The phylogeographic population structure of the Cape sea urchin, *Parechinus angulosus*

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Declaration

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Abstract

South Africa's coastline is in the region of 3650kms and encompasses many different and dynamic marine environments. To enhance our current understanding of the population structure and gene flow patterns of intertidal zone marine species in this region, this study sets out to investigate the phylogeographic population structure of the Cape sea urchin, *Parechinus angulosus,* using mitochondrial and nuclear DNA sequence data collected in 2007 and 2008. Individuals were sampled from 18 geographic locations between southern Namibia and Durban, covering nearly the full extent of the species range. Sequence data were obtained from a 790bp region of the COI mtDNA gene (n=510) and a 182bp region of the nDNA SpREJ9 gene (n=145), respectively. The mtDNA data revealed 283 polymorphic sites (36%) defining 195 haplotypes, of which 160 were unique and 35 shared among individuals. Haplotype diversity (h) was found to be high both overall (h=0.95) and for individual localities (h=0.75-0.98), with nucleotide diversity (π) being low overall (π=0.013) as well as for individual localities (π=0.0033-0.0254). AMOVA revealed significant population structure among sampling sites in the Namaqua Province biogeographical region, as well as between three of the four respective coastal biogeographic provinces/regions. Gene flow was bi-directional among sampling sites in the south coast Agulhas and East Coast Province biogeographical regions, while gene flow in the Namaqua Province appears to be dominated by northwards movement. BAPS identified a significant break in the Cape Point region, which was also reflected in the gene flow patterns and parsimony networks. This broadly corresponds to previously identified biogeographic regions as well as genetic breaks for other marine species found along this coast. Fu's Fs statistics showed strong signal(s) of population expansion for individual sampling localities as well as for the data set as a whole, while MDIV estimated a time since expansion ranging from 7733-4759 years ago. The nDNA data

revealed 54 variable sites (29.7%), defining 72 alleles of which 50 were unique and 22 shared among individuals. Many of the alleles (69.4%) were restricted to single sampling sites, with Betty's Bay on the south coast being the most diverse from a genetic viewpoint. Allelic diversity was high overall (h=0.86) while nucleotide diversity was low (π=0.025). No nuclear sub-group structure was identified by BAPS, although the parsimony network revealed shallow genetic structure between the Namaqua and Agulhas Provinces, with significant pairwise Φ_{ST} values also recovered between their individual coastal localities. This points to at least one major barrier to gene flow for *Parechinus angulosus* along the South African coast, namely Cape Point. Several additional, smaller hindrances to gene flow along the coast were also identified, most of which are congruent with findings from studies on both other and sea urchin species. As a standalone study this research elucidated many aspects regarding the phylogeography of the Cape sea urchin, *P. angulosus*. However, it is when viewed in the broader context of invertebrate phylogeography along the southern African coastline that this research will provide its most critical insight.

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As with any endeavor in life, there are many people who help you along the way towards the realisation of your goals. As such, I have many people to thank. A sincere thank you to my supervisors: Conrad Matthee, Sophie von der Heyden and Rauri Bowie for their ongoing assistance, guidance and advice spanning the period of this project. The many members of the Evolutionary Genomics Group for their help and support at various, often odd, times. Of course, many people assisted with sample collection in the field: Marlene Neethling, Sophie von der Heyden, Conrad Matthee, Nina du Toit, Brad Mardon and Rauri and Verna Bowie. For assistance with the gene flow analyses I would also like to thank the Centre for Proteomic and Genomic Research in Cape Town, especially Dane Kennedy who spent countless hours running the data and without whom the gene flow analyses would have been impossible. Last but not least, a very heartfelt thanks to my family and friends for their love, support and encouragement throughout, especially my partner Lorraine and my parents Neels and Magru, without whom I would have given up long ago. This study was funded by the South African National Research Foundation as well as by a Stellenbosch University Departmental bursary.

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Chapter 1: Introduction

1.1 Phylogeography in dynamic oceans

Phylogeography is a field of science that is highly integrative, as it employs genetic information to study the geographic distribution of genealogies in order to draw conclusions about historical patterns of gene flow (Avise 1994, Beheregaray 2008). It also aims to investigate both the spatial and temporal components of genetic structure of populations of a single or multiple species (i.e. comparative phylogeography), and by doing so explain how the observed genetic patterns became established. Never more so than at present have phylogeographic studies been imperative for marine species, especially in the southern Hemisphere, and more importantly in the oceans bordering developing countries, as they have been grossly neglected by the scientific community (Beheregaray 2008). Gaining this information will greatly aid the ability of scientists in these regions to make predictions and give advice regarding natural ecosystem functioning, which in turn will allow for more efficient and directed conservation planning in the future (Beheregaray 2008, von der Heyden 2009).

The physics of a liquid ocean on a rotating sphere, coupled to temperature differentiation (heated unequally at the poles and the equator), combine to set the scene for complex oceanic circulation (Schopf 1979, Palumbi 1994), i.e. oceanic currents. These currents have fluctuated over geological time and are also influenced by El Niño events that are in turn associated with climate which fluctuates on an annual basis (Meyers *et al.* 1983, Shannon 1985, Peterson and Stramma 1991). Schopf (1979) suggested that the basic oceanic circulation patterns (major currents), as well as heating and cooling regimes originated in the relatively recent past, and it is expected that biogeographic boundaries of marine organisms are typically set by these

physical forces. Although little data exist on the influence of these currents on species formation, gene flow across the oceans is probably constrained and directed by these circulation patterns to a large extent (Palumbi 1994, Weersing and Toonen 2009).

Along coastlines geographic features such as points, capes, bays and local ocean currents, upwelling and retention zones, can influence broad scale oceanic circulation, and this in turn results in variable modes of larval transport among species (Largier 2004). Disruptions in gene flow will ultimately result in distinct phylogeographic patterns in a region. Seasonal shifts in currents and episodic events such as the relaxation of upwelling, may also have significant consequences for the transport and recruitment success of marine larvae (Largier 2004, Levin 2006). In the study of a broadcast spawner, the green sea urchin (*Strongylocentrotus droebacheinsis*), it was suggested that the differences in oceanographic conditions and patterns of surface currents along the coast may be responsible for the differences in population structure (Addison and Hart 2004). On the east coast of North America, Cape Hatteras and Cape Cod define biogeographic boundaries set by near-shore currents and a steep temperature gradient (Schopf 1979). Along this coast, genetic differentiation seems to be over a far shorter geographic scale than expected from analyses of larval dispersal abilities and current patterns alone (Powers 1987, Palumbi 1994).

Interestingly, from a southern African perspective, recent phylogeographic studies on marine fauna with differing life histories have indicated that biodiversity (including cryptic species) along this coastline is considerably greater than previously thought, and in many instances show unexpected patterns of genetic structure, or lack thereof. For instance Teske *et al.'s* (2006) study on three different estuarine crustaceans (*Upogebia africana*, *Exosphaeroma hylecoetes*, *Iphinoe truncata*), as well as Teske *et*

al. (2007) on a caridean shrimp species (*Palaemon peringueyi*) and Teske *et al.* (2009) on an estuarine prawn (*Callianassa kraussi*); all studies showed various degrees of concordance between genetic structure and identified biogeographic provinces along the coast. Gopal *et al.* (2006) studied the spiny lobster (*Palinurus delagoae*) and argued for the importance of eddies and counter currents on the African east coast as being important to explaining the observed genetic structure between Mozambique and South African populations, concluding that genetic panmixia cannot be taken for granted even when a species' distribution is believed to be continuous with potential for high gene flow due to a long pelagic larval phase. Matthee *et al.* (2007) studied the west-coast rock lobster (*Jasus lalandii*) and found lower genetic diversity at the edges of the species' range, but high diversity at localised sampling sites and attributed this pattern to larval settling behaviour.

Zardi *et al.* (2007) found that the indigenous mussel (*Perna perna*) grouped into two lineages, one on the west coast and the other on the south-east coast, although some admixture occurred among lineages several hundreds of kilometers away, invoking current patterns and upwelling as explanations. Research on the clinid fish *Clinus cottoides* (a viviparous species) revealed significant population structure between east, south and west coast samples that broadly corresponded with the recognised biogeographic provinces along the southern African coastline (von der Heyden *et al.* 2008). This is adding to an increasing research collective that have identified several possible key areas along the southern African coast which could be acting as genetic breaks for several marine species.

From a conservation perspective it is important to realise that several areas that show considerable genetic diversity and structure across multiple taxa currently fall outside the existing marine protected area network of the country (von der Heyden 2009).

1.2 Southern African coastline

The coast of southern Africa extends to 35°S, with its southeastern side flanked by the warm waters of the southwesterly flowing Agulhas Current, which is also the most dominant oceanographic influence along this stretch of coastline (Shannon 1985, Lutjeharms and de Ruijter 1996, Reason *et al.* 2006). In comparison, the west coast of southern Africa is heavily influenced by the cold waters of the northward flowing Benguela Current from Cape Point to Lüderitz (Shannon 1985, Peterson and Stramma 1991, Reason *et al.* 2006, Fig. 1).

The biogeographic break between the Atlantic and Indian Oceans is caused by the cold Benguela Current and upwelling system, which comes close to the southwestern coast of South Africa at about 34°S (Shannon 1985, Reason *et al.* 2006). Temperature of the oceanic water changes markedly from ~24°C in the Agulhas system in the region of Durban (Heydorn 1978, Branch *et al.* 2002) to ~12°C in the Benguela system on South Africa's west coast. Along the south coast in the region of Cape Agulhas the water is closer to ~17ºC (Andrews and Cram 1969, Branch *et al.* 2002, Lutjeharms 2006). The position and interaction among the currents cause marked differences in marine faunal and floral diversity along the southern African coastline and this in turn has lead to the recognition of at least four major inshore biogeographic provinces: the 'cool-temperate Namaqua Province' (Lüderitz, Namibia – Cape Point), 'warm-temperate south coast Agulhas Province' (Cape Point – Algoa Bay), the

'subtropical East Coast Province' (Algoa Bay – Northern Kwazulu-Natal) and the 'tropical Mozambique Province' (north of Cape Vidal), (Emanuel *et al.* 1992, Awad *et al.* 2002, Teske *et al.* 2006, Teske *et al.* 2009, von der Heyden 2009, Fig. 1).

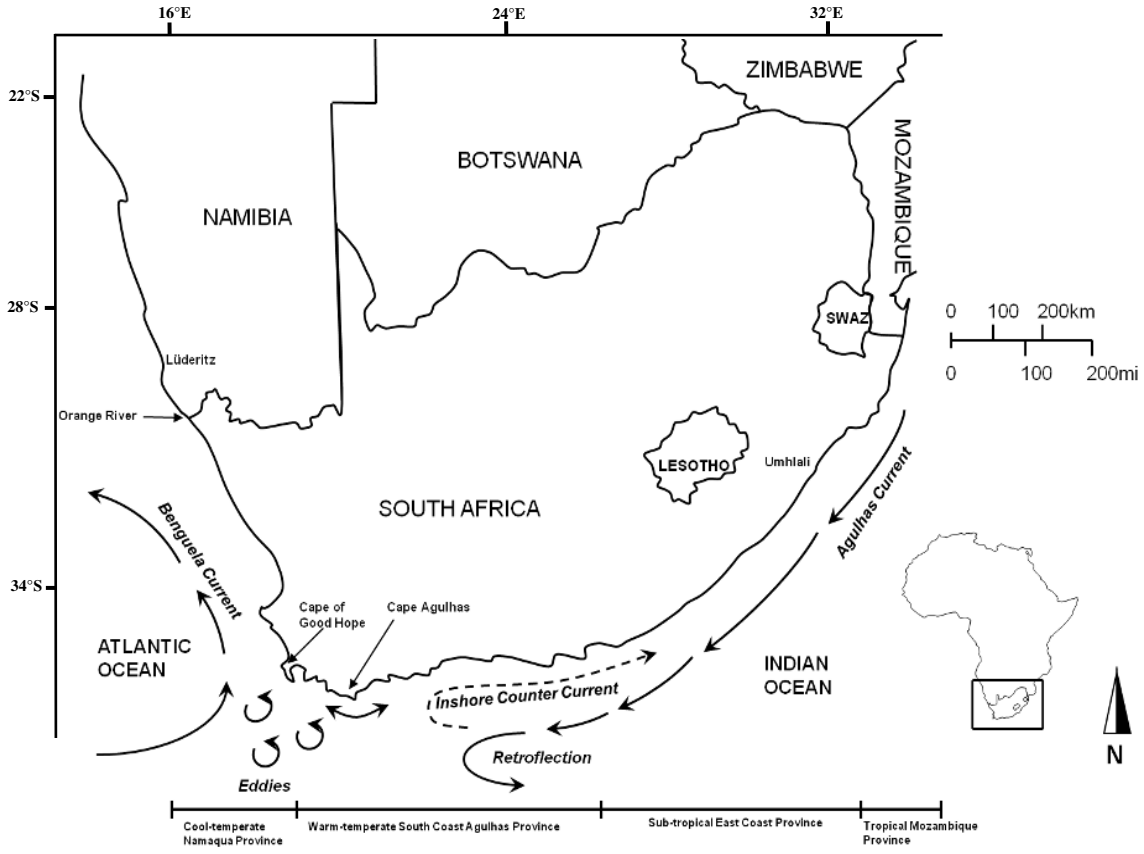

Fig. 1 Map showing the major oceanographical features along the southern African coastline that are likely to influence the gene flow patterns in *P. angulosus* and other southern African marine organisms. The four biogeographic provinces mentioned in the text are also indicated. Modified after von der Heyden *et al.* (2008).

The boundaries of the biogeographic provinces are a much debated topic among management and scientific authorities alike and are not clear cut, with transitional regions leading from one province into the next (Harrison 2002, Teske *et al.* 2009, von der Heyden 2009). However, it is clear that there are significant faunal differences between the Agulhas and Namaqua Provinces along the south-east and west coasts, respectively, separated by Cape Point (Branch *et al.* 2002) whereas the transition between the Agulhas and subtropical East Coast Province remains unclear

(Harrison 2002, Bolton *et al.* 2004). Furthermore, what adds to the complexity of the system is the fact that the position of the boundaries between these biogeographic provinces tends to shift seasonally and depends on the specific assemblage (e.g. fish, invertebrates) of species studied (Harrison 2002, Teske *et al.* 2009, von der Heyden 2009).

Given the dynamic nature of the southern African coastline as well as the outcome of the recent research focusing on this region, there would appear to be many biological (larval behavior, duration, etc.) and physical (localised currents and upwelling, of cold oceanic water, etc.) factors, extant and historical, that can contribute to population subdivision in this marine environment. Many genetic studies to date broadly reflect one or more of the coastal biogeographic provinces through their target species' genetic pattern (e.g. Harrison 2002, Evans *et al.* 2004, von der Heyden *et al*. 2008, 2009, Teske et al. 2006, 2009; von der Heyden 2009), but the complexity of the system suggests that if we are to improve our understanding further, studies particularly from species with different life histories are needed.

1.3 *Parechinus angulosus* natural history

The Cape sea urchin, *Parechinus angulosus*, has a geographical range extending from Lüderitz just north of the Orange River mouth to Umhlali in northern Kwazulu-Natal, (Branch *et al.* 2002, Day and Branch 2002,) and is the most widespread of southern African echinoids. The species forms very dense, sometimes continuous, populations of up to 90 animals per square meter (Fricke 1978). Their high population density and voracious feeding pattern (Greenwood 1975) make them key players in the ecology of kelp bed communities (Fricke 1978, Fricke 1980, Stuart and Field 1981, Branch *et al.*

2002). Cape sea urchins also provide shelter for commercially important species such as the juveniles of the abalone *Haliotis midae* (Branch *et al.* 2002, Personal observation), and they also serve as an important prey item for the rock lobster *Jasus lalandii* (Stuart and Field 1981).

The Cape sea urchin's life history remains poorly studied, with most work having been carried out decades ago, e.g. studies on its ontogeny e.g. Cram (1971) and genetic work on its sperm histone genes e.g. Reyes *et al.* (2001). Cram (1971) established in the laboratory that *P. angulosus* had a larval stage of several weeks (49-56 days) before metamorphosis, and could delay metamorphosis for at least 11 days if suitable substrate was not present, which it would actively select. The most rigorous study was conducted by Greenwood (1980) who focused on the population structure, growth, mortality, feeding and respiration of *P. angulosus* at two study sites around the Cape peninsula. His results revealed: 1) that *P. angulosus* reaches sexual maturity at 1-2 years (i.e. generation time), 2) it spawns twice per year, 3) recruitment can be highly stochastic, 4) size at sexual maturity ranges from 20-30mm (test diameter) and 5) mortality is highest early in its life cycle (shortly after metamorphosis). No predictions were made regarding its lifespan. In this study, the majority of adult *P. angulosus* individuals collected were between 30-50mm in diameter, with very few individuals reaching 60mm, which approximates to an age of 4-7 years (Greenwood 1980). Individuals of this diameter can possibly be older as growth slows significantly with age in favour of gonad development (Greenwood 1980).

Colour variation of *P. angulosus* poses an additional layer of enigmatic complexity to this species. Individuals within the same rock pool can display three distinct colour

forms, namely pink, purple and red (Fig. 2). Whether these colour polymorphisms represent distinct evolutionary lineages is currently not known.

Fig. 2 Photographs showing the three distinctive colour forms of *Parechinus angulosus* which occur along the southern African coastline (L-R: purple, red, pink).

Recent studies attempting to explain colour variation in related urchin species focused on sperm morphology (Manier and Palumbi 2008) and genetic work on the bindin gene that controls gamete recognition in sea urchins belonging to *Parecentrotus gaimardi* (Calderon *et al.* 2010). The later authors found a significant bindin differentiation between five colour morphs that occur sympatrically and they argued that this could potentially point to some form of assortative mating between colour morphs. As *P. angulosus* also shows sympatric distribution of its colour forms, it could form a basis for further investigation.

1.4 Phylogeography of sea urchins globally and within southern Africa

In generating hypotheses and explanations for the potential genetic structure in *P. angulosus* it is important to consider the outcome of studies performed on related taxa elsewhere. Studies of genetic structure in several marine organisms, including sea urchins with pelagic larvae (i.e. broadcast spawners), suggest that the simple assumption that species with long lived planktonic larvae will be genetically

homogenous is often unfounded (Debenham *et al.* 2000, Hart 2002, Gopal *et al.* 2006, Weersing and Toonen 2009). Some of the best studied cases are from the sea urchin genera *Diadema* (Lessios *et al.* 2001), *Echinometra* (Palumbi 1996) and *Strongylocentrotus* (Palumbi and Wilson 1990). In the case of the genus *Diadema*, Lessios *et al.* (2001) conducted a global phylogeography of all extant species. They found that species from this genus showed both remarkable genetic discontinuities as well as instances of high gene flow over thousands of kilometers of open ocean. Among their explanations were geographic boundaries e.g. the Isthmus of Panama and the cold water upwelling around Cape Point, as well as fresh water river outflows such as the Amazon and Orinoco Rivers. For *Echinometra* throughout the Pacific, patterns of isolation by distance were identified along with high heterogeneity of populations, despite dispersal potential (Palumbi 1996). Studies of the red sea urchin, *Strongylocentrotus franciscanus*, have shown that the larval pool is not well mixed geographically (even on spatial scales below 20km), despite long planktonic larval duration (Moberg and Burton 2000) and research on the pan-tropical sea urchin *Tripneustes* showed that one of the barriers important to the phylogenetic separation in *Tripneustes* is the cold water upwelling close to the tip of South Africa in the Cape Point region (Lessios *et al.* 2003). This upwelling has permitted relatively recent larval transport in certain sea urchin species from the genus *Diadema* (Lessios *et al.* 2001), but for other sea urchins, such as *Eudicaris* (Lessios *et al.* 1999) and *Echinometra* (McCartney *et al.* 2000), it has been an effective barrier at least since the Pliocene (Lessios *et al.* 2003). Thus the opportunities for gene flow could have fluctuated along with these cyclical occurrences (upwelling, river outflows, etc.), and could possibly have resulted in disparities in genetic homogeneity.

Although Fricke (1980) found that Cape sea urchin populations in his cold water study site (Robben Island, Western Cape) were on average smaller, more numerous, and included more size classes than his warm water study site (False Bay, Western Cape) any firm predictions on the expected phylogeographic structure of *P. angulosus,* is purely speculative. There are biotic (for example selective settling and larval duration) and abiotic (for example ocean currents, counter currents, eddies, gyres, temperature differences and upwelling) factors that can drastically reduce or promote larval dispersal (Debenham *et al.* 2000, Levin 2006, Zardi *et al.* 2007, Banks *et al.* 2007, Wing 2009, Weersing and Toonen 2009). The extremely dynamic and oceanographically unique southern African coastline as outlined above further enhances this interplay between biotic and abiotic factors. In addition, differential selection against particular genotypes to settle can separate populations with several sources of parent populations (Hartl and Clark 1989, Nosil *et al.* 2005). Biotic factors such as vertical migration, swimming, and benthic residence can also keep larvae closer than expected to parent populations (Raimondi and Keough 1990, Banks *et al.* 2007).

1.5 Molecular techniques and markers

1.5.1 Molecular approaches

The usefulness of molecular data and methods has become ever more apparent over the last couple of decades and, when utilised together with data from the target organism's biology, as well as relevant data from oceanography, can be dutifully employed to enhance the conservation and management of marine biodiversity (Banks *et al.* 2007, Bell 2008, Chatzimanolis and Caterino 2008, Selkoe *et al.* 2008, Waples *et al.* 2008, Sanford and Worth 2009, von der Heyden 2009). Using such a versatile

approach in the study of the marine environment is broadly referred to as "multifaceted seascape genetics" (Selkoe *et al.* 2008, p364), and is one of the best techniques available to scientists looking to distinguish patterns of population connectivity and structure in the marine environment (Selkoe *et al.* 2008, Waples *et al.* 2008).

Genetic data contain information not only about genetic relationships between populations, but also about past population sizes and thus demographic history (Felsenstein 1988, Avise *et al.* 1988, Avise 2009). Molecular data can also provide large independent data sets for phylogeographic and phylogenetic reconstruction, and can provide some insight into the timing of divergences (Avise 1994, Palumbi 1996, Koufopanou *et al.* 1999). These data can be used to understand the behaviour and unpredictability of species within oceanic basins (Palumbi 1996, Zink *et al.* 2000) and in turn can provide new insights into the series of evolutionary events leading to current biogeographic patterns (Palumbi 1996, Lessios *et al.* 2001, Avise 2009). For example the ages of clades/assemblages can be used to explain potential speciation due to abiotic factors (Koufopanou *et al.* 1999, Avise 2009) and genetic data are also useful to detect and characterise cryptic species (Knowlton 1993, Palumbi and Metz 1991, Palumbi 1996).

1.5.2 Molecular markers

1.5.2.1 Brief overview

An important limitation of a large number of phylogeographic studies is that their insights are based on data from a single locus, namely mtDNA (Beheregaray 2008). In the past disparate results have been obtained when using different classes of

molecular genetic markers, and it is thus very important to employ multiple loci in population genetic analyses (Bermingham and Lessios 1993, Edmands *et al.* 1996, Lessios *et al.* 2003, Zhang and Hewitt 2003, Near *et al.* 2004, Sparks and Smith 2004, Moyer *et al.* 2005, Addison and Hart 2005, Teske *et al.* 2006, Zardi *et al.* 2007, Bird *et al.* 2007, von der Heyden *et al.* 2008, Beheregaray 2008, Teske *et al.* 2009, Avise 2009, Calderon and Turon 2010). For example: a study by Teske *et al.* (2009) used both mtDNA (COI) and nDNA (18S) to validate the existence of distinct phylogroups that broadly corresponded with the four biogeographic provinces along the southern African coastline for the estuarine prawn, *Callianassa kraussi*. Their study showed that the four mtDNA phylogroups recovered comprised two Evolutionarily Significant Units (as in Moritz 1994) when combined with the nDNA data: a tropical/Mozambican clade and one in the remainder of South Africa.

1.5.2.2 Mitochondrial and Nuclear DNA

Mitochondrial DNA (mtDNA) was the original molecular marker used in animal phylogeographic studies, and today is still the most commonly used (Bermingham and Moritz 1998, Hare 2001, Avise 2009), it has a number of advantages over nuclear loci, for instance it has a simple sequence organisation in that there are none of the introns or long intergenic regions of non-coding spacer sequence present which tend to characterise nuclear genomes (Macaulay *et al.* 1999, Avise 2009 Galtier *et al.* 2009). Further, mtDNA is haploid as well as maternally inherited in most animal species (Avise 1994, 2009). However, recent studies have challenged these firm beliefs with the documentation of mtDNA gene duplication and recombination in a variety of taxa; although these studies still admit the usefulness of mtDNA, citing the use of a cautionary approach (Morris-Pocock *et al.* 2010 and references therein).

Despite the low expectation of monophyly for nDNA when used in genetic studies due to its four times larger effective population size and slower mutation rate (Palumbi *et al.* 2001, Avise 2009), it does not necessarily negatively affect the credibility of nuclear phylogeography: for questions regarding more ancient populations the comparatively deep coalescence times of nDNA are critical (Moore 1995, Hare 2001 and references therein, Zhang and Hewitt 2003).

The reduced degree of structuring generally revealed by nuclear DNA versus mitochondrial DNA data in phylogeographic studies can often be explained by ancestral polymorphism and incomplete lineage sorting between populations, as well as their mutation rate difference. As a result, genetic drift and the mutation rate difference will cause divergence between populations at the nuclear level to occur four times slower relative to the mitochondrial genome (Hare 2001, Zhang and Hewitt 2003, von der Heyden *et al.* 2008, Avise 2009). Thus, as a general rule, phylogeographic structure is anticipated to be less prominent at diploid nuclear loci when contrasted with mitochondrial loci due to their disparity in effective population sizes and evolutionary rates (Hare 2001, Avise 2009).

Essentially, mitochondrial and nuclear DNA markers work together in that they reveal different aspects of a multifaceted historical story at varying depths of perception (Hare 2001, Zhang and Hewitt 2003, Avise 2009). A more complete view and understanding of the assortment of genealogical patterns evolving in genomes can be obtained through the combination of nuclear and mitochondrial phylogeography, especially the way in which these patterns responded to the history and environments that populations have experienced (Hare 2001, Zhang and Hewitt 2003, Teske *et al.* 2009).

Furthermore, recovery of similar phylogenetic patterns from independent loci would strengthen the confidence that phylogenetically resolved gene trees are representative of the evolutionary relationships of species or populations (Bermingham and Moritz 1998, Near *et al.* 2004) and would thus advocate a more dependable inference of the population history (Bermingham and Moritz 1998, Zhang and Hewitt 2003, Avise 2009). Thus it is imperative, especially at a time where the generation of molecular genetic data is becoming more affordable and can be reliably duplicated, to focus research on loci from both the mitochondrial and nuclear genomes.

1.6 Molecular markers used in the present study

For the purpose of exploring ancient as well as more recent evolutionary processes and phylogeographic patterns of *P. angulosus* along the southern African coast, partial regions of two genes from both the mitochondrial and nuclear genomes of *P. angulosus* were sequenced: the mitochondrial gene COI (cytochrome oxidase subunit I; also see Bermingham & Lessios 1993, Edmands *et al.* 1996, Debenham *et al.* 2000, Lessios *et al.* 2003), which has been widely used in other sea urchin studies, and a nuclear Receptor for Egg Jelly Protein 9 (SpREJ9) from the PKD1 gene family of sea urchins (Gunaratne *et al.* 2007). The PKD1 family of proteins are large trans-membrane glycoproteins, which bind to smaller proteins to regulate signal transduction pathways and ion channel activities (Gunaratne *et al.* 2007). The subfamily of 10 SpREJ proteins are sperm plasma membrane proteins, involved with sperm cell recognition and the development of the fertilised egg (Gunaratne *et al.* 2007), and are thus hypothesised to be evolutionary important and stable.

1.7 Aims and Hypotheses

The main aim of this study was to investigate the phylogeographic population structure of the Cape sea urchin, *Parechinus angulosus*, along its range by employing the mtDNA (COI) and nDNA (SpREJ9) markers. By using it as an example this study will further our understanding of the demographic and evolutionary processes driving biogeographical structuring in the southern African marine environment, and will also provide species specific phylogeographic data for *P. angulosus*. These data are important for conservation planning and will provide new insights into the biology of sea urchins.

This study focused on the following hypotheses:

1) $\mathbf{H_0}$: *P. angulosus* is genetically homogenous along its range.

   $\mathbf{H_A}$: *P. angulosus* is genetically structured along its range.

2) $\mathbf{H_0}$: Genetic structuring (if any) in *P. angulosus* is not congruent with the recognised biogeographic provinces along the southern African coastline.

   $\mathbf{H_A}$: Genetic structuring (if any) in *P. angulosus* does show congruency with the recognised biogeographic provinces along the southern African coastline.

3) $\mathbf{H_0}$: There is no genetic basis for the different colour forms of *P. angulosus* at the molecular level tested.

   $\mathbf{H_A}$: *P. angulosus* does show genetic distinction between its three main colour forms as revealed by molecular data.

Chapter 2: Materials & Methods

2.1 Sampling protocol

Individuals were collected from tidal rock pools by hand and either frozen at -5ºC immediately, preserved in 100% ethanol or stored in sea water and frozen at -20ºC once back at the laboratory. A total of 18 localities along the southern African coastline were sampled, with 16-45 individuals collected per population/locality (Fig. 3)

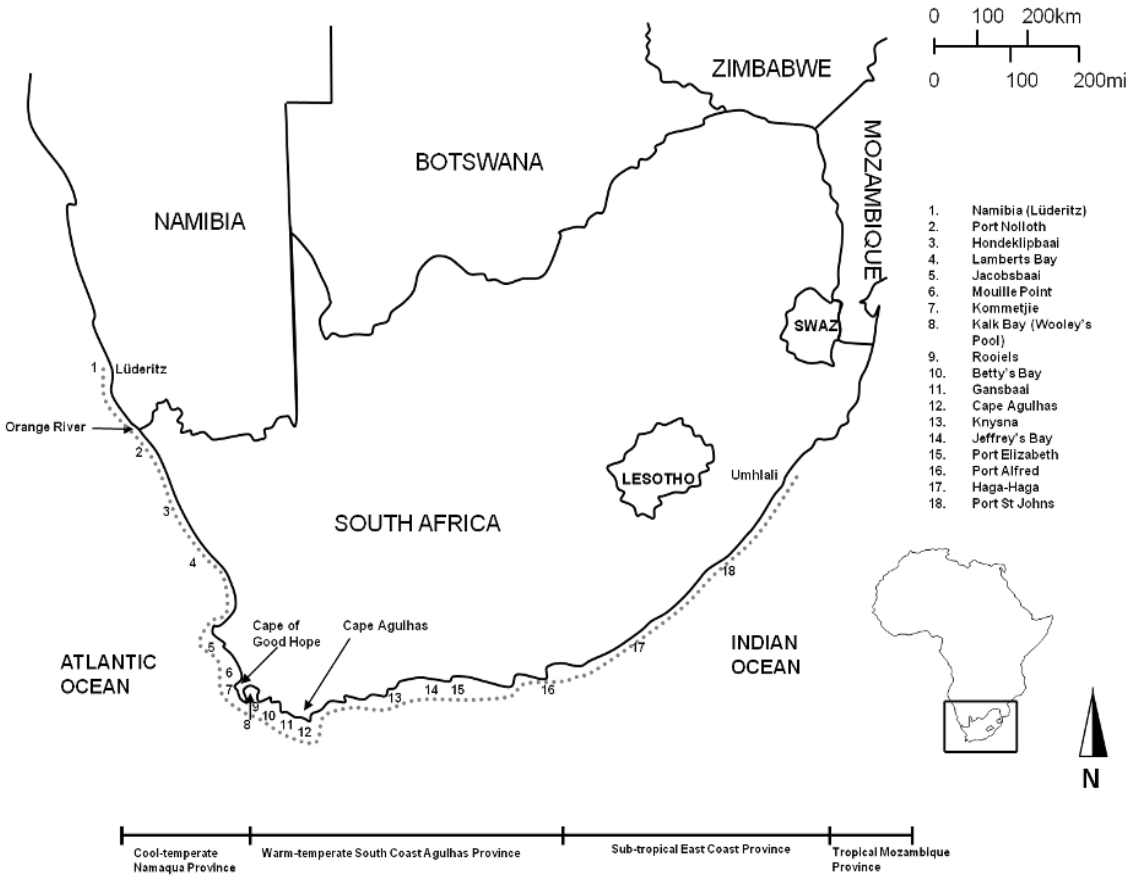

Fig. 3 Map showing the sampling localities covering the range of *Parechinus angulosus* (grey dotted line). Modified after von der Heyden *et al.* (2008). Number of individuals sampled at each locality were: 1=16; 2=26; 3=29; 4=26; 5=26; 6=31; 7=30; 8=28; 9=23; 10=45; 11=30; 12=30; 13=31; 14=20; 15=30; 16=22; 17=38; 18=29.

2.2 mtDNA and nDNA

2.2.1 Laboratory procedures

Gonad tissue was dissected from each specimen and total genomic DNA extracted with the DNEasy Kit (Qiagen) following standard protocols. The COI gene was amplified and sequenced using the universal reverse primer HCOI2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994) and the forward primer 16SB: 5`-ACGTGATCTGAGTTCAGACCGG-3` (Palumbi *et al.* 1991). The PCR reactions were performed in 25µl volumes containing 1µl of ≈25ng genomic DNA diluted by a ratio of 1:100 (DNA:H_2O), 2.5µl of 10xPCR reaction buffer, 1.25µl of each respective primer (0.1µM), 2.5µl dNTP's (0.2mM), 2µl Mg^{2+} (1.5-2.5mM), 0.1µl of Super-Therm BioTaq DNA polymerase (Super-Therm, JMR Holdings,. London, United Kingdom) and 14.5µl of distilled water. Cycling conditions were as follows: an initial denaturation of 2min at 95ºC, followed by 35 cycles of denaturation (30s at 95ºC), annealing (30s at 45ºC) and extension (45s at 72ºC), with a final extension at 72ºC for 5min. For nDNA the fragment was amplified using the primers SpREJ9f: 5`-TGCGAACAGACGGATGACAAC-3` and SpREJ9r: 5`-CCTGAAGTGGTATCAACAGTGGC-3`, from Gunaratne *et al.* (2007). PCR reactions were performed in 25µl volumes as for the mtDNA, however the 1µl of ≈25ng genomic DNA was not diluted. Cycling conditions were as for the aforementioned mtDNA, except an annealing temperature of 55ºC was used instead. Negative controls (template-free PCR reactions) were included for each reaction. PCR products were separated and visualised through 1% agarose gels containing ethidium bromide. Gel purification was done using the Wizard SV Gel and PCR Clean-Up System (Promega). The purified products were cycle sequenced using BigDye

Terminator v3.1 cycle sequencing kit (Applied Biosystems) and analysed on an Applied Biosystems 3100 automated sequencer.

2.2.2 Data analyses

Sequences were edited and aligned in BioEdit ver7.0.9.0 (Hall 1999), as well as checked against sea urchin COI and SpREJ9 in GenBank, using BLASTN (http://blast.ncbi.nlm.nih.gov), to ensure correct amplification. Sequences were translated to amino acids to obtain more confidence in the authenticity of the data (no stop codons were observed). The mtDNA data were then collapsed to haplotypes using the program Collapse 1.2 (http://darwin.uvigo.es). For the nDNA data, alleles were first separated for each individual using the program Phase2.1 (Stephens *et al.* 2001). The haplotypic phases of alleles were considered to be resolved if recovered with a degree of confidence of 90% or greater from a run of 1 million iterations. After this the nDNA alleles were collapsed to haplotypes using the program Collapse 1.2. The data sets were analysed in two ways: first all samples from all localities were combined in a panmixia model and, secondly, each of the 18 sampled localities (17 for SpREJ9) were grouped separately. Standard diversity indices in the form of haplotype/allelic (*h,* Nei and Tajima 1981) and nucleotide (π, Nei and Li 1979) diversity and the number of polymorphic sites were calculated for the data set as a whole and for each population/locality separately using Arlequin 3.1 (Excoffier *et al.* 2005).

2.2.3 Population structure

Analysis of molecular variance (AMOVA) using a non-parametric permutation approach with 10 000 iterations, as well as the calculation of pairwise Φ_{ST} values, were conducted in Arlequin 3.1 to ascertain the level of genetic structuring between sampled localities. Pairwise Φ_{ST} values are useful as they are an analogue of F_{ST} that considers both haplotype frequency and the extent of differentiation between haplotypes (Excoffier *et al.* 1992). An initial mtDNA sample of 69 sea urchins (consisting of individuals from each colour type) were selected for maximum parsimony analysis in PAUP 4.0 (Swofford 2000) using Heuristic search and TBR branch swopping, with the maximum number of trees saved limited to 5000. The same was done for the nDNA locus, with a total of 86 (172 alleles) sea urchins (individuals from each colour type) used for the colour analysis.

The latitude and longitude coordinates for each sampling location were obtained using Google Earth® and verified with Garmin®'s proprietary MapSource® software. Bayesian Analysis of Population Structure (BAPS; Corander *et al.* 2003, 2008) was used for both the mtDNA and nDNA data sets in order to identify any possible sub-populations within the sampled landscape, regardless of the geographic location of sampling localities. The BAPS model is derived using novel Bayesian predictive classification theory which is then applied to the population genetics context (Corander *et al.* 2003). It is particularly useful in that it can be used to cluster either individuals or groups of individuals in order to identify evidence for sub-groups that have genetically drifted apart (Corander *et al.* 2003 and program notes).

A simple distance matrix between sampling localities was constructed by measuring the geographical distances between them in kilometers - on a scaled map - as the

shortest distance along the coastline, excluding bays. This was then used to test for isolation by distance in the mtDNA and nDNA datasets via a Mantel test in Arlequin 3.1, using 10 000 permutations.

2.2.4 Statistical parsimony network

A statistical parsimony network was constructed among haplotypes for the mtDNA and among alleles for the nDNA data sets using the program TCS version 1.21 (Clement *et al.* 2000). Using such a network is more suitable for viewing evolutionary relationship between closely related haplotypes (rather than making use of bifurcating phylogenetic trees) where ancestry may not be strictly bifurcating (Posada and Crandall 2001). The network was then redrawn for clarity using the program CorelDraw verX3 (©2005 Corel Corporation).

2.2.5 Demographic history

To test for possible population expansion Fu's F_S (Fu 1997) test values were calculated along with mismatch distributions (Harpending 1994) using Arlequin 3.1. This was done for each of the datasets as a whole, as well as for each individual locality sampled.

The time of expansion was calculated with the formula $T=\tau/2\mu$, where μ = generation time x mutation rate for the marker used (Rogers and Harpending 1992), and τ being calculated in Arlequin 3.1. The generation time of *P. angulosus* is 1.5-2 years (Greenwood 1980), with other closely related sea urchin species having been given a conservative generation time estimated at two years. A mutation rate varying between

1.6 – 2.6% per million years is generally used for the sea urchin COI mtDNA gene region (Hickerson *et al.* 2003).

MDIV (Nielsen and Wakeley 2001) was used to calculate the divergence time between the two most genetically divergent mtDNA groups of *P. angulosus*. The program was used to estimate a variety of parameters: theta ($\theta=2N_{ef}\mu$), migration rate ($M=2N_{ef}\,m$), time of population divergence ($T=t\,/\,2N_{ef}$) and time to the most recent common ancestor (TMRCA=$t\mu$), where N_{ef} is the female effective population size, t is the generation time and μ is the mutation rate. Three simulations were run with 2 x 10^6, 5 x 10^6, and 10 x 10^6 generations, all had a 10% burn-in. A finite-sites model with upper bounds of 10 for the scaled migration rate and time of population divergence of 5 U were set. Scaled divergence time was converted into years where $T_{DIV}=T\theta/2\mu$, with T and θ being estimated from MDIV, and μ being calculated by multiplying with the estimated 1.6-2.6% per million year mutation rate for the sea urchin COI mtDNA gene region (Hickerson *et al.* 2003).

2.2.6 Migration between sampling localities

Migrate-n version 2.4 (Beerli and Felsenstein 1999, 2001) was utilised to calculate approximate past migration rates as well as directionality of gene flow between sampled localities. For the maximisation of statistical power for the gene flow analyses, unnecessary parameters were kept to a minimum by constructing a stepping-stone model with asymmetrical gene flow (see e.g. Bowie *et al.* 2006, von der Heyden *et al.* 2008). The linear nature of the southern African coastline lends itself particularly well to this type of model (von der Heyden *et al.* 2008). It is especially important to determine directional gene flow patterns as in the light of this a better

understanding can be obtained on how oceanographical features (e.g. currents, upwelling, etc.) affect such patterns (von der Heyden *et al.* 2008).

Two systematic runs were conducted: an initial short run, followed by a second longer run. For both runs, the starting values of the population mutation parameter and the ratio between the immigration rate and the respective population and mutation rate per generation were estimated from F_{ST} values (Beerli and Felsenstein 1999). For the long run, 10 short chains, each with a total of 25 000 generations and a sampling increment of 20 generations, and two long chains each with a total of 250 000 generations and a sampling increment of 50 generations were run twice. A total of 50 000 and 12 500 000 genealogies (recorded steps multiplied by the sampling increment) were visited by the short and long chains, respectively. For both the short and long chains, the first 10 000 genealogies were discarded (the burn-in). An adaptive heating scheme with four chains (starting values of 5.00, 2.50, 1.50, 1.00) and a swapping interval of one was used to ensure that efficient mixing occurred. Default values were implemented for all other settings.

Chapter 3: Results

3.1 Overview

3.1.1 mtDNA (COI)

A total of 505 sea urchins (16-45 per locality) for mtDNA were sampled from 18 locations distributed between southern Namibia and Durban covering nearly the full extent of the species range (Fig. 3, Table 1). After editing and alignment in BioEdit, 790 base pairs remained displaying 283 polymorphic sites (36.0%). A total of 195 haplotypes were recovered, of which 160 were unique and 35 shared among individuals (Table 1). Haplotype diversity (h) was found to be high both overall (h=0.95±0.01) and for individual sampling localities (h=0.75±0.11-0.98±0.02), with nucleotide diversity (π) being low overall (π=0.0134±0.0067) and for individual sampling localities (π=0.0025±0.0016-0.0254±0.0110, Table 2). Both the highest (h=0.98±0.02) and lowest (h=0.75±0.11) haplotype diversity within sampling localities was found to be on the west coast, namely in the cool-temperate Namaqua Province. Nucleotide diversity was lowest (π=0.0025±0.0016) at a locality in the sub-tropical East Coast Province, but the warm-temperate south coast Agulhas Province showed consistently lower values (Table 2). Samples from the Agulhas Province contained more unique haplotypes than any other coastal region (Table 1).

3.1.2 nDNA (SpREJ9)

An initial, longer stretch (~650 bp) of the SpREJ9 gene was amplified for *P. angulosus*. However due to low sequence yield, extreme and uncommon variability leading to alignment difficulty it was decided to use a shorter section of the exon of SpREJ9 for this study, which was obtained more readily and timely. Upon closer

inspection and preliminary analysis the shorter (182 bp) nuclear exon of the SpREJ9 gene region was deemed sufficient for population genetic analysis.

Nuclear DNA sequencing of the entire dataset (505 individuals) yielded 145 individuals (290 alleles) from 17 localities (the Namibian sampling site failed to amplify despite considerable efforts, Table 1). Sequence data from the 182 bp exon region of the SpREJ9 gene was obtained for analyses, which included 54 variable sites (29.7%). Seventy-two individual alleles were recovered of which 50 were unique and 22 shared among individuals. Many of the alleles (69.4%) were restricted to single sampling sites. Haplotype diversity was high overall (h=0.86±0.01) and for individual localities (h=0.57±0.14–1.00±0.50, Table 2), whereas nucleotide diversity remained low overall (π=0.0252±0.0137) as well as for individual sampling areas (π=0.0054±0.0054–0.0385±0.0411, Table 2). Betty's Bay in the Agulhas Province on the southern African south coast was observed to be the most diverse according to number of alleles (Table 1), but Jeffrey's Bay – also in the Agulhas Province – was observed to be the most diverse according to haplotype and nucleotide diversity (h=1.00±0.50, π=0.0385±0.0411; Table 2).

Table 1 Sampling localities, sample size, and frequencies of mtDNA haplotypes and nDNA alleles for *Parechinus angulosus*. *n* = number of individuals sampled for mitochondrial and nuclear DNA markers (and alleles). [ ], haplotypes unique to a specific population; ( ), haplotypes (mtDNA) and alleles (nDNA) shared between sampling areas. The no's 1-18 refer to the sampling localities depicted in Fig. 3

Locality	*n*	Haplotype (mtDNA)	*n*	Alleles (nDNA)
Cool-temperate Namaqua Province (West Coast)				
Namibia (1)	16	2(8) 8(2) 9(2) 197[1] 192[1] 194[1] 195[1]	-	-
Port Nolloth (2)	26	7(1) 8(3) 9(1) 121(1) 126(2) 128[1] 129[6] 130[1] 131[1] 132[1] 133[2] 134[1] 135[1] 136[1] 137[1] 138[1] 139[1]	11 (22)	1(1) 4(12) 5(2) 17(1) 51[2] 52[1] 53[1] 54[1] 55[1]
Hondeklipbaai (3)	29	2(3) 7(8) 8(9) 9(1) 140[1] 141[1] 142[1] 143[1] 144[1] 145[1] 146[1] 147[1]	2 (4)	4(1) 17(1) 56[1]
Lambertsbay (4)	26	2(1) 7(4) 8(1) 13(1) 70(1) 78(1) 112[1] 113[1] 114[1] 115[1] 116[1] 117 [2] 118[1] 119[1] 120[1] 122[1] 123[1] 124[1] 125[1] 126(1) 127[1]	3 (6)	4(2) 5(2) 29(2)
Jacobsbaai (5)	26	1[1] 2(3) 3[2] 4[1] 5[1] 6[1] 7(1) 8(3) 9(2) 10[1] 11[1] 12[1] 13(1) 14[1] 15(2) 16[1] 17(1) 18[1] 19[1]	9 (18)	1(2) 2[1] 3[1] 4(6) 5(2) 6[1] 7[1] 8[1] 9[1] 10[1] 11[1]
Mouille Point (6)	31	7(3) 8(6) 9(1) 13(4) 17(4) 21(3) 40(1) 50[1] 51[1] 52(1) 53[1] 54(1) 56[1] 57(1)	6 (12)	4(4) 5(4) 13(1) 24(1) 29(1) 30[1]
Kommetjie (7)	30	8(4) 9(2) 13(2) 17(3) 21(4) 37(1) 52(1) 54(1) 58[1] 59(3) 60[1] 61(1) 62(1) 63(1) 64[1] 65[1] 66(1) 67[1]	8 (16)	1(1) 4(1) 5(2) 13(5) 17(3) 31(2) 32[1] 33[1]
Warm-temperate Agulhas Province (South Coast)				
Wooley's Pool (8)	28	8(1) 13(1) 17(6) 21(7) 40(2) 57(1) 62(1) 101(1) 104[1] 105[1] 106[1] 107[1] 108[1] 109[1] 110[1] 111[1]	9 (18)	5(12) 13(1) 16(1) 31(1) 46(1) 49[1] 50[1]
Rooiels (9)	23	7(1) 13(2) 17(3) 21(7) 57(1) 59(1) 180[1] 181[1] 182[1] 183[1] 184[1] 185[1] 186[1] 187[1]	7 (14)	4(1) 5(5) 13(4) 17(1) 46(1) 63[1] 64[1]
Betty's Bay (10)	45	13(1) 15(1) 17(2) 20[1] 21(11) 22[1] 23[1] 24[1] 25[1] 26[1] 27[1] 28[1] 29(1) 30[1] 31[1] 32[1] 33[1] 34[1] 35[1] 36[1] 37(2) 38[1] 39[1] 40(1) 41[1] 42[1] 43[1] 44[1] 45[1] 46[1] 47[1] 48[1] 49[1]	25 (50)	5(18) 12(2) 13(13) 14[1] 15[1] 16(1) 17(1) 18[1] 19[1] 20[1] 21[1] 22[1] 23[2] 24(1) 25[1] 26[2] 27[1] 28[1]
Gansbaai (11)	30	13(2) 17(4) 21(5) 29(1) 54(1) 59(2) 63(1) 68[1] 69[1] 70(1) 71[1] 72[1] 73[1] 74[1] 75[1] 76[1] 77[1] 78(1) 79[1] 80[1] 81[2]	10 (20)	5(8) 12(1) 13(4) 16(1) 34[2] 35[1] 36[1] 37[2]
Cape Agulhas (12)	30	17(3) 21(8) 40(1) 59(1) 62(1) 82(1) 83[1] 84[1] 85[1] 86[1] 87[1] 88[1] 89[1] 90[1] 91[1] 92[1] 93[1] 94[1] 95[1] 96[1]	10 (20)	5(3) 13(9) 16(1) 24(1) 38[1] 39(1) 40[2] 41(1) 42[1]
Knysna (13)	31	13(3) 17(2) 21(10) 52(1) 57(2) 59(3) 61(1) 70(1) 82(1) 97[1] 98[1] 99[1] 100[1] 101(1) 102[1] 103[1]	9 (18)	1(1) 5(5) 13(6) 43[1] 44[1] 45[1] 46(1) 47[1] 48(1)
Jeffrey's Bay (14)	20	13(4) 17(2) 21(7) 57(3) 59(1) 177[1] 178[1] 179[1]	1 (2)	5(1) 13(1)
Port Elizabeth (15)	30	13(6) 17(2) 21(10) 55(2) 57(2) 59(1) 63(2) 101(2) 188[1] 189[1] 190[1]	10 (20)	1(1) 5(3) 13(6) 31(1) 46(3) 65[1] 66[1] 67[1] 68[1] 69[1] 70[1]
Subtropical Province (East Coast)				
Port Alfred (16)	22	17(3) 55(2) 162[3] 163[1] 164[1] 165[1] 166[1] 167[1] 168[1] 169[1] 170[1] 171[1] 172[1] 173[1] 174[1] 175[1] 176[1]	7 (14)	5(2) 12(1) 13(6) 41(2) 60[1] 61[1] 62[1]
Haga-Haga (17)	38	13(5) 15(3) 17(3) 21(18) 55(1) 101(1) 155(1) 156[1] 157[1] 158[1] 159[1] 160[1] 161[1] 162[1]	10 (20)	5(7) 12(1) 13(2) 24(2) 39(1) 48(2) 58[1] 59[1] 71[2] 72[1]
Port St Johns (18)	29	17(3) 21(6) 55(2) 66(3) 101(1) 148[1] 149[1] 150[1] 151[1] 152[1] 153[2] 154[1] 155(1)	8 (16)	4(1) 5(6) 13(4) 16(2) 17(1) 57[2]

Table 2 mtDNA (COI) and nDNA (SpREJ9) diversity indices for the eighteen sampling locations. *n,* the number of urchins sampled; *h*, haplotype diversity; π, nucleotide diversity; *significant Fu's F_S test ($P < 0.05$); NS, not significant. 1-18 refer to the sampling localities depicted in Fig. 3

	mtDNA (COI)				nDNA (SpREJ9)			
Gene Locality	*n*	*h*	π	Fu's *Fs*	*n*	*h*	π	Fu's *Fs*
Cool-temperate Namaqua Province (West Coast)								
Namibia (1)	16	0.75±0.11	0.0070±0.0041	NS	0			
Port Nolloth (2)	26	0.94±0.03	0.0059±0.0033	-7.3*	11	0.71±0.10	0.0121±0.0077	NS
Hondeklipbaai (3)	29	0.84±0.05	0.0033±0.0020	-4.1*	2	0.83±0.22	0.0054±0.0054	NS
Lambertsbay (4)	26	0.98±0.02	0.0254±0.0110	NS	3	0.80±0.12	0.0263±0.0173	NS
Jacobsbaai (5)	26	0.97±0.02	0.0114±0.0061	-5.1*	9	0.89±0.06	0.0273±0.0156	NS
Mouille Point (6)	31	0.92±0.03	0.0136±0.0071	NS	6	0.82±0.08	0.0203±0.0124	NS
Kommetjie (7)	30	0.95±0.02	0.0114±0.0060	NS	8	0.88±0.06	0.0230±0.0135	NS
Warm-temperate Agulhas Province (South Coast)								
Wooley's Pool (8)	28	0.90±0.04	0.0056±0.0032	-5.7*	9	0.57±0.14	0.0083±0.0059	-2.3*
Rooiels(9)	23	0.90±0.05	0.0090±0.0049	NS	7	0.82±0.08	0.0235±0.0139	NS
Betty's Bay (10)	45	0.94±0.03	0.0083±0.0045	-22.0*	25	0.81±0.04	0.0228±0.0128	NS
Gansbaai (11)	30	0.96±0.02	0.0058±0.0033	-11.8*	10	0.81±0.07	0.0259±0.0148	NS
Cape Agulhas (12)	30	0.92±0.04	0.0044±0.0026	-14.1*	10	0.79±0.09	0.0270±0.0153	NS
Knysna (13)	31	0.89±0.05	0.0043±0.0025	-6.9*	9	0.84±0.07	0.0274±0.0156	NS
Jeffrey's Bay (14)	20	0.84±0.06	0.0062±0.0035	NS	1	1.00±0.50	0.0385±0.0411	NS
Port Elizabeth (15)	30	0.85±0.05	0.0035±0.0021	NS	10	0.89±0.05	0.0310±0.0173	NS
Subtropical Province (East Coast)								
Port Alfred (16)	22	0.97±0.02	0.0124±0.0066	NS	7	0.81±0.09	0.0232±0.0138	NS
Haga-Haga (17)	38	0.77±0.07	0.0025±0.0016	-7.0*	10	0.87±0.06	0.0233±0.0135	NS
Port St Johns (18)	24	0.92±0.04	0.0043±0.0025	-4.7*	8	0.81±0.07	0.0258±0.0149	NS

3.2 Population structure

3.2.1 mtDNA

Pairwise Φ_{ST} values among sampled localities ranged from zero to extremely high (0-0.87, Table 3). Values were significant and included very high values between Namaqua Province populations (0.13-0.84), as well as between Namaqua Province and all other localities (Table 3). This could be indicative of some degree of isolation between these localities. In the Agulhas Province fewer population comparisons showed significant population differentiation among them, with significant Φ_{ST} values becoming apparent between East Coast Province localities (Table 3). Moving around

Cape Point from Kommetjie eastwards to Wooley's Pool, pairwise Φ_{ST} values increased on average by 0.2 between Namaqua Province localities (except Mouille Point and Kommetjie) and those in the Agulhas Province (versus those between individual Namaqua Province sampling areas, Table 3).

Also, pairwise Φ_{ST} values for Wooley's Pool (the last Agulhas Province locality before moving west around Cape Point) were not significantly different for localities to the east of it, except for Port Alfred (one of the three most easterly sampling sites). Interestingly, although pairwise Φ_{ST} values between Wooley's Pool and all Namaqua Province localities were significantly different, the Φ_{ST} values between it and the two immediate Namaqua Province sampling localities on the other side of the Cape peninsula (Kommetjie and Mouille Point) were orders of magnitude smaller than those from Jacobsbaai northwards (Table 3). Pairwise Φ_{ST} values between Jacobsbaai and the remaining two Namaqua Province sampling areas south of it are on average 0.25 higher than between Jacobsbaai and localities to its north (Table 3). These Φ_{ST} values suggest a gene flow anomaly both in the Cape Point region as well as from Mouille Point moving northwards to Jacobsbaai.

Also, a significant relationship between genetic and geographical distance was detected by the Mantel test for isolation by distance for the entire mtDNA dataset (0.58, $P < 0.05$). On a finer scale, when isolation by distance was carried out for each biogeographic region, it was found to be significant for the Namaqua and Agulhas Provinces (0.63, $P < 0.05$ and 0.47, $P < 0.05$, respectively), but not for the East Coast Province. The finer scale analysis of IBD was carried out to account for the transition zones – with features such as currents and environmental clines - between biogeographic regions that could act as barriers to many coastal invertebrates in

southern Africa and thus skew results obtained from a larger scale IBD analysis (e.g. Teske *et al.* 2007 and references therein, Teske *et al.* 2009).

Table 3 Pairwise Φ_{ST} values among sampling localities for *Parechinus angulosus*. Values below the diagonal are for the mtDNA (COI) data set, and above the diagonal for the nDNA (SpREJ9) data set. Numbers in bold with an * are significant. Localities 1-7: Namaqua Province; 8-15: Agulhas Province; 16-18: East Coast Province (Note: no data for Namibia was available for analyses for the nDNA)

Location	1. Namibia	2. Port Nolloth	3. Hondeklip-baai	4. Lamberts-bay	5. Jacobs-baai	6. Mouillie Point	7. Kommetjie	8. Wooley's Pool	9. Rooiels	10. Betty's Bay	11. Gansbaai	12. Cape Agulhas	13. Knysna	14. Jeffrey's Bay	15. Port Elizabeth	16. Port Aflred	17. Haga-Haga	18. Port St Johns
1. Namibia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2. Port Nolloth	**0.13***	-	0.08	**0.14***	0.05	**0.13***	**0.22***	**0.64***	**0.29***	**0.32***	**0.32***	**0.33***	**0.30***	0.32	**0.30***	**0.37***	**0.43***	**0.29***
3. Hondeklipbaai	**0.06***	**0.17***	**-**	0.07	0.00	0.11	0.11	**0.71***	**0.19***	**0.25***	0.23	0.23	**0.20***	0.27	**0.21***	**0.27***	**0.37***	0.19
4. Lambertsbay	**0.13***	**0.17***	**0.17***	-	0.00	0.00	0.00	**0.42***	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
5. Jacobsbaai	**0.11***	**0.20***	**0.15***	0.03	-	0.00	0.03	**0.38***	0.05	**0.13***	**0.09***	**0.10***	**0.08***	0.00	**0.10***	**0.12***	**0.20***	0.06
6. Mouillie Point	**0.42***	**0.49***	**0.50***	**0.09***	**0.19***	-	0.03	**0.33***	0.00	0.07	0.03	0.05	0.05	0.00	0.04	0.11	**0.11***	0.01
7. Kommetjie	**0.53***	**0.59***	**0.61***	**0.17***	**0.30***	0.00	-	**0.41***	0.00	0.02	0.03	0.01	0.00	0.00	0.04	0.00	**0.19***	0.00
8. Wooley's Pool	**0.75***	**0.77***	**0.81***	**0.34***	**0.55***	**0.16***	**0.07***	-	**0.24***	**0.23***	**0.19***	**0.22***	**0.28***	0.00	**0.15***	**0.40***	0.03	**0.24***
9. Rooiels	**0.67***	**0.71***	**0.74***	**0.26***	**0.46***	**0.09***	0.03	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.00
10. Betty's Bay	**0.69***	**0.72***	**0.73***	**0.33***	**0.50***	**0.14***	**0.06***	0.00	0.01	-	0.00	0.00	0.00	0.00	0.00	0.00	**0.09***	0.00
11. Gansbaai	**0.75***	**0.77***	**0.80***	**0.35***	**0.55***	**0.18***	**0.08***	0.01	0.02	0.00	-	0.00	0.00	0.00	0.00	0.02	0.04	0.00
12. Cape Agulhas	**0.79***	**0.81***	**0.84***	**0.38***	**0.60***	**0.22***	**0.12***	0.00	**0.04***	**0.02***	0.00	-	0.00	0.00	0.00	0.00	0.06	0.00
13. Knysna	**0.79***	**0.81***	**0.84***	**0.39***	**0.60***	**0.20***	**0.11***	0.00	0.02	0.02	0.02	0.03	-	0.00	0.00	0.00	**0.11***	0.00
14. Jeffrey's Bay	**0.75***	**0.78***	**0.82***	**0.33***	**0.55***	**0.17***	0.09	0.01	0.01	**0.04***	**0.03***	**0.05***	0.00	-	0.00	0.00	0.00	0.00
15. Port Elizabeth	**0.81***	**0.82***	**0.85***	**0.39***	**0.61***	**0.21***	**0.12***	0.01	0.02	**0.04***	**0.03***	**0.06***	0.00	0.00	-	0.03	0.03	0.00
16. Port Alfred	**0.68***	**0.72***	**0.75***	**0.33***	**0.52***	**0.22***	**0.16***	**0.12***	**0.09***	**0.14***	**0.11***	**0.15***	**0.16***	**0.10***	**0.15***	-	**0.18***	0.01
17. Haga-Haga	**0.84***	**0.85***	**0.87***	**0.43***	**0.66***	**0.25***	**0.14***	0.01	0.03	**0.03***	**0.03***	**0.03***	0.01	0.03	0.01	**0.20***	-	0.06
18. Port St Johns	**0.78***	**0.80***	**0.84***	**0.36***	**0.58***	**0.20***	**0.11***	0.00	0.02	0.02	0.00	0.01	0.02	0.03	0.03	**0.12***	0.01	-

No clear pattern between mtDNA (COI) and sea urchin colour was obtained from the parsimony tree analysis (Fig. 4), drawn from a sample of 69 individuals.

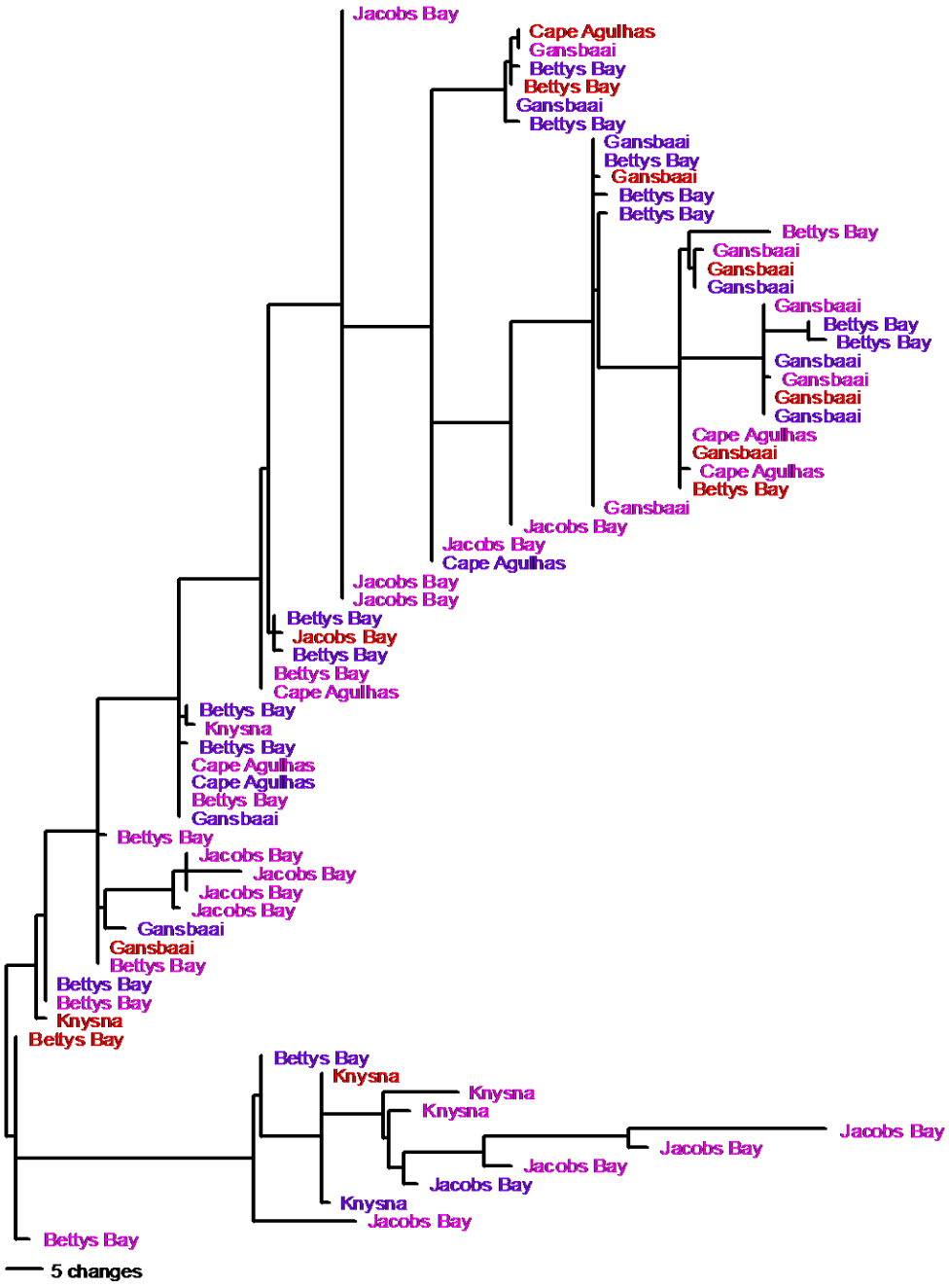

Fig. 4 Parsimony tree constructed using Heuristic search and TBR branch swapping; maximum number of trees constructed limited to 5000; shortest tree=352 steps (colour of text indicates colour of sea urchin, with locality specified)

BAPS analyses identified 2 sub-groups within the mtDNA data set (Fig. 5a): a west coast Namaqua Province group consisting of Jacobsbaai and all localities to its north, and a south coast Agulhas Province group which includes the two most southern Namaqua Province localities, as well as all other localities up to Port St Johns (the most easterly population) in the East Coast Province (Figs 3 and 5). The level of certainty – or goodness of fit - for the clustering of the individual sampling localities into the sub-groups in BAPS is reflected in a three dimensional rendering (Fig. 5b), where the height of each cell is equal to one minus the probability of its inclusion in that group (Corander *et al.* 2008). Thus, the lower the height of the cell, the better the fit. In this case, the cells are virtually flat, hence indicating a high level of confidence in the grouping.

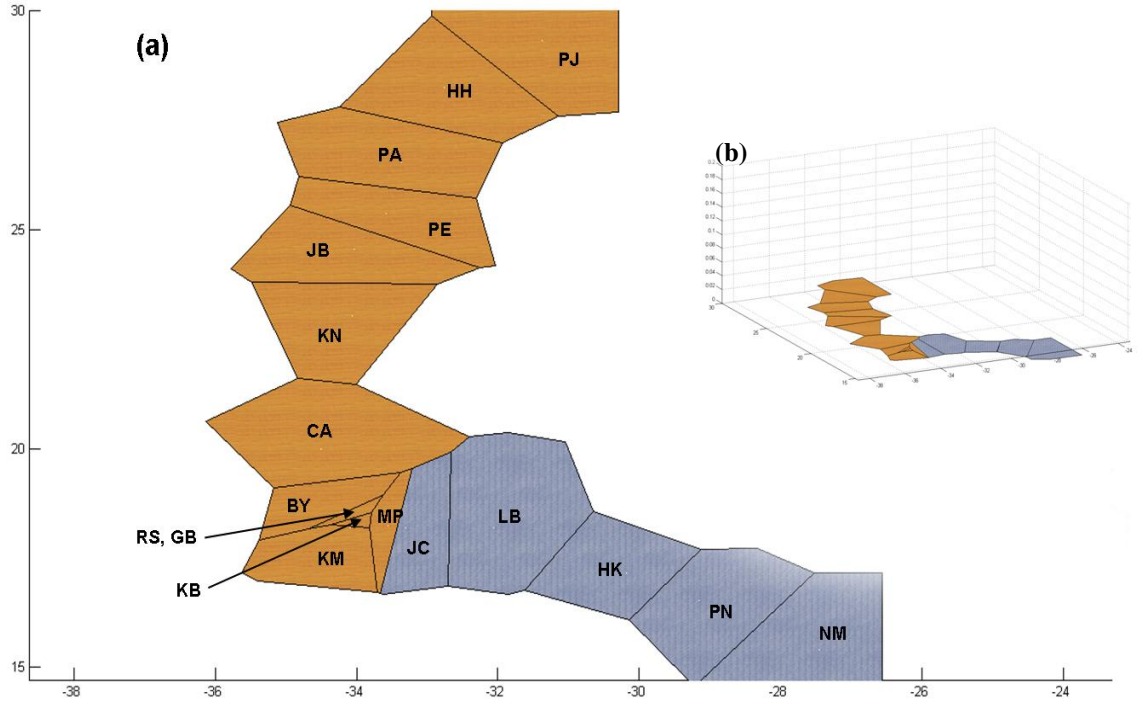

Fig. 5 (a) Voronoi tessellation, generated with the BAPS software, depicting the two sub-groups within the mtDNA data set; NM – Namibia, PN – Port Nolloth, HK – Hondeklip Bay, LB – Lamberts Bay, JC – Jacobsbaai, MP – Mouille Point, KM – Kommetjie, KB – Kalk Bay/Wooley's Pool, RS – Rooiels, GB – Gansbaai, BY – Betty's Bay, CA – Cape Agulhas, KN – Knysna, JB – Jeffrey's Bay, PE – Port Elizabeth, PA – Port Alfred, HH – Haga-Haga, PJ – Port St Johns. (b) Three dimensional probability rendering of the sub-group structure in (a), showing the goodness of fit; numbers on the axes refer to east (y-axis) and south (x-axis) longitude and latitude coordinates.

3.2.2 nDNA

The nuclear DNA data showed very little structure in the Namaqua and East Coast Provinces, with some structure being present in the Agulhas Province (Table 3). The Φ_{ST} values reflected values ranging from zero to fairly high (0 – 0.71, Table 3). However, the amount of significant structure evident was markedly less than that for the mtDNA data, which is to be expected due to the differences in the modes of evolution between nuclear versus mitochondrial DNA. Congruent with the mtDNA data, there was a clear distinction between Namaqua and East Coast Province localities: very little genetic structure was present between Namaqua Province sampling areas, except for Port Nolloth, which was the northern most locality for the nDNA data set. Port Nolloth was also significantly different from all other localities to its south and to the east of Mouille Point, except for Jeffrey's Bay and Jacobsbaai (Table 3).

Very little to no structure was evident in the Agulhas and East Coast Provinces, with the exceptions being Wooley's Pool and Haga-Haga, respectively (Table 3). Noticeably, as with the mtDNA, the significant Φ_{ST} values between Wooley's Pool and the Namaqua Province localities were markedly higher (0.33 – 0.71, Table 3) than between it and the other Agulhas Province localities (0.15 – 0.40, Table 3). This again suggests a possible gene flow anomaly in the Cape Point region, as was the case for the mtDNA.

The nDNA also did not show any clear pattern at the SpREJ9 locus for sea urchin colour, as can be seen from the parsimony tree analysis (Fig. 6), drawn from a sample of 172 alleles/haplotypes (86 individuals).

Fig. 6 Parsimony tree constructed using Heuristic search and TBR branch swapping; maximum number of trees constructed limited to 5000; the figure has been simplified for the sake of clarity; colour of text indicates colour of sea urchin, with locality specified.

The BAPS analyses identified no sub-group structure in the nDNA data set (i.e. one panmictic population), as might be expected at the nDNA level (Fig. 7a, following page), with the probability rendering showing a high level of confidence (Fig. 7b).

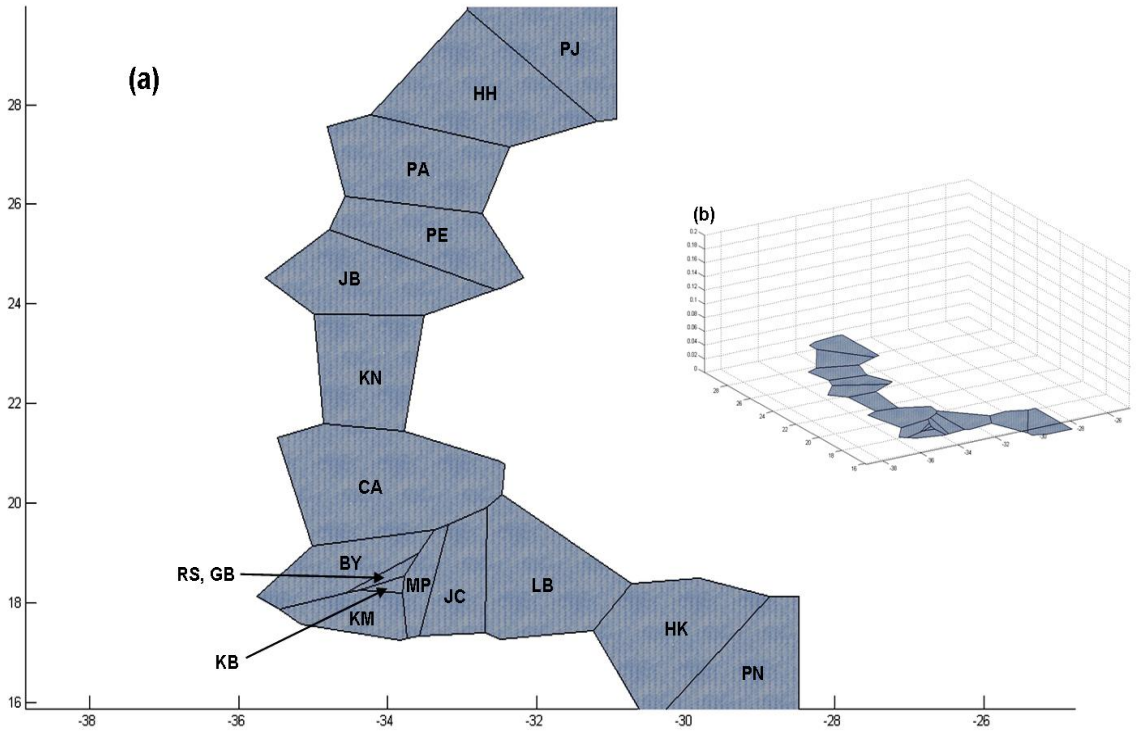

Fig. 7 (a) Voronoi tessellation, generated with the BAPS software depicting that no sub-groups were identified within the nDNA data set; NM – Namibia, PN – Port Nolloth, HK – Hondeklip Bay, LB – Lamberts Bay, JC – Jacobsbaai, MP – Mouille Point, KM – Kommetjie, KB – Kalk Bay/Wooley's Pool, RS – Rooiels, GB – Gansbaai, BY – Betty's Bay, CA – Cape Agulhas, KN – Knysna, JB – Jeffrey's Bay, PE – Port Elizabeth, PA – Port Alfred, HH – Haga-Haga, PJ – Port St Johns. (b) Three dimensional probability rendering of the sub-group structure in (a): the closer to zero the height of the cell, the better the fit; Numbers on the axes refer to east (y-axis) and south (x-axis) longitude and latitude coordinates.

3.3 Demographic history

3.3.1 mtDNA

Fu's F_S test for all sites combined was highly negative and significant (-23.79, $P < 0.05$). Fu's F_S values for some of the sampled localities were also negative as well as significant, with strong indications of population expansions on the south and west coasts (Table 2). The mismatch distribution for the entire dataset clearly shows two

peaks (Fig. 8), which indicates population differentiation between the Namaqua Province and the other regions, supported by the BAPS analysis. Harpending's raggedness index (R) also indicated a good fit. The mismatch distribution for each individual population showed instances of recent demographic change, more so on the south coast in the Agulhas Province and the East Coast Province than in the Namaqua Province (Fig. 9).

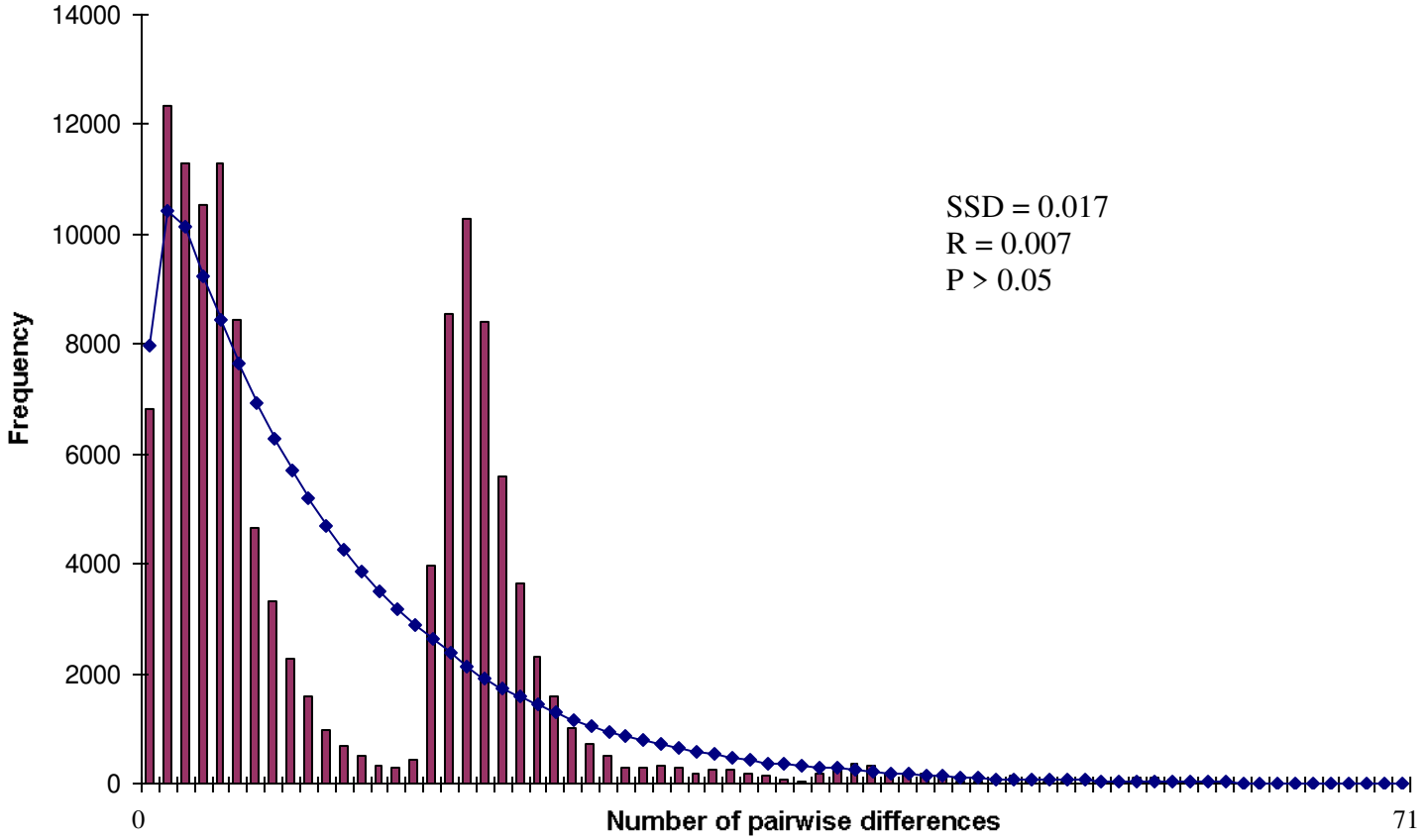

Fig. 8 Observed frequency distribution (purple bars) for the number of pairwise differences among all individuals sampled for the mtDNA data set for *P. angulosus* along the coast of southern Africa. The solid line shows the expected distribution given a population expansion. (Note: x-axis has been redrawn with numbers omitted for clarity).

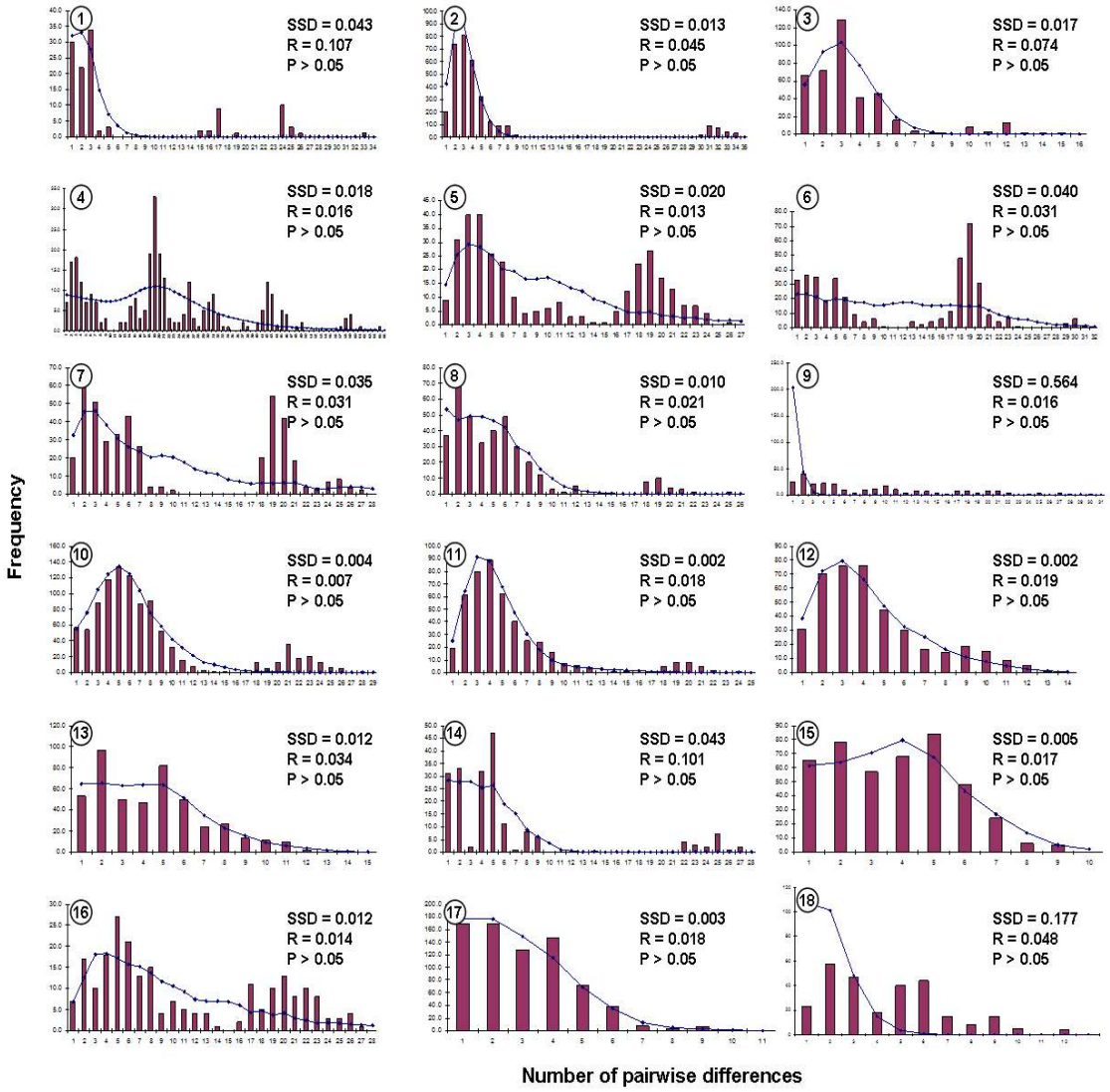

Fig. 9 Mismatch distribution for mtDNA (COI) of each sampling locality population for *P. angulosus*. Purple bars represent the number of pairwise differences among individuals of each locality, while the solid line shows the expected distribution given a population expansion. Sequential numbers at top left of each graph correspond to sampling localities as in Figure 3, p-values shown is that of the Raggedness-statistic, or goodness of fit.

The parsimony haplotype network showed phylogeographic structure between the Namaqua Province and the Agulhas and East Coast Provinces, with some degree of mixing, as Agulhas Province haplotypes were present in the Namaqua Province west coast (Figs 11 and 12). The two regions are isolated by several mutational steps indicating a fairly old split. The number of private haplotypes at each locality ranged from three at Jeffrey's Bay to 26 at Betty's Bay.

There were also ten singleton haplotypes (not connected to any other haplotype) recovered, with an additional pair connected to one another. This is possibly indicative of ancestral polymorphisms within the species (Figs 11 and 12). These singleton haplotypes were exclusively from the Namaqua and East Coast Provinces and sampled at the edges of *P. angulosus'* range (except for one individual at Jeffrey's Bay). This could perhaps be a further indication of the significant isolation by distance found for the mtDNA data set. One dominant haplotype (haplotypes that occur in more than 5% of the sampled population) was present in the Namaqua Province, with three dominant haplotypes in the Agulhas and East Coast Provinces (Fig. 11).

When using τ=0.402 and a two year generation time for *P. angulosus* as a closest approximation (Greenwood 1980, Hickerson *et al.* 2003), the time of population expansion is estimated to be between 4759 (2.6% per million year mutation rate for urchin COI) and 7733 (1.6% per million years mutation rate for urchin COI) years ago.

Using the 10 x 10^6 run from MDIV and the two sub-groups identified by BAPS for the mtDNA data set, results clearly suggest that the Namaqua Province populations north of Mouille Point are isolated to some degree, although not entirely, from the Agulhas and East Coast Province populations (Figs. 10 a, b). Utilising $T_{DIV}=T\theta/2\mu$, it is estimated that the partial divergence between the Namaqua Province populations (north of Mouille Point) and all other localities as estimated by BAPS occurred at least 4012 years ago. TMRCA was estimated to be at least 544 000 years ago.

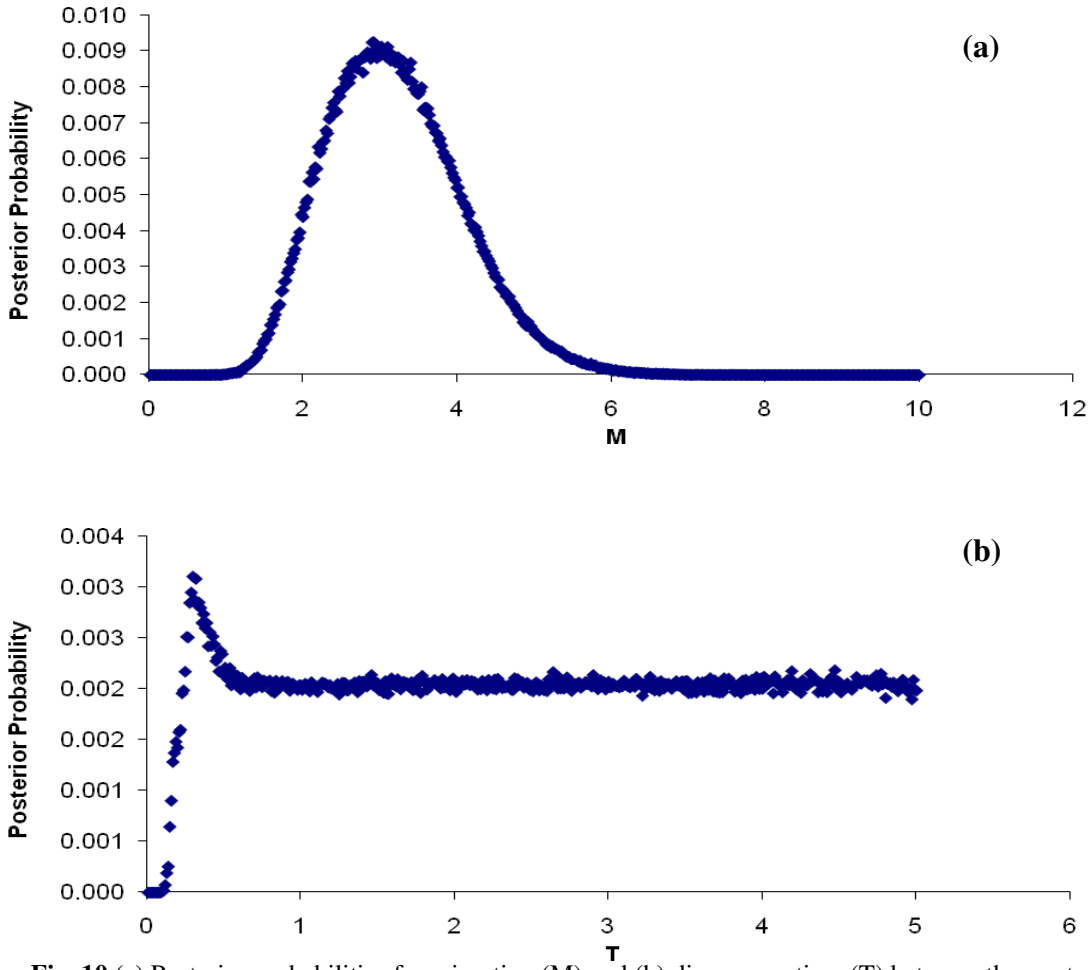

Fig. 10 (a) Posterior probabilities for migration (M) and (b) divergence time (T) between the west coast localities (north of Mouille Point) and all other sampling localities of *P. angulosus*.

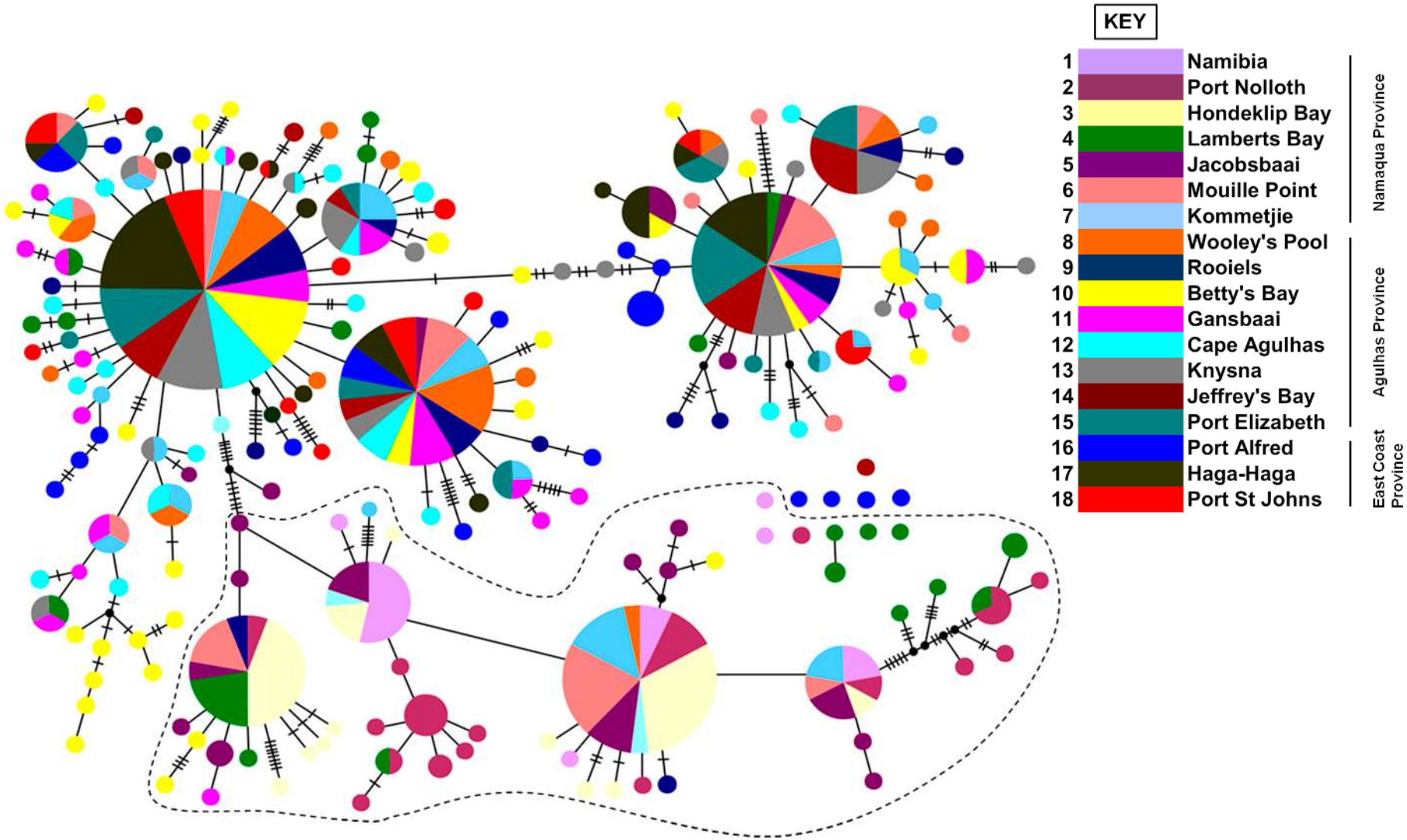

Fig. 11 A parsimony haplotype network for the mtDNA COI region data set. The size of the circles is proportional to the frequency of each haplotype; the smallest circles represent unsampled or extinct haplotypes. Each line represents one mutational step. A divergent, almost exclusive west coast clade is outlined (see also Fig. 12). Population numbers correspond to those in Fig. 3.

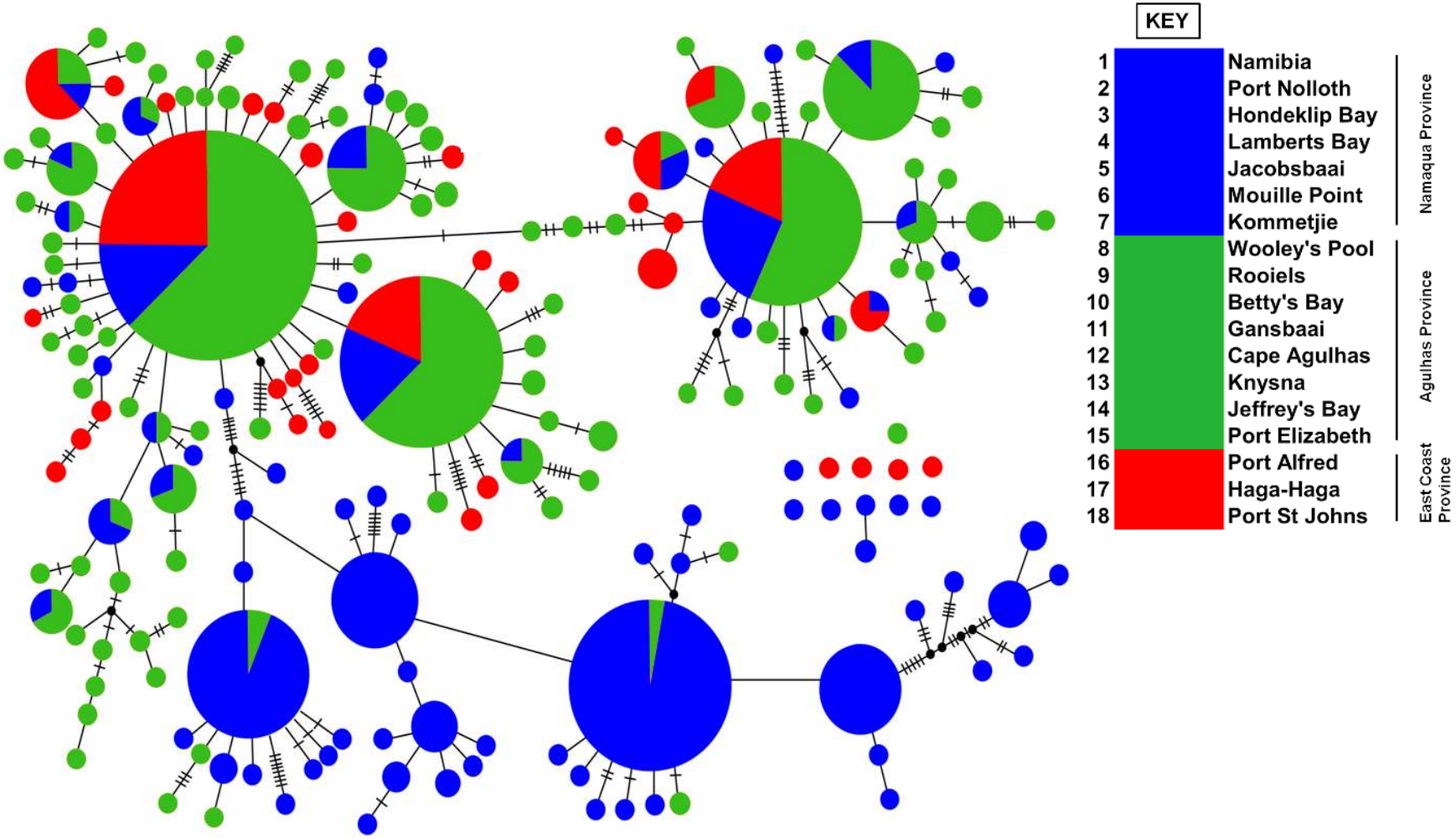

Fig. 12 A parsimony haplotype network for the mtDNA COI region data set as in Fig. 11, but drawn according to biogeographic province (Blue=Cool-temperate Namaqua Province, Green=Warm-temperate South Coast Agulhas Province, Red=Sub-tropical East Coast Province, as depicted in Fig. 3). The size of the circles is proportional to the frequency of each haplotype; the smallest circles represent unsampled or extinct haplotypes. Each line represents one mutational step. Population numbers correspond to those in Fig. 3.

3.3.2 nDNA

The mismatch distribution for the nDNA data set as a whole was indicative of population expansion, with Harpending's raggedness index (R) not deviating significantly from the observed pattern (Fig. 13). Fu's F_S test for selective neutrality was highly negative and significant overall (-25.15, $P < 0.05$), however on an individual locality level only the Wooley's Pool population exhibited a significant Fu's F_S value (-2.3, $P < 0.05$, Table 2).

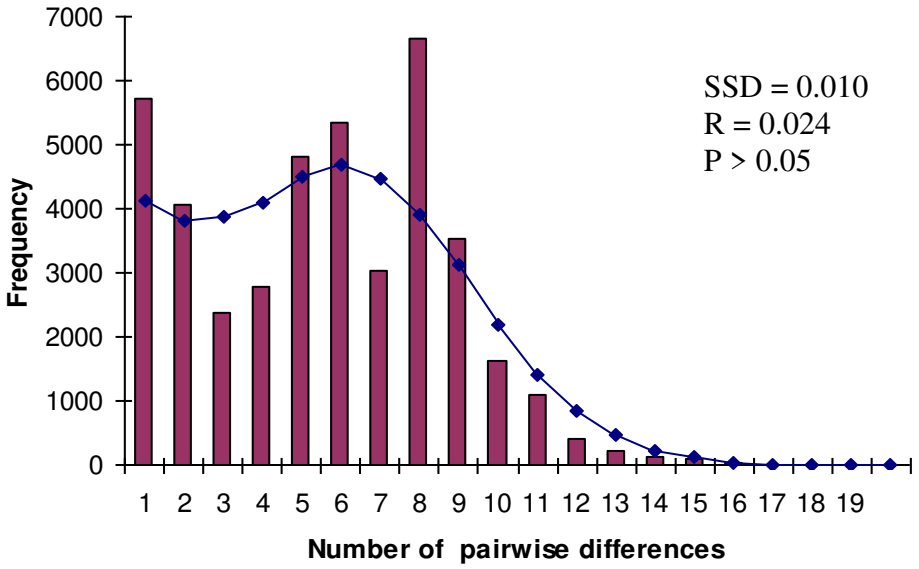

Fig. 13 Observed frequency distribution (purple bars) for the number of pairwise differences among all individuals sampled for the nDNA data set for *P. angulosus* along the coast of South Africa. The solid line shows the expected distribution given a population expansion.

The nDNA parsimony network analysis revealed a fairly similar pattern to that of the mtDNA network, although with very limited structuring between the Namaqua, Agulhas and East Coast Provinces, most likely due to the different evolutionary rates (Figs 14 and 15). As with the mtDNA, albeit to a much lesser extent, there is evidence for genetic structure between the Namaqua Province and the Agulhas and East Coast Provinces, with a small Namaqua Province clade visible - separated from the

remainder of the network by two mutational steps (Fig. 14, outlined), and of three dominant alleles (occurring in more than 5% of the population). Alleles from Betty's Bay and Jacobsbaai were the most widely distributed throughout the SpREJ9 network, and were shared by most individuals (Fig. 14). The number of private alleles at each locality ranged from 0 – 16, with Betty's Bay in the Agulhas Province again (as with the mtDNA) exhibiting the highest number at 16, double that of the locality with the next highest number of private alleles, namely Jacobsbaai in the Namaqua Province with eight (Table 1). However, this pattern could be skewed by the increased sample size at Betty's Bay. When viewed from a biogeographic province perspective (Fig. 15), the slight genetic structuring between the Namaqua and Agulhas/East Coast Provinces becomes clearer, although the small degree of mixing is still evident.

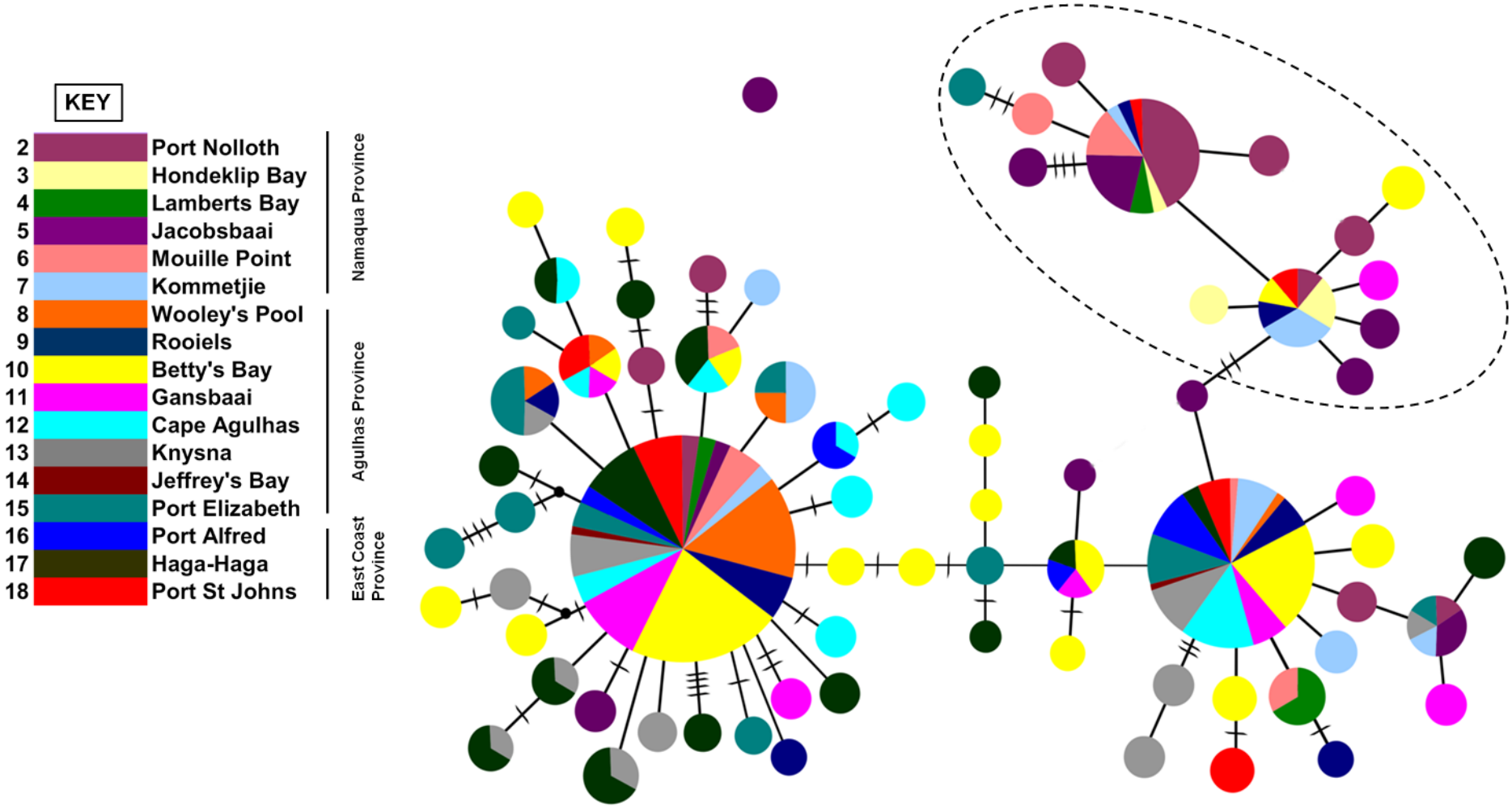

Fig. 14 A parsimony allele network for the nDNA SpREJ9 data set. The size of the circles is proportional to the frequency of each haplotype; the smallest circles represent unsampled or extinct haplotypes. Each line represents one mutational step. A potential west coast clade is outlined. Population numbers correspond to those in Fig. 3

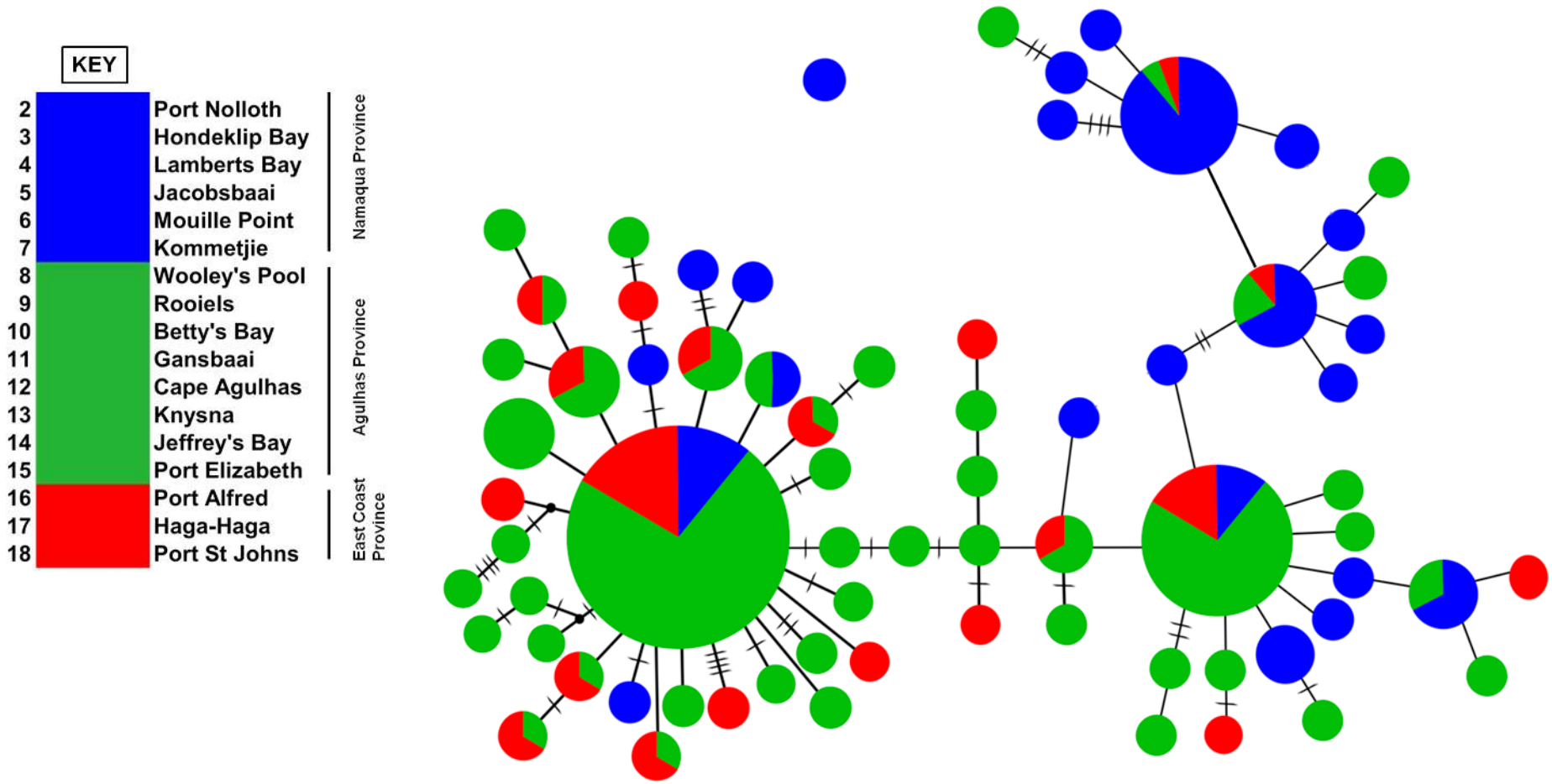

Fig. 15 A parsimony allele network for the nDNA SpREJ9 data set as in Fig. 14, but drawn according to biogeographic province (Blue=Cool-temperate Namaqua Province, Green=Warm-temperate South Coast Agulhas Province, Red=Sub-tropical East Coast Province, as depicted in Fig. 3). The size of the circles is proportional to the frequency of each haplotype; the smallest circles represent unsampled or extinct haplotypes. Each line represents one mutational step. Population numbers correspond to those in Fig. 3.

3.4 Migration between sampling localities

3.4.1 mtDNA

Interestingly, coalescent analyses revealed a bidirectional gene flow scenario for *P. angulosus* along the southern African coast (Table 4, Fig. 16). In the Namaqua Province gene flow appears to be in a predominantly northwards direction (*Nm*=2.4-23.3), following the direction of the Benguela Current, with very little migrant exchange between Namibia and Port Nolloth (which have the outflow of the Orange River into the Atlantic Ocean between them) in either direction (*Nm*=0.8-1.8). No gene flow occurs from Mouille Point south to Kommetjie (*Nm*=0) and very little onwards to Wooley's Pool (*Nm*=1.9) around Cape Point from west to east. In sharp contrast, there are high levels of gene flow (*Nm*=39) around Cape Point from east (Wooley's Pool) to west (Kommetjie, Fig. 16).

In the Agulhas Province Wooley's Pool appears to be a source population, as it receives very few migrants from localities to either its west or east (*Nm*=1.9-7), whereas high numbers of migrants appear to be going out from there to adjacent localities towards the east and west (*Nm*=30.1-39, Fig. 16). A similar source population scenario seems likely true for Haga-Haga on the east coast (Fig. 16). Furthermore, gene flow is occurring in both directions in the Agulhas Province, which is where the Benguela and Agulhas Currents mix and no particular current is dominant, with both in- and offshore components of the currents available for possible use to aid dispersal (Table 4, Fig. 16).

In contrast, in the East Coast Province gene flow appears to be occurring predominantly from west to east, with much fainter signals of east to west gene flow

(Fig. 16), perhaps due to the inshore counter currents in this region. Notably, there is very limited gene flow between the four eastern most populations (Port Elizabeth, Port Alfred, Haga-Haga and Port St Johns) and all other localities, with limited gene flow between Jeffrey's Bay and Port Elizabeth contributing to this.

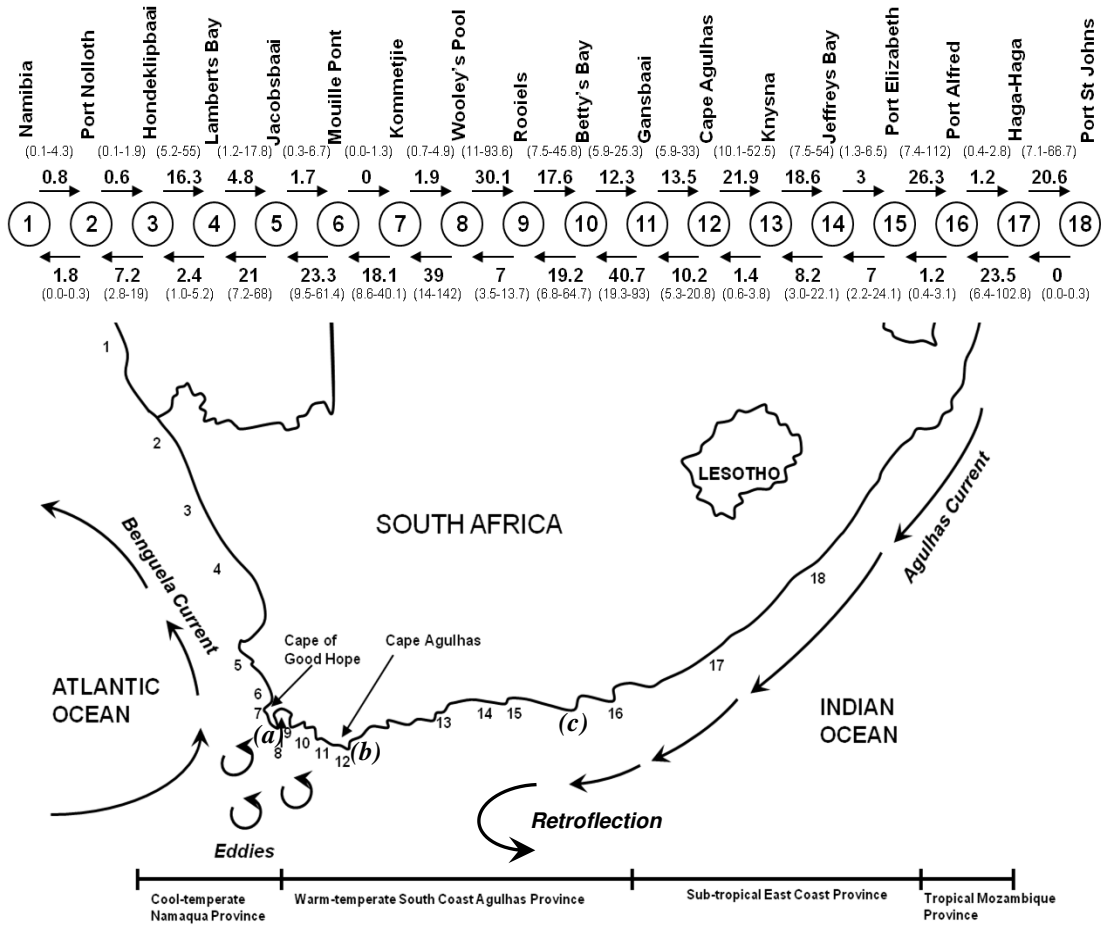

Fig. 16 Map of the southern African coastline showing directionality of gene flow for *Parechinus angulosus* mtDNA (COI). The four major marine biogeographical regions of the coast (see text) are indicated. Relative directionality of gene flow is indicated between populations by arrows; numbers above and below the arrows denote the relative migration rates (with their associated confidence intervals). Previously identified genetic break regions: (a) Cape Point, (b) Cape Agulhas and (c) Algoa Bay. Map modified after Von der Heyden *et al.* (2008).

3.4.2 nDNA

The gene flow scenario for the *P. angulosus* nuclear DNA appears to support that of the mtDNA (Fig. 17). In the Namaqua Province gene flow is in an almost exclusively northwards direction, following the direction of the Benguela Current, with zero gene

flow to the south - except between Lamberts Bay and Jacobsbaai. There is no gene flow from west to east around Cape Point, however it appears to be occurring from east to west around this land mark from Kalk Bay to Kommetjie (Fig. 17).

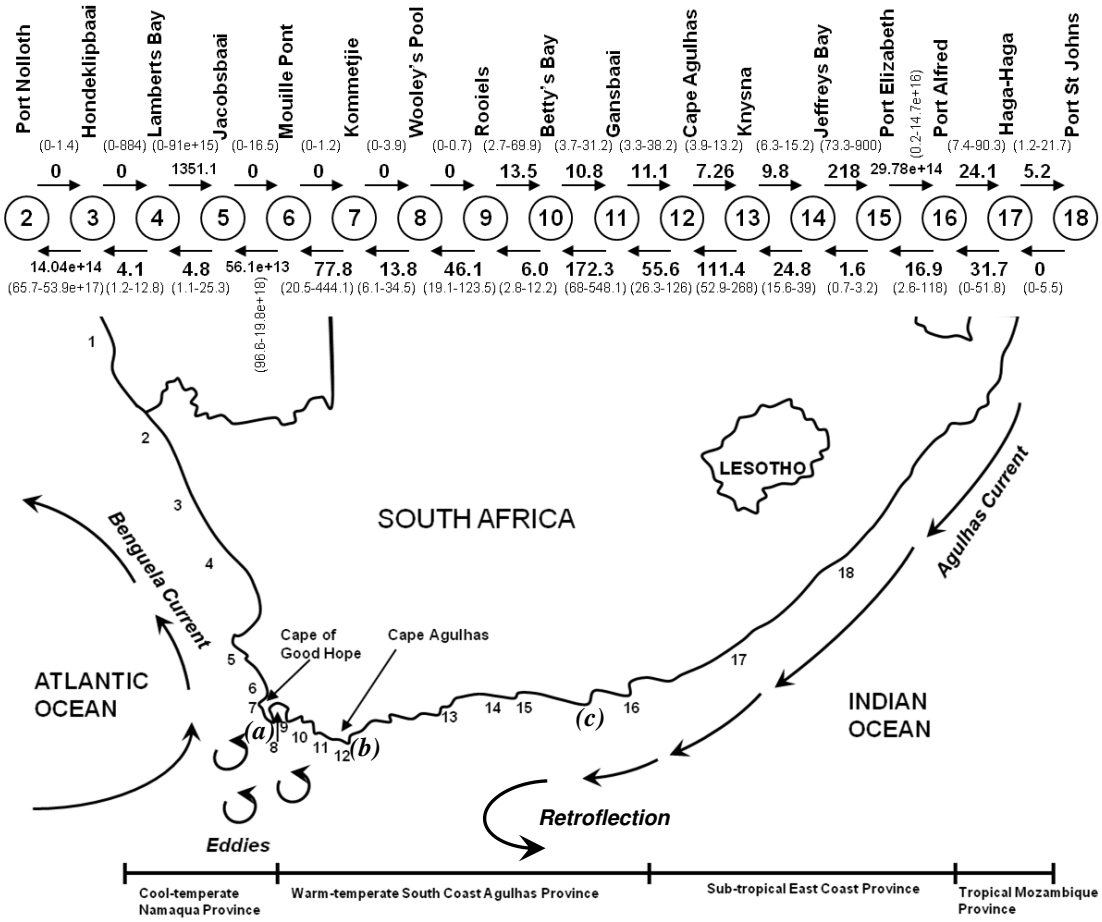

Fig. 17 Map of the southern African coastline showing directionality of gene flow for *Parechinus angulosus* nDNA (SpREJ9). The four major marine biogeographical regions of the coast (see text) are indicated. Relative directionality of gene flow is indicated between populations by arrows; numbers above and below the arrows denote the relative migration rates (with their associated confidence intervals). Previously identified genetic break regions: (a) Cape Point, (b) Cape Agulhas and (c) Algoa Bay. Modified after Von der Heyden *et al.* (2008).

Gene flow in the Agulhas Province – where no particular current is dominant - is once again shown to be of a bidirectional nature, similar to that of the mtDNA (Fig. 16). This is also evident in the East Coast Province (Fig. 17). The strongest signals of gene flow is from east to west (south and east coasts) and south to north (west coast), following the direction of the two major coastal currents along the southern African

coastline (Fig. 17). There are some very large gene flow values for the nDNA, namely between localities 1 and 3, 5 and 6 and 15 and 16 (Fig. 17). This is most likely due to very little signal in the data for those localities and thus it is possible that erroneous gene flow estimates could have been reported (Peter Beerli, pers. comm.). In addition, the nDNA gene flow data analysis is based on fairly small sample sizes, and thus should be treated with caution.

Chapter 4: Discussion

4.1 Phylogeographic structure of *Parechinus angulosus* along its range

4.1.1 General overview

The broad scale genetic pattern recovered by AMOVA analysis of the mtDNA data shows an unexpectedly high level of population structure for *P. angulosus* in the Namaqua Province, little to no structure in the Agulhas Province and intermediate population structure in the East Coast Province biogeographical regions (Table 3). Gene flow for the mtDNA is occurring in both directions along each coastal biogeographic region, but most strongly so in the Agulhas Province (Fig. 16). Bayesian analysis of population structure recovered two sub-groups within the mtDNA data, namely a Namaqua Province and Agulhas/East Coast Province group, with no sub-group structure found for the nDNA data (Figs 5 and 7). The mtDNA mismatch distribution is also indicative of underlying population structure between two groups or clades (Fig. 8). This was also true for the parsimony networks, where there is a clear distinction between the Namaqua Province north of Mouille Point (outlined in Fig. 11) and the Agulhas and East Coast Provinces in the mitochondrial network, which suggests that the region to the north of Cape Point acts as a potential barrier to gene flow between these two sea urchin genetic assemblages. The non-monophyly of the two assemblages point either to a low level of gene flow across this barrier, or the retention of ancestral polymorphisms (Figs 11 and 12). Furthermore, no correlation between either mtDNA or nDNA and sea urchin colour was found (Figs 4 and 6).

For the nDNA, AMOVA revealed little to no structure between individual sampling areas for the nuclear data in the Namaqua, Agulhas and East Coast Provinces, but a split between the Namaqua Province and the Agulhas/East Coast Provinces was revealed by the Φ_{ST} values (Table 3), which further highlights the significance of Cape Point as a potential barrier. The nDNA gene flow largely supports that of the mtDNA, except for the west coast Namaqua Province where it is occurring in an almost exclusive northerly direction, whereas the gene flow in the Agulhas and East Coast Provinces are bidirectional (Fig. 17). However the parsimony network still showed some level of structuring between the Namaqua and Agulhas/East Coast Provinces (Figs 14 and 15).

4.1.2 Two major clades: sub-group structure and gene flow

The bayesian analysis of population structure identified two major sub-groups within *P. angulosus* along its range, with the split being in the Cape Point region (Fig. 5). This finding was supported by the mtDNA AMOVA results (Table 3), mismatch distribution (Fig. 8), parsimony networks (Figs 11 and 12) and to a lesser extent the nuclear DNA data (Table 3, Figs 14 and 15). Strong determinants that can disrupt the dispersal ability of marine invertebrates with pelagic larvae are factors such as oceanic currents, larval behaviour (e.g. self recruitment, swimming ability), habitat availability, upwelling and fresh water plumes from rivers (Palumbi 1994, Lessios *et al.* 2003, Waters and Roy 2004, Banks *et al.* 2007, Quinteiro *et al.* 2007, Teske *et al.* 2008, von der Heyden 2009). Notably, a number of studies have demonstrated a genetic break or severely limited gene flow around Cape Point for marine species exhibiting a number of differing life histories with, among others, two southern African estuarine crustaceans (Teske *et al.* 2006), three species of clinid fish (von der

Heyden *et al.* 2008, von der Heyden 2009) and three long distance dispersing sea urchin species (Lessios *et al.* 1999, 2003, McCartney *et al.* 2000).

In the case of the *P. angulosus* urchin populations it is most likely that oceanic currents, along with upwelling and its associated dynamics as well as larval behaviour could have disrupted gene flow in the Cape Point region, which then gave rise to the observed pattern. Interestingly, there is very little to no gene flow from west to east around Cape Point for either the mtDNA or nDNA (Mouille Point – Kommetjie – Wooley's Pool, Figs 16 and 17), but orders of magnitude higher gene flow from east to west (Wooley's Pool – Kommetjie, Figs 16, 17). Pairwise Φ_{ST} values between the Agulhas/East Coast Province sampling localities and the two immediate Namaqua Province localities (Kommetjie and Mouille Point) are on average 0.30 lower than for the other Namaqua Province localities from Jacobsbaai northwards (Table 3). Also, the only haplotypes present in the Namaqua Province for COI are those from the Agulhas Province, which is geographically the most proximal.

This pattern fits with the ocean dynamics of the region and life history strategy of *P. angulosus*, providing further evidence of the Cape Point region as a genetic barrier in this species. Where the Agulhas Current veers away from the coast in the Agulhas Bank region on the southern African south coast (Agulhas Province biogeographic region), eddies often form that transport water masses, along with their associated biota (such as pelagic larvae), from the Indian Ocean around Cape Point and into the Atlantic (Fig. 16, Shannon 1985, Lutjeharms 2006, Reason *et al.* 2006), where it is then integrated into the Benguela system (Shannon 1985, Branch *et al.* 2002, Lutjeharms 2006, von der Heyden *et al.* 2008 and references therein, see also Fig. 1), which acts as a barrier to south and eastwards gene flow. *Parechinus angulosus* has

been demonstrated to have a pelagic larval phase of approximately 56 days, can delay metamorphosis and settling by up to 11 days as well as actively select a suitable substratum to metamorphose and settle onto (Cram 1971).

It is thus a possibility that larvae of *P. angulosus* can be transported around Cape Point via these eddies and settle on the west coast in the Namaqua Province, explaining the presence there of individual Agulhas Province haplotypes from the south coast. However, the rarity of such haplotypes (Figs 11 and 12) in the Namaqua Province would suggest that this is either not a common occurrence or that most larvae are lost during the transportation period – possibly due to their pelagic phase coming to an end before suitable substrate is found - and hence the genetic differentiation observed. The difference in water temperature – it is significantly colder on the west coast - might also inhibit larval settlement. This could also explain why Kommetjie and Mouille Point are associated with the Agulhas/East Coast Province sub-grouping (Fig. 5): as they are the two most proximal sampling localities on the opposite side of the Cape peninsula, they more readily receive migrants from the other coastal regions and thus remain genetically more similar over time. Furthermore, upwelling has been demonstrated as an effective barrier to larval transport in several marine species (e.g. Apte and Gardner 2002, Waters and Roy 2004), and in sea urchins in particular (e.g. Lessios *et al.* 2003, Banks *et al.* 2007), and is likely reinforcing this genetic discontinuity around Cape Point – due to the persistent upwelling in this region (Shannon and Nelson 1996, Laudien *et al.* 2003).

A significant pattern of isolation by distance was found for the entire mtDNA dataset as well as within two (Namaqua and Agulhas) of the three biogeographic provinces studied, but not for the nDNA. A pattern of isolation by distance results when

individuals of a species reproduce with partners from the same or nearby populations/localities, rather than with those from far away (Wright 1943, Handley *et al.* 2007). Thus, populations of a species grow genetically more similar to other, more proximal, populations versus those further away. Isolation by distance is not usually expected in a species with long-lived planktonic larvae that have the potential to disperse over large distances (Neethling *et al.* 2008 and references therein).

This appears to be true for this study, as the significant finding of isolation by distance for the mtDNA data shows. As *P. angulosus* larvae have the ability to spend almost two months in the water column, and thus have the potential to disperse over great distances, the isolation by distance finding could more likely point to the large role that the oceanography along the southern African coast plays in limiting dispersal in this species. The dynamic nature of the coastline with its many regional, seasonal and permanent upwelling cells as well as counter currents provide many opportunities for larvae to be prevented from passing through or deflected away. Other studies have found strong influences of physical oceanography, coastal topography and life-history on the genetic structuring of sea urchins (e.g. Banks *et al.* 2007). The significant isolation by distance finding within the Namaqua and Agulhas Provinces – especially for a potentially long distance disperser - reinforces the strong influence of the dynamic coastline mentioned above.

The gene flow analysis for the mtDNA (Fig. 16) potentially indicates where migrants might be lost in this dynamic environment and thus prevent mixing of geographically distant localities' populations. There are several instances where gene flow is markedly reduced (Jacobsbaai-Mouille Point, Kommetjie-Wooley's Pool, Jeffrey's Bay-Port Elizabeth) or absent altogether (Mouille Point - Kommetjie) in a west-east

direction and vice versa (Port St Johns - Haga-Haga, Port Alfred - Port Elizabeth, Knysna - Cape Agulhas). This could lead to migrant larvae being unsuccessful in reaching far away populations regularly and hence isolation by distance as they accumulate genetic differences. The Φ_{ST} values for the mtDNA also show a clinal increase from west to east (Table 3), which means that urchin populations at the sampled localities gradually become more genetically dissimilar the further apart they are along the southern African coast. The parsimony network for the mtDNA (Figs 11 and 12) reinforces the isolation by distance.

The reduced degree of structuring revealed by the nDNA data in the AMOVA, BAPS and parsimony analyses can be explained by ancestral polymorphism and incomplete lineage sorting between populations. Accordingly, phylogeographic structure is generally anticipated to be less prominent at diploid nuclear loci when contrasted with mitochondrial loci due to their disparity in effective population sizes and evolutionary rates (Hare 2001, Avise 2009). Also, the low level of variation in the nuclear DNA could explain the fuzzy signal for the gene flow analysis.

4.1.3 Namaqua Province (west coast) structure and gene flow

Phylogeographic analyses for *P. angulosus* in the Namaqua Province revealed results that indicated a high incidence of population genetic structure among the localities sampled. Analysis of molecular variance showed all these sampling areas to be significantly different from one another, except for Mouille Point and Kommetjie, the two southern most localities (Table 3). Gene flow is occurring to both the north and south within this biogeographic region, but unevenly (Fig. 16). There are several factors that have the potential to contribute to the observed genetic structuring in

marine organisms with free-swimming larvae, such as upwelling, oceanic currents, larval behaviour and recruitment stochasticity, freshwater plumes from rivers and habitat availability (e.g. Greenwood 1980, Waters and Roy 2004, Lessios *et al* 2003, Banks *et al.* 2007, Neethling *et al.* 2008, von der Heyden *et al.* 2008, Hodgson 2010). However, as the Namaqua Province has abundant intertidal rocky habitat, it is unlikely that habitat availability would be a limiting factor in dispersal for *P. angulosus*, and thus cannot be used to explain the large amount of structure present here (von der Heyden *et al.* 2008).

The west coast of southern Africa (Namaqua Province) possesses very dynamic oceanography, with the cold northward flowing Benguela Current dominating along with regional upwelling cells, sub-surface counter currents and steep bathymetry (Shannon 1985, Shannon and Nelson 1996, Laudien *et al.* 2003, Lutjeharms 2006). Upon closer inspection it appears that, for *P. angulosus*, ocean currents (both the Benguela and localised inshore currents), upwelling (large and small scale), larval behaviour and river plumes (such as the Orange River outflow) are the most likely determinants of genetic structure on the west coast of southern African.

Gene flow in the Namaqua Province is occurring in a northerly direction along with the flow of the Benguela Current (Figs 16 and 17), although there is some southwards gene flow - particularly between Hondeklipbaai and Lamberts Bay (Fig. 16) - which suggests smaller scale dynamics at play. The northward flowing Benguela Current has the potential to transport larvae up the coastline, with sub-surface counter currents transporting them in the opposite direction. This larval transport pattern can be markedly altered by factors such as larval behaviour, where larvae alter their position in the water column by active swimming and thus move into opposite travelling

currents. Besides the seasonal upwelling driven by the wind and Benguela current, there are also a number of upwelling cells on the southern African west coast within the Namaqua Province that could disrupt gene flow. These upwelling cells are in the region of Cape Columbine and Hondeklipbaai (Shannon 1985, Laudien *at al.* 2003), both where *P. angulosus* show decreases in gene flow (Fig. 16). These counter currents and periodic inshore coastal current reversals driven by wind and upwelling could potentially be influencing the gene flow between Hondeklipbaai and Lamberts Bay, along with southwards flowing sub-surface counter currents (Shannon 1985) transporting larvae between these two localities. Notably, there is a large increase in Φ_{ST} values for mtDNA (on average 0.4, Table 3) when moving from Lamberts Bay northwards to Hondeklipbaai, along with markedly reduced gene flow towards the north (Fig. 16). Interestingly, Hondeklipbaai has an upwelling cell - named after it - which may be preventing the import of larvae from further south. This barrier to gene flow appears to be significant for the sea urchin, as the large increase in Φ_{ST} values between the two localities suggests.

Self recruitment is another plausible contributor to the observed structure, as *P. angulosus* larvae have been shown to actively select substrate to metamorphose onto as well as delay metamorphosis if forced to do so (Cram 1971). This means that larvae actively select their native habitat to metamorphose and settle in, as it is already proven to be suitable (Teske *et al.* 2008), possibly attracted by olfactory cues given off by individuals already established there (Bowen *et al.* 2001). Recent reviews and studies (Levin 2006, Pineda *et al.* 2009) have highlighted the higher incidence of self recruitment than was previously thought possible for benthic marine organisms. Along with self recruitment, recruitment stochasticity may be another factor influencing settlement success in *P. angulosus* and thus its genetic population

structure. Greenwood (1980) reported that *P. angulosus* recruitment was highly variable and suffered from high mortality during early settlement, especially on the southern African west coast (Namaqua Province). Thus, it is possible that larvae of *P. angulosus* may, through their behavioural traits and recruitment success described above remain among - or close to - their natal habitats, with few migrants reaching other populations and thus resulting in the observed structuring.

Freshwater plumes from rivers have also been shown to inhibit larval transport across such barriers due to factors such as increased turbidity and decreased salinity in these regions (Rocha *et al.* 2002, Lessios *et al.* 2003). Echinoderms in particular have been shown to be sensitive to decreases in salinity during fertilisation and egg development, leading to reduced developmental success (Allen and Pechenik 2010). This could explain the markedly decreased gene flow north and south between Namibia and Port Nolloth (Fig. 16), as larvae from *P. angulosus* would have to traverse the Orange River outflow, which forms the border between South Africa and Namibia. Although the genetic difference between these two localities is significant (Table 3), the low Φ_{ST} value (0.13) as well as the fact that some, albeit very low, gene flow occurs (Fig. 16), would suggest that *P. angulosus* larvae from Port Nolloth and Namibia periodically move past this non-permanent barrier to maintain a genetic connection. Interestingly, the Orange River is cited as a possible cause of genetic discontinuity in the dispersive egg and pelagic larval stages of the deep water Cape hake, *Merluccius paradoxus* (von der Heyden *et al.* 2007b). Sampling on a finer scale both north and south of this geographic feature forming the political border between South Africa and Namibia will assist in shedding more light on the extent and validity of the Orange River as a barrier.

The reason for the gene flow anomaly from Mouille Point northwards to Jacobsbaai and the associated jump in Φ_{ST} values versus those to the south (Table 3) is not immediately clear. Even though BAPS analyses (Fig. 5) identified this break as well, there is no easily identifiable reason for the observed pattern. Interestingly, pairwise Φ_{ST} values between Jacobsbaai and the remaining two Namaqua Province localities south of it (Mouille Point and Kommetjie) are on average 0.25 higher than between Jacobsbaai and localities to its north (Table 3). This could be due to any of the number of factors already discussed, or more likely a combination of these (see below), with upwelling and ocean currents being the most likely vectors.

One probable explanation is that larvae of *P. angulosus* are transported from the Agulhas Province via eddies around Cape Point (as described previously) and then settle as quickly as possible – or once the limits of their larval duration is reached - once suitable habitat is found i.e. at Kommetjie or Mouille Point. Thus, the fact that these two southern most Namaqua Province sampling localities receive migrants more readily than those further north would maintain the genetic connection with them, resulting in them being grouped with the Agulhas/East Coast Province group in the BAPS analyses (Fig. 5), as well as have less genetic differentiation between them which is reflected in the lower Φ_{ST} values (0.03-0.25 vs 0.19-0.87, Table 3). Ocean temperatures on either side of Cape Point are also of a similar nature, and thus it may be easier for urchins in this Agulhas Province region to move around Cape Point and settle as they are semi-adapted to the cold. The reason as to why these two most southerly Namaqua Province sampling locality populations then do not propagate larvae further north is more than likely explained by the ocean dynamics: the upwelling cells at Cape Columbine and Hondeklipbaai discussed above, along with sub-surface counter currents (Shannon 1985, Laudien *et al.* 2003).

4.1.4 Agulhas Province (south coast) structure and gene flow

There appears to be very little to no population subdivision in the Agulhas Province biogeographic region, with the significantly different Φ_{ST} values that there are being very low (0-0.16, Table 3). Gene flow is also occurring in both western and easterly directions along the coast (Fig. 16). Importantly, the current patterns on this part of the coast have no unidirectional, dominant pattern, as it is here where the south westwards flowing Agulhas Current deflects away from the coast and mixing of the Atlantic and Indian Oceans occur (Shannon 1985, Schumann *et al.* 1988, Branch *et al.* 2002, Lutjeharms 2006, Reason *et al.* 2006). As a result smaller scale coastal processes tend to dominate e.g. wind driven currents, coastal counter currents and seasonal upwelling (Shannon 1985, Schumann *et al.* 1991, Branch *et al.* 2002, Lutjeharms 2006). This lack of genetic differentiation could thus point to an influence from the inshore Agulhas counter current that flows up the east coast of southern Africa in the opposite direction to the Agulhas Current proper, as has been hypothesised for other marine species in this region (von der Heyden *et al.* 2008).

This variability in marine circulation patterns could be the reason why *P. angulosus* exhibits less population structure in the Agulhas Province, as the stochastic nature of these patterns can result in pelagic larvae being transported bidirectionally and which consequently maintains the genetic connectivity among Agulhas Province sampling areas. A similar pattern of genetic homogeneity in this region of coastline has also been identified for the spiny lobster, *Palinurus gilchristi* (Tolley *et al.* 2005) and for the intertidal fish species, *Caffrogobius caffer* (Neethling *et al.* 2008). One factor that is likely to be contributing to the weak population differentiation observed is the upwelling cells off Cape Agulhas, Tsitsikamma and Algoa Bay (Shannon and Nelson 1996). However, since these upwelling cells are seasonal, wind dependent and not as

intense as on the southern African west coast in the Namaqua Province, larvae have a much better chance of surmounting these barriers and maintaining genetic connectivity within this biogeographic region.

The result that Wooley's Pool is functioning as a source population for Agulhas Province localities is re-enforced by the lack of genetic structure between this locality and all other Agulhas Province localities (Table 3), as well as the high number of migrants that move out from there (Fig. 16). The reason for this could be the stable environment inside False Bay: it faces away from the predominantly south westerly swell direction (calmer seas), has a relatively stable, warmer temperature regime in the northern side of the Bay (Atkins 1968, Cram 1971) and ample rocky shore habitat.

Further east, *Parechinus angulosus* does not seem to be affected by the genetic break identified at Cape Agulhas for other South African marine species (e.g. Evans *et al.* 2004, Teske *et al.* 2006, 2007, von der Heyden *et al.* 2008), as gene flow is occurring readily across this region (Figs 16 and 17). This surprising finding was also made by Neethling *et al.* (2008), who found no genetic discontinuity across this genetic barrier for *Caffrogobius caffer*. However, between Jeffreys Bay and Port Elizabeth gene flow is markedly low in either direction (Fig. 16). Thus, the population genetic boundary identified on the southern African south-east coast in the region of Port Elizabeth (von der Heyden *et al.* 2009) does appear to cause shallow genetic differentiation (Table 3) in *P. angulosus* as well as limit gene flow. Factors promoting this could be localised oceanographic features such as counter currents and the aforementioned upwelling cell in the region.

4.1.5 East Coast Province (east coast) structure and gene flow

Small but significant Φ_{ST} values between the three East Coast Province sampling areas (Table 3) indicates some degree of population structure in this region. Interestingly, a study by Teske *et al.* (2008) identified the upwelling in the region of Algoa Bay as a potential barrier to gene flow for mudprawn populations. This upwelling may occur as far north as Port St Johns (Teske *et al.* 2008 and references therein), and could thus contribute to the genetic differentiation between East Coast Province localities of *P. angulosus*. Another potential factor could be self recruitment due to scarcity of habitat, where *P. angulosus* settles in its native habitat due to favourable conditions, as has been shown for other marine benthic taxa (Levin 2006, Pineda *et al.* 2009).

The limiting of gene flow in *P. angulosus* in the East Coast Province appears to include the most easterly Agulhas Province sampling area, namely Port Elizabeth, as gene flow between here and localities to the west is very low (and vice versa). Thus it would seem that the genetic barrier for *P. angulosus* due to the upwelling is situated just to the west of Port Elizabeth, and could be due to seasonal upwelling (Fig. 16). Other coastal invertebrates that have shown a phylogeographic break along this stretch of coastline include the mussel *Perna perna* (Zardi *et al.* 2007), a cumacean species, *Iphinoe truncate* (Teske *et al.* 2007), an intertidal fish species, *Clinus cottoides* (von der Heyden *et al.* 2008) and, very interestingly, *Caffrogobius caffer*, that has not shown geographic structure anywhere else (Neethling *et al.* 2008).

The observed genetic structuring could also be a function of rocky shore habitat availability. As one moves around the southern African coast from west to east, such habitats become progressively more scarce and are interspersed with extensive sandy

beaches (von der Heyden *et al.* 2008, Neethling *et al.* 2008, personal observation), which could mean that larvae find it ever more difficult to migrate between localities. Although due to the shallow genetic structure evident larvae still appear to overcome this barrier readily possibly as a consequence of their longevity. This could be due to the bidirectional nature of the ocean currents which transport larvae readily in either direction along the coast, as well as the fluctuation of upwelling which is seasonal and primarily wind driven along the south (Agulhas Province) and east (East Coast Province) coasts, thus allowing free swimming larvae to pass through during periods of reduced upwelling.

4.2 Demographic history

The mitochondrial COI region demonstrated adequate variability for population genetic analysis in the Cape sea urchin, *Parechinus angulosus*. The significantly negative Fu's F_S value (-23.64, $P < 0.05$) for the entire mtDNA data set for *P. angulosus* strongly implies that as a species it has undergone recent demographic change. Interestingly, upon further investigation at the individual population/locality level, 10 of the 18 sampled localities' populations are shown to have undergone expansion (Table 2). Even though the individual locality mismatch distributions contrast with this by showing that every one of the locality populations has undergone expansion (good raggedness indexes, Fig. 9), it has been shown that Fu's F_S is a more powerful indicator and test for demographic expansion (Ramos-Onsins and Rozas 2002). The high haplotypic diversity and comparatively low nucleotide diversity (Table 2) is also typical of the signature of population expansion (Grant and Bowen 1998, Quinteiro *et al.* 2007, von der Heyden 2009). Contradictory to the mtDNA data, only one of the nDNA sampling localities, Wooley's Pool, is shown to be expanding

(Table 2), despite the significant Fu's F_S value (-25.15, $P < 0.05$) for the whole nDNA dataset. This overall signature of population expansion is in agreement with the mitochondrial data.

The estimated time of expansion is indicative of a growing population since the early to mid Holocene (7700 to 4700 years ago, depending on generation time and mutation rate used, see results section), which is after the last glacial maximum (LGM) at 20 000 years ago (Lambeck *et al.* 2002). In accordance with this, other southern African marine species have been found to show similar times of population expansion, for example the commercially important hake species, *Merluccius capensis* (von der Heyden *et al.* 2007b), *Caffrogobius caffer* (Neethling *et al.* 2008), as well as several lobster species: *Palinurus delagoae* (Gopal *et al.* 2006), *Palinurus gilchristi* (Tolley *et al.* 2005) and *Jasus tristani* (von der Heyden *et al.* 2007a). The changing sea levels and associated temperatures during this time period since the LGM most likely played a role in the observed demographic changes (Lambeck *et al.* 2002, Tolley *at al.* 2005).

The most dominant haplotype in the mitochondrial and nuclear parsimony networks is from Betty's Bay in the Agulhas Province (Figs 11 and 12). This locality, however, also has the most unique haplotypes of any of the sampled area, and haplotypes from here are also the most widely distributed (Figs 11 and 12, Table 1). This could point to it being an ancestral population from where most other populations were founded after initial population contraction (pre-LGM) and consequent expansion (post-LGM) as suitable habitat became available (Tolley *et al.* 2005, von der Heyden *et al.* in press). It is hypothesised that the Agulhas Bank served as a refuge for marine intertidal organisms during periods of low sea levels (Tolley *et al.* 2005) during the

Pleistocene up until the last glacial maximum, after which sea levels gradually began returning to present levels (Shannon 1985, Lambeck *et al.* 2002). The sea level drop during this time was in the region of 120-140m (Lambeck *et al.* 2002, von der Heyden *et al.* 2010), which would have meant that intertidal habitat on the west and east coasts of southern Africa, where the bathymetry is steep, would become unavailable due to the quick gaining of depth and thus most habitat would be beyond intertidal organisms' range.

In contrast, the Agulhas Bank with a maximum depth of 200m below current sea levels (Shannon 1985), would have been able to serve as a refuge for intertidal species such as sea urchins, harbouring the increased genetic diversity observed in the Agulhas Province sampling localities of *P. angulosus* today. This could also partly explain the strong signals of population expansion observed for the Agulhas Province sampling areas, particularly Betty's Bay (Table 2), as it is centrally situated on the Agulhas Bank region and would have been ideally placed to serve as one of the first resettlement localities when sea levels became elevated post-LGM 20 000 year ago. The lack of population genetic structure in the Agulhas Province could be due to this recent population expansion, such that the sampling localities' populations here have not yet differentiated genetically to a great extent. This would require a very recent expansion date (within the Holocene) for *P. angulosus*, due to the habitat on the Agulhas Bank only becoming available then (Tolley *et al.* 2005), and is indeed the case, with the dates of expansion posited being between 4700 – 7700 years ago.

The singleton haplotypes/alleles observed in the parsimony networks (Figs 11 and 14) could be a sampling artifact, i.e. due to the high observed genetic diversity for *P. angulosus*, however given the large sample size of >500 individuals this is not likely.

Alternatively, although leading to a similar result, it could be that these are from older populations that still harbour a large number of divergent haplotypes. The fact that all the singleton haplotypes are from either the Namaqua or East Coast Province (except one), which are the two extremes of *P. angulosus'* range, could also mean that after the initial settlement from Agulhas Province (south coast) sampling areas in these regions they diverged significantly enough to result in the observed pattern. An alternative possibility could be that this pattern reflects source-sink dynamics between localities along each coastal region, as some non-expanding localities definitely appear to have incoming migrants, with comparatively little to none emigrating (Fig. 16), e.g. Wooley's Pool in the Agulhas Province and Port Alfred and Haga-Haga in the East Coast Province, as well as high haplotype and low nucleotide diversities. The high number of private haplotypes and alleles (Table 1) – along with the singleton haplotypes/alleles (Figs 11, 12 and 14, 15) - observed in *P. angulosus* may also be the result of large effective population sizes (N_e). Large effective population sizes retain ancestral polymorphisms more readily than smaller populations over time (Avise *et al.* 1988, Beerli and Felsenstein 2001, Leberg 2005), resulting in increased genetic diversity, as is evident from the h and π indices of *P. angulosus* (Table 2).

The parsimony networks themselves may be pointing to a more complex past, coupled with large effective population sizes, in *P. angulosus*, as there is no central haplotype/allele to all the others, but rather several dominant alleles forming separate clades (Figs 11, 12 and 14, 15). This could point to several populations of *P. angulosus* that have survived over time, accumulating genetic differences, and upon reconnection (post-LGM) mixing would have occurred, but the genetic diversity would have been sufficient to result in the high degree of structure observed between sampling areas (Table 3). This means that more than one population of *P. angulosus*

would have had to survive in refuges during glacial cycles. Possible refuges exist on the southern African west coast (Namaqua Province) in the Jacobsbaai and Lamberts Bay areas, akin to that of the Agulhas Bank refuge described earlier. The bathymetry on here is steep as mentioned previously, however in places the 100-200m isobaths appear to be sufficient distances from the coast (Shannon 1985) to have been able to provide intertidal habitat during glacial cycles up to the LGM.

P. angulosus could thus have been fragmented into several smaller populations on both the west and south coast (Namaqua and Agulhas Provinces, respectively) of southern Africa during glacial periods, although not for sufficient amounts of time to completely diverge into separate species, which could have given rise to the high degree of genetic structuring seen in the present analyses. Interestingly, Lessios *et al.* (2001) points out that periods of glaciation were also periods of active speciation in the sea urchin genus *Diadema*, and that segregation between local populations as a result of lower sea levels and oceanic current variations were probable during glacial cycles. The high degree of upwelling, combined with oceanic currents and counter currents, along with river outflows and recruitment stochasticity, could possibly have maintained this genetic structure in the Namaqua Province up to the present day, after sea levels were raised post-LGM. In contrast, the lesser degree of mostly seasonal upwelling and bidirectional current patterns along the coasts in the Agulhas and East Coast Provinces probably assisted in the mixing of populations and resulted in reduced population structure (Table 3).

It is also not impossible that *P. angulosus* consisted of one panmictic population with no genetic structuring along the southern African coastline at some point in the past, as is alluded to by the parsimony networks (Figs 11, 12 and 14, 15). The mtDNA

network shows the presence of Agulhas Province haplotypes in the Namaqua Province, which could be due to gene flow. However, the nuclear data shows the presence of alleles from all three of these biogeographic regions throughout the network, with shallower structuring between coastal regions than for the mtDNA. This could mean that all the coastal regions were connected in the past by gene flow, but have subsequently become isolated over time (see section above), with East Coast Province mtDNA haplotypes (those furthest from Namaqua Province localities) disappearing first from the Namaqua Province, while they are still evident in the nDNA parsimony network. This is most likely due to the different modes of inheritance of mitochondrial and nuclear DNA. This is not unlikely, as the both the Benguela (along with its associated upwelling) and Agulhas Current systems have experienced fluctuations in intensity since the Pleistocene (Meyers *et al.* 1983, Shannon 1985), and could have been responsible for allowing mixing, then fragmenting and isolating a panmictic population into individual urchin populations over time. This would have given rise to some extent of genetic structuring and, evidently in *P. angulosus*, the level of structuring is very high in places.

4.3 Colour forms of *Parechinus angulosus*

The mitochondrial DNA COI marker, as a mainly neutral evolving marker, does not appear to be involved in differentiation between colour morphs of *P. angulosus*, as the absence of any correlation between mtDNA (COI) structure and sea urchin colour indicates (Fig. 4). The estimation of demographic parameters requires the use of largely neutral markers such as COI, however such markers may not be suitable for detecting adaptive changes over short periods (Calderon and Turon 2010, Calderon *et al.* 2009, 2010). This finding is supported by the nDNA data, which also did not show

any degree of structure or correlation between sea urchin colour forms at the SpREJ9 locus (Fig. 6), even though this genetic marker is part of the reproductive cycle in the sea urchin sperm plasma membrane, where it is speculated to function in regulating signal transduction pathways and ion channel activities (Gunaratne *et al.* 2007).

Notably, recent studies have found that both sea urchin sperm morphology, as well as the nuclear gene coding for the sperm protein bindin in sea urchins show differentiation among populations, cohorts and colour morphs (Manier and Palumbi 2008, Calderon and Turon 2010, Calderon *et al.* 2010). Bindin is directly involved with gamete recognition and is under positive selection, which can result in a temporal signal of genetic differentiation (Calderon *et al.* 2010), which appears to be more suitable to the study of colour differentiation. Thus, markers that are directly related to reproductive success will be able to put forward a more complete view of continuing differentiation processes (Calderon *et al.* 2010) than alternate markers like COI or indirect reproductive markers from the PKD1 family, like SpREJ9.

4.4 Conclusion

Parechinus angulosus has an extremely wide distribution from southern Namibia to KwaZulu-Natal, spanning the entire South African coastline. Throughout its range the marine environment changes dramatically from cold conditions on the west coast of southern Africa, to temperate conditions on the south coast and finally warm to sub-tropical conditions on the east coast, which has been recognized as separate biogeographic provinces. As such, and given its life history as a broadcast spawner with pelagic larvae and a sessile adult stage, it makes for a fascinating study subject from a molecular point of view.

In this regard it has certainly delivered. Instead of a species with no genetic structure present as was expected in this study, by employing mitochondrial and nuclear DNA, we found a number of anomalies for *P. angulosus* with regards to population genetic structure along the southern African coastline. In the Namaqua Province, where rocky habitat is plentiful and unlikely to be a limiting factor, the unexpectedly high genetic structure found for *P. angulosus* is likely due to the intense upwelling, larval behaviour, stochastic recruitment, ocean currents and potentially freshwater outflow from rivers, all of which affect larval survival and dispersal. The Agulhas Province is marked by little or no population structure, possibly due to weak upwelling, bi-directional current patterns and recent population expansion. *P. angulosus* in the East Coast Province exhibits population structure once more, albeit very shallow. Causes for this could be the upwelling cited by Teske *et al.* (2008) as well as habitat availability as rocky shore becomes ever scarcer.

This work adds to the growing body of knowledge, generated by scientists across the globe (e.g. Lessios *et al.* 2003, Banks *et al.* 2007, Quinteiro 2007, Waples *et al.* 2008, Weersing and Toonen 2009, Calderon and Turon 2010) and in southern Africa particularly (e.g. Evans *et al.* 2004, Matthee *et al.* 2007, von der Heyden *et al.* 2008, Neethling *et al.* 2008, von der Heyden 2009, Teske *et al.* 2009, von der Heyden *et al.* 2010), that is showing that the common expectation that marine invertebrate population genetic structure is dictated purely by their pelagic larval duration (Weersing and Toonen 2009) is false. Instead, these studies suggest that it is rather a combination of life history and the dynamic nature of the marine environment, coupled with historical factors. This study also highlights the west coast of southern Africa (cool-temperate Namaqua Province biogeographic region) as an important area

of genetic interest, but which currently is receiving no conservation input in the form of Marine Protected Areas (MPA's), and thus agrees with a recent review by von der Heyden (2009) that identified a number of genetically important regions along the southern African coast which fall outside current MPA networks.

Along with other research being carried out, this work adds to the multi-species approach advocated by scientists (e.g. Bermingham and Moritz 1998, Zink *et al.* 2000, von der Heyden 2009) when considering aspects such as the placement of MPA's and additional conservation efforts, and will hopefully add to the efforts being made in conserving the marine diversity found along the southern tip of Africa.

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