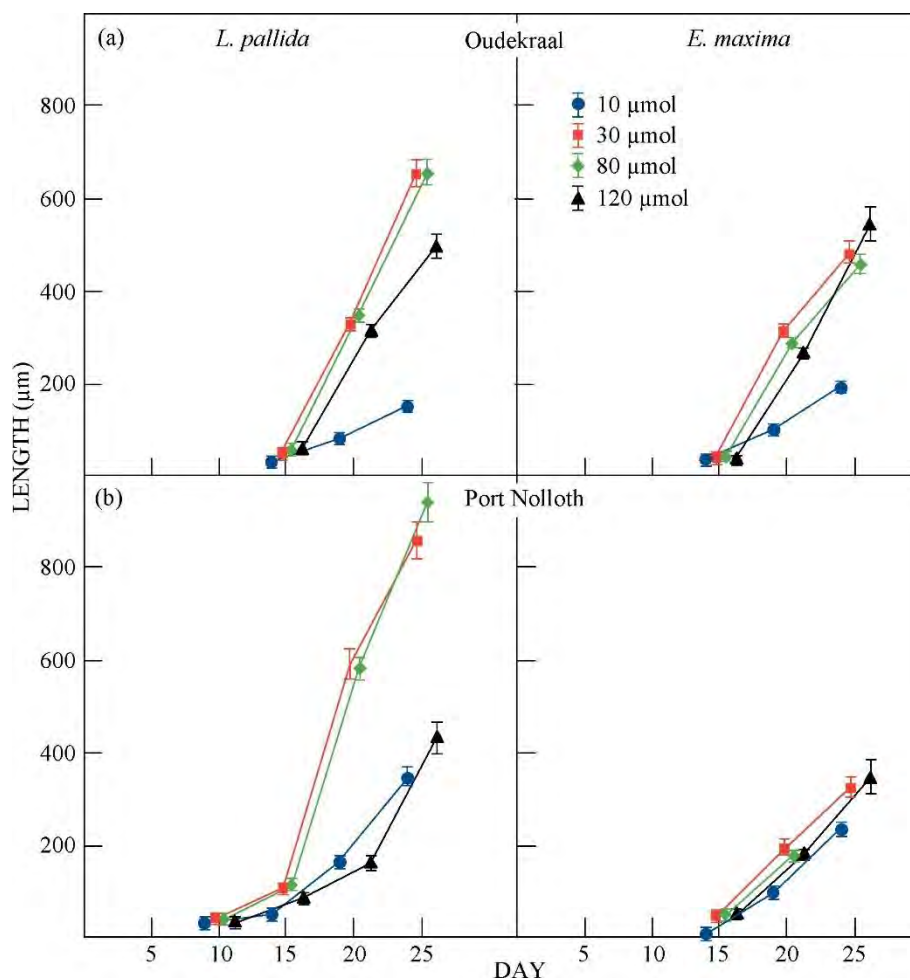
Fig. 3. Lengths of small sporophytes at days 10, 15, 20 and 25 at different light intensities. Graphs represent sporophytes from two sites (Oudekraal on left) and Port Nolloth (on right). From each site, two species are represented *(E. maxima* and *L. pallida)*. The statistics are represented in tables 1. a - b.
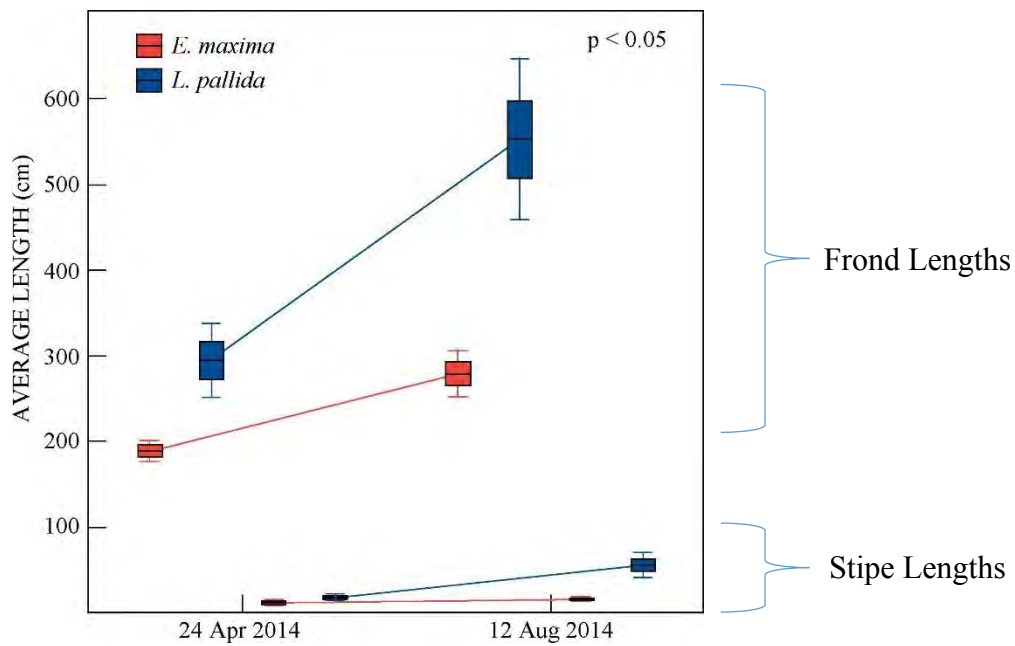
Fig. 4. Stipe length and frond length of *E. maxima* and *L. pallida* after 4 and 8 months after ropes were installed at Oudekraal, South Africa. Ropes at between 6 - 9 m deep. Box indicate Standard Error, line indicate mean values and whiskers indicate 95% confidence limits.
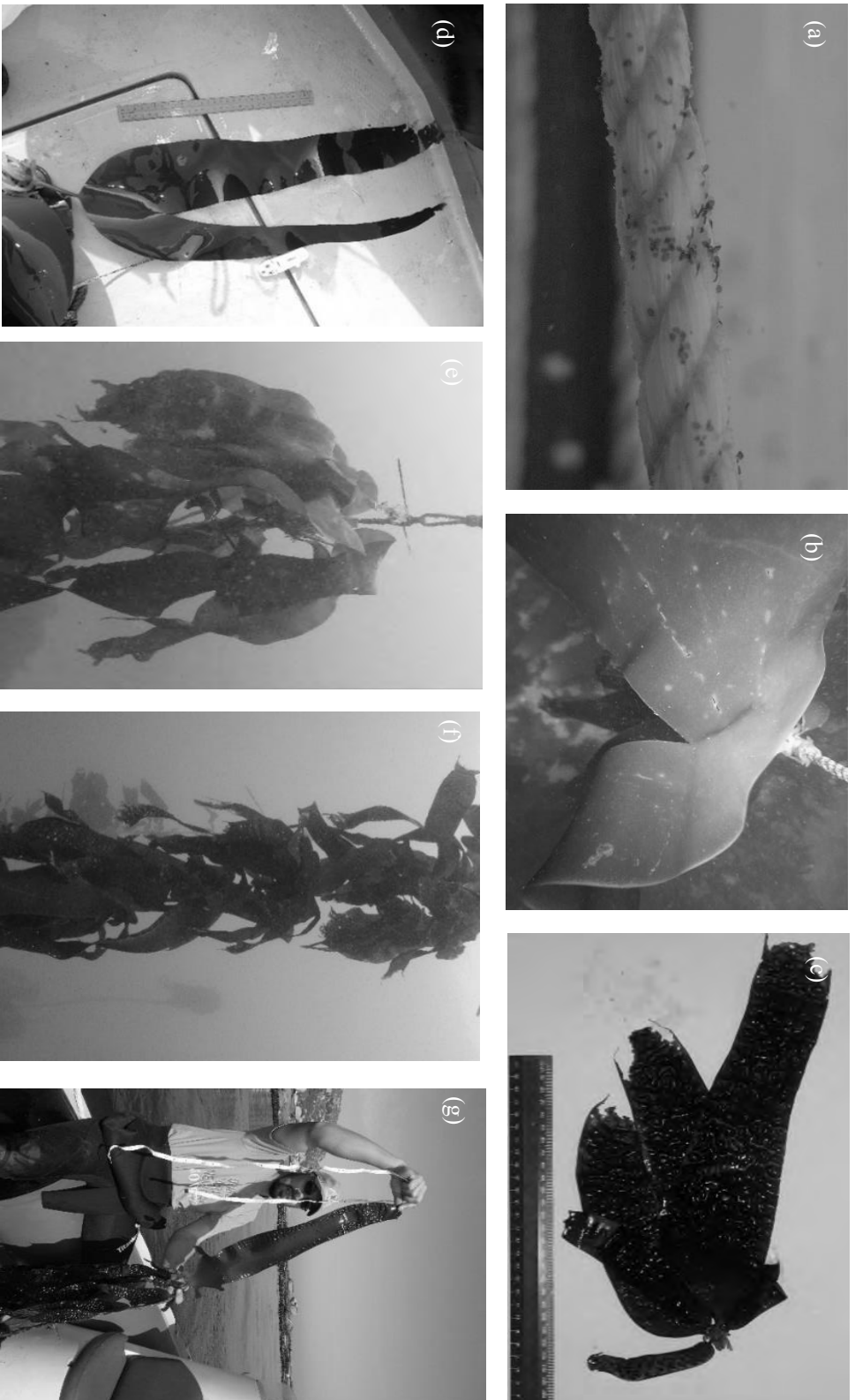
Fig. 5. (a) The small developing sporophytes after 3 months on the ropes in the aquarium; (b) A *L. pallida* sporophyte starting to split, 4 months after it has been put in the sea. Note the perforations in the blade where it will split next; (c) An *E. maxima* juvenile sporophyte after 4 months on the ropes in the sea; (d) A *L. pallida* after 8 months in the sea on the ropes; (e) Underwater picture of *L. pallida* sporophytes on the ropes; (f) Underwater picture of *E. maxima* sporophytes on the rope; (g) An *E. maxima* sporophyte beginning to develop secondary fronds after 8 months in the sea.

DISCUSSION

*Gametophytes.*

Both *Ecklonia maxima* and *Laminaria pallida* gametophytes, in culture, were unable to survive in darkness and died after 3 days. This was contrary to the results of tom Dieck and de Oliveira (1993) who showed that filamentous kelp gametophytes (which included *L. pallida*) could survive up to 18 months of darkness. However, their gametophytes had already developed vegetatively (they were cultured under red light, before being transferred to the dark), while the *E. maxima* and *L. pallida* spores used in this study were freshly released and had no period of growth in red (or any) light. Lüning (1980) also showed that *L. digitata* (Hudson) J.V. Lamouroux, *L. hyperborea* and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes. Druehl & G.W. Saunders (as *L. saccharina* (Linnaeus) J.V. Lamouroux) survive in total darkness for 5 months. However, in nature, these Northern Hemisphere species are subject to different irradiances as compared to the Southern Hemisphere and might also behave differently.

Barradas et al. (2011), who conducted *in situ* studies, speculated that *Laminaria ochroleuca*, the closest related *Laminaria* to *L. pallida* (see Ch 3), has banks of microscopic forms, in a state of suspended growth that can replace populations that undergo severe disturbances. Kinlan et al. (2003), also in *in situ* experiments, found that *Macrocystis* gametophytes could survived in 2 – 3 µmol photon m$^{-2}$ s$^{-1}$ light conditions for up to a month.

Here, for *L. pallida* and *E. maxima* in darkness, we observed the formation of a germination tube, but, at least for these Southern Hemisphere species light may be necessary for further development into the gametophyte stage. This would imply that the spores or very young gametophytes of these two species cannot survive in a state of arrested development in the dark.

In the Northern Hemisphere some *Laminaria* species have to survive in extreme light limited conditions, especially during winter months (*L. digitata* and *L. hyperborea* see Lüning 1990, *L. solidungula* see Dunton 1990). Extreme conditions like these do not occur in the shallow water of the distribution area of *L. pallida* in Southern Africa where a constant supply of light is available. However, Laminarian gametophytes have been shown to be extreme shade plants (Lee and Brinkhuis 1988), so they should be able to grow under very low light. The genus *Ecklonia* is distributed between latitudes where extreme light-limited conditions, such as those that some species of *Laminaria* experience, do not occur.

Carney (2011) found *Macrocystis* gametophytes to be viable after 7 months of nutrient-limited conditions at 80 µmol photons $m^{-2}$ $s^{-1}$ irradiance levels, an indication that nutrients may also affect the light responses of these early developmental stages. During the current study it was very difficult to draw any meaningful conclusions from the gametophyte growth data because the gametophytes developed so rapidly. On day 5 it was impossible to distinguish between the male and female gametophytes. Over the next 5 days the gametophytes differentiated in to male and female, became fertile, fertilization took place, and sporophytes started to develop in both species. From the speed at which these gametophytes develop into the next stage it would be improbable that this phase of the life history can give either species a competitive advantage, under the conditions tested here.

*Young sporophytes*. Generally early developmental stages of seaweeds are low-light adapted (Coelho et al. 2000), but here, under the lowest light conditions (10 µmol photons $m^{-2}$ $s^{-1}$) both species performed poorly. However, under the 30 – 80 µmol photons $m^{-2}$ $s^{-1}$ condition the elongation of early stage sporophytes (< 25 days) of *Laminaria pallida* was faster than those of *Ecklonia maxima*. Although young sporophytes of *E. maxima* are able to grow in low light, *L. pallida* grew faster under these conditions. Sporophytes of some other *Laminaria* species have been shown to increase in length well under low light conditions.

*Laminaria solidungula* survives for months in near darkness under ice in the Alaskan Beaufort Sea (Dunton 1990) during which sporophytes have their highest growth rate (Chapman and Lindley 1980). Dense *L. hyperborea* beds, on the other hand, generally do not grow in light conditions that are below 5% of surface light although scattered individuals have been found at 15 - 25 m in Helgoland where light percentage is around 1% (Lüning 1990).

Moving northwards along the Southern African west coast, the amount of light available to plants in the inshore decreases due to an increase in water turbidity (see Ch 3). This inverse relationship between turbidity and dominance of *L. pallida* indicates that it may be more shade tolerant. Young sporophytes (<25 days old) of *L. pallida* already showed a greater tolerance to low light than those of *E. maxima*. In areas further north, where the water is more turbid and there is less light available, *L. pallida* appears to out-compete *E. maxima* in the shallow sublittoral zone, probably because there it can grow faster. Here we see two kelp species competing for the same space and results indicate that where turbidity is higher (and less light is available), *L. pallida* perform better than *E. maxima*.

*Rope cultures*: Further evidence of faster elongation rates of small (but still relatively young) sporophytes of *Laminaria pallida* (at least at 6 – 9 m depth) was obtained from the *in situ* rope culture. *Laminaria pallida* sporophytes increased in length (frond length and stipe length) faster than those of *Ecklonia maxima* (Fig. 4). Rothman et al. (2006) showed that, at 3 m depth on the east coast of the Cape Peninsula, underwater irradiance can be less than 10% of surface illumination. Anderson (1982) showed that on the west coast of the Cape Peninsula (near Oudekraal) at 6 m depth, under upwelling and downwelling events, underwater irradiance is reduced to 26 and 9 % of surface levels respectively. Below 6 m underwater irradiance would be lower. Since these ropes were grown at 6 - 9 m the faster growth of *L. pallida* compared to *E. maxima* indicates a competitive growth advantage of the

former at these lower light levels. Since this is also the depth range over which *L. pallida* replaces *E. maxima* on the west coast of the Cape Peninsula, the result seems reasonable. However, this "competitive advantage" interpretation needs to be compared to growth data from shallow water (e,g, 2 - 4 m, where *E. maxima* dominates in the south), before this explanation can be accepted.

It would thus appear that light, certainly at depths between 6 - 9 m, seems to be important in the distribution of the two dominant Southern African kelps. One can conclude that it is the ability of *Laminaria pallida* to exploit low light conditions that enables it to become more dominant northwards and out compete *Ecklonia maxima* especially as underwater irradiance deceases and turbidity increases northwards (see Ch 4).

Some kelp species similarly replace or prevent other kelp species from dominating due to environmental conditions. For example, *Laminaria digitata* dominates wave-exposed upper sublittoral zone of the Arctic and cold temperate shores of the northern Atlantic because of its flexible stipe and deeply divided fronds while *L. hyperborea* has a rigid stipe which is more prone to snapping under high wave action. *Laminaria hyperborea* out competes *L. digitata* deeper down where it overshadows the latter (Kain 1979, Lüning 1990). Moreover, *L. hyperborea* is more low-light tolerant than *L. digitata* (Lüning 1990). In the southern part of *L. hyperborea* distribution range, it competes with *Saccharina latissima* (formerly *Laminaria saccharina*) (Kain, 1979). Studies by Walker (1947, 1950, 1954 in Kain 1979) showed that the competition between these two species depended on the type of substratum on which they were found. *Laminaria hyperborea* required solid rock and *S. latissima* was adapted to unstable conditions when it is sufficiently sheltered.

*Conclusions*. In the previous chapter it was shown that along the west coast turbidity increases northwards, indicating a gradient of decreasing underwater irradiance. The results here indicate that very young sporophytes as well as juvenile sporophytes of *Laminaria pallida* grow faster than those of *Ecklonia maxima* under low-light conditions. I conclude that juvenile *L. pallida* sporophytes grow faster than juvenile *E. maxima* sporophytes in low-light conditions.

CHAPTER 6

GENERAL DISCUSSION

Of the four kelp species present in Southern African waters, only *Ecklonia maxima* and *Laminaria pallida* have received significant scientific attention, doubtless because of their ecological and economic importance, and these are the main focus of the present study.

Only these two (*E. maxima* and *L. pallida*) form extensive beds that extend up the coast of Namibia, essentially occupying all or most of the Benguela Marine Province. It has never been established why *Macrocystis pyrifera* is restricted to a few isolated and rather sheltered bays (west coast of the Cape Peninsula, Robben Island, Melkbosstrand and Jacobs Bay) and extends no further up the west coast, or into Namibia: this would be an interesting although perhaps difficult question to answer. It is tempting to speculate that the reason may be related to water turbidity and light penetration, since *M. pyrifera* stops just south of Cape Columbine, which is also the point north of which the hollow-stiped *L. pallida* starts to become more prominent in the shallow inshore zone, as shown here.

Geographically, *Ecklonia radiata* is almost entirely restricted to the Agulhas Marine Province, occurring from east of Cape Agulhas to the southern KwaZulu-Natal border. This warm temperate geographical distribution relates to the temperature responses of gametophytes of this species in culture (Bolton and Anderson 1987). What is more difficult to explain is that this species on the south coast does not form large subtidal kelp beds, as does Australian *E. radiata*, with which this study shows it to be conspecific. This is even more surprising given that populations that match this species morphologically (but do not entirely match it in terms of DNA – Rothman et al. 2015), form subtidal beds in a small area in Buffels Bay, False Bay, and seem to occur in small numbers on some deep reefs (20 - 40 m) on the south and east coasts, although molecular confirmation of the latter kelp specimens

is still needed. Again, these broad ecological questions offer fascinating but challenging directions for future studies.

Recently it was found that *E. maxima* is spreading eastwards (Bolton et al. 2012) to an extent that *E. maxima* and *E. radiata* now co-exist at De Hoop Nature Reserve on the south coast. While the cause of this range extension is not certain, changes in water temperature are implicated (Bolton et al. 2012), indicating the potential for global climate change effects on South African kelps, with ecological and also socio-economic implications.

Along the west coast, where *E. maxima* and *L. pallida* co-exist, this study has improved our understanding of the change in dominance between the two species from south to north. Also, *L. pallida* was previously described as two species: *L. pallida* (solid-stiped form) and *L. schinzii* (hollow-stiped form). Based on morphology Stegenga et al. (1997) regarded the two synonymous but no molecular analysis had been done to confirm this. Unlike *L. pallida*, two species of *Ecklonia* are currently accepted for South Africa (Bolton and Anderson 1994, Stegenga et al. 1997), *E. maxima* and *E. radiata*. Bolton and Anderson (1994) also speculated that *E. maxima* evolved from *E. radiata* but there was no molecular evidence for this or that they are indeed two distinct species. Furthermore, a phylogeny for the genus *Ecklonia*, to show the relationship between the species of *Ecklonia* worldwide, was also lacking.

*The* Ecklonia *study.* Southern African *Ecklonia* initially had seven *Ecklonia* species attributed to it (*E. biruncinata* (Bory) Papenfuss (later renamed *E. maxima*), *E. buccinalis* (Linnaeus) Hornemann, *E. exasperata* (Turner) J. Agardh, *E. fastigiata* (Endlicher & Diesing) Papenfuss, *E. lanciloba* Sonder, *E. maxima*, and *E. richardiana* J. Agardh) (Seagrief 1984). *E. maxima* (also the type of the genus) was first described as *Fucus maxima* by Osbeck in 1757 but was later renamed by Papenfuss in 1940 to its current name.

Treatments of *E. radiata* in Australia by Womersley (1967) and Novaczek (1980) had a broader definition of the species which led Bolton and Anderson (1994) to include the different morphologies represented by all the above species, except *E. maxima*, in *E. radiata*. This means that in Southern Africa there were only two species of *Ecklonia* recorded.

Using Bayesian Inference phylogenetic analysis, we clearly showed that *E. maxima* is a single species in Southern Africa and is distinct from *E. radiata*. Furthermore, the species called *E. radiata* in the Southern Hemisphere was shown to be a single species. In addition the analysis showed that there are clear Northern and Southern Hemisphere clades in the genus *Ecklonia*. The Northern hemisphere clade could not be resolved further, and this requires further studies.

*Diversity and distribution of Ecklonia in the Southern Hemisphere.* Given the geographical distribution of both species and their phylogenetic relationship, a likely hypothesis is that the two *Ecklonia* species present in Southern Africa originated from an Australian *E. radiata* migrant. A similar hypothesis was discussed by Hommersand (1986) to explain similarities between Australian and South African red algae.

The South Atlantic Ocean, was much warmer at about 3.5 Ma than compared to the present (Marlow et al. 2000). These conditions would have been ideal for the migration of *E. radiata* westward from Australia. Around 2.5 Ma the SAO started cooling due to increased upwelling but it was only around 1.5 Ma that temperatures dropped below 20°C (Marlow et al. 2000). As upwelling intensified, the coastal temperatures cooled and become more nutrient rich. The population of *E. radiata* on the west coast could then have developed a longer stipe, larger frond mass and a bulb due to the high nutrient environment of the Benguela upwelling system that could sustain a larger morphology than on the east coast. The population on the west

coast could have evolved into a different species, *E. maxima,* but on the east coast, in the absence of new selection pressures, *E. radiata* may have remained.

In a study focussed on South African species, Bolton and Anderson (1987) showed that, in culture, the temperature tolerance of small *E. radiata* sporophytes is 2 - 3 °C higher than those of *E. maxima*. They crossed these species, with the resulting hybrids having intermediate temperature tolerances. In nature, a hybrid with intermediate temperature tolerance would undoubtedly be best suited to establish itself in the transition zone between the warm and cold marine provinces of South Africa: the south coast, where both species occur with almost no overlap (but see Bolton et al. 2012). During the present study, specimens with intermediate morphologies between *E. maxima* and *E. radiata* were collected from Bordjiesrif (> 8 m deep) in False Bay near Cape Point. The specimens were intermediate in size between the two species, with stipes that were either solid or hollow, *E. radiata*-like secondary fronds and *E. maxima*-like primary fronds. One specimen was sequenced during the present study. Interestingly, the analysis of the mitochondrial marker grouped the sequence for the specimen within the *E. radiata* clade whereas the analysis of the chloroplastic and nuclear markers recovered the specimen in the *E. maxima* clade (results were confirmed by a second extraction and sequencing). Both morphological and molecular results suggest the possible presence of wild hybrids at Bordjiesrif.

Druehl et al. (2005) showed that *Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, Druehl and G.W. Saunders and *Saccharina angustata* (Kjellman) C.E. Lane, C. Mayes, Druehl and G.W. Saunders (as *Laminaria* spp.) hybrids had the genomes of both parents. Their conclusions indicate that it would be very difficult to show hybridization in nature and that molecular confirmation is essential. To confirm the occurrence of wild hybrids at Bordjiesrif, more specimens must be sequenced and a hybridization experiment coupled with a molecular population genetic study should be conducted. A search for potential

hybridization could also be conducted at De Hoop where eastward migrating *Ecklonia maxima* now occur (see Bolton et al. 2012) side-by-side on the same shore where the westernmost population of *E. radiata* occur.

The calculation of a molecular clock to estimate the age of the ancestor of *E. maxima* and *E. radiata* and paleo-niche modelling (e.g. Spellman et al. 2010) would be required to test the hypothesis on the origin of *E. maxima*. It should be noted that the other major kelp in South Africa, *Laminaria pallida*, which also shows a hollow-stiped form further north along the west coast (Stegenga et al. 1997), may have evolved this trait in response to the same environmental factors that may have caused the evolution of the hollow-stiped *Ecklonia maxima*.

Both *Ecklonia* (in South Africa) and *Ecklonia arborea* (in the Eastern Pacific) have hollow and solid stiped forms. *Ecklonia arborea* exhibits stipe morphological variation along a latitudinal gradient (Matson and Edwards 2006) as well as in exposed vs protected sites (Parada et al. 2012). Roberson and Coyer (2004) reported that *E. arborea* exhibited different frond morphology depending on the water motion variability, with wide bullate fronds in low-moving water while being flat and narrow in high-flow areas. They further showed, by using M13 DNA fingerprinting, that these traits are genetically fixed and suggested that this represented an example of "nascent speciation".

There are few taxa in the Laminariales that exist as hollow and solid stiped forms: *Saccharina longicruris* (Miyabe) C.E. Lane, C. Mayes, Druehl & G.W. Saunders is a hollow stiped form that is now considered conspecific with the solid stiped *Saccharina latissima* (Miyabe) C.E. Lane, C. Mayes, Druehl et G.W. Saunders (McDevit and Saunders 2010). It appears that the current study of *Ecklonia* involving the separation within a geographical region of two closely related species of Laminariales with solid and hollow stipes,

demonstrated by molecular methods, is currently the only example of this in the
Laminariales.

*The* Laminaria *study*. The second molecular study was focused on the species of *Laminaria*.
This genus is primarily distributed in the Northern Hemisphere with only two species found
in the Southern Hemisphere (*L. abyssalis* and *L. pallida*). Our results confirm the decision of
Stegenga et al. (1997)  to consider *L. pallida* and *L. schinzii* conspecific and indicate further
that the Northern Hemisphere species, *L. ocroleuca*, is its sister species. This agrees with
Lüning and tom Dieck (1990) and tom Dieck and de Oliveira (1993) who speculated that *L.
ochroleuca* crossed the equator and developed a larger stipe in the cool nutrient-rich
environment of the Benguela current. These two species are very similarly distributed in the
Northern and Southern Hemispheres with *L. pallida* occurring between 30° - 49° S and *L.
ochroleuca* between 30 ° - 50° N (Kain 1979), which may strengthen this hypothesis.

Marins et al. (2012) reported that the other Southern Hemisphere *Laminaria*, *L. abyssalis*, is
sister to *L. digitata*, which would indicate that two Northern Hemisphere species had to
migrate south across the tropics. However, their analysis did not include *L. pallida* nor *L.
ochroleuca* which, if they did, would likely have changed the picture. The ITS tree, which
contains most of the available sequences for species of *Laminaria*, and was used in the
current study, showed a close relationships between the Southern Hemisphere species of
*Laminaria* and *L. ochroleuca*. This would indicate that an *L. ochroleuca*-like ancestor may
have arrived on two sides of the South Atlantic and evolved into two different species in
these new environments: the Brazilian coast to give rise to *L. abyssalis* and the Southern
African coast to give rise to *L. pallida*. An alternative hypothesis is that the *L. ochroleuca*-
like ancestor landed on the Southern African coast, gave rise to *L. pallida* which in turn,
crossed the Atlantic to give rise to *L. abyssalis*, or vice versa. However, the finding is only
based on a single marker and to fully elucidate this relationship, we would require more

samples of *L. ochroleuca* and *L. abyssalis* to be sequenced. This would also shed more light on the timing of the origin of these species.

Current research trends are focused on the phylogeny of kelp species (Kawai 2014, Chi et al. 2014) as well as the population genetics and gene flow in kelps (Brennan 2014). The population genetics and gene flow in *L. pallida* would be a very interesting direction of research, especially considering the different morphologies the species exhibits along the coastline. Such studies would allow investigation of connectivity between populations of *L. pallida* as well as the closely related *L. abyssalis* and *L. ochroleuca*.

*Ecology*. The ecology of South African kelp beds has received a fair amount of research attention, particularly at the systems level and mostly as a result of the "Kelp Bed Project" in the late 1970s, which resulted a number of publications on their kelp bed communities (eg. Allen and Griffiths 1980, Field et al. 1980, Jarman and Carter 1981, Velimirov and Griffiths 1979, Velimirov et al. 1977). Much of this work was summerised in the review of Field and Griffiths (1991). While their studies provided an important understanding of kelp bed function at local and even international levels, they left many questions unanswered and raised many new questions, particularly with respect to the biology of individual species. This project was essentialy system based, since it stressed the value of the system to important commercial species like rock lobster and abalone.

Despite the evidence in the present study indicating that it is likely that water turbidity is behind this ecological changeover in species hollowness, clearer evidence is needed to confirm this. One method to test this idea would be by doing reciprocal transplant studies. Due to the success with which in situ rope cultures were done during this study, this method could be extended. If ropes, seeded with the hollow form *L. pallida* from the north, still produced hollow stiped sporophytes, when transplanted to the south, or *vice versa*, the

conclusion would be that the hollowness might be a genetic response to environmental variables. Common garden experiments (growing sporophytes from different parts of the coast under the same conditions) would also provide useful information about the interactions between genes and the environment and this kind of experiment is logistically less complicated. I already gathered good results from an out-plant experiment where ropes seeded with kelp spores were put in the sea. Early indications were that the *L. pallida* sporophytes out-performed *E. maxima* sporophytes with frond and stipe elongation indicating that *L. pallida* might be more low-light adapted. However, these experiments should be repeated in shallow water, as well as at other sites on the west coast for comparison to give definitive answers. Also the turbidity measurements, which were extracted from satellite imagery, indicating that turbidity increases gradually along the Southern African coast. Direct turbidity measurements (using *in situ* instruments).

 The evidence points to water clarity being the likely environmental variable responsible for *L. pallida* progressively becoming more dominant along the Southern African west coast, but the heteromorphic life cycle of brown kelps is complex and competitive advantage between two kelp species can occur at any of the life stages. Culture experiments studying juvenile life stages of *L. pallida* and *E. maxima* indicated that *L. pallida* grew faster than *E. maxima* in low-light conditions. This supports the idea that the dominance of *L. pallida* along the west coast is related to the irradiance levels in the inshore environment. In the Northern Hemisphere some *Laminaria* species have to survive in extreme light limited conditions, especially during winter months (*L. digitata* and *L. hyperborea* see Lüning 1990, *L. solidungula* see Dunton 1990). Extreme conditions like these do not occur in the inshore distribution area of *L. pallida* in Southern Africa where a constant supply of light is available.

Laminarian gametophytes have been shown to be extreme shade plants (Lee and Brinkhuis 1988). The genus *Ecklonia* is distributed between latitudes where extremely light limited

conditions do not occur, unlike those experienced by some species of *Laminaria*. Therefore it would be reasonable to assume that *Ecklonia* never had to develop the ability to be in a state of arrested development which would also explain why spores could not survive in darkness for more than three days.

Our knowledge of age-specific data for Southern African kelps are lacking. These kelps cannot be aged, unlike *E. radiata* (Novaczek 1981), *Saccharina latissima* (Parke 1948), and *Ecklonia cava* (Hayashida 1977). During this study, I tried to age *E. maxima* and *L. pallida*, but with no success because no definitive growth rings could be observed. This is in spite of both *E. maxima* and *L. pallida* showing seasonal growth rates (Dieckmann 1980, Rothman et al. 2006). It might be that the winter/summer extremes in the Southern African marine environment are not marked enough for the kelps to sufficiently slow their metabolism in winter, and produce growth rings.

For the first time in South Africa kelp was grown, on ropes, in the sea. These rope cultures could provide basic age-specific information about the kelps' fertility, growth patterns, fecundity and longevity – this is the subject of an ongoing study. It was already observed that *L. pallida* sporophytes' fronds started splitting when they are about seven months old, something that previously was not known nor recorded.

While the present study has improved understanding of the systematics of *Ecklonia* and *Laminaria*, and gone some way to explaining the geographical relationship between these species in Southern Africa, it leaves many questions unanswered. Further studies should, however, be encouraged by the ecological and economic importance of these two large Southern African kelps.

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