PHYLOGEOGRAPHY AND EPIFAUNA OF TWO INTERTIDAL SEAWEEDS ON THE COAST OF SOUTH AFRICA

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ABSTRACT

Southern African biogeographic boundaries delimit the phylogeographic distribution of some coastal and estuarine invertebrates. This study investigated the impact of these boundaries on the phylogeographic distribution of two intertidal red seaweeds, *Gelidium pristoides* and *Hypnea spicifera* using the mitochondrial *Cox2-3* spacer and the nuclear *ITS1* regions. *G. pristoides* spores have short distance-dispersal, while long distance-dispersal is more likely in *H. spicifera* via spores and drifting fertile thallus fragments. Both markers revealed a south-western and south-eastern lineage within *G. pristoides* but the breaks between lineages do not coincide with any recognised biogeographic limits.

The *Cox2-3* spacer revealed a boundary between the two lineages at the Alexandria Coastal Dunefield (ACD) and *ITS1* at the Gamtoos-Van Stadens Dunefields (GVD) which is approximately 80km west of the ACD. The minor difference between the two markers regarding location of the phylogeographic boundary is probably due to the dating differences between the two dunefields. The ACD as developed currently is superimposed on the ancient dunefields which formed during the Pleistocene, coinciding with the *Cox2-3* spacer sequences divergence which dates back 500,000 - 580,000 years. The GVD formed during the Holocene (6,500 - 4,000 years ago), coinciding with the *ITS1* sequences divergence which dates 4,224 - 4,928 years ago. Thus, these phylogeographic boundaries probably appeared without the influence of biogeographic boundaries, but rather due to the lack of suitable habitat in the dunefields, coupled with short dispersal-distances of the spores. Analysis of the *ITS1* and *Cox2-3* spacer regions in *H. spicifera* revealed that the species is characterized by uniform genetic structure along the coastline. This reflects the species`s potential for long range expansion as it inhabits both the intertidal and subtidal zones; and this presumably leads to high gene flow among populations. The *ITS1* sequences showed minimal genetic variation of one substitution between the gametophyte and tetrasporophyte generations within *H. spicifera*. This suggests the predominance of asexual reproduction, which reduces gene flow and fixes alleles between generations.

ANOSIM and Bray-Curtis cluster analyses showed scale-dependant variation in the abundances of epifauna (mainly amphipod, isopod, mollusc and polychaete species) on *G. pristoides*. At small local (within site) and large (among sites) scales, there were weak and no structure in epifaunal abundances respectively. However, at larger, biogeographic scales, samples from the same biogeographic region tended to be clustered together. Thus, there was a group containing predominantly south coast samples and a group containing east coast samples mixed with the remaining south coast samples. Such scale-dependant variation in epifaunal abundances is probably due to the effects of factors driving species richness at small local (within site) scales (e.g. wave exposure, seaweed biomass) and at larger, biogeographic scales (e.g. surface sea temperature).

Moreover, at very small (individual samples) scales; there was no correlation between epifauna composition and genotype of the seaweed. Seaweed samples characterized by distinct *ITS1* or *Cox2-3* spacer sequences did not show any significant differences in epifaunal composition. Although the distributional pattern of the epifaunal community observed at large biogeographic scale is not clear, it seems to be associated with the biogeographic regions. However, phylogeographic distribution of *Gelidium pristoides* is not connected to biogeographic regions. Thus, at larger, biogeographic scales, there is no correlation between phylogeographic distribution of *G. pristoides* and distribution of the associated fauna.

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CHAPTER ONE: GENERAL INTRODUCTION

1.1. Southern African bioregions and Oceanographic currents

The pioneering intertidal research by Stephenson (1939, 1944 and 1948) divided the South African coastline into three distinct biogeographic regions (Figure 1.1a): (1) the cold-temperate Benguela region which stretches from Oranjemund to Cape Peninsula, (2) the warm-temperate Agulhas region which stretches from Cape Agulhas to Port Elizabeth and (3) the subtropical Natal region which stretches from Port Edward eastwards up to the vicinity of Cape St. Lucia. The region between Cape Peninsula and Cape Agulhas comprises the western overlap while the region between Port Elizabeth and Port Edward comprises the eastern overlap. The largest Tropical Indo-West Pacific region stretches from the vicinity of Cape St. Lucia eastwards.

Subsequent to Stephenson`s studies, many authors have investigated marine and estuarine biogeographic patterns along the South Africa coastline (Stephenson & Stephenson 1972; Brown & Jarman 1978; Emanuel *et al.* 1992; Stegenga & Bolton 1992; Bustamante & Branch 1996; Bolton & Anderson 1997; Turpie *et al.* 2000; Bolton *et al.* 2004; Sink *et al*. 2005). In all of the above-mentioned studies, at least three main biogeographic regions were identified based on the analysis of the distribution of different taxa. Although those studies were mostly zoological, there appears to be a broad agreement that the South African coastline includes at least three main biogeographic regions.

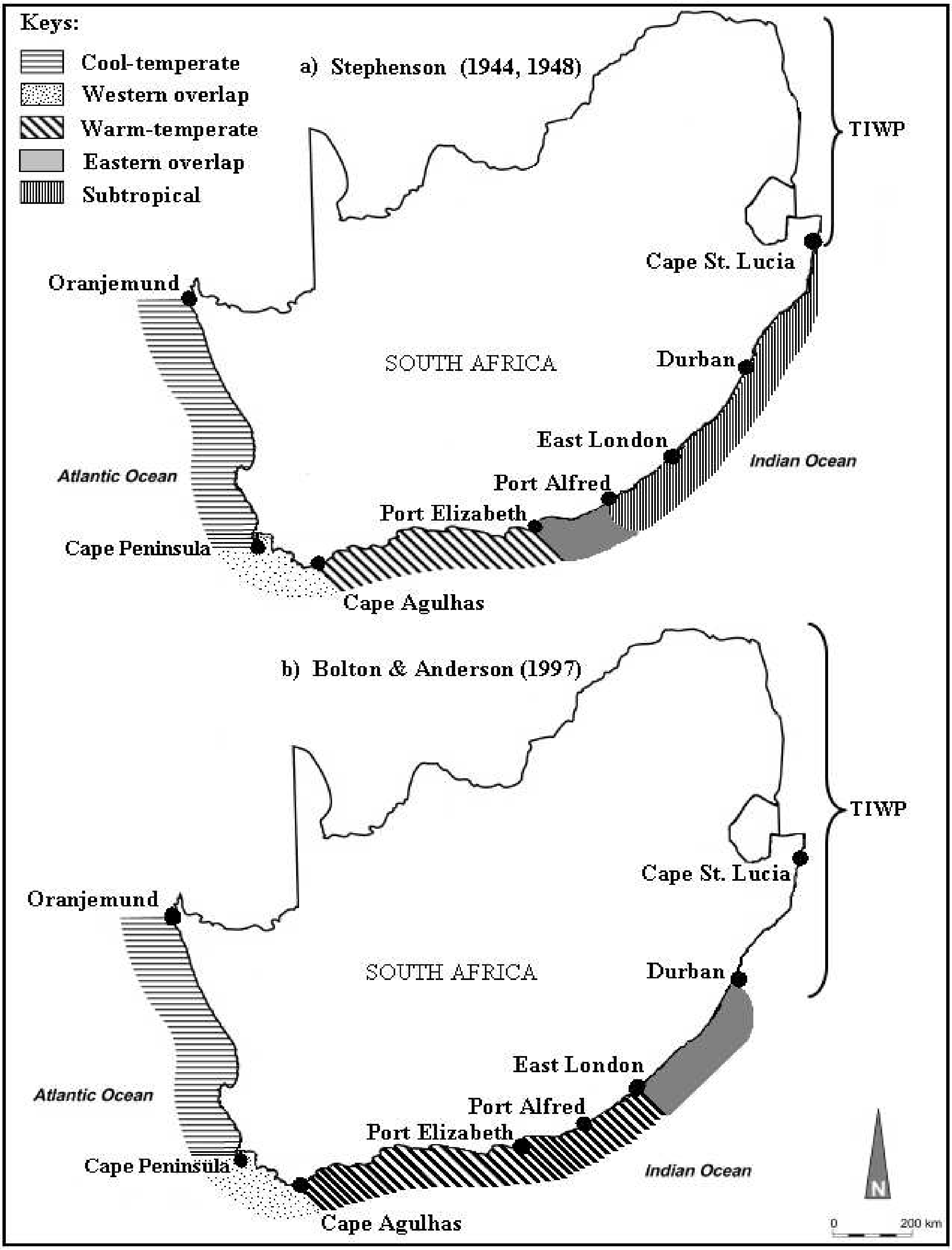

Figure 1.1. Map of South Africa showing biogeographic regions recognised by a) Stephenson (1948) and b) Bolton & Anderson (1997). TIWP = Tropical Indo-West Pacific region.

However, local phycologists argue that the distribution of South African seaweeds does not exactly follow the pattern described by Stephenson (1944, 1948). Bolton (1986) analysed seaweed distributions along the South African coastline and suggested only two biogeographic regions: the warm-temperate Agulhas region comprising the west and south coasts and the subtropical Natal region on the east coast. But subsequent studies had consistently defined the south coast as an independent biogeographic region (Stegenga & Bolton 1992, Emanuel *et al.* 1992, Bustamante & Branch 1996, Sink *et al*. 2005).

Bolton & Anderson (1997) in their review of marine vegetation in South Africa argued that the subtropical Natal region does not deserve recognition as a distinct biogeographic region for seaweeds. They argued that the seaweed flora shows an eastwardly decrease in the number of temperate south coast Agulhas region species, as they are replaced largely by Indo-West Pacific species. As a result, they suggested that there is an extended eastern overlap region from the coastline around East London to northern Kwazulu-Natal/southern Mozambique (Figure 1.1b). The extended eastern overlap region comprises a mixture of species from the Agulhas and Indo-West Pacific marine bioregions (Bolton & Anderson 1997).

Therefore, from the seaweed perspective, the subtropical Natal region is not recognized as a distinct biogeographic region. It is rather regarded as an extended eastern overlap region between the warm temperate Agulhas flora and the flora of the tropical Indo-West Pacific region (Bolton & Anderson 1997; Bolton *et al.* 2004). This extended eastern overlap region has low endemicity and similar numbers of seaweeds with affinities to the temperate Agulhas flora and the tropical flora of the Indo-West Pacific region (Bolton & Anderson 1997). The tropical Indo-West Pacific region is the largest coastal biogeographic region on earth (Briggs 1974).

Thus, in this study only two biogeographic regions (plus the two overlap regions) are considered in reference to seaweed biogeography (Figure 1.1b): (1) the cold-temperate Benguela region comprising the western coastline from Oranjemund southwards up to the Cape of Good Hope and (2) the warm-temperate Agulhas region from Cape Agulhas eastwards to the vicinity of East London. The boundary between these two regions is placed either at the tip of the Cape Peninsula, where a portion of the Agulhas current meets the current from the Southern Ocean, or at Cape Agulhas, the southernmost tip of the continent Africa (Hommersand 1986).

The waters along the southern African coastline are influenced by two major current systems (Figure 1.2), the Agulhas current on the east coast and the Benguela current on the west coast (Payne & Crawford 1989). The south-flowing Agulhas current lies at latitude of 39° 30`S south of Africa, increasing slowly downstream to a latitude of 44° 30`S at 60°E (Lutjeharms & Ansorge 2001). The current is about 60 to 100 km wide and usually lies 14 to 38 km offshore (Goschen & Schumann 1990; Lutjeharms 1998). The current is supplied with tropical waters of the Indian Ocean; as a result the waters of the Agulhas current are relatively warm and become cooler as the current moves southwards.

The warm Agulhas current follows the edge of the continental shelf. The continental shelf is narrow off the coastline of northern Kwazulu-Natal and the current flows close to the shore. The continental shelf widens to 50 km just south of Cape St. Lucia and the current flows closer inshore, forming an elongated system of eddies between Cape St. Lucia and Durban, called the Natal Bight (Lutjeharms *et al*. 2000). South of Durban the continental shelf is narrow and the current flows close to the shore. The continental shelf increases in width as the current move southward toward Port Elizabeth, pushing the warm water away from the coast, resulting in cooler water temperatures (Lutjeharms & Ansorge 2001). The Agulhas Current turns backward and then flows eastwards and northwards as the Agulhas Return Current when it reaches the southern limit of the Agulhas Bank (Shannon 1989).

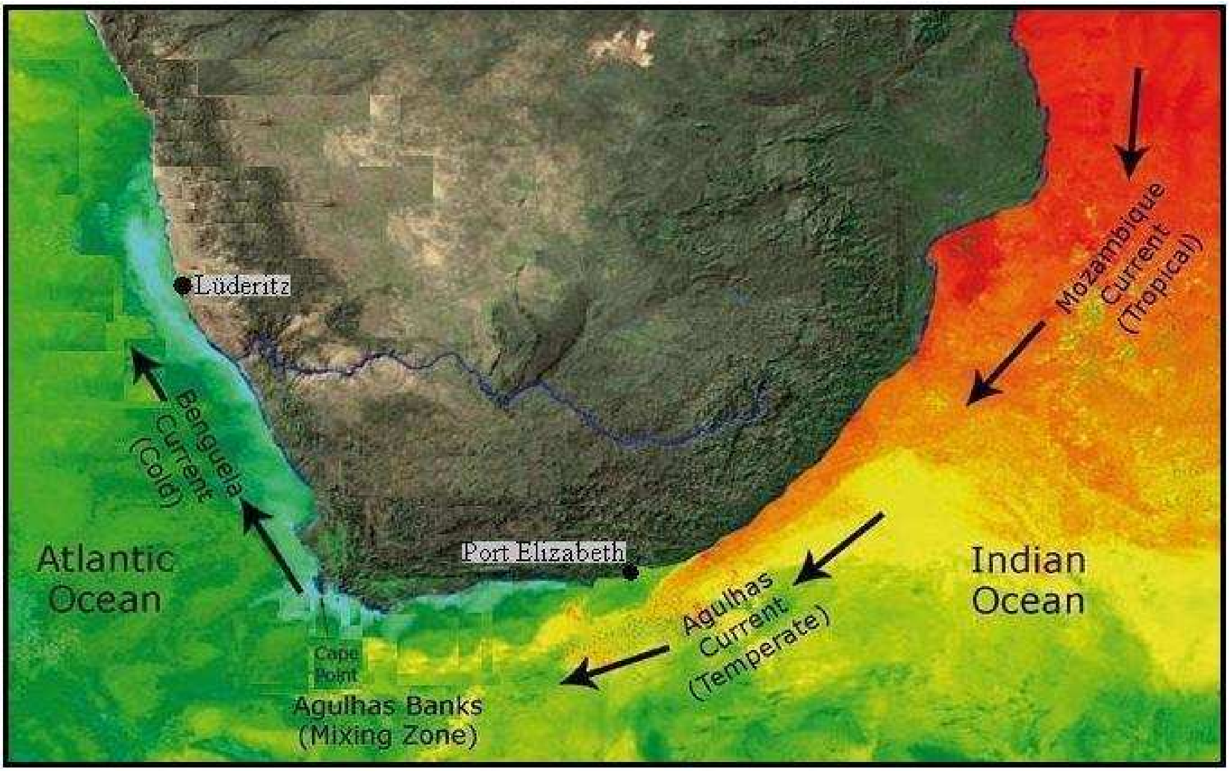

Figure 1.2. Satellite image of mean sea-surface temperatures showing the two major current systems influencing the coastal waters of southern Africa (adapted from Payne & Crawford 1989). Note the temperate Agulhas current (yellow) and the cool Benguela current (green).

The west coast is influenced by the cold, north-flowing Benguela Current. The Benguela Current flows from Cape Point northward to Lüderitz in Namibia (Peterson & Stramma 1991). The average surface water temperature of the Benguela Current is between 13 and 15 °C (Shannon 1989). Off the Lüderitz coast in southern Namibia, a cell of intense and consistent upwelling separates the Northern Benguela from the Southern Benguela and creates a semi-permanent environmental barrier (Boyd & Cruickshank 1983; Agenbag & Shannon 1988). According to Shannon (1985) the Benguela upwelling system is believed to have been in existence at its present intensity for 2 to 3 million years. Thus, Bolton & Levitt (1987) suggested that the distinct seaweed flora of the west coast evolved in the late Pliocene, 2 - 3 millions years ago.

1.2. Distribution of seaweeds along the South African coastline

Factors affecting the geographical distribution of seaweeds differ in relation to the scale of investigation (Bolton & Anderson 1990). At small scales, on any bay or short stretch of coast, factors such as wave exposure, substratum, exposure to air, salinity and competition were shown to be influential in different areas. For example, McQuaid & Branch (1984), from detailed investigations of biomass and species composition of intertidal communities around the Cape Peninsula, showed that wave exposure is the most influential factor for seaweed distribution. At larger, biogeographic scales, seawater temperature is consistently highlighted as the most important factor regulating the distribution of seaweed floras (Van de Hoek 1982; Breeman 1988; Lüning 1984, 1990). Gaines & Lubchenco (1982) suggested that biological factors may influence large scale distribution, although there is no evidence supporting this.

The evidence for seawater temperature as a regulating factor for seaweed distribution is both correlative and experimental. Correlatively, the distribution of the South African seaweed flora shows discontinuities associated with changes in seawater temperature patterns (Isaac 1938; Bolton 1986; Bolton & Stegenga 1987; Bolton & Anderson 1990). Experimentally, laboratory studies on South African seaweeds have shown that there is a correlation between a seaweed`s physiological temperature tolerance and seawater temperature in its native habitat (Bolton & Anderson 1987; Anderson & Bolton 1989).

Therefore, like intertidal invertebrates, South African seaweed floras are distributed around the coastline in patterns that closely follow patterns of the prevailing seawater temperature. As a result, the three biogeographic regions show differences in species diversity patterns and richness (Bolton & Stegenga 2002). The distribution pattern of Rhodophyte species in terms of species richness along the South African coastline is shown in Figure 1.3 below.

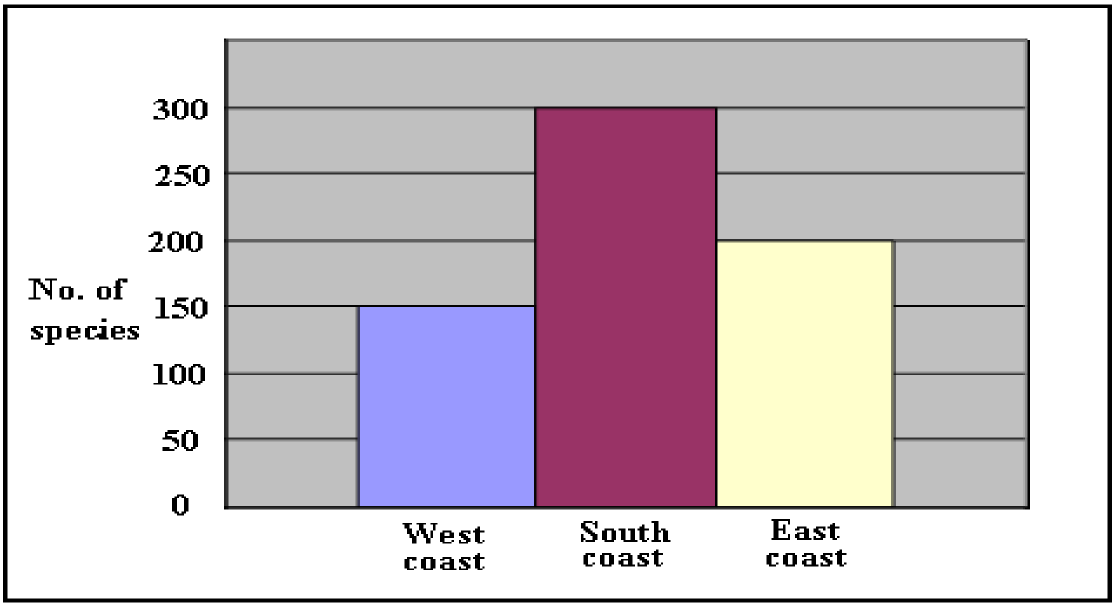

Figure 1.3. Graphical representation of red seaweed species richness along a 50km section of the West coast, South coast and East coast (modified from Bolton & Stegenga 2002).

The west coast or Benguela Region is species-poor, but high in endemism. According to Stegenga *et al.* (1997), of the almost 400 species they described from the west coast, 58% of Rhodophyceae, 33% of Phaeophyceae and 28% of Chlorophyceae were endemic to temperate southern Africa. However, with a record of 130 - 200 species per 50km section, the west coast is poor in species relative to the south and east coasts (Bolton & Stegenga 2002). The west coast shows a considerable drop-off in seaweed species from the Cape Peninsula northwards. The reduction in species diversity along the west coast is attributed to the stretches of unfavourable habitat and extreme upwelling on the west coast (Bolton 1996). The west coast is also less rich in intertidal invertebrate species than either the south or east coasts (Branch & Griffiths 1988; Bustamante *et al*. 1997).

The south coast or Agulhas Region, although not as well studied as the west coast, is species-rich and again high in endemism (Bolton 1999). A 50km coastal section on the south coast can contain up to 300 species, of which around 125 species are endemic to southern Africa (Bolton & Stegenga 2002). Thus, both the west and south coasts have high levels of seaweeds endemism. Approximately 38.5 % (327 seaweed species) of South African seaweeds are endemic to these regions, but most species are endemic to the richer south coast flora (Bolton & Stegenga 2002).

The south coast has long been found to be rich in marine invertebrates (Stephenson 1948). The level of species endemism for intertidal invertebrates is higher on the south coast than on the west and east coasts (Griffiths & Prochazka 1999). The numbers of endemic species are high between Cape Point and Port Elizabeth and decrease rapidly eastwards from Plettenberg Bay to Port Alfred (Griffiths & Prochazka 1999). The high richness and endemism of the south coast flora and fauna represents a clear indication of the influence of temperature on species distributions. The south coast flora and fauna is a mixture of warm- and cold- water species that has come about as a result of the mixing of the warm-water of Agulhas current and the cold-water of the Benguela current (Stephenson & Stephenson 1972).

The east coast or extended Eastern Overlap with 200-240 species per 50km section is also rich in species compared to the west coast. Whether the east coast is poor in species per coastal section compared to south coast is open to debate. Although Bolton & Stegenga (2002) reported a higher number of species per 50km section on the south coast than on the east coast. Studies on the Kwazulu-Natal (KZN) coast described more than twenty species of Rhodophyceae that had not previously been recorded in South Africa (Norris & Aken 1985; Norris 1992*a*; Bolton *et al*. 2001). Moving eastwards along the KZN coast, the numbers of species decrease and the coastline is progressively dominated by the tropical Indo-West Pacific species (Hommersand 1986; Bolton 1999; Bolton & Stegenga 2002).

There is a high level of species diversity in overlapping regions between the three major biogeographic regions (Bolton & Stegenga 2002). False Bay on the eastern side of the Cape Peninsula represents a classical example; over 200 species of seaweeds have been recorded in False Bay alone (Bolton *et al.* 1991). The seaweed richness in False Bay is attributed to the variety of habitats in the bay and the biogeographic position of the bay, as an overlap region between the seaweed floras of the warm-temperate Agulhas region and the cool-temperate Benguela region (Bolton *et al.* 1991).

The geographical distribution of seaweeds is not only a reflection of each species` temperature tolerance, but also a result of dispersal and vicariance (Van de Hoek 1984). Therefore, the present distribution of seaweeds along the South African coastline is also the result of their dispersal, introduction or displacement over geological time periods (Lüning 1990). Interestingly, various authors have independently discovered that the majority of the South African seaweed flora originated in Australasia (Norris & Aken 1985; Lüning 1990; Hommersand & Fredericq 2001; Bolton *et al.* 2004).

1.3. Phylogeography of seaweeds and community of their associated fauna

It is well known that the southern African biogeographic regions can be characterized by species composition which each region comprising distinct community. However, the subtropical Natal region on the eastern coast (generally accepted as a bioregion for invertebrates) is not recognised as a distinct biogeographic region with regards to seaweed flora. Bolton & Anderson (1997) indicated that the seaweed flora along this region comprises a mixture of species from both the Agulhas region and the Indo-West Pacific region. This study will use in particular the south-eastern coast of South African to address a general biogeographic problem that still trouble marine ecologists and phycologists in South Africa. Studies that investigated the distribution of the coastal invertebrates along this coastline were based either on the community distribution or phylogeographic distribution. There has been no previous study that investigated in particular the phylogeographic patterns of seaweeds along this coastline. This is one of the first studies (if not the first one) to integrate both the phylogeographical analysis and epifaunal community analysis to determine the correlation between the phylogeographic distribution of seaweeds and their associated fauna.

CHAPTER TWO: PHYLOGEOGRAPHY OF TWO RED SEAWEED SPECIES

2.1 INTRODUCTION

2.1.1 Phylogeography

It is common in the natural sciences for a new word or phrase coined to explain a particular concept to become part of the working terminology in the discipline. The term “phylogeography” was introduced just more than two decades ago due to the need to explain striking phylogenetic patterns (Avise *et al.* 1987). The analyses of mitochondrial DNA (mtDNA) revealed phylogenetic trees which displayed remarkable geographic patterns (Avise *et al.* 1987).

Following the coining of the term, any study concerned with within-species variation in mtDNA and the geographical distribution of phylogenetic groupings was termed intraspecific phylogeography (Avise *et al.* 1987). More precisely, phylogeography can be defined as a sub-discipline of biogeography that applies phylogenetic techniques to achieve a comprehensive understanding of how biological (i.e. evolutionary) and physical (i.e. ecological) processes in the past have shaped the current spatial distribution of genealogical lineages within species and among closely related taxa (Avise 2000; Avise 2004). Thus, phylogeographic patterns simply refer to relationships between genealogical lineages and geographical locations.

The analysis and interpretation of genealogical distribution usually requires extensive knowledge from various disciplines such as molecular genetics, population genetics, phylogenetic biology, ecology, geology, paleontology and historical geography (Avise 2000). Thus, phylogeography is an integrated field that involves various microevolutionary and macroevolutionary disciplines (Avise 2000). Phylogeographical analysis covers alternative historical scenarios to account for the spatial distribution of genealogical lineages. Dispersal and vicariance are two often-competing historical scenarios invoked to account for the origins of spatially disjunct genealogical lineages (Ronquist 1997). Under the dispersal interpretation, lineages came to occupy their present ranges through active or passive dispersal from one or more ancestral centers of origin (Briggs 1974). Under the vicariance scenario, lineages became separated when the more-or-less continuous ranges of ancestral forms were split apart by natural events such as mountain formation (Croizat *et al.* 1974). Thus, phylogeographical analysis weighs the influence of both dispersal and vicariance phenomena in shaping the geographical distribution of genealogical lineages.

There are many studies that have investigated phylogeographical patterns of seaweeds from various coastlines around the world (e.g. van Oppen *et al*. 1994; Fredericq & Ramirez 1996; Strate van der *et al.* 2002; Zucarrello & West 2002; Leskinen *et al.* 2004; Provan *et al.* 2005*b*; Verbruggen *et al.* 2005; Hu *et al.* 2007*b*). This is the first study to investigate intraspecific phylogeographical patterns of seaweeds from the South African coastline. Previous studies on the geographical distributions of South African seaweeds were exclusively focused on phytogeographical patterns (e.g. Stephenson 1948; Bolton 1986; Hommersand 1986; Bolton & Stegenga 1987; Bolton & Anderson 1990; Bolton *et al.* 2004).

Several studies have investigated the phylogeographical patterns of some of the coastal and estuarine invertebrates of South Africa. These studies found that some coastal and estuarine invertebrates exhibit phylogeographical disjunctions that often coincide with the boundaries between the three main biogeographic regions (e.g. Ridgway *et al.* 1998; Evans *et al.* 2004; Teske *et al.* 2006; Edkins *et al.* 2007; Teske *et al.* 2007*a*; Teske *et al*. 2007*b*; Zardi *et al*. 2007). However, some studies have refuted the long-held believe that the genetic structure of marine organisms is dependent on the period the larvae spend in planktonic stage, whereby species with long-lived larvae should show limited phylogeographic structure (Avise *et al.* 1987). For example, Teske *et al.* (2006; 2007*a*) and Zardi *et al.* (2007) found that some of coastal invertebrates with long-lived planktonic larvae are characterized by phylogroups.

As a result, in an effort to understand the biological processes that shape such phylogeographical structures, Teske *et al.* (2007*c*) analysed the implications of life history for genetic structure and migration rates of five southern African coastal invertebrate species. Based on mtDNA CO1 analyses of these species, they concluded that the period the larvae spend in the planktonic stage has no or little effect on genetic structure. They asserted the genetic structure within marine biogeographic regions strongly depends on the presence or absence of a free-swimming larva.

2.1.2. Study species

2.1.2.1. *Gelidium pristoides* (Turner) Kützing 1843

Gelidium pristoides (Turner) Kützing 1843 was once the subject of taxonomic problems in the order Gelidiales. Akatsuka (1986) erected the new genus *Onikusa* for two species formerly included in the genus *Gelidium* (*Gelidium pristoides* and *Gelidium japonica*). As a result, *Gelidium pristoides* was changed to *Onikusa pristoides* and *G. japonica* changed to *O. japonica.*

Following this, Norris (1992*b*) described *Onikusa foliacea* from samples in Japan and South Africa. However, Tronchin *et al.* (2002) reassessed the classification of the species in the newly formed genus *Onikusa*. From their findings, based on both morphological and molecular data (*rbcL* spacer region), all the species described in the genus *Onikusa* were re-placed into the genus *Gelidium*. As a result, *Onikusa pristoides* was renamed as *Gelidium pristoides.* Therefore, the species referred to herein as *G. pristoides* was once known as *O. pristoides.* Carter (1986) provides a comprehensive description of the life history of this species.

G. pristoides (Turner) Kützing 1843 (Figure 2.1) is a Floridean Rhodophytan species endemic to South Africa. It is characterised by flattened narrow fronds with serrated margins and its blades are pale and branch irregularly, with small expanded lateral leaflets (Branch *et al.* 1994). It is found in the littoral zone on the high shore, attached to various substrata such as rocks, tube-worms, limpet and barnacle shells (Anderson *et al*. 1991). The species distribution range is shown in Figure 2.1 following Anderson *et al.* (1991). However, Day (1969) reported the western limit of the species to be little bit further north of Kommetjie at Sea Point.

Studies of *G. pristoides* contributed to the successful long term commercial harvesting of the species. One of the reasons for more studies on this species was the public concern for how harvesting might affect the species and other plants and animals in symbiosis with it. Anderson *et al.* (1991) investigated the extent to which *G. pristoides* might be affected by harvesting on the east coast. They found that harvesting has no significant biological effect on the species as harvested seaweeds recover their biomass within two to three months in summer and four to five months in winter period (Anderson *et al.* 1991).

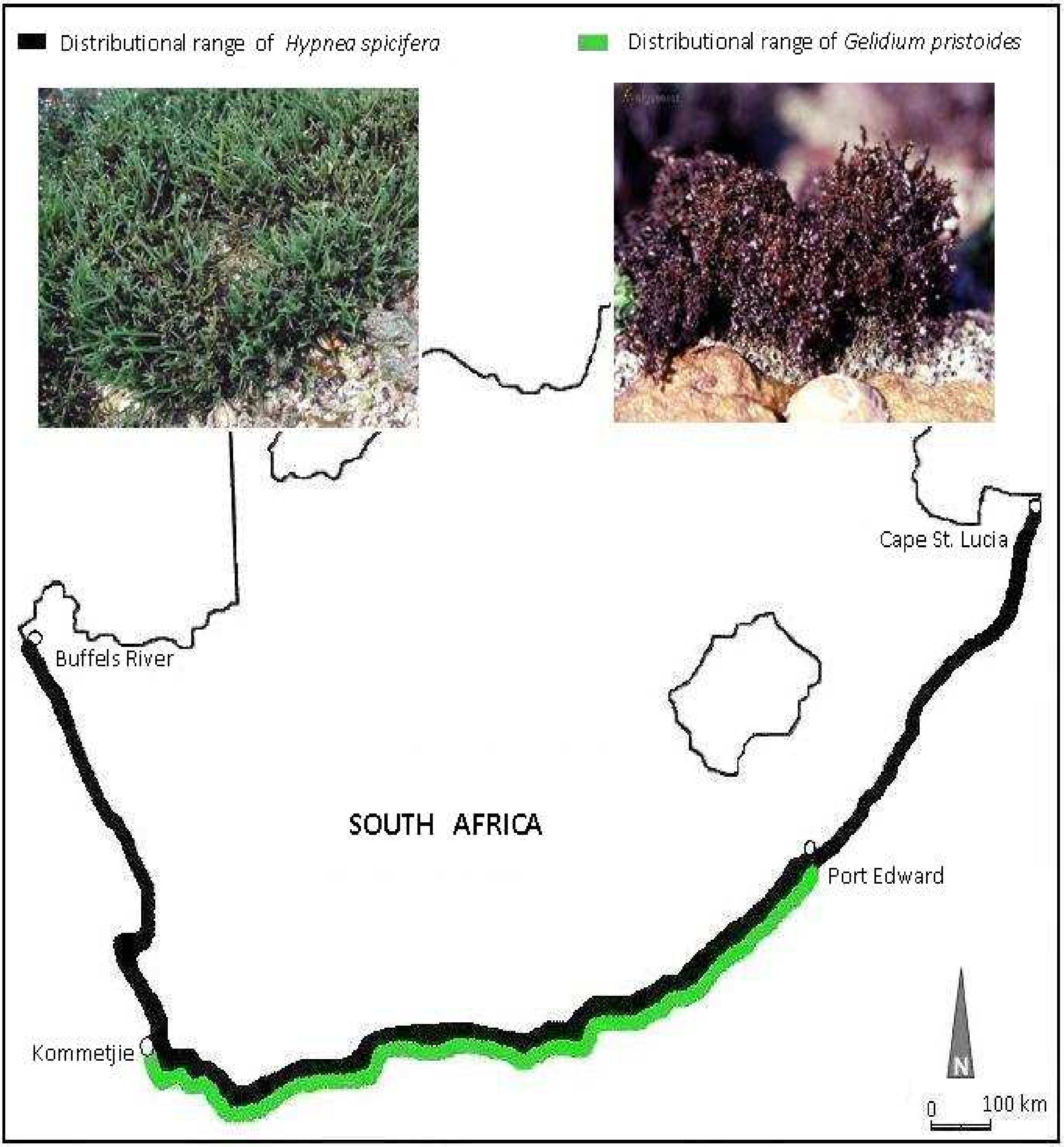

Figure 2.1. Map of South Africa showing distributional ranges of *Hypnea spicifera* (black line) and *Gelidium pristoides* (green line) along the coast following Hewitt (1955) and Anderson *et al.* (1991). Pictures of the species by Anderson RJ (2001).

Additionally, Anderson *et al*. (1991) also investigated whether *Gelidium pristoides* harvesting affects animals serving as an attachment substratum, such as limpets and barnacles. They showed that harvesting has no significant effect through the accidental removal of limpets and barnacles. According to their findings, the accidental removal of limpets and barnacles is negligible for two reasons. Firstly, most of the limpets and barnacles removed with the seaweeds are dead and if alive, they are very small and would be reproductively inactive. Secondly, *Gelidium* harvesters are instructed to tap limpet shells before picking *G. pristoides*, allowing the limpet to cling tightly onto the substratum.

2.1.2.2. *Hypnea spicifera* (Suhr.) Harvey 1847

Hypnea spicifera (Suhr.) Harvey 1847 (Figure 2.1) is a red seaweed that is often green and may be often thought to be a green alga. This species is one of the most widely distributed seaweeds along the South African coastline; its range is shown on the map in Figure 2.1 following Hewitt (1955). Unlike *G. pristoides*, *H. spicifera* is not endemic to the South African coastline; it also occurs in Namibia and Borgesen (1934) also recorded the species at Karachi on the north-west coast of India. This species has been known to occur only in tidal estuaries and in the lower intertidal zone (Hewitt 1955); however it has recently been observed in the subtidal zone (Anderson RJ, pers. comm.). In the lower intertidal zone, *H. spicifera* is much more common than any of the other *Hypnea* species (Hewitt 1955). Details of the life history and ecology of this species are provided by Hewitt (1955) as part of her M.Sc. project at Rhodes University. It is well established that *H. spicifera* has the potential for commercial harvesting of agar in South Africa (Hewitt 1955; Anderson *et al.* 1989; Branch *et al.* 1994). However, to my knowledge this species is not yet harvested commercially in South Africa.

2.1.3 Life histories of the two study species

G. pristoides and *H. spicifera* are Floridean red seaweeds, and this implies that they have a *Polysiphonia*-type life history. Species with such life histories exhibit two morphologically similar but separate, free-living, isomorphic phases that differ in ploidy levels (Thornber 2006). The two isomorphic phases (the diploid sporophyte and the dioecious haploid gametophyte) are morphologically similar yet distinguishable by their reproductive structures, provided they are fertile (Hewitt 1955, Carter 1985).

In *G. pristoides*, the fertile male gametophyte is distinguished by translucent patches on the surface of determinate branches, while fertile female gametophytes can be distinguished by the presence of the bilocular cystocarp (Carter 1985). The bisporophyte generation can be distinguished from the gametophyte generation by conspicuous sporangia on the fertile branches (Carter 1985). In *H. spicifera*, fertile male gametophytes are distinguishable with the naked eye by the pale fertile branchlets termed antheridia whereas fertile female gametophyte plants produce prominent cystocarps which, when mature, are distinguishable by the naked eye as minute red globules on the fertile branchlets (Hewitt 1955). The tetrasporophyte generation is distinguished from the gametophytes by the prominent tetrasporangia swollen in fertile branchlets termed stichidia (Hewitt 1955).

Like seaweeds in general, members of the Florideophycidae have a highly complicated, variable life history (Dixon 1973; Maggs 1988). Diagrammatic illustrations of the life histories of *G. pristoides* and *H. spicifera* are shown in Figure 2.2. The mature, multicellular male haploid gametophytes release haploid non-flagellated spermatia into the water column (Thornber 2006).

The spermatium is non-motile, unable to swim freely and relies on random water motion to fertilize the carpogonium (egg cell) attached onto the haploid female gametophyte (Maggs 1988). Each carpogonium has a trichogyne, a hair-like receptive structure involved in receiving the spermatium (Dixon 1973).

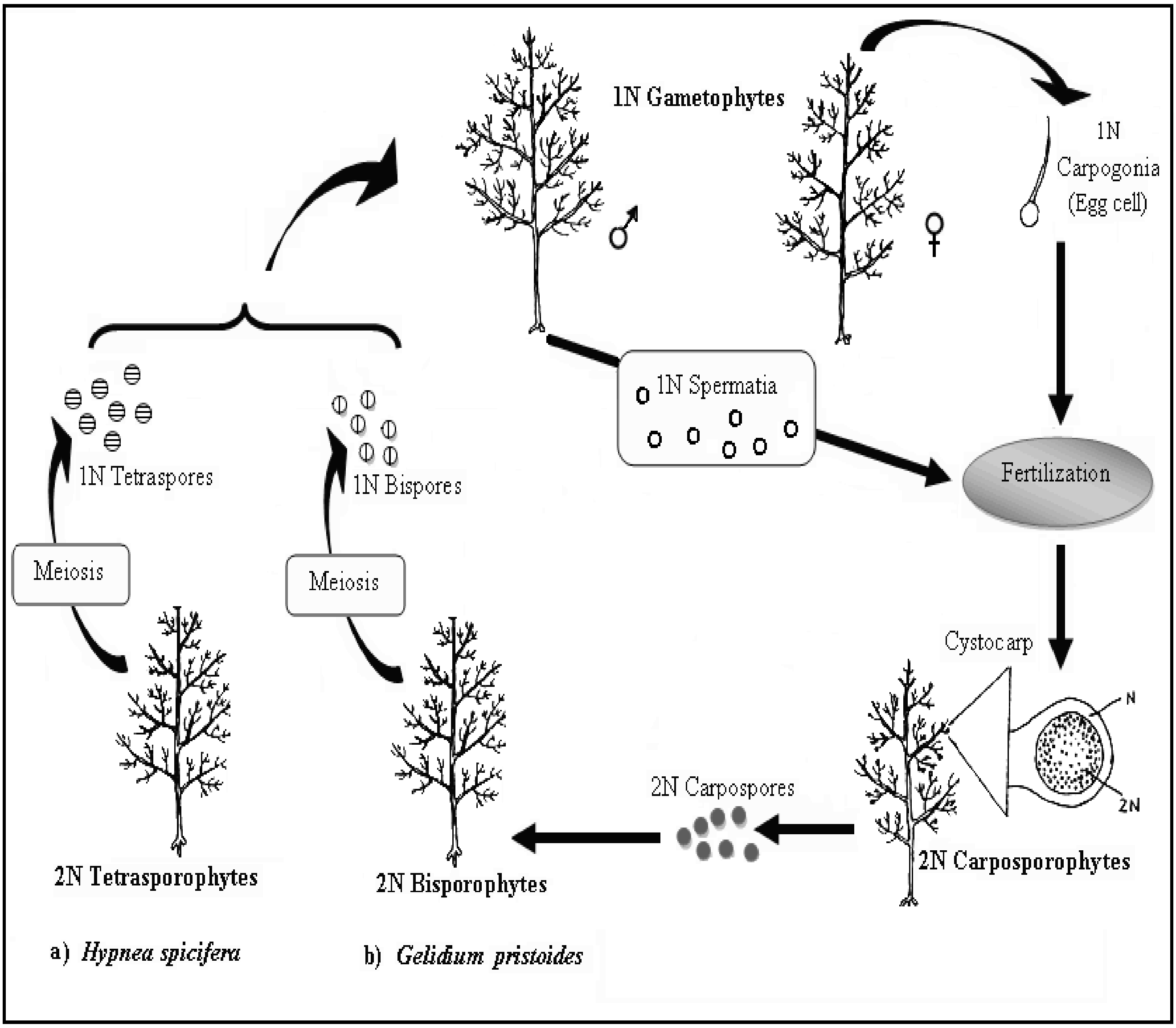

Figure 2.2. Diagrammatic illustration of the life history of *Hypnea spicifera* (a) and *Gelidium pristoides* (b) (After Maggs 1988 and Thornber 2006).

The trichogyne receives the spermatium which fertilizes the carpogonium to form an intermediate, short-lived diploid stage called a carposporophyte (Thornber 2006). The tiny carposporophyte lives attached to the female gametophyte in a cystocarp (Dixon 1973). The carposporophyte acquires nourishment from the cystocarp until it becomes a mass of diploid spores (Kamiya & Kawai 2002). The carposporophytes release diploid carpospores into the water column; carpospores settle and grow into a free-living diploid phase called a tetrasporophyte (spores packaged in groups of four) (Maggs 1988). However, in *G. pristoides* bisporophytes (spores packaged in groups of two) are produced rather than tetrasporophytes (Carter 1986). Mature diploid tetrasporophytes (*H. spicifera*) or bisporophytes (*G. pristoides*) under go meiosis to release haploid tetraspores or bispores respectively (Hewitt 1955, Carter 1985). In the Florideophycidae, tetraspores and bispores may either be zonately, tetrahedrally or cruciately divided (Dixon 1973). In *H. spicifera* the tetraspores are zonately divided (Hewitt 1955). It is uncertain how bispores in *G. pristoides* are divided; however Carter (1986) suggested that they are cruciately divided. *G. pristoides* produces three types of bispores (mononucleate, binucleate and tetranucleate) and binucleate bispores are the most common and abundant in natural populations (Carter 1986; Maggs 1988). The haploid tetraspores or bispores are released into the water column, settle, and grow into an equal number of haploid male and female gametophytes (Dixon 1973). Therefore, in *H. spicifera* each tetraspore will give rise to two haploid male gametophytes and two haploid female gametophytes. While in *G. pristoides* each bispore will give rise to one haploid male gametophyte and one haploid female gametophyte.

Seaweed species characterized by a *Polysiphonia*-type life history often show an unequal ratio of gametophyte to tetrasporophyte generations in their natural populations (Kong & Ang 2004). For example, the gametophyte generation was found to be dominant in natural populations of *Gigartina skottsbergii* Setchell *et* Gardner (Piriz 1996; Zamorano & Westermeier 1996). The dominance of tetrasporophyte over gametophyte generations has been reported for a range of Rhodophyceae species, including species of the Gelidiales (Johnstone & Feeney 1944, Montalva & Santelices 1981). The dominance of the tetrasporophyte generation has been commonly reported within populations of *Hypnea* species (Mshigeni 1976; Rama Rao 1977; Mshigeni & Lorri 1977; Mathieson 1989; Reis & Yoneshigue-Valentin 2000).

Isaac & Hewitt (1953) in detailed studies of *H. spicifera* at Port Alfred showed that the gametophyte generation always constituted a very small proportion of the population. However, no quantitative data were given. It is believed this phase dominance is part of the life history strategy of these seaweeds (Kong & Ang 2004) and several hypotheses have been suggested to explain such a strategy. It is thought that since the diploid phase usually dominates, there might be inherent advantages of diploidy over haploidy, resulting in a greater success rate of sporophyte populations (Mshigeni 1976; Kain 1982).

Mshigeni (1976) found that the tetrasporophyte generation is always more abundant than the gametophyte generation within populations of *H. cervicornis* and *H. chordacea* from Oahu Island (Hawaii). The author suggested that the carpospores of these species have more vigorous germling success than tetraspores, leading to greater recruitment into the tetrasporophyte generation. Rama Rao (1977) and Mathieson (1989) also attributed the dominance of tetrasporophytes within *H. musciformis* populations from India to poor germling success of tetraspores, leading to a failure to recruit into the gametophyte generation. Reis & Yoneshinge-

Valentin (2000), however, suggested that the rarity of tetrasporophytes within *H. musciformis* populations from the Rio de Janeiro coast is probably due to the replacement of sexual reproduction by asexual reproduction via apomixis or vegetative reproduction.

Bispores are the product of meiosis from the bisporophyte in *G. pristoides* (Carter 1993). As a result, it was expected that bisporophyte and gametophyte generations should be present in the same proportions in natural populations of *G. pristoides* (Hoyle 1978; Kain 1982). However, Carter (1986) found that in natural populations of *G. pristoides*, this is not so. He found that in populations from Sharks Bay, Kenton-On-Sea and St James, the bisporophyte generation was dominant over the gametophyte generation, in a ratio of 3:1. The same degree of imbalance between the two generations was believed to persist throughout the coast, suggesting that bisporophyte dominance is biologically determined in *G. pristoides* (Carter 1985).

Carter (1985) suggested two main reasons for this dominance. Firstly, in *G. pristoides*, bispores are produced rather than tetraspores. So, one might expect a disproportionate ratio of bisporophytes to gametophytes. Secondly, there is a difference in germling success between the bispores and carpospores. Carter suggested that increased vigor of carpospores germlings might be advantageous in the intertidal habitat of *G. pristoides*, resulting in more recruitment into the bisporophyte generation.

Although it is well known that *G. pristoides* produces bispores rather than tetraspores, the reason behind this is still not clearly understood. Van der Meer (1977) suggested that larger bispores are better able to survive under certain conditions, than smaller tetraspores. Okuda & Neushul (1981) agreed that larger spores may be able to survive better in habitats that are only suitable for germling growth during certain times, but believe this need not lead to phase dominance. Nevertheless, Carter (1985) accepts Van der Meer`s suggestion that larger bispores are produced in *G. pristoides* because bisporophytes can achieve greater success in the intertidal zone than tetrasporophytes.

2.1.4. Dispersal potential of the spores

The only known evidence for long-distance dispersal of seaweeds is circumstantial. Two classic examples of such circumstantial evidence are: (1) the similarity of the seaweed floras of Islands of the West Wind Drift (i.e. Gough, Crozet, Kerguelen, Bouvet and Marion Islands.) and (2) the mid-oceanic Islands of volcanic origin (i.e. Tristan da Cunha and the Faeroes) are inhabited by well-developed seaweed flora which could reach these Islands only by long distance dispersal from continental areas (Hommersand 1986 & Van den Hoek 1987). The central question one may ask is whether such long-distance dispersal of seaweeds occurs via propagules or not? Do the propagules of seaweeds remain suspended and viable long enough for long-distance dispersal?

Outstanding empirical phycological studies were done to elucidate the mechanisms of spore dispersal, lifespan of the floating spores, range of spore dispersal and the probability of spores colonizing a new area (Santelices 1990; Norton 1992; Gaylord *et al.* 2002). These studies provide several convincing reasons that long-distance dispersal of seaweeds via propagules (spore or zygote) is unlikely.

Firstly, most seaweed propagules have a brief phytoplanktonic stage and settle within days after being released (Santelices 1990). Secondly, the viability of free-floating spores (bispores, tetraspores and carpospores) is very low (Santelices 1990; Lobban & Harrison 1994). Thirdly, if the propagules have to travel far, they need stored energy during the journey. However, seaweeds propagules lack the larva`s bloated yolk sac (Gaylord *et al*. 2002). Fourthly, the reproductive cells of the red seaweeds are devoid of flagella and thus cannot swim (Thornber 2006). Overall, the implication is that most seaweed spores have a relatively short-dispersal distance and long-distance dispersal via propagules is very unlikely.

However, Gaylord *et al.* (2002) found that the propagules of four nearshore seaweed species (*Macrocystis pyrifera, Sargassum muticum; Ectocarpus siliculosus* and *Sarcodiotheca gaudichaudii*) have a greater potential for long-distance dispersal. These authors used the sinking speed of the propagules to determine the potential dispersal distance of the propagules for each of the four species. They found that the propagules of the four species have a slow sinking speed and concluded that the propagules are likely to remain suspended in the water column longer and have a greater potential for long-distance dispersal than those of other species. However, Gaylord *et al.*`s (2002) findings are species-specific. The sinking speed of a propagule is determined by its density and size, and propagule size in seaweeds is highly variable among species (Coon *et al*. 1972; Clayton 1992).

In fact, long distance-dispersal in seaweeds is believed to occur via drifting plant fragments containing viable or developing spores rather than directly via propagules (Hommersand 1986, Chapman 1986, Van den Hoek 1987, Santelices 1990). Long distance-dispersal of seaweeds via drifting fertile plant fragments is like long distance-dispersal of larvae in marine invertebrates because both are largely controlled by the prevailing currents (Chapman 1986, Van de Hoek 1987, Gaylord *et al*. 2002). *Hypnea* tufts are easily swept away by strong waves and thus drift in the water column (Hewitt 1955; Reis & Yoneshigue-Valentin 2000; Kong & Ang 2004).

The spores of *Gelidium* species do not disperse over long distances and have their strongest capacity for attachment immediately after being released; it is believed they settle and grow into the next phase within days (Santelices 1990). Alberto *et al.* (1999) also suggested poor long-distance dispersal of spores in *Gelidium* species. They further suggested that dispersal strategies of *Gelidium* species rely more on the transport and re-attachment of loose fronds than on spores. However, *G. pristoides* has a strong thallus and also the rhizoid attaches strongly onto the substratum (Carter 1985). This hinders the dislodgment of the tufts by waves. For example, complex of seaweeds species were found washed-away by the swell at Cape Hangklip, Masbaai (Western Cape) but no *G. pristoides* was observed (personal observation).

Additionally, it has been shown that free-floating fragments of *Gelidium* spp. do not seem to be very successful in establishing new populations (Coon *et al.* 1972; Amsler & Searles 1980; Okud & Neushul 1981; Santelices 1990). Therefore, both the bispores or carpospores and free-floating fertile fragments of *G. pristoides* do not seem to be effective for long-distance dispersal.

Apparently less is known concerning the dispersal potential of spores in *H. spicifera*. Hewitt (1955) showed that the tetraspores in *H. spicifera* are denser than water and tend to sink after being released. However, it is not known at what speed the tetraspores sink and thus, it is not certain if the spores remain suspended in the water column long enough for long distance-dispersal. However, Mshigeni & Lorri (1977) found that the spores of *H. musciformis* can remain viable and healthy in laboratory cultures for a period of 3 months. This suggests that *in situ*, the spores of this species can remain planktonic long enough to be dispersed long distances via currents. As a result, we can neither support nor refute the suggestion of long distance-dispersal of *H. spicifera* via spores, but it may be more likely than in *G. pristoides*. Furthermore, *H. spicifera* has the potential for asexual reproduction by the spreading of the holdfast (Mshigeni & Lorri 1977).

2.1.5. Molecular markers

The accuracy and reliability of any phylogenetic study depends on the DNA marker/s used to reconstruct the evolutionary history of the species. There is a wide variety of DNA markers that can be utilized, depending on the depth of evolutionary level to be resolved. The lack of appropriate DNA markers has been particularly problematic for studies concerned with within-species genetic variation (Zuccarello *et al.* 1999; Provan *et al*. 2004). In the past, studies concerned with within-species genetic variation in red seaweeds mainly used allozyme electrophoresis, randomly amplified polymorphic DNA (RAPDs), restriction fragment length polymorphism (RFLPs) and microsatellite DNA (Sosa & Garcia-Riena 1992; van Oppen *et al*. 1994; Alberto *et al*. 1999; Provan *et al*. 1999).

Specific molecular markers have proved useful for determining within-species genetic variation in both the Chlorophyceae and the Rhodophyceae. Such markers include: (1) the internal transcribed spacer regions (*ITS1* and *ITS2* regions) in the ribosomal cistron of the nuclear genome; (2) the large and small subunits of the Ribulose-1,5-bisphosphate carboxylase –

RuBisCo genes (*RbcL* and *RbcS*) encoding regions on the chloroplast and (3) the mtDNA control region for the Cytochrome oxidase encoding gene (*Cox 2-3* spacer region).

2.1.5.1. Nuclear ribosomal markers (i.e. *rRNA, ITS1 and ITS2*)

The ribosomal RNA (*rRNA*) genes are highly conserved and can therefore successfully differentiate organisms at the family level to genus level (Iyer *et al.* 2005). The most commonly used *rRNA* genes include the highly conservative *18S rRNA* and *5.8S rRNA* gene sequences (Yeh & Chen 2004; Provan *et al.* 2005*b*; Iyer *et al.* 2005; Verbruggen *et al.* 2007). As a result of the highly conservative nature of the genes and their low resolution power at the intraspecific level, the regions located between the ribosomal genes, *ITS1* and *ITS2* (Figure 2.3) are often preferred for intraspecific genetic studies (Bakker *et al.* 1995; Coleman & Mai 1997; Hu *et al.* 2007*a*).

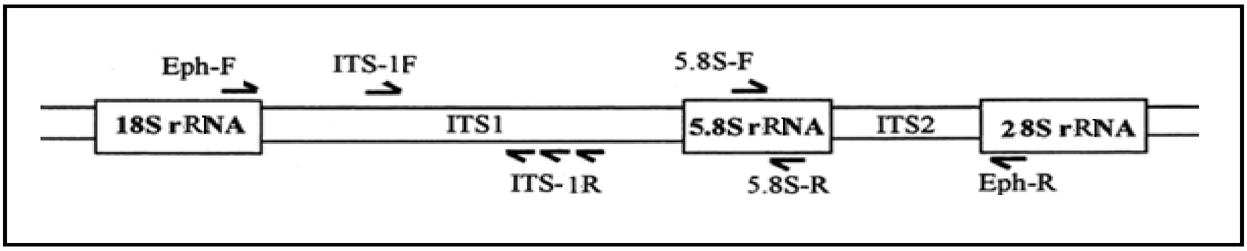

Figure 2.3. Diagrammatic illustration of the ribosomal *nrRNA* genes and the *ITS* spacer regions (adapted from Bakker *et al.* 1995). Note the ITS-1F and ITS-1R primers within the *ITS1* region.

The utilization of the *ITS* regions in intraspecific genetic studies can be attributed to their relatively high rate of nucleotide substitution, permitting comparison of relatively recently diverged taxa (Leskinen *et al.* 2004; Provan *et al.* 2005*a*). Additionally, the *ITS* regions can be readily PCR-amplified and sequenced with conserved primers positioned in the cistronic regions (Provan *et al.* 2004). Furthermore, the *ITS* regions are non-coding and thus evolve freely without functional constraints (Bakker *et al.* 1995). The *ITS* regions have so far proved to be the most appropriate markers for determining intraspecific genetic variation in the Chlorophyceae, Phaeophyceae and Rhodophyceae (Bakker *et al.* 1995; Coleman & Mai 1997; Pillmann *et al.* 1997; Coyer *et al.* 2002; Strate van der *et al.* 2002; Leskinen *et al.* 2004; Yeh & Chen 2004; Provan *et al.* 2005*a*; Hu *et al.* 2007*b*).

2.1.5.2. Plastid markers (i.e. *RbcL* and *Cox2-3* spacer regions)

The most commonly used plastid marker in the molecular systematics of seaweeds is the Ribulose-1,5-bisphopate carboxylase or *RuBisCo* gene. In red seaweeds, the large and small subunits of the RuBisCo enzyme are encoded by the *RbcL* gene. The *RbcL* spacer region, a non-coding control region downstream of the *RbcL* gene, has so far proved to be useful in determining within- or among-species genetic variation in the Rhodophyta (Freshwater *et al.* 1994; Fredericq & Ramirez 1996; Yamagishi & Masuda 2000, Zuccarello & West 2002; Zuccarello *et al.* 2002; Iyer *et al.* 2005).

Mitochondrial markers have been useful in phylogenetic and population studies of animals due to their haploid nature, uniparental inheritance and lack of recombination. The animal mitochondrial cytochrome oxidase 1 gene (*CO1*) has been used frequently and very successfully in systematic, phylogeographic and population studies because of its high mutation rate (Avise 1994). But there is no gene encoding region in the mtDNA of higher plants or seaweeds that has been found to have the same level of variation as the *CO1* gene in animals (Maggs *et al*. 2007).

Due to a lack of appropriate mtDNA markers for resolving within-species genetic variation in seaweeds, Zuccarello *et al.* (1999) designed a set of primers that can span the non-coding intergenic region within the mtDNA. The primers designed can successfully span the non-coding intergenic region located between the mitochondrial cytochrome oxidase subunit 2 (*Cox2*) and the cytochrome oxidase subunit 3 (*Cox3*) genes in various red seaweed orders including the Bonnemaisoniales, Ceramiales and Gracilariales. The *Cox2-3* spacer region has so far proved to be useful for determining intraspecific genetic variation in red seaweeds (Zuccarello & West 2002; Zuccarello *et al.* 2002).

Although these various markers have proved to be informative in particular cases, often they do not provide enough resolution when used solely. Therefore, phylogenetic and phylogeographic studies usually apply two or more sets of markers, aimed at resolving different depths of evolutionary relationship. Likewise, most studies of phylogeographical patterns of seaweeds utilise a combination of nuclear rDNA control regions or *ITS* regions; *Cox2-3* spacer region and *RbcL* or *RbcS* spacer regions (Sherwood & Sheath 1999; Zuccarello & West 2002; Yen & Chen 2004; Iyer *et al.* 2005; Verbruggen *et al.* 2005; Provan *et al*. 2005*b*).

2.1.6. The aim of the study

One central tenet of phylogeography is that phylogeographic boundaries discovered across a species range often co-incide with known biogeographic boundaries (Avise 1994). The most recent phylogeographical studies on coastal and estuarine South African invertebrates (Teske *et al.* 2006; Teske *et al.* 2007(*a*); Teske *et al*. 2007(*b*); Edkins *et al.* 2007 and Zardi *et al.* 2007) support this concept.

The aim of this study was to test whether the phylogeographical patterns of two South African intertidal red seaweeds (*Gelidium pristoides* and *Hypnea spicifera*) are also characterized by phylogeographic boundaries that co-incide with the known biogeographic boundaries.

2.2 MATERIALS AND METHODS

2.2.1 Sampling and preservation

One to five samples of each species were collected at the sampling sites shown on Figures 2.4. The samples were immediately preserved in 70% ethanol upon collection.

2.2.2 DNA extraction

Total genomic DNA was extracted following a modified hot CTAB DNA extraction protocol from Doyle & Doyle (1987) (Appendix 1). To make sure that each sample corresponded to a different individual, only one erect thallus fragment was used for DNA extraction from each tuft. Each selected thallus fragment was cleaned with 70% ethanol before DNA extraction to remove any contaminants such as epiphytes.

2.2.3 Primers

A nuclear ribosomal gene control region (*ITS1*) and mitochondrial control region (*Cox2-3* spacer) were amplified through the polymerase chain reaction (PCR) using the primer sequences presented in Table 2.1 below.

Table 2.1 The details about the *ITS1* and *Cox2-3* spacer regions

Primer	Length(bp)	Sequence	References
ITS-1F	19	5` -TCC GTA GGT GAA CCT GCG G- 3`	Bakker *et al.* (1995)
ITS-1R	20	5`-GCT GCG TTC TTC ATC GAT GC- 3`	Bakker *et al.* (1995)
Cox-2F	29	5`-GTA CCW TCT TTD RGR RKD AAA TGT GAT GC- 3`	Zuccarello *et al*. (1999)
Cox-3R	25	5`-GGA TCT ACW AGA TGR AAW GGA TGT C- 3`	Zuccarello *et al*. (1999)

2.2.4 Amplification – PCR

PCR amplifications were conducted using a ThermoHybaid PCR Sprint Temperature Cycling System under the following cycling parameters: initial denaturation at 95°C for three minutes, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 54-56°C for 45 seconds and extension at 72°C for 3 minutes. A final extension of 10 minutes at 72°C was included at the end of the PCR run. Annealing temperature was manipulated to obtain optimal PCR products. The PCR reagents and their volumes are presented in Appendix 2 for amplification of both *ITS1* and *Cox2-3* spacer regions.

2.2.5 Visualization of PCR products

The PCR products were run on 1% agarose gels (solutions given in appendices). Before loading, 5µl of each PCR product was mixed with 5µl of loading buffer (Bromophenol blue and xylene cyanol in glycerol). Then, the mixtures were loaded onto a gel and left to run in a gel rig for at least five minutes at 150 volts. PCR products were visualized using a UV transilluminator. A clean bright band was taken as a positive result. Smearing indicated a negative result and in these cases PCR conditions were manipulated (changing annealing temperature or number of PCR cycles) to obtain optimal bands.

Figure 2.4. Map of South Africa showing the sampling sites for *Hypnea spicifera* (a) and *Gelidium pristoides* (b).

2.2.6 Purification of PCR products

PCR products were purified using the Promega Magic PCR Preps TM, QIAGEN© QIAquick^{TM} or Promega Wizard ® kits following the manufacturers' instructions. The purified PCR product was finally eluted with 10µl nuclease free water or 10µl of elution buffer. The eluted purified PCR-product was checked for brightness (i.e. product concentration) by running 2µl of the product, with 5µl water and 5µl of a loading buffer on a 1% agarose gel. Purified PCR products were visualised by UV transilluminator. Clear bright bands were taken as positive results indicating purified PCR products.

2.2.7 Cycle sequencing

Purified PCR products were used for cycle sequencing reactions prepared for both forward and reverse primers. The amounts of the DNA template and water were manipulated depending on the quality of the DNA template (i.e. brightness of the PCR product under UV light). The final reaction volume was always 20µl. The reagents and their volumes used for sequencing reactions are presented in Appendix 3 for both markers. Sequencing reactions were conducted on a ThermoHybaid PCR Sprint Temperature Cycling System under the following cycling parameters: 95°C for 45 seconds, 50°C for 45 seconds and 60°C for 3 minutes repeated for 30 cycles. Cycle sequencing products were precipitated using an EDTA/Sodium Acetate procedure (Appendix 4). Sequences were then generated from both directions on an automated ABI Prism BigDye Terminator v3.0 or v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystematics) at Rhodes University's Sequencing Unit.

2.2.8 Sequence editing and alignment

Both forward and reverse sequences were assembled, checked and edited using Sequencher^{TM} version 3.1.1 and version 4.2.2. (Gene Codes Corporation). Consensus sequences were exported into MacClade version 4.06 (Maddison & Maddison 2000). In phylogenetic analyses, sequence alignment is crucial because sequences that are incorrectly aligned will give misleading results. Non-gene encoding DNA regions such as the *Cox2-3* spacer region are known to have a higher mutation rate than gene encoding DNA regions (Zuccarello *et al*. 1999). Such non-gene encoding DNA regions experience a high frequency of insertion/deletion mutations (indels) of varying lengths, making sequence alignment problematic (Small *et al*. 1998). Therefore, it is important that the indel mutations which cause length variation, are recognized and used during the alignment of length-variable sequences (Asmussen & Chase 2001). Final *ITS1* and *Cox2-3* sequence alignments are shown in Appendix 8 for both *G. pristoides* and *H. spicifera*.

2.2.9 Phylogenetic reconstruction

The aligned sequence matrices were used to reconstruct phylogenetic trees. Converting the information in nucleotide sequences into an evolutionary tree is the most important step in phylogenetics. Inferring an evolutionary relationship is an estimation procedure in which the best estimate of the evolutionary history is made on the basis of incomplete information (i.e. *ITS1* and *Cox2-3* spacer region data). Therefore, it is important to emphasize that an inferred tree is only as good as the assumptions on which the method of phylogenetic reconstruction is based (Smith 1998).

The Neighbor Joining (NJ) method (Saitou & Nei 1987) was used for phylogenetic reconstruction. The NJ analyses were conducted using the program PAUP* version 4.0b10 (Swofford 2002). NJ is a distance method that converts the aligned sequences into a distance matrix of pairwise differences/distances between the sequences (Swofford *et al*. 1996). Then it uses the pairwise distances between the sequences to group the sequences that are most similar (Langdale & Harrison 2006). NJ analyses can handle large data sets in less computational time than can parsimony methods (Page & Holmes 1998). However, the NJ method does not give information on which characters contribute to particular groupings (Langdale & Harrison 2006). NJ trees were generated based on the Jukes-Cantor model of DNA sequence evolution (Jukes & Cantor 1969). This model assumes that all base substitutions are equally likely and that all characters have equal rates of substitution (Huelsenbeck & Kirkpatrick 1996). The missing data were ignored for pairwise comparisons.

The trees were rooted using the midpoint rooting because this study was concerned with the grouping of specimens from geographical locations, rather than observing the ancestor-descendant relationship. The robustness of the topology of the trees was tested through bootstrap analyses with 1,000 replicates. The bootstrap values indicate the percentage of times that a particular branch appears when characters from the original character matrix are sampled randomly to create a new character matrix (Graur & Li 2000).

2.3 RESULTS

Phylogeographic analyses of Gelidium pristoides

The *Cox2-3* spacer region data analyses included a total of 74 ingroup taxa. The marker produced an aligned data set of 399 characters of which 262 were constant, 38 were variable but parsimony uninformative and 99 were parsimony informative. The data set had unequal frequency of bases (A = 29%; C = 12%; G = 28%; T = 36%). The *ITS1* region data analyses included a total of 70 ingroup taxa. The marker produced an aligned data set of 291 characters of which 263 were constant, 27 were variable but parsimony uninformative and only 1 was parsimony informative. The data set had unequal frequency of bases (A = 29%; C = 16%; G = 15%; T = 40%).

The Neighbor Joining analyses of both the *ITS1* and *Cox2-3* spacer region data sets revealed two lineages within *G. pristoides* on the south coast: south-eastern and south-western lineages. The Neighbor Joining trees for the *ITS1* region and *Cox2-3* spacer are presented in Figures 2.5a and 2.5b respectively. The Neighbor Joining tree generated from the *Cox2-3* spacer region resolved two samples from Still Bay separately from all the samples comprising the south-western lineage (Figure 2.5b). However, the bootstrap value of 52% suggests that too much reliance should not be placed on that branching node. Nevertheless, some of the observed branching nodes were not strongly supported by the bootstrap values (68% and 65%) (Figure 2.5).

The two markers showed minor differences in the phylogeographic boundaries between their south-eastern and south-western lineages. The *ITS1* region revealed the phylogeographic boundary between the two lineages at the Gamtoos-Van Stadens Dunefields (Figure 2.6a) while the *Cox2-3* spacer revealed a phylogeographic boundary at the Alexandria Coastal Dunefields (Figure 2.6b). The sequence divergence between the south-eastern and south-western lineages was 0.358% and 0.00352% for the *Cox2-3* spacer region and *ITS1* region respectively. The evolutionary divergence between the south-eastern and south-western lineages was substantial for *Cox2-3* spacer (52 nucleotide difference) and very slight for the *ITS1* region (1 nucleotide difference).

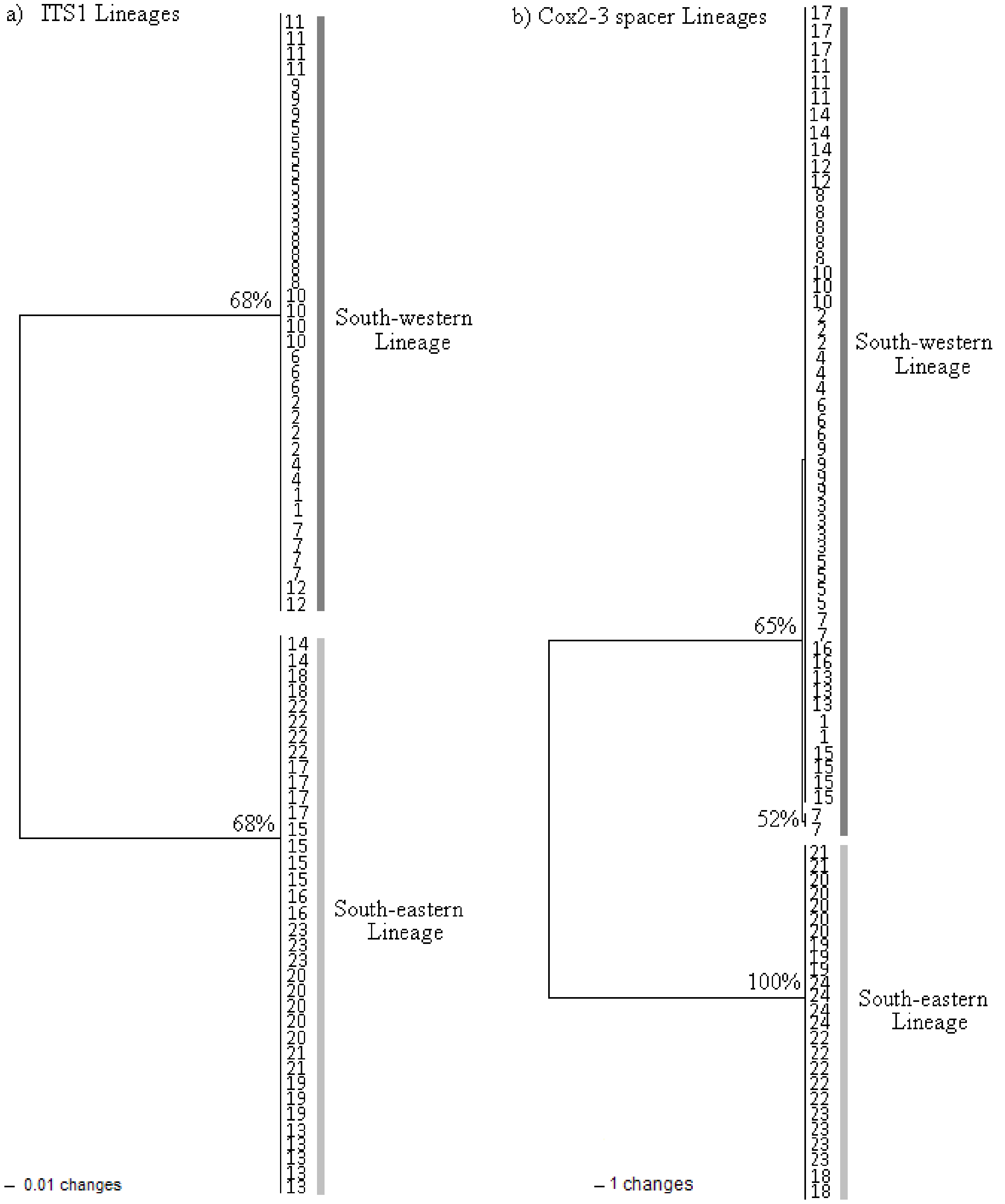

Figure 2.5. The Neighbor Joining trees of *Gelidium pristoides* generated from the *ITS1* data (a) and the *Cox2-3* spacer data (b). The bootstrap values (1000 replicates) are shown above the branches.

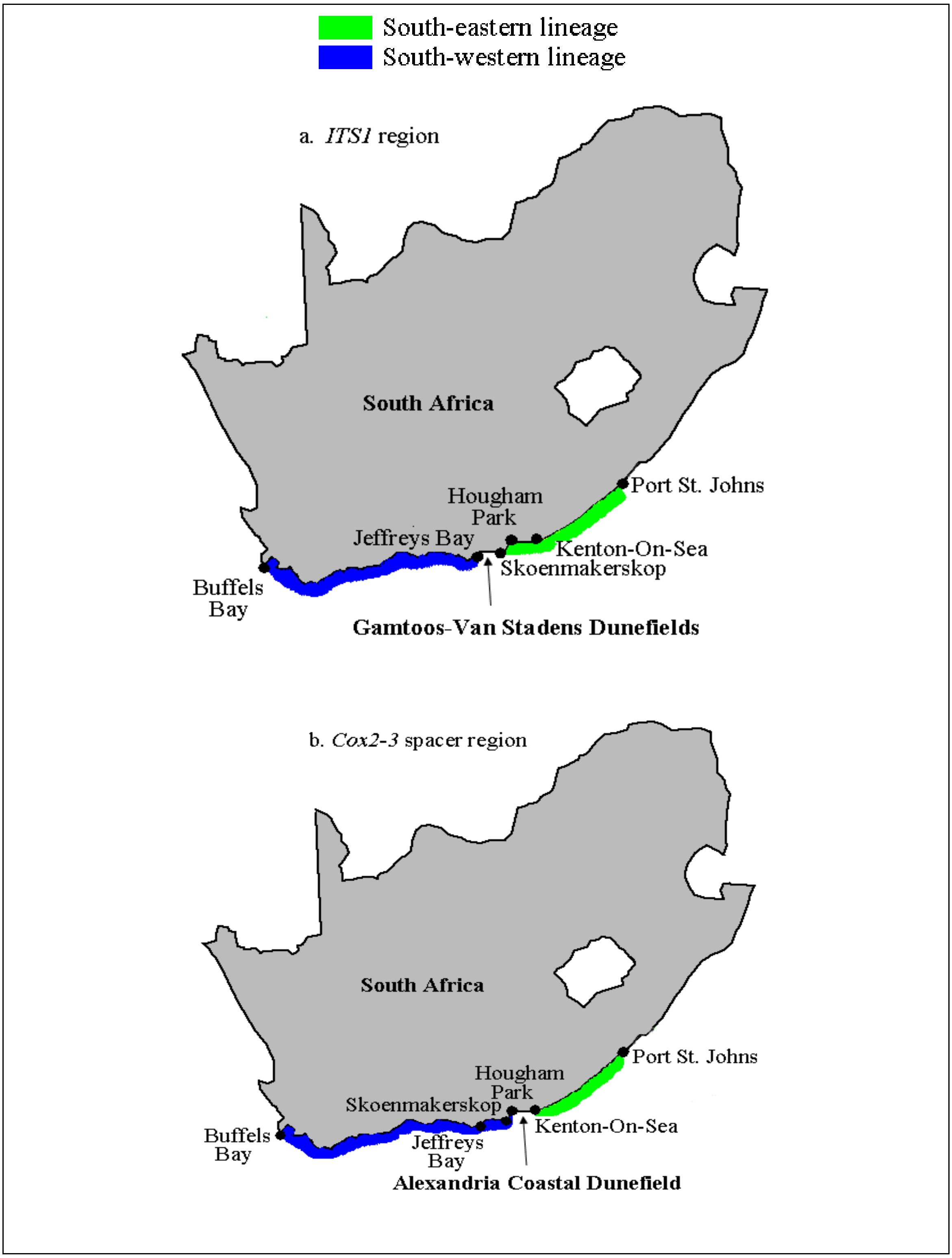

Figure 2.6. Phylogeographic disjunctions within *G. pristoides* revealed by: (a) the *ITS1* at the Gamtoos-Van Stadens Dunefields and (b) *Cox2-3* spacer at the Alexandria Coastal Dunefields.

Phylogeographic analyses of Hypnea spicifera

The *ITS1* region data analyses included a total of 80 ingroup taxa. The marker produced an aligned data set of 238 characters of which 150 were constant, 87 were variable but parsimony uninformative and only 1 was parsimony informative. The data set had unequal frequency of bases (A = 32%; C = 20%; G = 18%; T = 30%). The Neighbor Joining tree generated from the *ITS1* data resolved thirteen samples (from six sites) as a separate lineage from the remaining samples (Figure 2.7a). These thirteen samples were female gametophytes of *H. spicifera*, identified by the presence of cystocarps which were clearly visible on the branchlets. Samples on the main clade had no cystocarps (Sixty-five samples examined). The tetrasporophyte plants from the same localities as those thirteen samples in cystocarpic generations were resolved in the main clade (Figure 2.7a). Thus, the *ITS1* region shows genetic differences only between the tetrasporophyte and cystocarpic generations within *H. spicifera* (Figure 2.7a). The *Cox2-3* spacer region did not resolve the samples in cystocarpic generations separately from the tetrasporophyte generations.

The *Cox2-3* spacer region data analyses included a total of 69 ingroup taxa. The marker produced an aligned data set of 354 characters of which 303 were constant, 50 were variable but parsimony uninformative and only 1 was parsimony informative. The data set had unequal frequency of bases (A = 35%; C = 10%; G = 12%; T = 43%). The Neighbor Joining analyses of the *Cox2-3* spacer region resolved most samples on the same clade except two samples from Haga-Haga and one from Port St. Johns (Figure 2.7b). These samples differed from the other samples by one substitution, probably accumulated through random mutation.

The Neighbor Joining analysis from both the *ITS1* and *Cox2-3* spacer data sets showed insignificant genetic variation within *H. spicifera* samples collected from Camps Bay (west coast) to Shaka`s Rock (East coast). Both markers indicated that *H. spicifera* comprise a single genetic lineage distributed along the coast across two of the biogeographic regions recognized by Bolton & Anderson (1997). However, it must be noted that the Benguela region was represented by only one locality (Camps Bay) from which five samples were analysed.

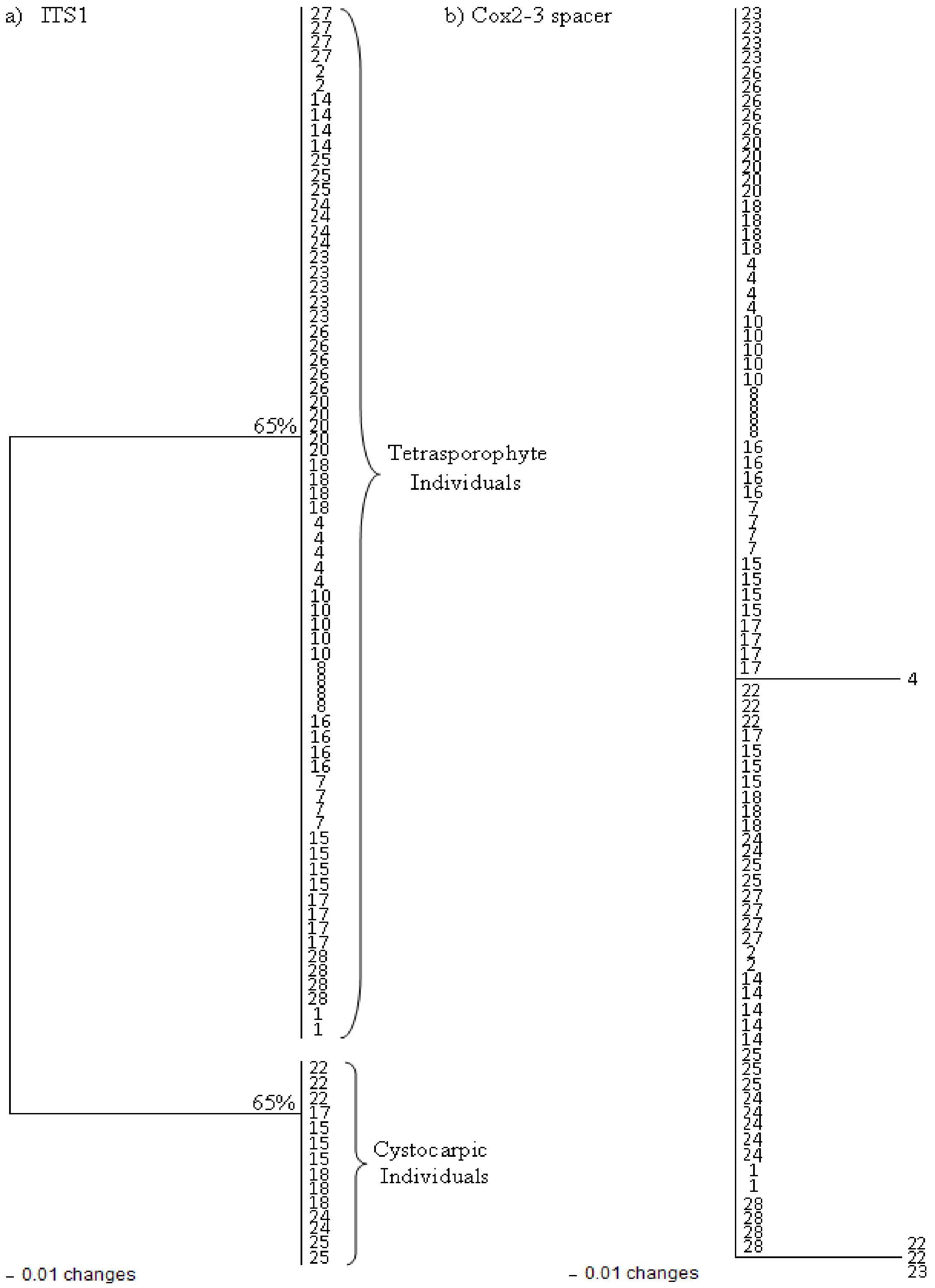

Figure 2.7. The Neighbor Joining trees of *Hypnea spicifera* generated from the *ITS1* data (a) and *Cox2-3* spacer data (b). The bootstrap values (1000 replicates) are shown above the branches.

2.4 DISCUSSION

2.4.1 Phylogeographic pattern of *Gelidium pristoides*

Phylogeographic boundaries

The South African coastline comprises approximately 80% coastal dunefields and sandy beaches distributed intermittently along the coastline (Tinley 1985). This study suggests that the Alexandria Coastal Dunefield and the Gamtoos-Van Stadens Dunefields (Figure 2.8) act as a barrier to gene flow in *G. pristoides*. The former dunefield was also found to act as a barrier to gene flow for the estuarine cumacean *Iphinoe truncata* (Teske *et al*. 2006). The Alexandria Coastal Dunefields, as currently located, dates back 6,500 years and is the largest coastal dunefield in South Africa (Illenberger & Rust 1988; Lubke & Moore 1998). The dunefield stretches approximately 50km eastwards from Sundays River mouth to Cape Padrone (Illenberger & Rust 1988).

The two lineages revealed by the *Cox2-3* spacer diverged 0.50 - 0.58 Ma using the percentage divergence of 1.8% per 3 - 3.5 Ma for red seaweeds estimated by Zuccarello & West (2002) using the closing of the Isthmus of Panama as a calibration point. Although this implies the lineages diverged hundreds of thousands of years before the formation of the present Alexandria Coastal Dunefields, it is probable that there were dunefields in the same area during past glacial periods (Tinley 1985).

Coastal dunefields in this region were formed during the Mio-Pliocene when strong wind systems blasted across massive deposits of exposed shelf sediment (Tinley 1985). These dunefields, however, were subsequently destroyed or submerged by rising sea levels during the warm interglacial periods (Tinley 1985), and subsequently new dunefields were formed over the old ones during the Holocene (Illenberger & Verhagen 1990).

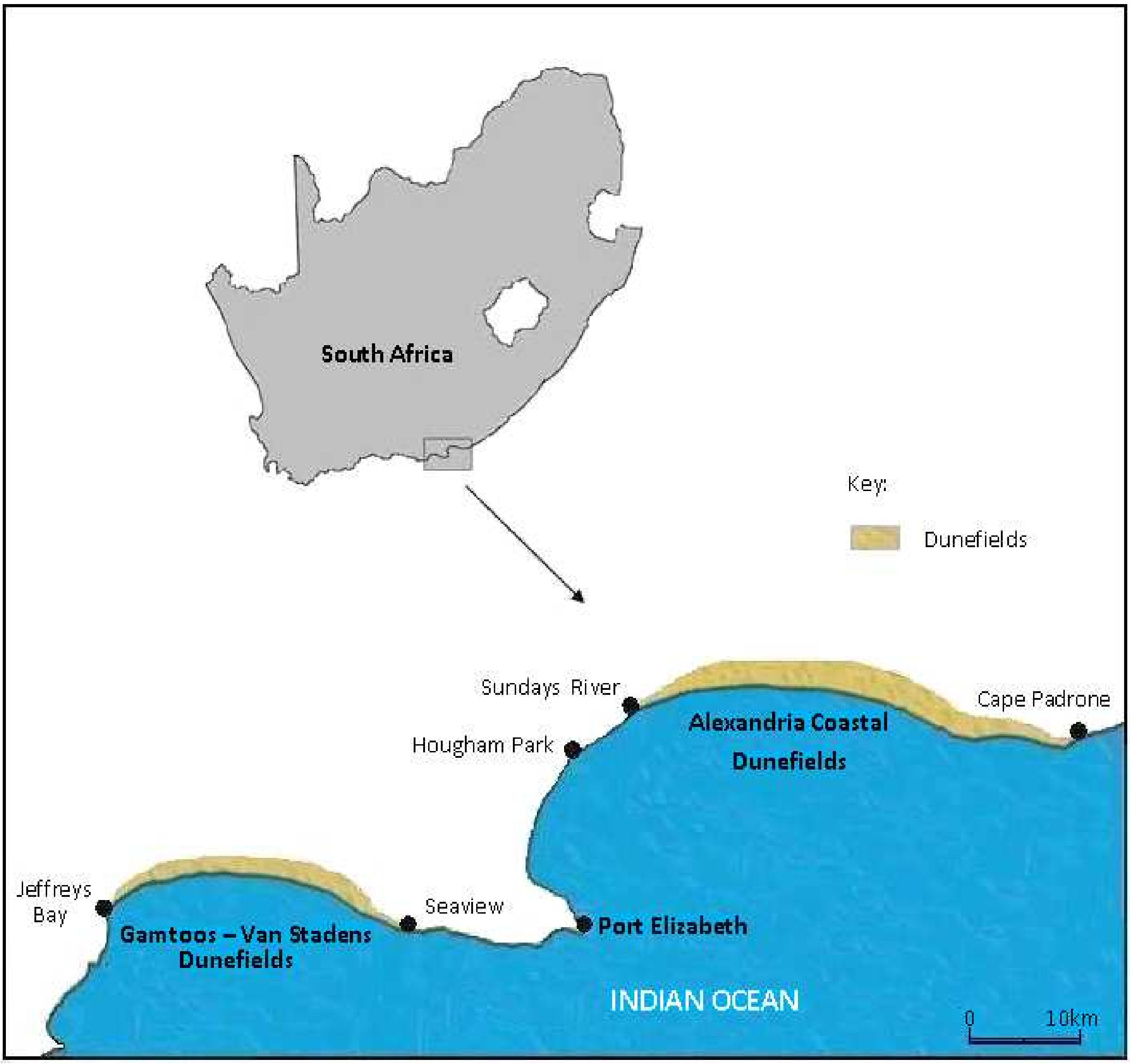

Figure 2.8. Map of South Africa showing location of the Alexandria Coastal Dunefield and the Gamtoos-Van Stadens Dunefields which act as barriers to gene flow for *Gelidium pristoides.*

Thus, the Alexandria Coastal Dunefield as developed currently is superimposed on the ancient dunefields which may have acted as a dispersal barrier to gene flow for *G. pristoides*. This is supported by the *Cox2-3* spacer sequence divergence which dates back 500, 000 – 580, 000 years, a period when the ancient dunefields was probably still in existence. Teske *et al*. (2006) also found that sequence divergence between two lineages of the cumacean *Iphinoe truncata* dates back millions of years before the existence of the Alexandria Coastal Dunefield. Similarly, they suggested a possible dispersal barrier to have been the existence of the coastal dunefields in the same area during the last interglacial periods.

The Gamtoos-Van Stadens Dunefield (Figure 2.8) is relatively small compared to the Alexandria Coastal Dunefield, it covers approximately 30km coastline between Seaview and Jeffreys Bay (Lubke & Moor 1998). This dunefield started forming during the Holocene (6,500 – 4,000 years ago) based on the dating of the carbonate component of the sands (Illenberger & Verhagen 1990). The two lineages revealed by the *ITS1* diverged 4,224 – 4,928 years ago using the *ITS1* percentage divergence of 2.5% per 3 - 3.5 Ma for red seaweeds (Zuccarello & West 2002), indicating the barrier is of Holocene origins. Thus, the dispersal barrier at the Gamtoos-Van Stadens Dunefield was formed relatively recently compared to the barrier at the Alexandria Coastal Dunefield.

The minor difference regarding the location of the phylogeographic boundary between the *ITS1* and *Cox2-3* spacer regions is probably due to the dating differences between the two dispersal barriers. The dispersal barrier at the Alexandria Coastal Dunefield has been in existence much longer (more than a million years ago) than the dispersal barrier at the Gamtoos-Van Stadens Dunefield which appeared just less than 6,500 years ago.

Furthermore, the evolutionary divergence of only one character in the *ITS1* sequences strongly supports the suggestion that the boundary at the Gamtoos-Van Stadens Dunefield appeared relatively recently. Such a close range in the *ITS1* sequences divergence has been observed before in recently diverged seaweed species (Chopin *et al*. 1996; Leclerc *et al*. 1998, Shimada *et al*. 1999; Shimada & Masuda 2003). Additionally, it is well known that the *ITS1* is the most appropriate marker for revealing relatively recently diverged taxa due to its rapid evolutionary rate (Yeh & Chen 2004; Provan *et al.* 2005*a*; Hu *et al*. 2007*a*).

The appearance of the two phylogeographic boundaries can be strongly associated with two factors: the coastal topography and poor habitat conditions in the dunefields. The former factor possibly limits the chances of fertile thallus fragments crossing the two dunefields. The flow of the Agulhas Current is directed by the coastal topography (Lutjeharms & Ansorge 2001). The continental shelf increases in width around the two dunefields, as a result the Agulhas Current shifts gradually offshore when it approaches the dunefields (Lutjeharms & Ansorge 2001) (Figure 2.9). This limits the chances of fertile thallus fragment crossing the dunefields successfully and remaining viable, as there is a suggestion of water retention within the large bays where these dunefields lie (Roughan *et al*. 2005).

Furthermore, the west-southwest wind that supplies the dunefields with sands moves perpendicular to the boundaries (Illenberger & Rust 1988), counter-acting southward dispersal of fertile thallus fragments across the boundaries. Also, *G. pristoides* has a strong thallus and attaches tightly onto its substrata (Carter 1985). Thus, it is not easy for the tufts to be dislodged by strong waves. Overall, the chances are very slim if not nil that drifting fertile thallus fragments of *G. pristoides* can cross the Alexandria Coastal Dunefield or the Gamtoos-Van Stadens Dunefields.

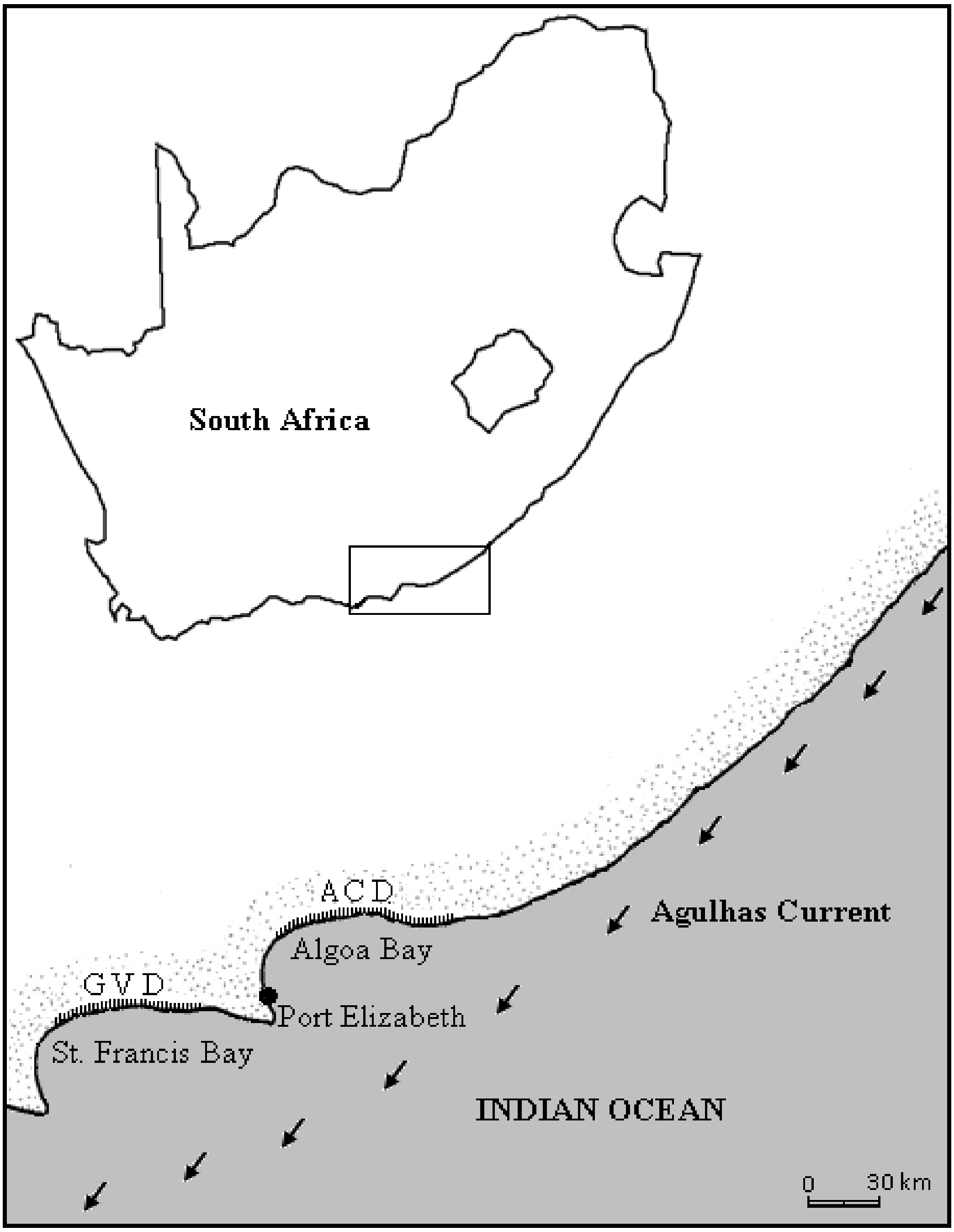

Figure 2.9. Map showing direction of the Agulhas Current along the two dunefields.

ACD = Alexandria Coastal Dunefield, GVD = Gamtoos- Van Stadens Dunefields.

It has been well documented that continuously distributed species with low dispersal-distances can show phylogeographic boundaries that appear without any historical biogeographic barrier to gene flow (Saunders *et al*. 1986; Neigel & Avise 1993; Avise 2000; Irwin 2002; Kou & Avise 2004). This idea was extensively analysed and explained thoroughly by Irwin (2002) and Kou & Avise (2004) using computer simulations.

Irwin (2002) indicated that the probability of observing phylogeographic boundaries in the absence of historical biogeographic barriers to gene flow is high under the following conditions: (1) the species has a very low dispersal-distance, (2) there is slight habitat variation across the species linear distributional range, (3) the species has very low population size or (4) the species has experienced local extinction.

Thus, it is probable that the observed phylogeographic boundaries within *G. pristoides* appeared without any historical biogeographic barrier to gene flow because: (1) the spores of *Gelidium* species do not disperse over long distances and have the strongest capacity for attachment immediately after being released (Santelices 1990). It is believed the spores settle and grow into the next phase within days (Santelices 1990). If by any chance the spores are dispersed, it can only be for few meters after which they become non-viable (Alberto *et al.* 1999). Presumably the spores of *G. pristoides* behave in the same way as in the genus in general.

(2) the Alexandria Coastal Dunefield and the Gamtoos-Van Stadens Dunefield do not provide suitable habitats for *G. pristoides*. These dunefields stretches for approximately 50km and 30km respectively and *G. pristoides* spores can only disperse for a few meters. Therefore, it would be difficult for the species to maintain gene flow across the two dunefields. For example, in order to

maintain gene flow across the Alexandria Coastal Dunefield, the spores of *G. pristoides* must be dispersed for a distance of at least 50km to find the most immediate available suitable habitat which is at Hougham Park.

(3) the effective population size for mtDNA is always low within *G. pristoides* populations due to the relatively low number of gametophytes (Carter 1985).

(4) the *ITS1* and *Cox2-3* spacer regions revealed no intermediate lineages. In short-distance dispersal species characterized by phylogeographic breaks, mtDNA will tend to evolve through bifurcating lineages due to its non-recombining inheritance (Irwin 2002). As a result, molecular analyses of the marker will show no intermediate lineages and individual samples can belong to either of the two deepest lineages (Irwin 2002). In this study, the *Cox2-3* spacer analyses showed no intermediate samples/individuals within *G. pristoides*; all samples analysed through Neighbor Joining were resolved either as south-western or south-eastern lineages. Even the Neighbor Joining analyses of the *ITS1* data resolved samples into either the south-western or south-eastern lineage, with no intermediate lineages.

Generally both the *ITS1* and *Cox2-3* spacer resolved two genetic lineages within *G. pristoides*: the south-eastern and south-western genetic lineages. The phylogeographic boundary between the two lineages is in the vicinity of Port Elizabeth. The map in Figure 2.10 shows that the south-eastern lineage revealed by *ITS1* and the south-western lineage revealed by *Cox2-3* spacer overlap/co-occur in the vicinity of Port Elizabeth. Individual samples collected within the overlapping region were resolved differently by each marker. The *Cox2-3* spacer region resolved 15 samples collected from five sites within the overlapping region in the south-western lineage.

However, the *ITS1* resolved the very same 15 samples in the south-eastern lineage. This is probably because the two lineages evolved at different evolutionary time scales.

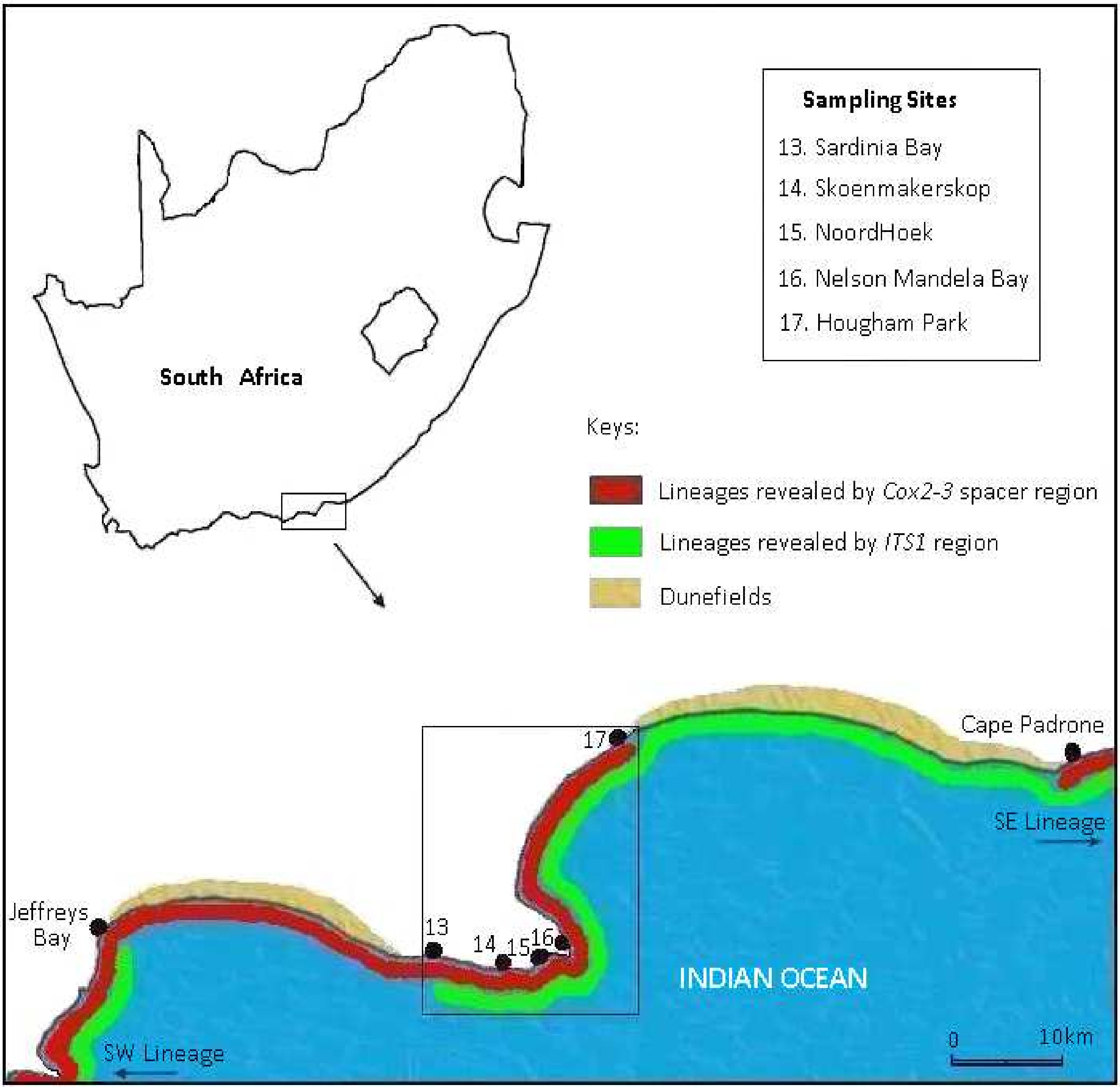

Figure 2.10. Map showing the overlapping region between the *ITS1* south-eastern lineage and the *Cox2-3* spacer region south-western lineage. The two lineages overlap in the vicinity of Port Elizabeth. SW lineage = south-western lineage, SE lineage = south-eastern lineage.

The *Cox2-3* spacer south-western lineage evolved during the Pleistocene (500, 000 – 580, 000 years ago) whereas the *ITS1* south-eastern lineage evolved during the Holocene (4,224 – 4,928 years ago). The time interval since the evolution of the *Cox2-3* spacer south-western lineage until the evolution of the *ITS1* south-eastern lineage is very long, more than 400,000 years. As a result, the lineages are so evolutionarily divergent that within an individual sample the south-eastern lineage is represented by the *ITS1* and the south-western lineage is represented by the *Cox2-3* spacer where the two lineages overlap (Figure 2.10).

Phylogeographic boundaries incongruent with Biogeographic boundaries

The two phylogeographic boundaries do not co-incide with the known biogeographic boundaries of seaweeds described by Bolton & Anderson (1997) and it is suggested the identified lineages appeared in the absence of historical biogeographic barriers to gene flow.

The interpretation of phylogeographic structure seems straightforward when the phylogeographic boundaries co-incide with the known biogeographic boundaries. In such cases, researchers usually assume that the phylogeographic boundaries are due to historical biogeographic barrier to gene flow, boundaries between cryptic species or regions of contact between historically allopatric populations (Irwin 2002). As a result, theoretical analyses of gene trees have generally focused on how phylogeographic breaks can appear as a result of biogeographic barrier to gene flow (Nei & Takahata 1993; Wakeley & Hey 1997). However, it has been shown that sometimes phylogeographic boundaries do not co-incide with known biogeographic boundaries (Burton 1998; Irwin 2002; Teske *et al*. 2006). Burton (1998) further indicated that different biogeographic boundaries may have less or greater effect on the phylogeographic patterns of different species.

It has long been known that phylogeographic breaks can appear even in the absence of longstanding biogeographic barriers to gene flow (Neigel & Avise 1993; Burton 1998; Avise 2000). However, most phylogeographic studies that discover genetic disjunctions assume that the disjunction appeared due to longstanding historical biogeographic barrier to gene flow (Irwin 2002). Furthermore, Irwin (2002) emphasized that sometimes genealogical distributions can be influenced by chance, such as in the case of genetic drift. Thus it should be considered that sometimes genealogical breaks in a continuously distributed species can arise as a result of stochastic causes rather than historical biogeographic barriers to gene flow (Irwin 2002).

It seems the *Cox2-3* spacer phylogeographic break at the Alexandria Coastal Dunefield reflects a historical biogeographic barrier to gene flow because: (1) the phylogeographic boundary at the Alexandria Coastal Dunefield co-incides with the biogeographic boundary for seaweeds between the warm-temperate Agulhas Region and the subtropical Natal Region described by Stephenson (1948). According to Stephenson (1948) the biogeographic boundary between the two biogeographic regions is within the transitional zone between Port Elizabeth and Port Alfred. (2) the *Cox2-3* spacer is inherited maternally without recombination (Zuccarello *et al*. 1999; Zuccarello & West 2002) and thus gives the evolutionary history of the haploid female gametophytes within *G. pristoides*. The marker revealed two distinct lineages of female gametophyte within *G. pristoides*.

However, it is also possible that the phylogeographic boundary at the Alexandria Coastal Dunefield does not reflect a historical biogeographic barrier to gene flow because: (1) currently the subtropical Natal Region is not recognized as one of the biogeographic regions for seaweeds (Bolton & Anderson 1997); so the Alexandria Coastal Dunefield does not co-incide with any of the biogeographic boundaries recognized by Bolton & Anderson (1997).

(2) maternally inherited and non-recombining markers like the mitochondrial *Cox2-3* spacer are more likely to show deep phylogeographic boundaries that appeared without historical biogeographic barriers to gene flow (Avise 2000). These markers have one-fourth effective population of the nuclear genes, do not go under recombination and lineage sorting is completed rapidly (Irwin 2002). The probability of observing phylogeographic boundaries that appeared in the absence of biogeographic barriers increases with decreasing population size, and thus maternally inherited markers are more likely than nuclear markers to show such boundaries (Irwin 2002). Furthermore, there are generally far fewer individuals of the female gametophyte generation within *G. pristoides* populations (Carter 1985).

The idea that the two phylogeographic boundaries appeared without a historical biogeographic barrier to gene flow is further supported by Aspect II of genealogical concordance. Aspect II is one of the four distinct aspects of genealogical concordance (Avise 2000; Kou & Avise 2004). The latter authors indicated that according to Aspect II of genealogical concordance …

> “there must be agreement across independent loci with respect to the geographic positions of salient genealogical breaks within a species” (pp 184).

Furthermore, Kou & Avise (2004) concluded that in phylogeographic inference Aspect II should be a key consideration when inferring the existence of historical biogeographic barriers to gene flow. Two independently evolving markers (*ITS1* and *Cox2-3* spacer regions) were not congruent regarding the location of a phylogeographic boundary between the two lineages. This implies the results are not in accordance to Aspect II of genealogical concordance. Thus, it is suggested that the two phylogeographic boundaries discovered within *G. pristoides* appeared due to lack of suitable habitat coupled with short dispersal-distances of the spores rather than a biogeographic boundary to gene flow.

Therefore, the biogeographic boundary discovered by Stephenson (1948) in the vicinity of Port Elizabeth between the warm temperate Agulhas and the subtropical Natal Regions does not influence phylogeographic patterns of *G. pristoides and H. spicifera*. This is probable considering that Bolton & Anderson (1997) in a review on marine vegetation of Southern Africa did not recognize any biogeographic boundary of seaweed in the vicinity of Port Elizabeth.

In fact, there is a combination of biological factors (i.e. very short dispersal-distance, low gametophyte generations) and physical factors (i.e. poor habitat conditions, the west south-west wind and the Agulhas current) that together led to the appearance of the observed phylogeographic boundaries at the Alexandria Coastal Dunefield and the Gamtoos-Van Stadens Dunefield.

2.4.2 Phylogeographic pattern of *Hypnea spicifera*

Both the *ITS1* and *Cox2-3* spacer region results indicate that *H. spicifera* populations from Camps Bay to Shaka`s Rock have a fairly uniform genetic structure. It is very probable that the observed uniform genetic structure within *H. spicifera* populations is due to the species`s potential for long range expansion. *H. spicifera* was thought to inhabit only the intertidal zones (Hewitt 1955). But the species has recently been observed from the subtidal (Anderson RJ, pers.comm). This makes even more sense considering that *H. spicifera* is one of the most widely distributed seaweed species along the coastline. Hewitt (1955) also indicated that the spores of this species are denser than water and sink onto the subtidal after being released. Thus, by virtue of inhabiting both the intertidal and subtidal zones, the level of gene flow among *H. spicifera* populations is probably higher than that of *G. pristoides*. Such a persistent level of gene flow ultimately resulted in a fairly uniform genetic structure among the populations.

It is plausible that long range expansion of the species might be due to the species` potential for long distance-dispersal via spores and drifting fertile thallus fragments. Apparently it is not clear whether *H. spicifera* spores have the potential for long distance-dispersal, but studies from other *Hypnea* species have shown that their spores can remain viable *in vitro* for 3 months (Mshigeni & Lorri 1977). Probably *H. spicifera* spores could be dispersed for long distances if they can also remain viable *in situ* for such long periods. The spores could be dispersed for long distances via oceanic currents if their sinking velocity is relatively slow as indicated by Gaylord *et al*. (2002) for several nearshore seaweed species.

Long distance-dispersal of *H. spicifera* via fertile thallus fragment is more probable because *Hypnea* tufts are easily dislodged by strong waves (Hewitt 1955; Reis & Yoneshigue-Valentin 2000; Kong & Ang 2004). For example, tufts of *H. spicifera* were observed washed-up by the swell at Cape Hangklip (Personal observation). It is not known how far they were dispersed to end up there.

The *ITS1* data analyses showed genetic variation between the cystocarpic and the tetrasporophyte individuals (Figure 2.11). The *ITS1* sequences of the two generations were very similar, differentiated by only one character. Genetic variation between sporophyte and gametophyte individuals has been reported before within seaweeds populations, mainly through isozyme electrophoresis (Fujio *et al*. 1985; Sosa & Garcia-Reina 1992; Sosa *et al*. 1998). These studies found that the predominance of asexual reproduction combined with genetic drift (due to lower numbers of gametophytes than tetrasporophytes) reduces gene flow and fixes alleles between the two generations, resulting in genetic variation (Sosa & Garcia-Reina 1992; Sosa *et al*. 1998). The observed genetic variation between the cystocarpic and tetrasporophyte individuals within *H. spicifera* might also be due to predominance of asexual reproduction, reducing gene flow between the two generations.

The fact that only the *ITS1* marker showed sequence variation between the two generations might suggests the variation is due to the differences in ploidy level between the two generations. The nuclear *ITS1* is inherited biparentally and the cystocarpic generations are haploid while the tetrasporophytes generations are diploid. As a result, the *ITS1* sequence is different between the haploid cystocarpic and diploid tetrasporophyte generations within *H. spicifera*. Though, this defies the random mixture of genotypes between the two generations during meiosis. However, Sosa & Garcia-Reina (1992) indicated that seaweed populations that reproduce asexually for several years can show non-random mixture of genotype due to reduced gene flow and fixation of alleles between the haploid and diploid generations.

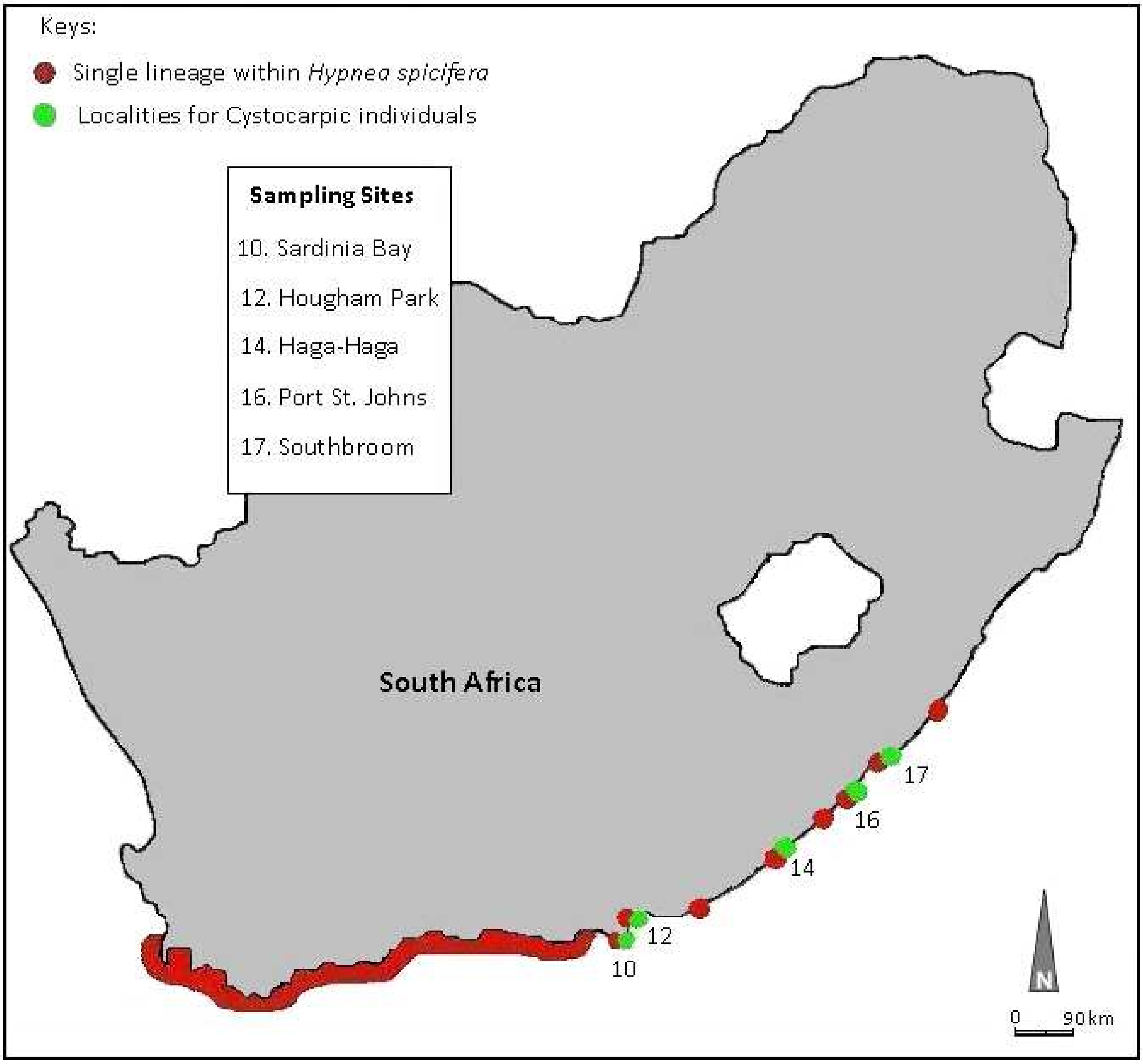

Figure 2.11. Map showing the genealogical distribution within *H. spicifera* from the *ITS1* data. The numbers on the map shows the localities where the cystocarpic individuals were found, only east of Sardinia Bay.

Nevertheless, the possibility exists that natural selection might be acting in both generations, thus selecting a specific copy of the *ITS1* for each generation. Natural selection has also been suggested as possible factors driving differences in allelic frequency between the gametophyte and sporophyte generations in several *Gelidium* spp (Sosa *et al*. 1998).

The separate resolution of only thirteen cystocarpic individuals can be attributed to the imbalance ratio between the gametophyte and tetrasporophyte generations. In natural populations of *H. spicifera*, the gametophyte generation always occurs at lower abundances than the tetrasporophyte generation (Hewitt 1955). Due to the imbalanced ratio between the two generations, only thirteen samples in cystocarpic generation were collected from all the sampling sites. The samples from each of the five sites shown in Figure 2.11 were collected at the same time, raising a possibility of temporal dominance variation in abundance of one generation.

Only the cystocarpic generation was observed; no male gametophytes were observed. Reis & Yoneshigue-Valentin (2000) also did not find any male gametophytes of *H. musciformis* in three populations from the Rio de Janeiro coast (Brazil). The absence of male gametophyte and the rare occurrence of cystocarpic individuals has been commonly reported within *Hypnea* populations (Mshigeni 1976; Rama Rao 1977; Schenkman 1989). Several explanations have been suggested for this. Rama Rao (1977) and Mathieson (1989) attributed this to the fact that tetraspores have low viability and do not germinate successfully, leading to a failure of recruit into the gametophyte generation. Reis & Yoneshigue-Valentin (2000) suggested that male gametophytes are elusive in natural populations because they manifest themselves for only a short period of time.

It is interesting to note that, the cystocarpic individuals came from Sardinia Bay eastwards up to Southbroom (Figure 2.11). Also, the south-eastern lineage of *G. pristoides* revealed from the *ITS1* data starts to appear at Sardinia Bay. The implication here is that the same factors may have led to the appearance of the south-eastern lineage of *G. pristoides* and separate resolution of *H. spicifera* cystocarpic generations. But more cystocarpic individuals will have to be sampled (especially west of Sardinia Bay) to confirm this hypothesis because the sampling in this study was limited by the availability of the cystocarpic individuals in the field. Interestingly, according to the results there were no cystocarpic individuals sampled west of Sardinia Bay. Presumably because they are very elusive in the field (Mshigeni 1976; Rama Rao 1977; Mathieson 1989; Reis & Yoneshigue-Valentin 2000).

The *ITS1* data showed genetic variation of one substitution among generations (gametophyte and tetrasporophyte) within *H. spicifera* and among two lineages within *G. pristoides*. The variation observed among *G. pristoides* lineages is ambiguous because the lineages were not sorted into generations. Thus this might reflect either genetic variation among the generations or within one generation.

However, it is probable that the observed *ITS1* variation in *G. pristoides* is mainly or exclusively within the bisporophyte generation, owing to its dominance in natural populations. The low level of genetic diversity within red seaweed can be indicative of the lack of gene flow between the haploid and diploid generations (Sosa *et al*. 1998). Thus, the possibility exists of genetic variation between the two generations within *G. pristoides* considering the observed low level of genetic diversity (two haplotypes).

2.5 CONCLUSION AND FUTURE RESEARCH

The Alexandria Coastal Dunefields and the Gamtoos-Van Stadens Dunefields act as dispersal barriers to gene flow for *G. pristoides*. The phylogeographic boundary at the latter dunefields appeared relatively recently compared to the boundary at the Alexandria Coastal Dunefields.

The mitochondrial *Cox2-3* spacer region did not reveal a relatively recent phylogeographic boundary revealed by the *ITS1* at the Gamtoos-Van Stadens Dunefields. Thus, based on this it can be deduced that *Cox2-3* spacer is not the appropriate marker for investigating the most recent evolutionary history within red seaweeds.

The study found that *H. spicifera* has almost uniform genetic composition along the coastline; however revealed genetic variation between the gametophyte and tetrasporophyte generations. Thus this study was in consistent with previous studies which found genetic variation between the gametophyte and tetrasporophyte generations in several *Gelidium* spp. (Fujio *et al*. 1985; Sosa & Garcia-Reina 1992; Sosa *et al*. 1996; Sosa *et al*. 1998). The lack of genetic variation within *H. spicifera* populations from Camps Bay to Shaka`s Rock is presumably due to long range expansion of the species as it inhabits both the intertidal and the subtidal zones.

In conclusion, the phylogeographical patterns of *G. pristoides* and *H. spicifera* are not characterized by phylogeographical structuring associated with the known biogeographic regions or phylogeographic boundaries coinciding with the known biogeographic boundaries of seaweeds. Thus, the accepted southern African biogeographic boundaries seem to have no impact on the phylogeographic structure of the two red seaweed species examined; in contrast to what has been found in some coastal invertebrates. Burton (1998) indicated that biogeographic boundaries may have strong or weak effects on the phylogeographic patterns of different species.

G. pristoides (short-distance disperser) and *H. spicifera* (long-distance dispersal seems to be more likely than in *G. pristoides*) show different phylogeographic patterns; presumably due to their different modes of dispersal. It is recommended future phylogeographical studies of seaweeds focus on species with relatively long-distance dispersal to establish if seaweeds are characterized by different phylogeographic patterns depending on the species` mode of dispersal. In invertebrates, it is known that southern African biogeographic boundaries delimit phylogeographic distribution of the species differently depending on their modes of dispersal (Teske *et al*. 2006, Teske *et al*. 2007*c*).

CHAPTER THREE: DISTRIBUTION OF THE EPIFAUNAL COMMUNITY ON *Gelidium pristoides.*

3.1. Introduction

The biological and physical factors regulating the distribution and community structure of seaweeds and intertidal invertebrates on South African rocky shores are well understood (Stephenson 1948; Brown & Jarman 1978; Emanuel *et al.* 1992; Bolton & Anderson 1997). As a result, it is well known that there are differences in species composition between the three main southern African biogeographic regions.

The warm-temperate Agulhas region has a high level of species richness, diversity and endemism (Griffiths & Prochazka 1999, Bolton & Stegenga 2002). The region has high species diversity of amphipods and isopods, and high endemism of mollusc species between Cape Point and Port Elizabeth (Griffiths & Prochazka 1999). The populations of *Gelidium pristoides* stretch for approximately 1000km covering the entire Agulhas region and partially the eastern and western coasts.

It is very important in ecology that factors affecting species richness and diversity of animals living in symbiosis with others are well understood (Pereira *et al.* 2006). The factors affecting the epifaunal species richness and diversity on seaweeds differ in relation to the scale of investigation. At large scales of investigation, factors such as geographical changes in seaweed composition and seasonal cycles have been found to affect the epifaunal community structure on seaweeds (Arrontes & Anadón 1990; Hull 1997; Russo 1997; Pereira *et al*. 2006).

At small scales, factors such as seaweed biomass, surface area and branching patterns have been found to influence epifaunal community structure (Russo 1990; Knowles & Bell 1998; Christie *et al*. 2003).

For example, Arrontes & Anadón (1990) found that the three dominant isopod species associated with three seaweed species (*Fucus vesiculosus*, *Gelidium latifolium* and *Cystoseira baccata*) show seasonal variation in abundances on the northern coastline of Spain. Russo (1997) found differences in the relative abundances of three epifauna taxa (amphipods, molluscs and polychaetes) on four sublittoral seaweed species (*Cystoseira barbata, Jania rubens, Laurencia obtusa* and *Padina pavonica*) among different regions in the eastern Mediterranean. The differences in hydrodynamic and physical conditions between the regions were thought to be influential in determining relative abundances of the epifauna (Russo 1997). Furthermore, Pereira *et al*. (2006) found differences in abundance and diversity of the major epifauna taxa (amphipods, isopods and tanaids) on several dominant seaweed species between the northern and southern coastlines of Portugal. Sea temperature, water currents and wave exposure were suggested as the main factors driving the distribution of the epifauna along the Portuguese coast (Pereira *et al*. 2006).

Marine invertebrates associated with seaweeds often adapt to the locally dominant seaweed hosts across their distributional range (Sotka 2005). Such local adaptation can result in the evolution of genetic variation among populations due to selection for the locally abundant seaweed host (Fox & Morrow 1981). Thus, the distribution of the epifaunal community can be genetically mediated due to preferences for locally abundant host species (Sotka 2005).

Gelidium pristoides is one of the dominant seaweed species on shores along the South African coastline (Anderson *et. al*. 1991). The biomass of *G. pristoides* in natural populations changes from season to season (Anderson *et al*. 1991), being high in late summer, January to March, and low in late winter, July to August (Carter & Anderson 1986). This species is densely tufted and thus its fronds and holdfast provide favourable shelter for the associated fauna (Beckley & McLachlan 1980). The epifaunal communities that live in symbiosis with the species comprise mainly amphipods, annelids, acari, copepods, gastropods, foraminiferans, harpacticoids, isopods, nematodes, nemerteans, pelecypods, ostracods, tardigrades and nauplian larvae (Beckley & McLachlan 1980; Beckley 1982; Anderson *et al*. 1991; Lubke & Moor 1998).

Beckley (1982) indicated that harvesting of *G. pristoides* holdfasts can detrimentally affect recruitment of the associated fauna. As a result, she suggested legislation be implemented to allow the harvesting of the fronds only as this would leave the holdfast intact, providing shelter for the associated fauna. However, a decade later without such legislation being implemented, Anderson *et al.* (1991) indicated that *G. pristoides* harvesting poses no habitat threat to the epifaunal community living in symbiosis with the species.

Approximately 2.8% of the total seaweed epifaunal species on this coast live on *G. pristoides* and none are specific to this seaweed (Anderson *et al*. 1991; Lubke & Moor 1998). The remaining epifaunal species live on other seaweeds in the lower eulittoral zone, including *Hypnea spicifera* (Lubke & Moor 1998). During harvesting, an average of 25% of *G. pristoides* biomass is removed from a particular shore (Anderson *et al.* 1991). This implies that only a negligible fraction of the total percentage of the epifaunal community is removed simultaneously (Lubke & Moor 1998).

Globally, most studies have investigated distributional relationships between epifaunal community structure and different seaweed species, seaweed architecture, seaweed biomass and different geographic regions (Russo 1997; Chemello & Milazo 2002; Christie *et al*. 2003; Pereira *et al*. 2006). Such studies are part of an all important multidisciplinary approach to biogeography, since they investigate factors driving the distribution of animals in symbiosis with seaweed.

However, apparently there are no local studies that have investigated distributional relationships between the seaweed epifaunal community structure and different locations along the coastline. The first study to investigate the epifaunal composition and abundances on *G. pristoides* thoroughly was on the south coast on St Croix Island (Figure 3.1) by Beckley & McLachlan (1980). These authors found that different littoral seaweed species on St Croix Island show variation in epifaunal composition and abundances due to their differences in architecture. Subsequent epifaunal studies on *G. pristoides* focused on local scales (sites) rather than biogeographic scales.

For example, Beckley (1982) analysed abundances of the epifauna on *G. pristoides* at St Croix Island. She found that amphipods, annelids, isopods, marine mites, molluscs and nematodes were the most abundant epifauna taxa per gram dry mass of *G. pristoides*.

Gibbons (1988) investigated the impact of wave exposure on the meiofauna associated with *G. pristoides* between exposed and sheltered shores in False Bay on the eastern side of the Cape

Peninsula (Figure 3.1). He found that copepods and ostracods were abundant on sheltered shores while amphipods and bivalves were abundant on exposed shores.

On the eastern coast, Anderson *et al.* (1991) analysed the abundances of the epifaunal community within *G. pristoides* samples collected from Glengariff (Figure 3.1) in March 1986, a time when seaweed biomass was high. They found that amphipods, gastropods and isopods were the most abundant epifauna taxa per gram dry mass of *G. pristoides*. However, Anderson *et al.* (1991) did not identify the epifauna to the species level but rather grouped the animals into those higher taxa.

3.2. The aims of the study

The main aims of this study were to:

(i) Compare the epifaunal species richness and diversity on *G. pristoides* among different localities along the coastline.

(ii) Describe distribution patterns of the epifaunal communities on *G. pristoides* along the coastline.

(iii) Determine if there is a relationship between distribution patterns of the epifaunal community and phylogeographic patterns in *G. pristoides*.

3.3. Materials and Methods

3.3.1 Study area

The study area comprised 10 sites along the South African coastline (Figure 3.1). The localities sampled were also sampled for the study of phylogeography on *Gelidium pristoides*. Sites were not characterized by similar levels of wave action. Unfortunately there were samples missing in the vicinity of Port Elizabeth where two phylogeographic boundaries were subsequently discovered within *G. pristoides*.

3.3.2 Sampling procedure

Most samples were collected between June and July 2007 (middle winter) except samples from Haga-Haga and Port St. Johns, which were collected in December 2007 (early summer) and samples from Port Alfred which were collected in March 2007 (late summer). At least two samples of *G. pristoides* tufts were collected at each site. Selected tufts were covered with a plastic bag before collection during low tide to retain as many epifaunal taxa as possible. The entire tufts (including the holdfast) were preserved in 70% ethanol upon collection. In the laboratory, the epifaunal species were isolated by washing the seaweed tufts with tap water in a 1368mm^3 container. The fraction of the epifaunal species retained within the seaweed was checked under a binocular dissecting microscope. All the epifaunal species were identified to the lowest possible taxonomic level (using identification keys in Day 1967; Day 1969; Griffiths 1976; Kensley 1978) and counted. After isolation and identification of the epifauna, the seaweed tufts were hand-dried with a cloth and the wet weight recorded in grams (Appendix 6). The same seaweed samples analysed for epifauna were also sequenced for *ITS1* and the *Cox2-3* spacer regions.

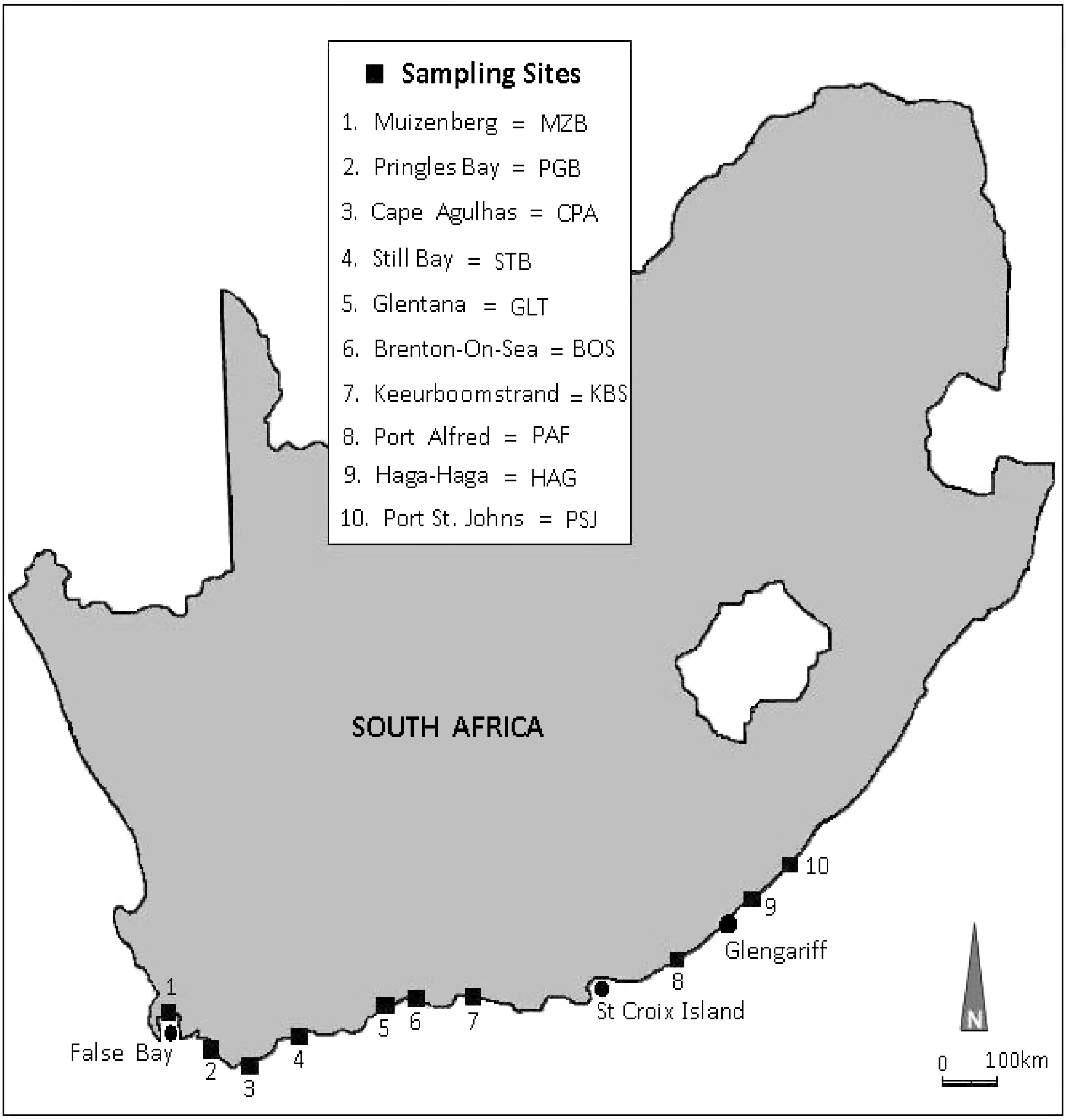

Figure 3.1.: Map of South Africa showing the sampling sites of the epifauna on *Gelidium pristoides*.

3.3.3 Data analyses

The final epifaunal data set used for analysis is presented in Appendix 7. The multivariate analyses of the samples were done using the PRIMER (Plymouth Routines In Multivariate Ecological Research) software package of the Plymouth Marine Laboratory (Clarke & Gorley 2006) to check differences in epifaunal abundances amongst different localities. The routine ANOSIM (analysis of similarity) which carries out a standard univariate 1-way ANOVA was used to check any grouping in epifaunal abundances among the samples (Clarke & Gorley 2006). Two analyses of the epifaunal data were performed, first with the epifaunal abundance data transformed into presence/absence data and secondly with the epifaunal abundance corrected to the biomass of the samples (i.e. species abundance per gram wet mass). Non-parametric multidimensional scaling (nMDS) ordination of the samples was performed based on the Bray-Curtis similarity index (Bray & Curtis 1957). The cut-off values for Bray-Curtis similarity were obtained by identifying the Bray-Curtis similarity value at which the greatest similarity among the groups (on the dendrogram) was observed. Samples were clustered using the hierarchical agglomerative clustering technique (Single-linkage grouping), which uses the similarity matrix to fuse samples into groups and the groups into larger clusters. The species that contributed significantly to dissimilarities between the observed groups were identified using the program SIMPER. The program creates a factor (SprofGps factor) into which the main groups identified from cluster analysis are entered. Then it gives average dissimilarity percentages of species discriminating among the groups based on a cut off value of 80% Bray-Curtis similarity.

3.4. Results

A total of 9 major taxa and 51 species were identified (Table 3.1). Of the nine taxa identified, the species diversity was highest for the following four major taxa: Mollusca, Polychaeta, Isopoda and Amphipoda. The most common groups were the amphipods: *Atylus swammerdamei* (recorded at all localities but Port St. Johns), *Guernea rhomba* and *Stenothoe adhaerens* (recorded at all localities); the isopod: *Dynamenella huttoni* (recorded at all localities) and the polychaetes: *Nereis caudata* and *Syllis prolifera* (recorded in seven localities). The amphipods and isopods had the greatest abundances of epifauna at most sites. These taxa were also found to be most abundant per gram dry mass of *G. pristoides* on St Croix Island and at Glengariff by Beckely (1982) and Anderson *et al*. (1991) respectively. A total of 132 individuals of *Atylus swammerdamei* were recorded in one sample from Brenton-on-Sea (BOS3). A total of 563 individuals of *Guernea rhomba* and 134 individuals of *Stenothoe adhaerens* were recorded in one sample from Haga-Haga (HAG1). At total of 81 individuals of *Dynamenella huttoni* were recorded in one sample from Cape Agulhas (CPA3). The polychaetes and molluscs were mostly recorded in samples from the south coast. The isopods and amphipods were recorded in most samples from most localities, but in different abundances.

The results of ANOSIM and Bray-Curtis cluster analysis of epifauna abundance with data transformed into presence/absence, showed that there were significant differences in epifaunal community structure amongst localities ($R = 0.45$, $P = 0.001$). The Dendrogram in Figure 3.2 shows that the analysis clustered most samples from the south and east coasts separately, based on a cut-off Bray-Curtis similarity value of 38%. Most importantly, there was no clear distributional pattern among the observed groups. For example, the samples from the east coast were clustered together with several south coast samples in group B. Group A comprised samples from the south coast plus one sample from Port Alfred (PAF4). Four samples from Glentana (GLT1, GLT2, GLT3, and GLT5) and one from Keeurboomstrand (KBS2) were clustered together (group C) but separately from the other south coast samples. One sample from Haga-Haga (HAG1) and one from Muizenberg (MZB2) did not cluster with any samples. However, the nMDS ordination in Figure 3.3 has a stress value of 0.24, which suggests that not too much confidence should be placed on the distributional pattern in the plot (Clarke & Gorley 2006).

Table 3.1. An overview of epifaunal species diversity on *Gelidium pristoides* pooled from all samples from all localities. ? = Identification of the animals to the species level was not possible.

Epifauna taxa	**Total No. of species**
Acarina	?
Amphipoda	6
Echinodermata	1
Isopoda	11
Mollusca	16
Nematoda	?
Ostracoda	3
Polychaeta	12
Tanaidacea	2
Total No. of identified species	**51**

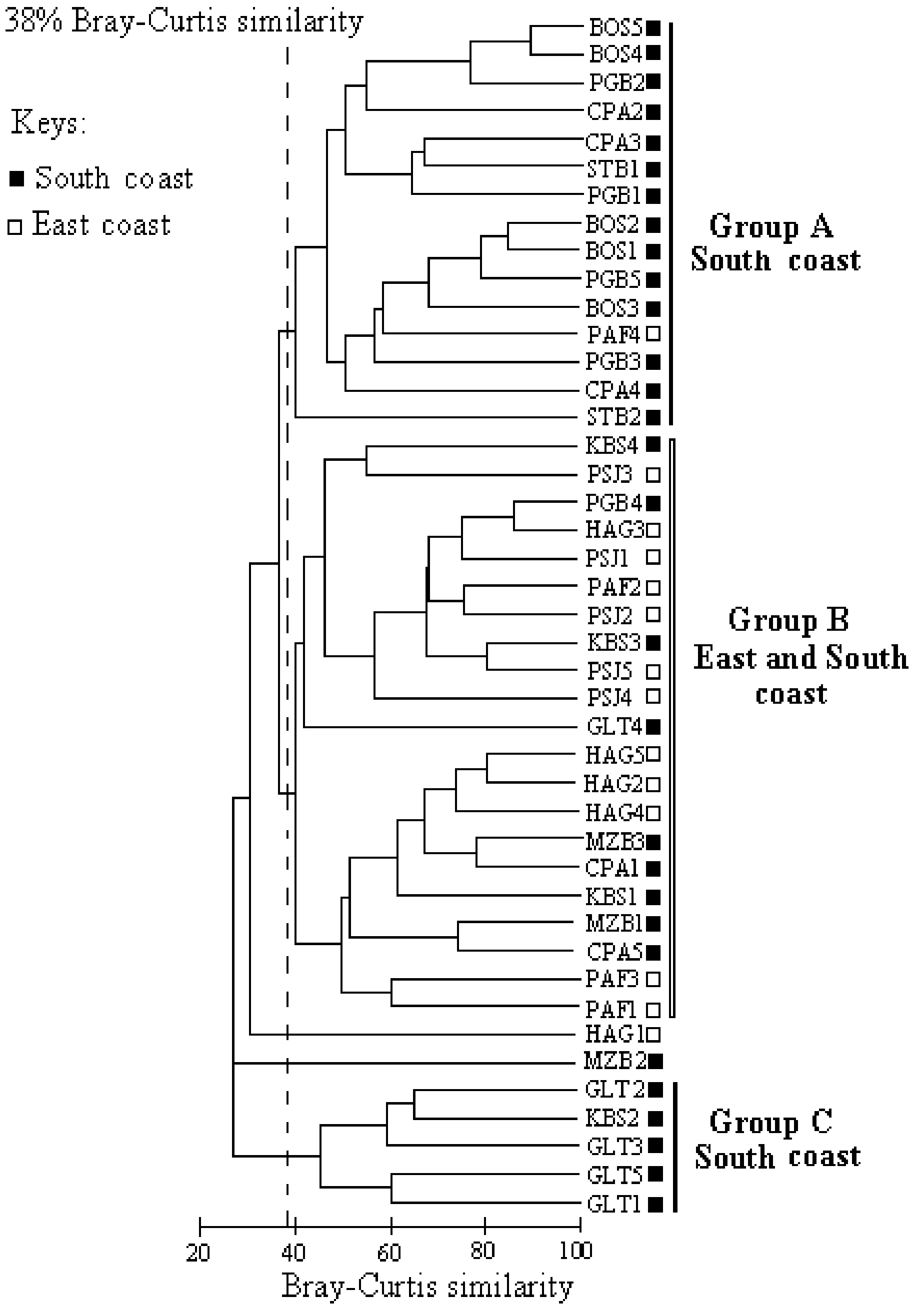

Figure 3.2. Dendrogram showing the percentage similarity of the epifauna samples from different localities along the coast, based on presence/absence data.

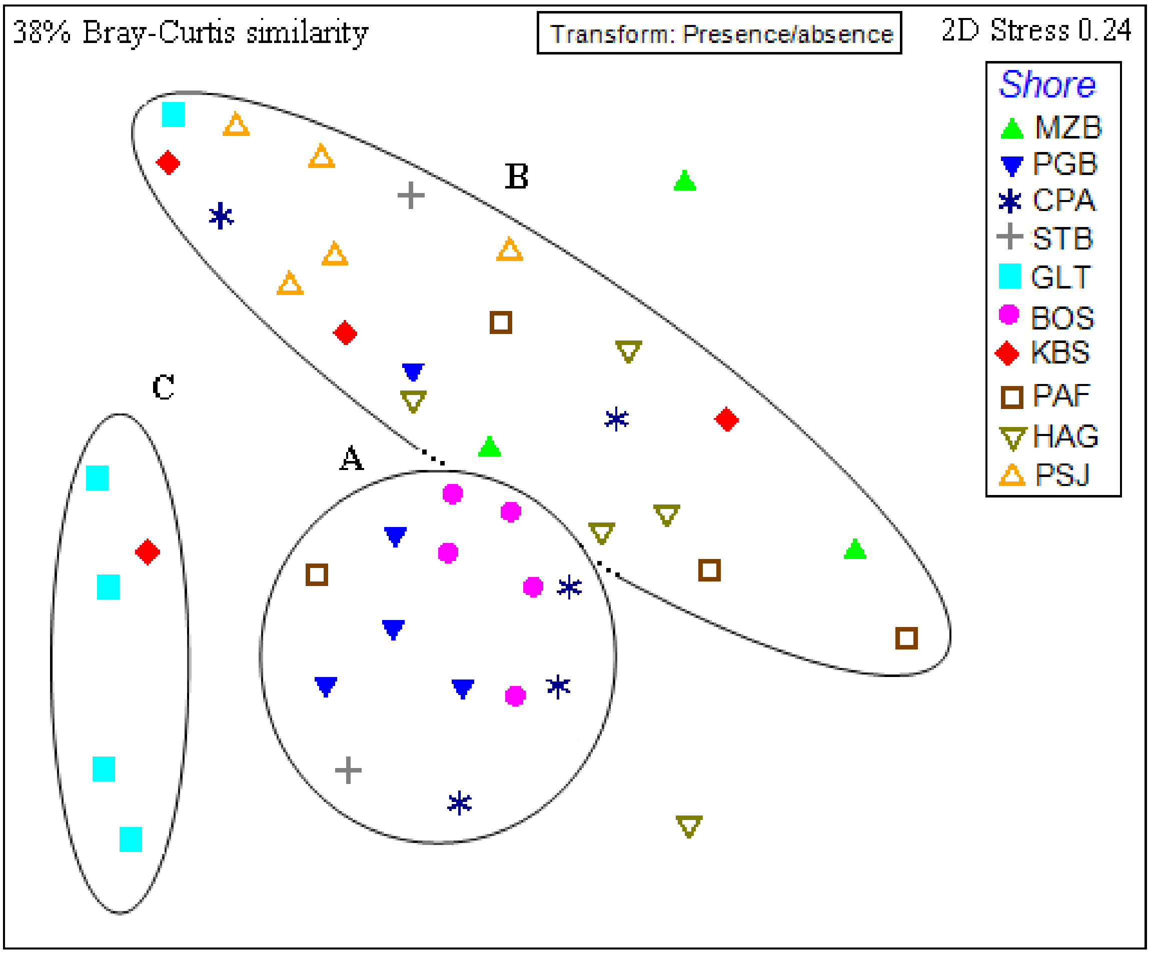

Figure 3.3. Multidimensional scaling ordination of the samples, data transformed into presence/absence. The localities are indicated by different grades, empty and shaded grades indicate eastern and southern localities respectively. The letters A, B and C correspond to groups identified in Figure 3.2.

The results of ANOSIM and Bray-Curtis cluster analysis of epifauna abundance corrected to biomass of *Gelidium pristoides* also showed significant differences in epifaunal community structure between the eastern and southern localities (*R = 0.43, P = 0.001*). The analysis showed considerable stringing of the samples, further sub-clustering them into 7 groups based on a cut-off Bray-Curtis similarity value of 35% (Figure 3.4). However, there was no clear distributional pattern among the observed groups. Groups 1, 4 and 5 contained samples mainly from the south coast, which included one sample from Haga-Haga (HAG2) and one from Port Alfred (PAF2). Groups 4 and 5 comprised samples from Glentana plus one sample from Cape Agulhas (CPA5). Groups 2, 3, 6 and 7 comprised samples from the east coast, clustered with a few samples from the south coast. Groups 2 and 3 contained samples mainly from Port St. Johns, whereas group 7 contained three samples from Haga-Haga. Group 6 contained two samples from Port Alfred (PAF1 and PAF3) and one from Muizenberg (MZB1). One sample from Haga-Haga (HAG5) and one from Muizenberg (MZB2) were not clustered with any samples. The samples from the three sites on the east coast (Port Alfred, Haga-Haga and Port St. Johns) were clustered almost separately from each other. The nMDS ordination (Figure 3.5) also showed less resolution of the samples, though the stress level was lower (0.18).

Interestingly, the results show a striking pattern in epifaunal abundances as a function of scale. At small local (within site) scales, samples from the same sites, collected at least 5m from each other, were in some instances clustered together, for example, samples from Glentana, Haga-Haga and Port St Johns (Figure 3.2 and 3.4). However, this was not the case for most samples which were scattered across the dendrogram (Figures 3.2 and 3.4). At large physical (among sites) scales, sites were not similar to their most adjacent neighbor.

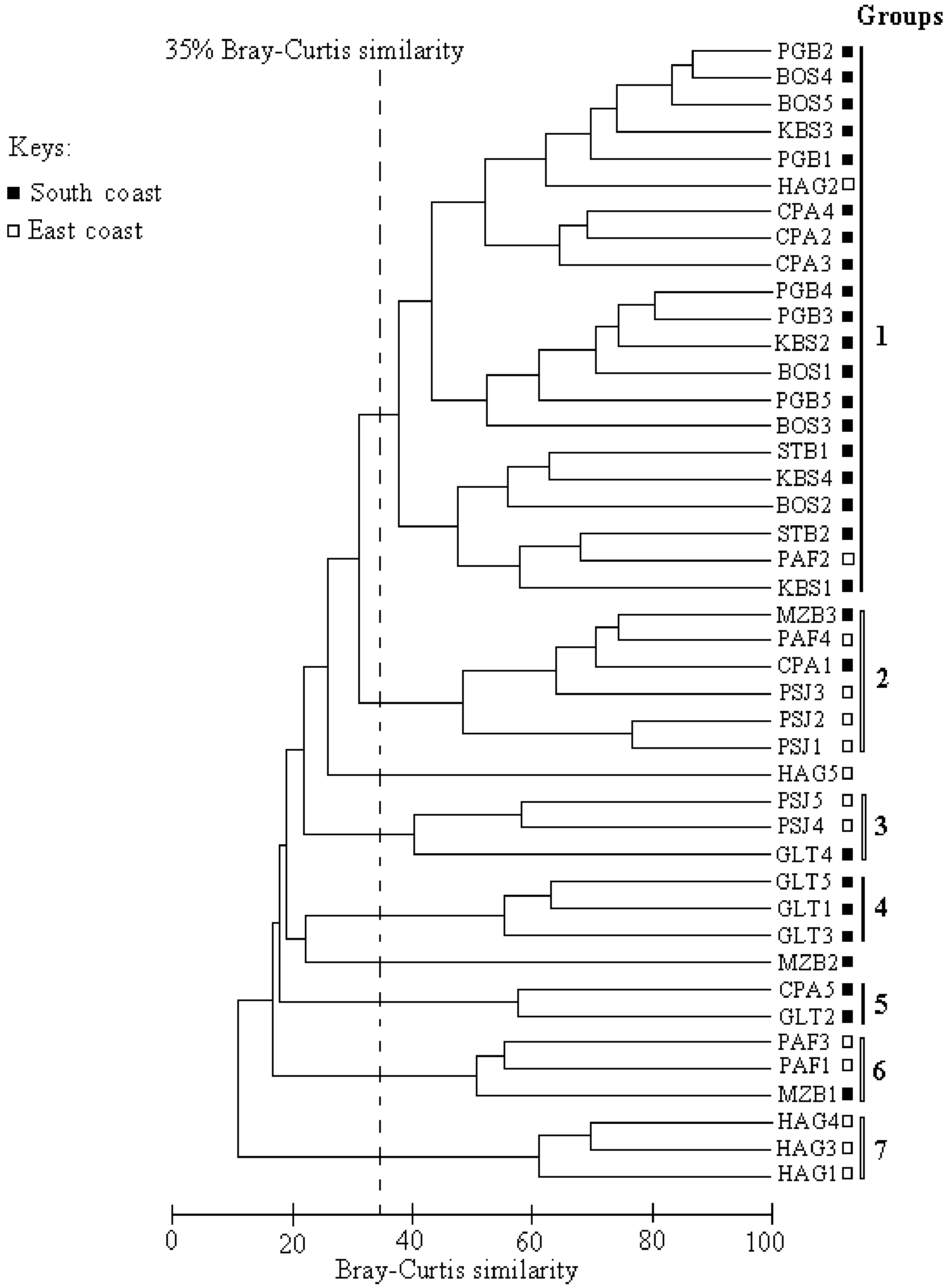

Figure 3.4. Dendrogram showing the percentage similarity of the epifauna samples from different localities along the coast. The epifaunal abundance was corrected to the biomass of the samples.

However, at larger, biogeographic scales, the samples showed spatial structuring in that samples from the same coastal region tended (with many exceptions) to be clustered together. Thus there was group A and group 1 in Figures 3.2 and 3.4 respectively, which contained predominantly south coast samples, while the east coast samples tended to be clustered together (mixed with the remaining south coast samples) in group B (Figure 3.2) and groups 2, 3, 6 and 7 (Figure 3.5).

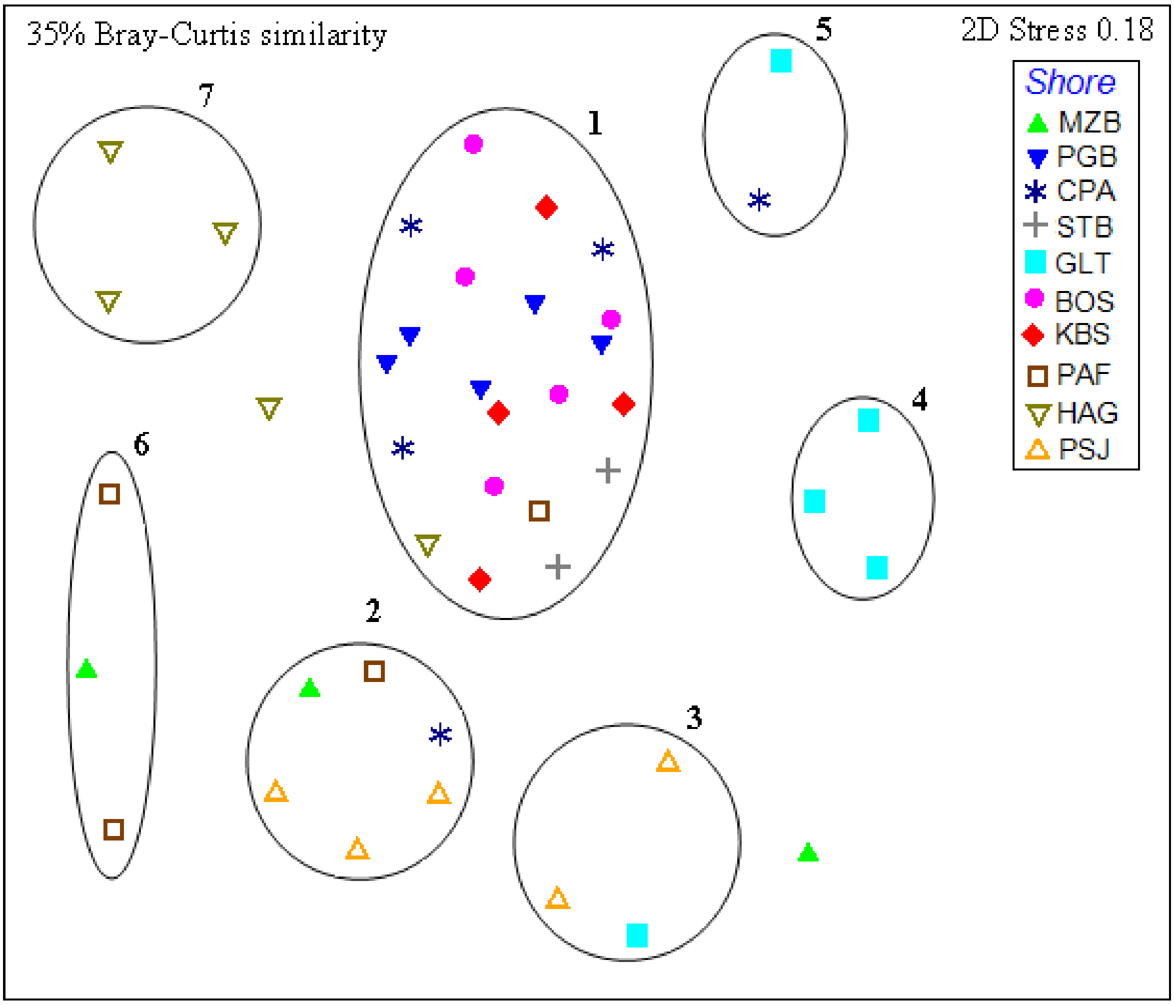

Figure 3.5. Multidimensional scaling ordination of the sample with epifaunal abundance corrected to the biomass of the samples. The localities are indicated by different grades, empty and shaded grades indicate the eastern and southern localities respectively. The numbers 1 – 7 correspond to the groups identified in Figure 3.4.

The SIMPER analysis showing the species discriminating between groups A and B is presented in Table 3.2 and between groups A and C in Table 3.3. A fairly large number of species (19 and 15 in Tables 3.2 and 3.3 respectively) contributed significantly to the observed differences among and between these groups. These species were predominantly in the four major epifaunal taxa identified (molluscs, polychaetes, isopods and amphipods) and the nematodes.

The SIMPER analysis showing the species discriminating between groups 1 and 2 is presented in Table 3.4 and between group 1 and groups 4 plus 5 in Table 3.5. Interestingly, far fewer species were involved (5 and 6 in Tables 3.4 and 3.5 respectively), and they included only the amphipods and isopods. Previous studies have also found the amphipods and isopods to be abundant epifauna on *G. pristoides* at small local (within site) scale investigations (Beckley 1982; Gibbons 1988; Anderson *et al*. 1991). The distributional range of most of these amphipods and isopods species covers the study area in this study. Thus, the differences between groups are primarily due to different levels of epifauna abundances on both coasts. However, one *Exosphaeroma* species, identified as *E. truncatitelson*, was found on the east coast though its recorded distributional limit is from Hermanus (west coast) to Lüderitz in Namibia. *E. truncatitelson* was abundant on the east coast (Tables 3.3 and 3.4).

Several species were only found on either the east or the south coast and in some cases these species were represented by a single individual found in one sample from one locality. Such species did not contribute to the differences in epifaunal abundances between the two regions. For example, a total of 17 marine mites (Acarina) were only found in samples from Haga-Haga. Three species of ostracods were only found in one sample from Haga-Haga (Hag1). Two tanaids species (*Aspeudomorpha avicularia* and *Tanais philetaerus*) were only found in one sample from Haga-Haga (Hag1). One individual each of *Choromytilus meridionalis* and Barnacle sp. where found only in one sample from Glentana (GLT3). One individual of *Scutellastra barbara* was found in one sample from Glentana (GLT5), one individual of *Marphysa corallina* in one sample from Glentana (GLT2), one individual each of *Fissurella mutabillis* and Bivalve sp. in one sample from Pringles Bay (PGB3), one individual of *Cirolana hirtipes* in one sample from Cape Agulhas (CPA1) and one individual of *Cymodocella sublevis* in one sample from Haga-Haga (HAG1). Thus Glentana and Haga-Haga had relatively large numbers of species that were unique to these sites, resulting in tight clustering of samples from these sites (Figure 3.2 and 3.4).

Table 3.2. Average abundance of species which contributed significantly (*P = 0.001*) to the dissimilarity between groups A and B (Figure 3.2). The average dissimilarity between the two groups was 61.55% (SIMPER, cut-off 80%).

Species	**Taxon**	**Average Abundance**		**Average Dissimilarity**
		Group B	Group A	
Syllis prolifera	Polychaeta	0.14	0.67	4.56
Exosphaeroma pallidum	Isopoda	0.67	0.07	4.56
Nereis caudata	Polychaeta	0.62	0.07	4.33
Atylus swammerdamei	Amphipoda	0.43	0.73	4.20
Guernea rhomba	Amphipoda	0.76	0.47	4.20
Exosphaeroma truncatitelson	Isopoda	0.48	0.33	3.61
Nematodes	Nematoda	0.14	0.40	3.19
Dynamenella huttoni	Isopoda	0.71	1.00	2.49
Platynereis dumerilii	Polychaeta	0.00	0.33	2.15
Mytilus galloprovincialis	Mollusca	0.00	0.27	2.06
Parisocladus stimpsoni	Isopoda	0.33	0.00	2.0
Caprella natalensis	Amphipoda	0.24	0.07	1.96
Phyllodoce castanea	Polychaeta	0.10	0.20	1.74
Naineris laevigata	Polychaeta	0.10	0.20	1.68
Eatoniella nigra	Mollusca	0.14	0.13	1.65
Pseudonereis variegata	Polychaeta	0.05	0.20	1.63
Janira extans	Isopoda	0.19	0.00	1.45
Lumbrineris cavifrons	Polychaeta	0.10	0.13	1.3
Parisocladus perforatus	Isopoda	0.19	0.00	1.11

Table 3.3. Average abundance of species which contributed significantly (*P = 0.001*) to the dissimilarity between groups A and C (Figure 3.2). The average dissimilarity between the two groups was 66.44% (SIMPER, cut-off 80%).

Species	**Taxon**	**Average Abundance**		**Average Dissimilarity**
		Group A	Group C	
Stenothoe adhaerens	Amphipoda	0.93	0.00	7.03
Caprella natalensis	Amphipoda	0.07	0.80	5.54
Perna perna	Mollusca	0.13	0.80	5.45
Syllis prolifera	Polychaeta	0.67	0.00	4.83
Mytilus galloprovincialis	Mollusca	0.27	0.80	4.81
Hemiaegina minuta	Amphipoda	0.00	0.60	4.39
Guernea rhomba	Amphipoda	0.47	0.60	3.91
Nematodes	Nematoda	0.40	0.00	3.00
Atylus swammerdamei	Amphipoda	0.73	0.80	2.87
Exosphaeroma truncatitelson	Isopoda	0.33	0.00	2.57
Platynereis dumerilii	Polychaeta	0.33	0.00	2.16
Phyllodoce castanea	Polychaeta	0.20	0.20	2.15
Exosphaeroma pallidum	Isopoda	0.07	0.20	1.66
Pseudonereis variegata	Polychaeta	0.20	0.00	1.42
Choromytilus meridionalis	Mollusca	0.00	0.20	1.37

Table 3.4. Average abundance of species which contributed significantly (*P = 0.001*) to the dissimilarity between groups 1 and 2 (Figure 3.4). The average dissimilarity between the two groups was 76.63% (SIMPER, cut-off 80%).

Species	**Taxon**	**Average Abundance**		**Average Dissimilarity**
		Group 1	Group 2	
Dynamenella huttoni	Isopoda	4.55	31.70	25.85
Stenothoe adhaerens	Amphipoda	21.55	9.25	16.00
Atylus swammerdamei	Amphipoda	1.64	17.80	12.15
Exosphaeroma truncatitelson	Isopoda	3.84	7.65	7.06
Guernea rhomba	Amphipoda	3.64	3.85	4.44

Table 3.5. Average abundance of species which contributed significantly (*P = 0.001*) to the dissimilarity between group 1 and groups 4 and 5 (Figure 3.4). The average dissimilarity between the three groups was 79.32% (SIMPER, cut-off 80%).

Species	**Taxon**	**Average Abundance**		**Average Dissimilarity**
		Group 1	Group 4 and 5	
Dynamenella huttoni	Isopoda	4.55	13.50	22.17
Caprella natalensis	Amphipoda	0.30	27.50	13.52
Atylus swammerdamei	Amphipoda	1.64	0.17	2.37
Stenothoe adhaerens	Amphipoda	21.55	2.00	6.77
Hemiaegina minuta	Amphipoda	0.15	15.67	6.38
Guernea rhomba	Amphipoda	3.64	2.83	4.80

3.5. Discussion and conclusion

The results revealed no clear distributional pattern in epifauna abundances, however they did show variation in epifauna abundances as a function of scale. At small local (within site) scales, samples from some sites were clustered together (e.g. samples from Glentana, Haga-Haga and Port St Johns). However, in some cases samples from the same site were scattered across the dendrogram (Figures 2.3 and 3.4).

At large physical (among sites) scales; there was no structure in epifauna abundances among the most adjacent sites. For example, adjacent sites on the east coast (Port Alfred, Haga-Haga and Port St. Johns) clustered separately (Figure 3.5) while on the south coast Glentana was distinct from its neighbouring sites (Figures 3.3 and 3.5).

However, at larger, biogeographic scales (biogeographic regions); samples from the same coastal region tended to be clustered together. Thus, there was one group containing predominantly south coast samples and another group containing most of the east coast samples mixed with the remaining south coast samples (Figure 3.2 and 3.4).

The lack of or weak structure observed at small local (within site) scales is probably due to the effects of different factors (i.e. wave exposure, seaweed biomass, interspecific interactions and chance effects) operating within each site. Locally, several studies have shown the effects of wave exposure and seaweed biomass on the epifauna abundances on *G. pristoides* at small local (within sites) scales (Beckley 1982; Gibbons 1988; Anderson *et al*. 1991). At such local (within site) scales, wave exposure can indirectly influence the epifauna abundances by affecting the seaweed biomass and structural complexity (Gibbons 1988). Samples in this study were collected at least 5m apart from each other within each site. While samples from the same site may have been exposed to differences in wave action, such effects would have been on extremely small scales. Nevertheless, such small-scale effects have been shown to affect mussel growth rates (McQuaid unpublished data) and could have been responsible for weak or inconsistent structure within sites. Beckley (1982) found that sheltered shores on St Croix Island are characterized by significantly higher abundances of epifauna per gram dry mass of *G. pristoides* than exposed shores. Gibbons (1988) also found variation between sheltered and exposed shores in epifauna abundances per gram dry mass of *G. pristoides* in False Bay.

At large biogeographic scales there was weak or little spatial structuring in epifaunal abundances, resulting in two groups which comprised predominantly south coast and east coast samples. This suggests that the epifaunal species respond differently to a variety of environmental factors prevailing within each geographic region. It is probable that physical factors (i.e. surface sea temperature) and interspecific interactions (i.e. predation, competition) might be driving the observed variation in epifauna abundances between the two coasts. Many studies have dwelt on abundance and diversity of animals in symbiosis with seaweeds, but how these animals interact biologically is still unclear (Russo 1997). Thus, it is possible that regional abundance of other taxa (i.e. predators) can limit the abundance of some taxa (i.e. prey) or interspecific competition for food and space can result in variation in abundance among different taxa (Broitman *et al*. 2001).

Though it remains unclear how strong the correlation is between the distribution of the epifauna and these biogeographic regions owing to the lack of epifauna samples in the vicinity of Port Elizabeth. Epifaunal species that showed variations in abundances over large biogeographic scale were primarily in the four major epifaunal taxa identified (amphipods, isopods, molluscs and polychaetes). At small local (within site) scales, there is no consistent distributional structuring in epifauna abundance due to different factors (wave exposure, seaweed biomass, interspecific interactions and chance effects) operating within each site. These factors also can affect epifauna abundance differently from site to site (Gibbons 1988; Anderson *et al*. 1991) and thus, there was no structure observed at large physical (among sites) scales.

The fact that the distribution patterns in epifauna abundance varied as a function of scale makes it difficult to correlate the observed patterns to the phylogeographic pattern exhibited by *G. pristoides*. Rahbek (2005) indicated that studies that show how results vary as a function of scale should also search for consistent patterns in these scale effects. The distributional pattern observed at small local (within site) scales in this study was inconsistent while at large physical (among sites) scales, there was no structure. Thus, these patterns cannot be accurately correlated to phylogeographic patterns in *G. pristoides*. The distributional pattern of the epifauna observed at large biogeographic scales do not correspond with phylogeographic pattern of the seaweed.

In conclusion, there was no clear pattern in the distribution of the epifauna within *G. pristoides*. This suggests that the fauna associated with this species are not host-specific and thus, their distribution is not linked to the phylogeographic distribution of the seaweed host. Anderson *et al*. (1991) also indicated that of all the epifaunal taxa in symbiosis with *G. pristoides*, none is specific to this seaweed. At a larger, biogeographic scale, the epifauna distribution is probably regulated by physical factors operating at these scales, such as temperature, rather than being associated with particular host species.

CHAPTER FOUR: SYNTHESIS

Gelidium pristoides and *Hypnea spicifera* populations showed very low genetic diversity compared to coastal and estuarine invertebrates in this region. Phylogeographical studies on coastal and estuarine invertebrates' species found a high level of genetic diversity with some species characterized by more than five haplotypes (Zardi *et al*. 2007, Teske *et al*. 2007*b*). However, only two haplotypes were discovered within *G. pristoides* populations, whereas *H. spicifera* populations showed a fairly uniform genetic composition. Low genetic diversity within red seaweeds has been reported before using isozyme electrophoresis (Cheney & Babbel 1978; Sosa & Garcia-Reina 1992; Sosa *et al*. 1996; Sosa *et al*. 1998). In *Gelidium* species such a low level of genetic diversity has been attributed to self fertilization (Sosa & Garcia-Reina 1992; Sosa *et al*. 1996).

Gelidium spp. are slow-growing perennial seaweeds that reproduce new generations from an established holdfast (Sosa & Garcia-Reina 1992) and thus the genetic composition of a particular year would remain relatively uniform (Innes 1987). This, combined with predominantly asexual reproduction, has been suggested as the main reason for low genetic diversity among various *Gelidium* spp. (Sosa & Garcia-Reina 1992; Sosa *et al*. 1998). Therefore, the observed low level of genetic diversity might indirectly suggest that populations of *G. pristoides* and *H. spicifera* reproduce predominantly asexually.

The lack of gene flow combined with predominance of asexual reproduction can result in a fairly low level of genetic diversity within seaweeds (Sosa *et al*. 1998). Gene flow is very limited among *G. pristoides* populations due to short dispersal-distances of the spores (Alberto *et al*. 1999). Thus, the lack of gene flow, combined with the observed low level of genetic diversity, again supports the suggestion that the species reproduces mainly asexually.

In *H. spicifera*, predominance of asexual reproduction is more probable considering the genetic differentiation detected between the gametophyte and tetrasporophyte generations. In species characterized by haplo-diploid generations, predominance of asexual reproduction can significantly reduce the effective population size ($N_{e)}$, resulting in genetic drift between asexually and sexually reproducing populations (Sosa *et al*. 1998). Ultimately, genetic variation can develop between haploid and diploid generations if the N_e of the sexually reproducing populations is lower than that of asexually reproducing populations (Sosa *et al*. 1998). Thus, based on significant genetic variation detected between gametophyte and sporophyte generations within *H. spicifera* populations, it can be deduced that the species reproduces mainly asexually. Additionally, studies on the reproductive biology of *Hypnea* species have shown that they maintain their populations through asexual reproduction (Rama Rao 1977).

The relationship between distribution of the epifaunal community and phylogeographic distribution of *Gelidium pristoides* varied as a function of scale. At small, local (within localities) scales, there was no correlation between the epifauna abundance and the genetics of the samples. For example, samples from Haga-Haga, Glentana and Muizenberg have a significantly higher numbers of epifauna species that were unique to these sites but the samples themselves were genetically identical to other samples in the same lineage. Also, samples from Still Bay were characterized by *Cox2-3* spacer sequence variation of one character compared to the rest of the samples on the south-western lineage. However, Still Bay samples did not show any significant difference in epifauna abundance from the other south coast samples. Thus, it can be concluded that the abundance of the epifauna is not genetically mediated by the genotype of *G. pristoides*.

However, at larger physical (among localities) scales, there was a correlation between the distribution of the epifaunal community and the phylogeographic distribution of *G. pristoides*. For example, all the localities that comprised the eastern and southern epifaunal communities also comprised the south-eastern and south-western genetic lineages respectively. This is ambiguous owing to the lack of epifaunal samples in the vicinity of phylogeographic disjunctions. It might imply that the distribution of the epifauna is associated with either distribution of genetic lineages or biogeographic regions. The latter possibility is favoured given the lack of correlation between epifauna abundance and genotype of the seaweed. Thus, the boundary between the eastern and southern epifaunal communities is more likely to fall within the transitional zone (Eastern overlap) between Port Elizabeth and Port Alfred; but not necessarily coincide with the phylogeographic boundaries discovered within this transitional zone.

The fauna associated with seaweeds can evolutionary adapt to the abundant host and such adaptations can result in genetic variation among populations (Sotka 2005). It is recommended future studies investigate phylogeographic patterns of the fauna associated with *Gelidium pristoides* and *Hypnea spicifera* to determine if they show correlation to the observed phylogeographic distribution.

This study integrated both intraspecific phylogeny and current epifaunal community distribution to help understand species distribution much better than studies centered on single approach (phylogeographical analysis or epifaunal community analysis). The study has been innovative by combining both phylogeography of seaweeds and analysis of their epifaunal community to help understand better biological and physical processes underlying species distribution.

The phylogeographic pattern discovered within *G. pristoides* did not conform to the biogeographic regions described by Stephenson (1948), while the distribution of the epifauna does seem to be associated with these biogeographic regions. Thus, seemingly, at larger, biogeographic scales, there is no association between phylogeographic distribution of *G. pristoides* and distribution of the associated fauna. This suggests that community distribution of the fauna associated with *G. pristoides* is not mediated by distribution of genetic lineages within *G. pristoides*. The epifaunal community associated with *G. pristoides* can be found in symbiosis with a variety of seaweed host. Presumably, distribution of this epifaunal community is not associated with phylogeographic distribution of a specific seaweed host. Furthermore, the temperature gradients along the coast appear to have biological consequences that range from the level of the community to the level of intraspecific phylogenetics.

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APPENDICES

Appendix 1: ***DNA Extraction Protocol***

1. Grind 5-6 pencil-tip sizes of thallus into fine powder using pestle and mortar in the presence of 1ml CTAB extraction buffer (check solutions) and a drop of 0.2 %(v/v) β- Mercaptoethanol.

2. Decant the ground material into 1.5ml Eppendorf tube and incubate at 60°C water bath for 10-30 minutes.

3. Add 500µl CIA (check solutions) and vortex.

4. Centrifuge at 13, 000 rpm for 1 minute.

5. Remove 600µl of clear aqueous phase and transfer into clean 1.5ml Eppendorf tube.

6. Add 400µl of Isopropanol; shake gently and leave the tube on ice for 10 minutes.

7. Centrifuge at 13,000 rpm for 10 minutes.

8. Pour off the supernatant and leave off the DNA pellet behind.

9. Add 750µl of 70% Ethanol.

10. Pour off Ethanol and air-dry the pellet in the fume hood.

11. Resuspend the DNA in 300µl distilled water.

Appendix 2: *Solutions*

CIA

Chloroform: isoamyl alcohol (24:1)

CTAB extraction buffer

For 100ml:

- 10ml 1M Tris [Tris(Hydroxymethyl) aminomethane]
- 28ml 5M NaCl
- 4ml 0.5M EDTA [Ethylene Diamine Tetra-acetic acid Di-sodium salt]
- 2g CTAB powder [Cetyltrimethylammonium bromide]
- 1g PVP [Polyvinyl pyrrolidine]
- 57ml water

[pH = 8]

Agarose gel

For 1%:

- 0.5g agarose
- 50ml TBE buffer
  - 10.8g Tris(Hydroxymethyl) aminomethane
  - 5.5g Boric Acid
  - 0.93g EDTA
  - Made up to 1L distilled water
- 10µl ethidium bromide

Appendix 3: *PCR reagents and their volumes for amplifying ITS1 and Cox2-3 spacer regions*

ITS1 amplification

PCR reagents	**Volumes(µl)**
H_2O	31
10x PCR buffer	5
ITS-1F(Forward primer)	2
ITS-1R(Reverse primer)	2
dNTPs	2
$MgCl_2$	3
Bio Taq	0.1
DNA template	5

Cox2-3 spacer amplification

PCR reagents	**Volumes(µl)**
H_2O	29
10x PCR buffer	5
Cox-2F (Forward primer)	2
Cox-3R(Reverse primer)	2
dNTPs	2
$MgCl_2$	5
Bio Taq	0.1
DNA template	5

H_2O = distilled PCR-quality water

10x PCR buffer = 10x Bioline NH_4 Dilution Buffer ($MgCl_2$ free) with enzyme

dNTPs = deoxynucleotriphosphates

$MgCl_2$ = 50mM of $MgCl_2$ provided with enzyme

Bio Taq = Bioline Taq Polymerase

Appendix 4: ***Reagents and volumes used for sequencing reaction***

Sequencing reagents	**Volumes(µl)**
H_2O	12.5
Sequencing Mix	2
5x Sequencing buffer	3
Primers	0.5
DNA template	2

Primers = ITS-1F/ITS1-1R or Cox-2F/Cox-3

DNA template = purified PCR-product

Appendix 5: ***Reagents and volumes used for precipitation***

Precipitation reagents	**Volumes(µl)**
100% Ethanol	50
125mM EDTA	2
3M Na Acetic Acid	2
DNA template	20

Appendix 6: ***The wet weight (grams) of the epifauna samples measured after isolating the epifaunal species.***

Sites	**Samples**				
PSJ	**1**	**2**	**3**	**4**	**5**
Weight (g):	6.54	7.89	8.65	8.02	6.55
HAG	**1**	**2**	**3**	**4**	**5**
Weight (g):	28.56	27.88	23.24	22.83	23.56
PAF	**1**	**2**	**3**	**4**	**-**
Weight (g):	12.60	13.34	17.19	14.32	
KBS	**1**	**2**	**3**	**4**	**-**
Weight (g):	17.21	10.99	11. 28	14.58	
BOS	**1**	**2**	**3**	**4**	**5**
Weight (g):	13.83	18.89	14.82	15.03	14.82
GLT	**1**	**2**	**3**	**4**	**5**
Weight (g):	20.60	24.43	18.97	19.98	27.12
STB	**1**	**2**	**-**	**-**	**-**
Weight (g):	10.38	8.06			
CPA	**1**	**2**	**3**	**4**	**5**
Weight (g):	10.22	11.56	16.95	19.91	21.06
PGB	**1**	**2**	**3**	**4**	**5**
Weight (g):	14.79	11.20	19.32	8.79	24.11
MZB	**1**	**2**	**3**	**-**	**-**
Weight (g):	16.01	18.39	13.78		

Appendix 7: ***Final epifauna data set***

Epifauna taxa	**Samples**									
	PSJ1	PSJ2	PSJ3	PSJ4	PSJ5	HAG1	HAG2	HAG3	HAG4	HAG5
ACARINA	-	-	-	-	-	4	1	5	5	2
AMPHIPODS										
Atylus swammerdamei	-	-	-	-	-	20	-	2	23	6
Caprella natalensis	-	-	2	-	-	-	-	-	-	-
Hemiaegina minuta	-	-	-	-	-	-	-	-	-	-
Guernea rhomba	13	3	-	1	5	563	5	191	321	15
Proboloides rotunda	-	-	-	-	-	-	-	-	-	-
Stenothoe adhaerens	20	21	50	8	5	134	10	101	57	12
ECHINODERMATA										
Patiriella exigua	-	-	-	-	-	-	-	-	-	1
ISOPODS										
Cirolona hirtipes	-	-	-	-	-	-	-	-	-	-
Cymodocella sublevis	-	-	-	-	-	-	5	-	1	10
Dynamenella huttoni	-	1	6	-	1	18	35	7	14	11
Dynamenella macrocephala	-	-	-	-	-	-	3	-	-	-
Dynamenalla ovalis	-	-	1	-	-	-	-	-	-	-
Exosphaeroma pallidum	1	-	-	-	-	-	11	5	1	38
Exosphaeroma truncatitelson	-	-	-	-	-	15	3	-	1	1
Janira extans	1	1	-	-	-	-	-	-	1	-
Paranthura punctata	-	-	-	-	-	-	-	-	-	-
Parisocladus perforatus	-	-	-	-	-	-	2	-	-	25
Parisocladus stimpsoni	-	-	-	-	-	-	1	-	-	18
MOLLUSCA										
Arcidae	-	-	-	-	-	-	-	-	-	-
Barnacle sp.	-	-	-	-	-	-	-	-	-	-
Gastropod sp.1	-	-	-	-	-	3	-	-	-	-
Gastropod sp.2	-	-	-	-	-	11	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	PSJ1	PSJ2	PSJ3	PSJ4	PSJ5	HAG1	HAG2	HAG3	HAG4	HAG5
Acanthochitona garnoti	-	-	-	-	-	-	-	-	2	-
Choromytilus meridionalis	-	-	-	-	-	-	-	-	-	-
Eatoniella nigra	-	-	-	-	-	9	-	-	1	2
Fissurella mutabillis	-	-	-	-	-	-	-	-	-	-
Helcion dunkiri	-	-	-	-	-	-	-	-	-	-
Littorina natalensis	-	-	-	-	-	-	-	-	-	-
Mytilus galloprovincialis	-	-	-	-	-	-	-	-	-	-
Perna perna	-	-	-	-	-	-	-	-	-	-
Siphonaria concinna	-	-	-	-	-	-	-	-	-	-
Scutellastra barbara	-	-	-	-	-	-	-	-	-	-
Tricolia neritina	-	-	-	-	-	-	-	-	-	-
Tricolia capensis	-	-	-	-	-	-	-	-	-	-
NEMATODA	-	-	2	-	-	-	-	-	-	1
OSTRACODA										
Ostracod sp.1	-	-	-	-	-	1	-	-	-	-
Ostracod sp.2	-	-	-	-	-	1	-	-	-	-
Ostracod sp.3	-	-	-	-	-	7	-	-	-	-
POLYCHAETA										
Lumbrineris cavifrons	-	-	-	-	-	-	-	-	-	-
Marphysa corallina	-	-	-	-	-	-	-	-	-	-
Naineris laevigata	-	-	-	-	-	2	-	1	-	-
Nereis caudata	2	-	2	-	1	-	1	4	1	1
Odontosyllis polycera	-	-	-	-	-	-	-	-	-	-
Phyllodoce castanea	-	-	-	-	-	-	-	-	-	-
Platynereis dumerilii	-	-	-	-	-	2	-	-	-	-
Pomatoleios kraussii	-	-	-	-	-	-	-	-	-	-
Pseudonereis variegata	-	-	1	-	-	-	-	-	-	-
Syllis prolifera	-	-	-	-	-	2	-	-	-	-
Frubricinae	-	-	-	-	-	-	-	-	-	-
Polynoidae	-	-	-	-	-	-	-	-	-	-
TANAIDACEA										
Apseudomorpha avicularia	-	-	-	-	-	1	-	-	-	-
Tanais philetaerus	-	-	-	-	-	1	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	PAF1	PAF2	PAF3	PAF4	KBS1	KBS2	KBS3	KBS4	BOS1	BOS2
ACARINA	-	-	-	-	-	-	-	-	-	-
AMPHIPODS										
Atylus swammerdamei	-	-	7	5	-	39	3	-	52	8
Caprella natalensis	-	-	-	-	-	2	-	3	1	-
Hemiaegina minuta	-	-	-	-	-	3	-	-	-	-
Guernea rhomba	4	-	6	4	-	14	3	6	6	2
Proboloides rotunda	-	-	-	-	7	-	-	-	-	-
Stenothoe adhaerens	5	4	4	34	12	-	8	-	6	2
ECHINODERMATA										
Patiriella exigua	-	-	-	-	-	-	-	-	-	-
ISOPODS										
Cirolona hirtipes	-	-	-	-	-	-	-	-	-	-
Cymodocella sublevis	-	-	-	-	-	-	-	-	-	-
Dynamenella huttoni	9	-	-	19	11	34	47	16	21	18
Dynamenella macrocephala	-	-	-	-	-	-	-	-	-	-
Dynamenalla ovalis	-	-	-	-	-	-	-	-	-	-
Exosphaeroma pallidum	2	3	5	-	1	-	-	-	-	-
Exosphaeroma truncatitelson	-	15	30	-	2	-	-	-	-	-
Janira extans	-	-	-	-	-	-	-	-	-	-
Paranthura punctata	-	-	-	-	-	-	-	-	-	-
Parisocladus perforatus	-	-	-	-	-	-	-	-	-	-
Parisocladus stimpsoni	-	-	-	-	2	-	-	-	-	-
MOLLUSCA										
Arcidae	-	-	-	-	-	-	-	-	-	-
Barnacle sp.	-	-	-	-	-	-	-	-	-	-
Gastropod sp.1	-	-	-	1	-	-	-	-	-	-
Gastropod sp.2	-	-	-	-	-	-	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	PAF1	PAF2	PAF3	PAF4	KBS1	KBS2	KBS3	KBS4	BOS1	BOS2
Acanthochitona garnoti	-	-	-	-	-	-	-	-	-	-
Choromytilus meridionalis	-	-	-	-	-	-	-	-	-	-
Eatoniella nigra	-	-	1	3	-	-	-	-	-	-
Fissurella mutabillis	-	-	-	-	-	-	-	-	-	-
Helcion dunkiri	-	-	-	-	-	-	-	-	-	-
Littorina natalensis	-	-	-	-	-	-	-	-	-	-
Mytilus galloprovincialis	-	-	-	-	-	-	-	-	-	4
Perna perna	-	-	-	1	-	-	-	-	-	-
Siphonaria concinna	-	-	-	-	-	-	-	-	-	-
Scutellastra barbara	-	-	-	-	-	-	-	-	-	-
Tricolia neritina	-	-	6	-	-	-	-	-	-	-
Tricolia capensis	-	-	-	-	-	-	-	-	-	-
NEMATODA	-	-	-	-	1	-	-	-	2	1
OSTRACODA										
Ostracod sp.1	-	-	-	-	-	-	-	-	-	-
Ostracod sp.2	-	-	-	-	-	-	-	-	-	-
Ostracod sp.3	-	-	-	-	-	-	-	-	-	-
POLYCHAETA										
Lumbrineris cavifrons	-	-	-	-	-	-	-	-	-	-
Marphysa corallina	-	-	-	-	-	-	-	-	-	-
Naineris laevigata	-	-	-	-	-	-	-	-	-	3
Nereis caudata	-	-	-	-	1	-	1	1	-	-
Odontosyllis polycera	-	-	-	-	-	-	-	-	-	-
Phyllodoce castanea	-	-	-	-	-	-	-	-	1	1
Platynereis dumerilii	-	-	-	4	-	-	-	-	3	3
Pomatoleios kraussii	-	-	-	-	-	-	-	-	-	-
Pseudonereis variegata	-	-	-	-	-	-	-	-	-	-
Syllis prolifera	-	-	-	-	-	-	-	-	4	1
Frubricinae	-	-	-	-	-	-	-	-	-	-
Polynoidae	-	-	-	-	-	-	-	-	-	-
TANAIDACEA										
Apseudomorpha avicularia	-	-	-	-	-	-	-	-	-	-
Tanais philetaerus	-	-	-	-	-	-	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	BOS3	BOS4	BOS5	GLT1	GLT2	GLT3	GLT4	GLT5	STB1	STB2
ACARINA	-	-	-	-	-	-	-	-	-	-
AMPHIPODS										
Atylus swammerdamei	132	3	2	1	7	-	-	5	-	-
Caprella natalensis	-	-	-	-	69	7	13	3	-	-
Hemiaegina minuta	-	-	-	-	37	4	-	-	-	-
Guernea rhomba	12	-	-	-	32	1	2	-	-	-
Proboloides rotunda	-	-	-	-	-	-	-	-	-	-
Stenothoe adhaerens	11	3	2	-	-	-	6	-	3	8
ECHINODERMATA										
Patiriella exigua	-	-	-	-	-	-	-	-	-	-
ISOPODS										
Cirolona hirtipes	-	-	-	-	-	-	-	-	-	-
Cymodocella sublevis	-	-	-	-	-	-	-	-	-	-
Dynamenella huttoni	23	31	40	6	8	7	-	6	18	8
Dynamenella macrocephala	-	-	-	-	-	-	-	-	-	-
Dynamenalla ovalis	-	-	-	-	-	-	-	-	-	-
Exosphaeroma pallidum	-	-	-	-	-	-	-	1	-	-
Exosphaeroma truncatitelson	-	2	1	-	-	-	-	-	-	-
Janira extans	-	-	-	-	-	-	-	-	-	-
Paranthura punctata	-	-	-	-	-	-	-	-	-	1
Parisocladus perforatus	-	-	-	-	-	-	-	-	-	-
Parisocladus stimpsoni	-	-	-	-	-	-	-	-	-	-
MOLLUSCA										
Arcidae	-	-	-	-	-	-	-	-	-	-
Barnacle sp.	-	-	-	-	-	1	-	-	-	-
Gastropod sp.1	-	-	-	-	-	-	-	-	-	-
Gastropod sp.2	-	-	-	-	-	-	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	BOS3	BOS4	BOS5	GLT1	GLT2	GLT3	GLT4	GLT5	STB1	STB2
Acanthochitona garnoti	-	-	-	-	-	-	2	-	-	-
Choromytilus meridionalis	-	-	-	-	-	1	-	-	-	-
Eatoniella nigra	-	1	-	-	-	-	-	-	-	-
Fissurella mutabillis	-	-	-	-	-	-	-	-	-	-
Helcion dunkiri	1	-	-	-	-	-	-	-	-	-
Littorina natalensis	-	-	-	-	-	-	-	1	-	-
Mytilus galloprovincialis	-	-	-	1	2	1	-	1	1	-
Perna perna	-	-	-	27	7	3	-	13	-	-
Siphonaria concinna	-	-	-	-	-	-	-	-	-	1
Scutellastra barbara	-	-	-	-	-	-	-	1	-	-
Tricolia neritina	-	-	-	-	-	-	-	-	-	-
Tricolia capensis	-	-	-	-	-	-	2	3	-	-
NEMATODA	-	-	-	-	-	-	-	-	-	1
OSTRACODA										
Ostracod sp.1	-	-	-	-	-	-	-	-	-	-
Ostracod sp.2	-	-	-	-	-	-	-	-	-	-
Ostracod sp.3	-	-	-	-	-	-	-	-	-	-
POLYCHAETA										
Lumbrineris cavifrons	-	-	-	-	-	-	1	-	-	-
Marphysa corallina	-	-	-	-	1	-	-	-	-	-
Naineris laevigata	-	-	-	-	-	-	-	-	-	-
Nereis caudata	-	-	-	-	-	-	2	-	-	-
Odontosyllis polycera	-	-	-	-	-	-	-	-	-	-
Phyllodoce castanea	1	-	-	-	1	-	3	-	-	-
Platynereis dumerilii	-	-	-	-	-	-	-	-	-	-
Pomatoleios kraussii	-	-	-	-	-	-	-	-	-	-
Pseudonereis variegata	-	-	-	-	-	-	-	-	-	-
Syllis prolifera	3	-	-	-	-	-	2	-	4	-
Frubricinae	-	-	-	-	-	-	-	-	-	-
Polynoidae	-	-	-	-	-	-	-	-	-	-
TANAIDACEA										
Apseudomorpha avicularia	-	-	-	-	-	-	-	-	-	-
Tanais philetaerus	-	-	-	-	-	-	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	CPA1	CPA2	CPA3	CPA4	CPA5	PGB1	PGB2	PGB3	PGB4	PGB5
ACARINA	-	-	-	-	-	-	-	-	-	-
AMPHIPODS										
Atylus swammerdamei	5	6	-	-	42	11	1	41	32	26
Caprella natalensis	-	-	-	-	73	-	-	-	-	-
Hemiaegina minuta	-	-	-	-	53	-	-	-	-	-
Guernea rhomba	7	20	-	3	-	-	-	5	2	-
Proboloides rotunda	-	-	-	-	-	-	-	-	-	-
Stenothoe adhaerens	41	16	52	-	6	11	2	15	22	3
ECHINODERMATA										
Patiriella exigua	-	-	-	-	-	-	-	-	-	-
ISOPODS										
Cirolona hirtipes	-	-	-	-	-	-	-	-	-	-
Cymodocella sublevis	-	-	-	-	-	-	-	-	-	-
Dynamenella huttoni	20	63	13	71	54	29	31	30	39	14
Dynamenella macrocephala	-	-	-	-	2	-	-	-	-	-
Dynamenalla ovalis	-	-	-	-	-	-	-	-	-	-
Exosphaeroma pallidum	2	-	5	-	13	-	-	-	1	-
Exospaheroma truncatitelson	17	3	3	-	5	-	1	-	-	-
Janira extans	-	1	-	-	-	-	-	-	1	-
Paranthura punctata	-	-	-	-	-	-	-	-	-	-
Parisocladus perforatus	1	-	-	-	-	-	-	-	-	-
Parisocladus stimpsoni	1	-	-	-	5	-	-	-	-	-
MOLLUSCA										
Arcidae	-	-	-	-	-	-	-	1	-	-
Barnacle sp.	-	-	-	-	-	-	-	-	-	-
Gastropod sp.1	-	-	-	-	-	-	-	-	-	-
Gastropod sp.2	-	-	-	-	-	-	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**									
	CPA1	CPA2	CPA3	CPA4	CPA5	PGB1	PGB2	PGB3	PGB4	PGB5
Acanthochitona garnoti	-	1	-	-	-	-	-	-	-	-
Choromytilus meridionalis	-	-	-	-	-	-	-	-	-	-
Eatoniella nigra	-	-	-	-	-	-	-	-	-	-
Fissurella mutabillis	-	-	-	-	-	-	-	1	-	-
Helcion dunkiri	-	-	-	-	-	-	-	-	-	-
Littorina natalensis	-	-	-	-	-	-	-	-	-	-
Mytilus galloprovincialis	-	-	1	-	-	1	-	1	-	-
Perna perna	-	-	-	-	-	-	-	-	-	-
Siphonaria concinna	-	-	-	-	-	-	-	-	-	-
Scutellastra barbara	-	-	-	-	-	-	-	-	-	-
Tricolia neritina	-	-	-	-	-	-	-	-	-	-
Tricolia capensis	-	-	-	-	-	-	-	-	-	-
NEMATODA	-	-	1	1	-	-	-	-	-	2
OSTRACODA										
Ostracod sp.1	-	-	-	-	-	-	-	-	-	-
Ostracod sp.2	-	-	-	-	-	-	-	-	-	-
Ostracod sp.3	-	-	-	-	-	-	-	-	-	-
POLYCHAETA										
Lumbrineris cavifrons	-	1	-	-	1	-	1	-	-	-
Marphysa corallina	-	-	-	-	-	-	-	-	-	-
Naineris laevigata	-	2	-	-	-	-	-	-	1	1
Nereis caudata	2	-	-	-	-	-	-	1	1	-
Odontosyllis polycera	-	1	-	-	-	-	-	-	-	-
Phyllodoce castanea	-	-	-	-	-	-	-	-	-	-
Platynereis dumerilii	-	-	-	-	-	-	-	2	-	1
Pomatoleios kraussii	-	3	-	-	-	-	-	-	-	-
Pseudonereis variegata	-	-	1	-	-	-	-	2	-	-
Syllis prolifera	-	1	2	2	2	-	5	1	-	2
Frubricinae	-	-	-	-	-	-	-	-	-	-
Polynoidae	-	-	-	-	-	-	-	-	-	-
TANAIDACEA										
Apseudomorpha avicularia	-	-	-	-	-	-	-	-	-	-
Tanais philetaerus	-	-	-	-	-	-	-	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples**		
	MZB1	MZB2	MZB3
ACARINA	-	-	-
AMPHIPODS			
Atylus swammerdamei	-	-	1
Caprella natalensis	2	-	-
Hemiaegina minuta	-	-	-
Guernea rhomba	-	-	1
Proboloides rotunda	-	-	-
Stenothoe adhaerens	15	-	37
ECHINODERMATA			
Patiriella exigua	-	-	-
ISOPODS			
Cirolona hirtipes	-	-	-
Cymodocella sublevis	-	-	-
Dynamenella huttoni	-	3	13
Dynamenella macrocephala	2	-	-
Dynamenalla ovalis	-	-	-
Exosphaeroma pallidum	17	2	2
Exospaheroma truncatitelson	21	-	1
Janira extans	-	-	-
Paranthura punctata	-	-	-
Parisocladus perforatus	2	2	1
Parisocladus stimpsoni	3	-	3
MOLLUSCA			
Arcidae	-	-	-
Barnacle sp.	-	-	-
Gastropod sp.1	-	-	-
Gastropod sp.2	-	-	-

Appendix 7 continued

Epifauna taxa	**Samples** MZB1	 MZB2	 MZB3
Acanthochitona garnoti	-	-	-
Choromytilus meridionalis	-	-	-
Eatoniella nigra	-	-	-
Fissurella mutabillis	-	-	-
Helcion dunkiri	-	-	-
Littorina natalensis	-	-	-
Mytilus galloprovincialis	-	-	-
Perna perna	-	-	-
Siphonaria concinna	-	-	-
Scutellastra barbara	-	-	-
Tricolia neritina	-	-	-
Tricolia capensis	-	-	-
NEMATODA	-	1	-
OSTRACODA			
Ostracod sp.1	-	-	-
Ostracod sp.2	-	-	-
Ostracod sp.3	-	-	-
POLYCHAETA			
Lumbrineris cavifrons	-	-	-
Marphysa corallina	-	-	-
Naineris laevigata	-	-	-
Nereis caudata	-	1	-
Odontosyllis polycera	-	-	-
Phyllodoce castanea	-	6	2
Platynereis dumerilii	-	-	-
Pomatoleios kraussii	-	-	-
Pseudonereis variegata	-	-	-
Syllis prolifera	2	-	-
Frubricinae	-	-	-
Polynoidae	-	2	-
TANAIDACEA			
Apseudomorpha avicularia	-	-	-
Tanais philetaerus	-	-	-

Appendix 8: ***Final sequence alignments***

Gelidium pristoides ITS1

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[                                   10         20         30        40         50         60]

[                                   .          .          .          .          .         .

Hougham Park              CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
East London               ccTTCCGTAG GTGAACcTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Haga-Haga x 3             CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Nelson Mandela Bay x 5    CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Skoenmakerskop x 2        CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCaT TTTGATTTAA   [60]
Sardinia Bay x 5          CCTTCCGTAG GTGAACCTGc GGAAGGATCa TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
NoordHoek x 2             CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Port Alfred x 5           CCTTCCGTAG GTGaACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Kenton-On-Sea x 2         CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Port St. Johns x 5        CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Keeurboomstrand x 5       CCTTCCGTAG GTGAaCCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Still Bay x 5             CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGAtTTAA   [60]
Cape St. Francis x 4      CcTTCCGTAG GTGAACCTGC GGAaGGAtCA TTCAAAGAAA AAACTAtCAT TTTGATTTAA   [60]
Brenton-On-Sea x 4        CCttccgtag gtgaacCtGC GGAAGGaTCA TTCaAAGaAA AAACTaTCaT TTTGATTTAA   [60]
Mossel Bay x 4            CCtTCCGTAG GTGAAcCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Muizenberg x 4            CCtTCCGTAG GTGAAcCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Pringles Bay x 2          CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Jeffreys Bay x 2          CCTtCcGtaG GtGAaCctgc GGAaGGatCA TtCAAagAAA AAActATCAT TTTGATTTAA   [60]
Glentana x 4              CCTTCCGTAG GTGAACCtGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Buffels Bay x 2           CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAAAGAAA AAACTATCAT TTTGATTTAA   [60]
Beacon Island x3          CCTtCcGtaG GtGAaCctgc GGAaGGatCA TtCAAagAAA AAActATCAT TTTGATTTAA   [60]

[                                   70         80         90         100        110       120]
[                                   .          .          .          .          .         .]

Hougham Park              AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
East London               AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Haga-Haga x 3             AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Nelson Mandela Bay x 5    AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Skoenmakerskop x 2        AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Sardinia Bay x 5          AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
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NoordHoek x 2             AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Port Alfred x 5           AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Kenton-On-Sea x 2         AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Port St. Johns x 5        AAA-CATCTT AGTATT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Keeurboomstrand x 5       AAA-CATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Still Bay x 5             AAA-CATCTt AGTTtt-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Cape St. Francis x 4      AAAACATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [117]
Brenton-On-Sea x 4        AAAACATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [117]
Mossel Bay x 4            AAAACATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [117]
Muizenberg x 4            AAA-CATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Pringles Bay x 2          AAA-CATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Jeffreys Bay x 2          AAA-CATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Glentana x 4              AAAACATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [117]
Buffels Bay x 2           AAA-CATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]
Beacon Island x 3         AAA-CATCTT AGTTTT-GAG CCGAAAA-TT CTGTTTTCTG TGCTCCTATT TTGTTTTT-A   [116]

[                                   130        140        150        160        170       180]
[                                   .          .          .          .          .         .]
Hougham Park              AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
East London               AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Haga-Haga x 3             AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Nelson Mandela Bay x 5    AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Skoenmakerskop x 2        AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Sardinia Bay x 5          AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
NoordHoek x 2             AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Port Alfred x 5           AATCATATGA TATTGTTTTT AATTttTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Kenton-On-Sea x 2         AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Port St. Johns x 5        AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Keeurboomstrand x 5       AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Still Bay x 5             AATCATATGA TATTGTTTTt AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Cape St. Francis x 4      AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [177]
Brenton-On-Sea x 4        AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [177]
Mossel Bay x 4            AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [177]
Muizenberg x 4            AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Pringles Bay x 2          AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Jeffreys Bay x 2          AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Glentana x 4              AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [177]
Buffels Bay x 2           AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
Beacon Island x 3         AATCATATGA TATTGTTTTT AATTTTTCGT GCTCGAATTC AATCCACTTT TATTGTTTTT   [176]
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[                                   190        200        210        220        230       240]
[                                   .          .          .          .          .         .]
Hougham Park              ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
East London               ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Haga Haga x 3             ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Nelson Mandela Bay x 5    ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Skoenmakerskop x 2        ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Sardinia Bay x 5          ACTAtTAAAt ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
NoordHoek x 2             ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Port Alfred x 5           ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Kenton-On-Sea x 2         ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Port St. Johns x 5        ACTATTAAAT ACTTTATTTT TTTTTATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Keeurboomstrand x 5       ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [234]
Still Bay x 5             ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATAtAA   [234]
Cape St. Francis x 4      ACTATTAAAT ACTTTATTTT TTTT-AtATG tTTTTCTTGA CaATTT-AAC CaAGAtATAA   [235]
Brenton-On-Sea x 4        ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTtCTTGA CAATTT-AaC CAAgAtATAA   [235]
Mossel Bay x 4            ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Muizenberg x 4            ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [234]
Pringles Bay x 2          ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [234]
Jeffreys Bay x 2          ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [234]
Glentana x 4              ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [235]
Buffels Bay x 2           ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [234]
Beacon Island x 3         ACTATTAAAT ACTTTATTTT TTTT-ATATG TTTTTCTTGA CAATTT-AAC CAAGATATAA   [234]

[                                   250        260        270        280        290]
[                                   .          .          .          .          . ]
Hougham Park              AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [286]
East London               AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCgaTGAA GAACGCAGCA a   [286]
Haga-Haga x 3             AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [286]
Nelson Mandela Bay x 5    AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGAtgAA GAACGCAGCA A   [286]
Skoenmakerskop x 2        AACTCGTAAC GGtGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [286]
Sardinia Bay x 5          AACTCGTAAC GGtGGATGTC TTGGCTCCCG CATCgATgAA gAACGCAgcA A   [286]
NoordHoek x 2             AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [286]
Port Alfred x 5           AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGAtGAA GAACGCAGCA A   [286]
Ke             x 2        AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [286]
Po            s x 5       AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [286]
Keeurboomstrand x 5       AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [285]
Still Bay x 5             AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [285]
Cape St. Francis x 4      AACTcGTAAC GgtgGatgtC ttGGCtCCCg CATCgatGAA GAACGCaGCA A   [286]
Brenton-On-Sea x 4        AACtCGtAac GGtGGAtGtC TtGGCtCCCG CatcgaTGAA GAACGCAGCA A   [286]
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Appendices

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Mossel Bay x 4           AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCgatGAA GAACGCAGCa a   [286]
Muizenberg x 4           AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCgatGAA GAACGCAGCA A   [285]
Pringles Bay x 2         AACTCGTAAC GGTGGATGTC TTGGCTCCCg CATCGATGAA GAACGCAGCA A   [285]
Jeffreys Bay x 2         AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [285]
Glentana x 4             AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGatGAA GAACGCAGCA A   [286]
Buffels Bay x 2          AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [285]
Beacon Island x 3        AACTCGTAAC GGTGGATGTC TTGGCTCCCG CATCGATGAA GAACGCAGCA A   [285]

END;
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Gelidium pristoides Cox2-3 spacer region

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[                                  10        20        30        40        50        60]
[                                  .         .         .         .         .         .]
Gonubie                  GGGGAAATGTGATGCCATTCCAGGTCGTTTAaaCCAAACTTCTC-ttttATAAAAAGAGA   [59]
East London              GGGGAAATGTGATGCCATTCCAGGTCGTTTAaACCAAACTTCTC-TTTTATAAAAAGAGA   [59]
Port Alfred x 5          GGGGAAATGTGATGCCATTCCAGGTCGTTTAAACCAAACTTCTCTTTTTATAAAAAGAGA   [60]
Kenton-On-Sea x 3        GGGGAAATGTGATGCCATTCCAGGTCGTTTAAACCAAACTTCTCTTTTTATAAAAAGAGA   [60]
Port St. Johns x 5       GGGGAAATGTGATGCCATTCCAGGTCGTTTAAACCAAACTTCTCTTTTTATAAAAAGAGA   [60]
Haga Haga x 5            GGGGAAATGTGATGCCATTCCAGGTCGTTTAAACCAAACTTCTCTTTTTATAAAAAGAGA   [60]
Sardinia Bay x 4         GGGGAAATGTGATGCAATTCCaGGAAGATTAAATCaAACTTCTTTATTTATTAAACGTGA   [60]
Hougham Park             GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
Cape St. Francis x 3     GGGGAAATGtGATGCAATTCCaGGaAGAtTAAATCaAACTTCTTTATTTaTTaAACGTGA   [60]
NoordHoek x 2            GGGGAAATGtGATGCAATtCCaGGaAGATtaAATCaAACTTCtTtATTTATTAAACGTGA   [60]
Still Bay x 4            GGGGAAAtGtGatGCaAtTCCaGGAAGatTAAATCaAACTTCTTTaTTTaTTaAACGTGA   [60]
Pringles Bay x 4         gggGaAATGtGATGCaAtTCCaGGaAGATTAAATCaAACTTCTTTATTTATTAAACGTGA   [60]
Muizenberg x 4           GGGGAAATGtGAtGCAATTCCaGGAAGATTAAATCaAACTTCTTTATTTATTAAACGTGA   [60]
Glentana x 4             GGGGAAATGTGAtGCAATTCCaGGAAGatTaAATCaAACTTCTTTATTTATTAAACGTGA   [60]
Cape Agulhas x 3         GGGGAAATGTGATGCAATTCCaGGAAGATTAAATCaAACTTCTTTaTTTaTTAAACGTGA   [60]
Buffels Bay x 2          GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
Brenton-On-Sea x 3       GGGGAAATGTGATGCaATTCCaGGAAGATTAAATCaAACTTCTTTATTTATTaAACGTGA   [60]
Mossel Bay x 5           GGGGAAATGtGATGCaATTCCaGGAAGATTAAaTCaAACTTCTTTATTTATTAAACGTGA   [60]
Ke[illegible]nd x 3      ggggaaatgtgatGCaAtTCCaGGaAGatTAAATCaAACtTCtTTaTTTaTTaAACgtGa   [60]
Je[illegible] x 2        GGGGAAATGTGATGCAATTCCaGGAAGATTAAATCaAACTTCTTTATTTATTaAACGTGA   [60]
Beacon Island x 3        GGGGAAATGTGATGCaATTCCaGGAAGaTTAAATCaAACTTCTTTATTTATTAAACGTGA   [60]
Skoenmakerskop x 2       GGgGAAATgtGATGCAAtTCCaGGaAGAtTaAATCaAACTTctttAttTAttAaACGTGA   [60]
Nelson Mandela Bay x 3   GGGGAAATGTGATGCAATTCCaGGAAGATTAAATCaAACTTCTTTATTTaTTaAACGTGA   [60]
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[                                  70        80        90        100       110       120]
[                                  .         .         .         .         .         .]
Gonubie                   AGGTGTtTACTACGGGCAATGCAGTGAAATTTGTGGTATAAATCATGGGTTTATGCCTAT   [120]
East London               AGGTGTtTACTACGGGCAATGCAGTGAAATTTGTGGTATAAATCATGGGTTTATGCCTAT   [120]
Port Alfred x 5           AGGTGTTTACTACGGGCAATGCAGTGAAATTTGTGGTATAAATCATGGGTTTATGCCTAT   [120]
Kenton-On-Sea x 4         AGGTGTTTACTACGGGCAATGCAGTGAAATTTGTGGTATAAATCATGGGTTTATGCCTAT   [120]
Port St. Johns x 5        AGGTGTTTACTACGGGCAATGCAGTGAAATTTGTGGTATAAATCATGGGTTTATGCCTAT   [120]
Haga Haga x 5             AGGTGTTTACTACGGGCAATGCAGTGAAATTTGTGGTATAAATCATGGGTTTATGCCTAT   [120]
Glentana x 4              GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Sardinia Bay x 4          GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Hougham Park              GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Cape St. Francis x 3      GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
NoordHoek x 2             GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Still Bay x 4             GGGTTTATATTATGGCCaAtGtAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Pringles Bay x 4          GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Muizenberg1 x 4           GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Glentana x 3              GGGTTTATAtTatGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Cape Agulhas x 3          GGGTTTATATTATGGCCaATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Buffels Bay x 2           GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Brenton-On-Sea x 3        GGGTTTATATTATGGCCaATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Mossel Bay x 5            GGGTTTATATTATGGCCaATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Keeurboomstrand x 3       GGGTTTATATTATGGCCaATGtAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Jeffreys Bay x 2          GGGTTTATATTATGGCCaATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Beacon Island x 3         GGGTTTATATTAtGGCCaATGtAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Skoenmakerskop x 2        GGGttTaTaTTATGGCCaATGtAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Nelson Mandela Bay x 3    GGGTTTATATTATGGCCaATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]

[                                  130       140       150       160       170       180]
[                                  .         .         .         .         .         .]
Gonubie                   TGTTGTGGAAGCCGTTTCCTTACCGCAATATATTTCTTGGGTTTCAAATAAATTGAGTGA   [180]
East London               TGTTGTGGAAGCCGTTTCCTTACCGCAATATATTTCTTGGGTTTCAAATAAATTGAGTGA   [180]
Port Alfred x 5           TGTTGTGGAAGCCGTTTCCTTACCGCAATATATTTCTTGGGTTTCAAATAAATTGAGTGA   [180]
Kenton-On-Sea x 4         TGTTGTGGAAGCCGTTTCCTTACCGCAATATATTTCTTGGGTTTCAAATAAATTGAGTGA   [180]
Port St. Johns x 5        TGTTGTGGAAGCCGTTTCCTTACCGCAATATATTTCTTGGGTTTCAAATAAATTGAGTGA   [180]
Haga Haga x 5             TGTTGTGGAAGCCGTTTCCTTACCGCAATATATTTCTTGGGTTTCAAATAAATTGAGTGA   [180]
Glentana x 4              AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Sardinia Bay x 4          AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Hougham Park              AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Cape St. Francis x 3      AGTAGTTGAAGCAGTTACTTTACCAAATTATATACaTTGGATTTTTAATAAAATTAATGA   [180]
NoordHoek x 2             AGTAGTTGAAGCAGTTACTTTACCAAATTATATACaTTGGATTTTTAATAAAATTAATGA   [180]
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Still Bay x 4             AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Pringles Bay x 4          AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Muizenberg x 4            AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Cape Agulhas x 3          AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Buffels Bay x 2           AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Brenton-On-Sea x 3        AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Mossel Bay x 5            AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Keeurboomstrand x 3       AGTAGTTGAAGCAGTTACTTTACCaAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Jeffreys Bay x 2          AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Beacon Island x 3         AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]
Skoenmakerskop x 2        AGTAGTTGAAGCAGTTACTTTACCAAATTATATACatTGGATTTTTAATAAAATTAATGA   [180]
Nelson Mandela Bay x 2    AGTAGTTGAAGCAGTTACTTTACCAAATTATATACATTGGATTTTTAATAAAATTAATGA   [180]

[                                  190       200       210       220       230       240]
[                                  .         .         .         .         .         .]
Gonubie                   ATAATTTATATGAGATTATCAATCTCCCAAATCATATTTCtAGGATTTATCTTTTTTATA   [230]
East London               ATAATTTATATGAGATTATCAATCTCCCAAATCATATTTCTAGGATTTATCTTTtTTATA   [230]
Port Alfred x 5           ATAATTTATATGAGATTATCAATCTCCCAAATCATATTTCTAGGATTTATCTTTTTTATA   [230]
Kenton-On-Sea x 4         ATAATTTATATGAGATTATCAATCTCCCAAATCATATTTCTAGGATTTATCTTTTTTATA   [230]
Port St. Johns x 5        ATAATTTATATGAGATTATCAATCTCCCAAATCATATTTCTAGGATTTATCTTTTTTATA   [230]
Haga Haga x 5             ATAATTTATATGAGATTATCAATCTCCCAAATCATATTTCTAGGATTTATCTTTTTTATA   [230]
Glentana x 4              ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Sardinia Bay x 4          ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Brenton-On-Sea x 3        ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Sardinia Bay x 3          ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Hougham Park              ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Cape St. Francis x 3      ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
NoordHoek x 2             ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTtG   [226]
Still Bay x 4             ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Pringles Bay x 4          ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Muizenberg x 4            ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Glentana x 3              ATAA---A-ATGAAATTATCTTTATTTCAAtTTTTTTTATTAAtAGtAATTTTTTTTTTG   [226]
Cape Agulhas x 3          ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Buffels Bay x 2           ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAAtTTTTTTTTTG   [226]
Mossel Bay x 5            ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Keeurboomstrand x 2       ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Jeffreys Bay x 2          ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Beacon Island x 3         ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
Skoenmakerskop x 2        ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAAtAGTAATTTTTTTTTTG   [226]
Nelson Mandela Bay x 3    ATAA---A-ATGAAATTATCTTTATTTCAATTTTTTTTATTAATAGTAATTTTTTTTTTG   [226]
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[                                  250       260       270       280       290       300]
[                                  .         .         .         .         .         .]
Gonubie                   CTGATTTACCCTAATAAAATTTTTCCCGCAAAGATATCAAAATCTTTGCGGGGTCTTATC   [290]
East London               CTGATTTACCCTAATAAAATTTTTCCCGCAAAGATATCaaAATCTTTGCGGGGTCTTATC   [290]
Port Alfred x 5           CTGATTTACCCTAATAAAATTTTTCCCGCAAAGATATCAAAATCTTTGCGGGGTCTTATC   [290]
Kenton-On-Sea x 4         CTGATTTACCCTAATAAAATTTTTCCCGCAAAGATATCAAAATCTTTGCGGGGTCTTATC   [290]
Port St. Johns x 5        CTGATTTACCCTAATAAAATTTTTCCCGCAAAGATATCAAAATCTTTGCGGGGTCTTATC   [290]
Haga Haga x 5             CTGATTTACCCTAATAAAATTTTTCCCGCAAAGATATCAAAATCTTTGCGGGGTCTTATC   [290]
Glentana x 4              TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Sardinia Bay x 4          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Brenton-On-Sea x 3        TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTtAAAATTTTTTAAAAAAATATAT   [290]
Hougham Park              TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Cape St. Francis x 3      TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
NoordHoek x 2             TTTTTTTATTCTAAAGGtTTTTTTATTCGAACTATTTTAAAATTTTTTAAaAAAATaTat   [290]
Still Bay x 4             TTTTTTTATTCTAAAGGTTTTTTTaTTCGAaCTATTTTAAAATTTTTTAAAAAAATATaT   [290]
Pringles Bay x 4          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATaT   [290]
Muizenberg x 4            TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Cape Agulhas x 3          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTtAAAAtTTTTTAAAAAAATATAt   [290]
Buffels Bay x 2           TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Mossel Bay x 5            TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Keeurboomstrand x 3       TTTTTTTATtC-AAAGGTTTTTTTaTTCGAaCTATTTtAAAATTTTTtAAAAAAATATaT   [290]
Jeffreys Bay x 2          TTTTTTTATTCTAAAGGTTTTTTTATTCGAaCTATTTtAAAATTTTTTAAAAAAAtATat   [290]
Beacon Island x 3         TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [290]
Skoenmakerskop x 2        TTTTTTTATTCTAAAGGtTTTTTTATtCGAACTATTTtAAAATTTtTTAAAAAAATaTat   [290]
Nelson Mandela Bay x 3    TTTTTTTATTCTAAAGGTTTTTTTATTCGAaCTATTTtAAAATTTTTTAAAAAAATATat   [290]

[                                  310       320       330       340       350       360]
[                                  .         .         .         .         .         .]
Gonubie                   AAAT----GGTTTTTAAAATCAA-TTTCTAAAAATTCGAAATAAACTTAATATGACTCTT   [350]
East London               AAAT----GGTTTTTAaAATCAA-TTTCTAAAaATTCGAAATAAACTTAATATGACTCTT   [350]
Port Alfred x 5           AAAT----GGTTTTTAAAATCAA-TTTCTAAAAATTCGAAATAAACTTAATATGACTCTT   [350]
Kenton-On-Sea x 4         AAAT----GGTTTTTAAAATCAA-TTTCTAAAAATTCGAAATAAACTTAATATGACTCTT   [350]
Port St. Johns x 5        AAAT----GGTTTTTAAAATCAA-TTTCTAAAAATTCGAAATAAACTTAATATGACTCTT   [350]
Haga-Haga x 5             AAAT----GGTTTTTAAAATCAA-TTTCTAAAAATTCGAAATAAACTTAATATGACTCTT   [350]
Glentana x 4              AAATAATCAtGTCTTTATCaCAAGTTTCTAAAAAT-------------------------   [325]
Sardinia Bay x 4          AAATAATCATGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
Brenton-On-Sea x 3        AAATAATCAtGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
Hougham Park              AAATAATCATGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
Cape St. Francis x 3      AAATAATCAtGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
NoordHoek x 2             AAATAaTCAtGtCTTTATCaCAAGTTTCTAAAAAt-------------------------   [325]
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Still Bay x 4             AAATAAtCaTGTCTTTATCaCAATTTTCTAAAAAT-------------------------   [325]
Pringles Bay x 4          AAATAATCAtGTCTTTATCaCAAGTTTCTAAAAAT-------------------------   [325]
Muizenberg x 4            AAATAATCATGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
Cape Agulhas x 3          AAATAAtCAtGTCTTTATCaCAAGTTTCTAAAAAT-------------------------   [325]
Buffels Bay x 2           AAATAATCATGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
Mossel Bay x 5            AAATAATCATGTCTTTATCaCAAGTTTCTAAAAAT-------------------------   [325]
Keeurboomstrand x 3       AAATAAtCatGtCTTTAtCaCAAGTTtCtAAAAAt-------------------------   [325]
Jeffreys Bay x 2          AAATAATCaTGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]
Beacon Island x 3         AAATAATCATGTCTTTATCACAAGTTTCTAAAAAt-------------------------   [325]
Skoenmakerskop x 2        AAAtAaTCATGTCTTTATCaCAAgTTTCTAAAAAT-------------------------   [325]
Nelson Mandela Bay x 3    AAATAAtCaTGTCTTTATCACAAGTTTCTAAAAAT-------------------------   [325]

[                                  370       380       390       ]
[                                  .         .         .         ]
Gonubie                   TTATCTCAAaTTTCTAAATCTGTACAGCGACATCCATTC   [389]
East London               TTATCTCAAATTTCTAAATCtGTACAGCGACATCCATtc   [389]
Port Alfred x 5           TTATCTCAAATTTCTAAATCTGTACAGCGACATCCATTC   [389]
Kenton-On-Sea x 3         TTATCTCAAATTTCTAAATCTGTACAGCGACATCCATTC   [389]
Port St. Johns x 5        TTATCTCAAATTTCTAAATCTGTACAGCGACATCCATTC   [389]
Haga-Haga x 5             TTATCTCAAATTTCTAAATCTGTACAGCGACATCCATTC   [389]
Kenton-On-sea5            TTATCTCAAATTTCTAAATCTGTACAGCGACATCCATTC   [389]
Glentana x 4              ---------------------ATACAACGACaTCCATTC   [368]
Sardinia Bay x 4          ---------------------ATACAACGACATCCATTC   [368]
Brenton-On-Sea x 3        ---------------------ATACAACGACaTCCATTC   [368]
Hougham Park              ---------------------ATACAACGACaTCCATTC   [368]
Cape St. Francis x 3      ---------------------ATACAACGACaTCCATTC   [368]
NoordHoek x 2             ---------------------ATACaACGaCaTCCATTC   [368]
Still Bay x 4             ---------------------ATaCAaCgaCatCCaTTC   [368]
Pringles Bay x 4          ---------------------ATACAACGACaTCCATtC   [368]
Muizenberg x 4            ---------------------ATACAACGACATCCATTC   [368]
Cape Agulhas x 3          ---------------------ATACAACGACaTCCATTC   [368]
Buffels Bay x 2           ---------------------ATACAACGACATCCATTC   [368]
Mossel Bay x 5            ---------------------ATaCAaCGaCAtCCaTtC   [368]
Keeurboomstrand x 3       ---------------------ATaCAaCGaCaTccattc   [368]
Jeffreys Bay x 2          ---------------------ATACAACGACaTCCATTC   [368]
Beacon Island x 3         ---------------------ATaCAACGaCatCCaTtC   [368]
Skoenmakerskop x 2        ---------------------ATACaACGaCatCCATtC   [368]
Nelson Mandela Bay x 3    ---------------------ATACAACGACaTCCATTC   [368]

END;
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Hypnea spicifera ITS1

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[                                   10         20         30         40         50        60]
[                                   .          .          .          .          .         .]
Buffels Bay x 2           CCTTCCGTAG GTGAACCtGc GGAaGGatCA TTCataGtGt GtGaGCGAGC GTGTTGATTC   [60]
Haga-Haga x 4             CCTTCCGTAG GTGAACCTGc GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Nelson Mandela Bay x 2    CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Sardinia Bay x 5          CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Ngazana x 4               CCtTCCGTAG GtGAACCTGc GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Hougham Park x 4          CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Keeurboomstrand x 5       CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Camps Bay x 5             CCTTCCGTAG GTGAACCtGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Glentana x 5              CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGtGT GTGAGCGAGC GTGTTGATTC   [60]
Brenton-On-Sea x 5        CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCAtAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Cape Agulhas x 5          CCTTcCGtAG GTGAACCtGC GGAAGGaTcA tTcaTAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Muizenberg x 5            CCTTCCGTAG GTGAACCTGC GGAAGGAtcA tTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Cape Hangklip x 5         CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Kenton-On-Sea x 4         CCTTCCGTAG GTGAACCTGC GGaaGgaTCA TTcATAGtGt GTGAGCGAGC GTGTTGATTC   [60]
Shaka’s Rock x 4          CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Coffee Bay x 5            CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Port St. Johns x 5        CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]
Southbroom x 4            CCTTCCGTAG GTGAACCTGC GGAAGGATCA TTCATAGTGT GTGAGCGAGC GTGTTGATTC   [60]

[                                   70         80         90         100        110       120]
[                                   .          .          .          .          .         .]
Buffels Bay x 2           TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCt   [119]
Haga-Haga x 4             TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Nelson Mandela Bay x 2    TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Sardinia Bay x 5          TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Ngazana x 4               TTTCGGGAGT CGCATACTTG CAAATACTTT TTTATTTCCG tGAACtATTt ATaCAACTCT   [120]
Hougham Park x 4          TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Keeurboomstrand x 5       TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
Camps Bay x 5             TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Glentana x 5              TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
Brenton-On-Sea x 5        TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
Cape Agulhas x 5          TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
Muizenberg x 5            TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
Cape Hangklip x 5         TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
Kenton-On-Sea x 4         TTTCGGGAGT CGCATACTTG CAAaTACTTT TT-ATTTCCG TGAACTATTT ATACAACTCT   [119]
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Shaka’s Rock x 4          TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Coffee Bay x 5            TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Port St. Johns x 3        TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]
Port St. Johns x 2        TTTCGGGAGT CGCATACTTG CAAATACTTT TTTATTTCCG TGAACTATTT ATACAACTCT   [120]
Southbroom x 4            TTTCGGGAGT CGCATACTTG CAAATACTTT TT-ATTTCCG TGAACTATTT ATACAACTC-   [118]

[                                   130        140        150        160        170       180]
[                                   .          .          .          .          .         .]
Buffels Bay x 2           TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACaACAC ACAAATAATT   [173]
Haga-Haga x 2             TTTTTTTA-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [173]
Haga-Haga x 2             TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [174]
Nelson Mandela Bay x 2    TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [172]
Sardinia Bay x 4          TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [172]
Sardinia Bay              -TTTTTTA-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [172]
Ngazana x 2               TTTTTTTt-- ATTaCAa-CC aTtGAaCCCA AAAAAAA--- CATACAACAC ACAAATAATT   [174]
Ngazana x 2               TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [174]
Hougham Park x 3          TTTTTTTA-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [173]
Hougham Park              TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [174]
Keeurboomstrand x 5       TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [173]
Camps Bay x 5             TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [173]
Glentana x 4              TTTTTTTT-- ATtACAa-CC aTtGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [174]
Glentana                  TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAAAA CATACAACAC ACAAATAATT   [176]
Brenton-On-Sea x 5        TTTTTTTT-- ATTACAa-CC aTtGAaCCCA AAAAAAAa-- CATACAACAC ACAAATAATT   [174]
Cape Agulhas x 5          TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [173]
Muizenberg x 4            TTTTTTTT-- ATTACAa-CC ATtGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [173]
Muizenberg                TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAA---- CATACAACAC ACAAATAATT   [172]
Cape Hangklip x 5         TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAA--- CATACAACAC ACAAATAATT   [173]
Kenton-On-Sea x 2         TTTTTTTT-- ATTACaA-CC aTTGaACCCA AAAAAAa--- CaTaCAaCAc aCAAATaATt   [173]
Kenton-On-Sea x 2         TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [174]
Shaka’s Rock x 2          TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [173]
Shaka’s Rock x 2          -TTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAAAA CATACAACAC ACAAATAATT   [174]
Coffee Bay x 5            TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [173]
Port St. Johns x 3        TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAAA- CATACAACAC ACAAATAATT   [174]
Port St. Johns x 2        TTTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [175]
Southbroom x 2            -TTTTTTT-- ATTACAA-CC ATTGAACCCA AAAAAAAA-- CATACAACAC ACAAATAATT   [172]
Southbroom x 2            TTTTTTTA-- ATTACAA-CC ATTGAACCCA AAAAAAAAA- CATACAACAC ACAAATAATT   [174]
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[                                   190        200        210        220        230]
[                                   .          .          .          .          .]
Buffels Bay x 2           ATTATACaAC CCGtGaCGGt GgataActaG GtTTCaGCaT CgaTGaAgAA CgCaGCAA    [231]
Haga-Haga x 5             ATTATACAAC CCGtGACGGt GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGcAA    [231]
Nelson Mandela Bay x 2    ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [230]
Sardinia Bay x 5          ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [230]
Ngazana x 4               ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGcAa    [232]
Hougham Park x 4          ATTATACAAC CCGtGACGGt GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGcAA    [231]
Keeurboomstrand x 5       ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [231]
Camps Bay x 5             ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [231]
Glentana x 5              ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [232]
Brenton-On-Sea x 5        attaTACAaC CCgTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [232]
Cape Agulhas x 5          ATTATACAAC CCGtGaCGGt GGaTAaCTaG GTTtCaGCat CGatGAAGAA CGCAGCAA    [231]
Muizenberg x 5            ATTATACAAC CCGtGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [231]
Cape Hangklip x 5         ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [231]
Kenton-On-Sea x 4         ATTatACAac ccGtgAcGGt GGatAactaG GTttcagcaT CgatGAAGAA cGcaGCAA    [231]
Shaka’s Rock x 4          ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [231]
Coffee Bay x 5            ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [231]
Port St. Johns x 5        ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [232]
Southbroom x 4            ATTATACAAC CCGTGACGGT GGATAACTAG GTTTCAGCAT CGATGAAGAA CGCAGCAA    [230]

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Hypnea spicifera Cox2-3 spacer region

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 [                                 10        20        30        40        50        60]
 [                                 .         .         .         .         .         .]
 Haga-Haga x 5            GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Brenton-On-Sea x 3       GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Buffels Bay x 2          GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Camps Bay x 4            GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Hougham Park x 4         GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Cape Agulhas x 4         GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Glentana x 4             GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Cape Hangklip x 4        GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Muizenberg x 4           GGGGAAATGTGATGCAATTCCaGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Kenton-On-Sea x 3        ggggaaaTGTGATGCaATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Sardinia Bay x 4         GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
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 Ngazana x 4              GGGGAAATGtGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Coffee Bay x 4           GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Southbroom x 4           GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Port St. Johns x 5       GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Keeurboomstrand x 4      GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]
 Shaka’s Rock x 5         GGGGAAATGTGATGCAATTCCAGGAAGATTAAATCAAACTTCTTTATTTATTAAACGTGA   [60]

[                                  70        80        90        100       110       120]
[                                  .         .         .         .         .         .]
Haga-Haga x 5             GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Brenton-On-Sea x 3        GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Buffels Bay x 3           GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Camps Bay x 4             GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Hougham Park x 4          GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Cape Agulhas x 4          GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Glentana x 4              GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Cape Hangklip x 4         GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Muizenberg x 4            GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Kenton-On-Sea x 3         GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Sardinia Bay x 4          GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Ngazana x 4               GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Coffee Bay x 4            GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Southbroom x 5            GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Port St. Johns x 5        GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Keeurboomstrand x 4       GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]
Shaka’s Rock x 5          GGGTTTATATTATGGCCAATGTAGTGAAATTTGTGGTATTAATCATGGATTTATGCCTAT   [120]

[                                  130       140       150       160       170       180]
[                                  .         .         .         .         .         .]
Haga-Haga x 5             AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Brenton-On-Sea x 3        AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Buffels Bay x 2           AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Camps Bay x 4             AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Hougham Park x 4          AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Cape Agulhas x 4          AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Glentana x 4              AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Cape Hangklip x 4         AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Muizenberg x 4            AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Kenton-On-Sea x 3         AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Sardinia Bay x 4          AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
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Ngazana x 4               AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Coffee Bay x 4            AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Southbroom x 5            AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Port St. Johns x 5        AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Keeurboomstrand x 4       AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]
Shaka’s Rock x 5          AGTAGTTGAAGCAGTTACTTTACCAAATTATATA-CATTGGATTTTTAATAAAATTAA-T   [180]

[                                  190       200       210       230       240       250]
[                                  .         .         .         .         .         .]
Haga-Haga x 5             GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Brenton-On-Sea x 3        GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Buffels Bay x 2           GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Camps Bay x 4             GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Hougham Park x 4          GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Cape Agulhas x 4          GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAACTTTTTTTTG   [248]
Glentana x 4              GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Cape Hangklip x 4         GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Muizenberg x 4            GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Kenton-On-Sea x 3         GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Sardinia Bay x 4          GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Ngazana x 4               GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Coffee Bay x 4            GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Southbroom x 4            GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Port St. Johns x 5        GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Keeurboomstrand x 4       GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]
Shaka’s Rock x 5          GAATAAAATGAAATTATCTTTATTTCAATTTTTTTTTATT--AATAGTAATTTTTTTTTG   [248]

[                                  260       270       280       290       300       310]
[                                  .         .         .         .         .         .]
Haga-Haga x 5             TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Brenton-On-Sea x 3        TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Buffels Bay x 2           TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Camps Bay x 4             TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Hougham Park x 4          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Cape Agulhas x 4          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Glentana x 4              TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Cape Hangklip x 4         TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Muizenberg x 4            TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Kenton-On-Sea x 3         TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
Sardinia Bay x 4          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTAAAAAAAATATAT   [310]
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Ngazana x 4               TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [310]
Coffee Bay x 4            TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [310]
Southbroom x 5            TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [310]
Port St. Johns x 3        TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [310]
Keeurboomstrand x 3       TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [310]
Shaka’s Rock x 5          TTTTTTTATTCTAAAGGTTTTTTTATTCGAACTATTTTAAAATTTTTTAAAAAAATATAT   [310]

[                                  320       330       340       350       360]
[                                   .         .         .         .         . ]
Haga-Haga x 2            AAATAATCATGTCTTTAGCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Haga-Haga x 3            AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Brenton-On-Sea x 3       AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Buffels Bay x 2          AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACaTCCTTTC   [363]
Camps Bay x 4            AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Hougham Park x 4         AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Cape Agulhas x 4         AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Glentana x 4             AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Cape Hangklip x 4        AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Muizenberg x 4           AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Kenton-On-Sea x 3        AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTtC   [363]
Sardinia Bay x 4         AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Ngazana x 4              AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Coffee Bay x 4           AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Southbroom x 5           AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Port St. Johns x 4       AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Port St. Johns           AAATAATCATGTCTTTAGCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Keeurboomstrand x 4      AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]
Shaka’s Rock x 5         AAATAATCATGTCTTTATCAC-AAGTTTCTAAAAATATACAACGACATCCTTTC   [363]

END;
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