Evolutionary and ecological connectivity at hierarchical scales from sperm to species in the reef-building coral genus *Seriatopora*

Thesis submitted by

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Statement on the contribution of others

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Abstract

Determining the spatial scales of ecological connectivity for marine populations, which typically exist as metapopulations of spatially isolated subpopulations linked by demographic processes (e.g., larval dispersal, reproduction), is critical for foundational taxa, yet poorly understood for the reef-building corals. Connectivity of populations on ecological timescales is becoming increasingly relevant to conservation and management efforts, as the pace of changing climate and anthropogenic impacts accelerates and the need to understand processes sustaining the resilience of coral populations becomes more urgent. In this thesis, I investigated three hierarchical divisions (species, populations and individuals) within the coral genus *Seriatopora* to resolve spatial scales of genetic linkages and elucidate the ecological and evolutionary processes that determine connectivity in the group, and interpret these results in light of population recovery and resilience.

To determine if cryptic diversity occurs in the abundant coral morphospecies *Seriatopora hystrix,* as suggested by inconsistent results from reproductive, population genetics and recent mitochondrial studies, multiple lines of evidence (genetic, ecological and morphological) were combined to compare diversity within *S. hystrix* to that found between *S. hystrix* and its only known congener on the Great Barrier Reef (GBR), *Seriatopora caliendrum* (Chapter 2). Based on hierarchical Bayesian clustering of microsatellite data, four distinct clusters were identified within *S. hystrix* that demonstrate genetic cohesion across two regions of the GBR separated by 450 km in both sympatric and allopatric populations. Correlations between mtDNA phylotypes, combined with habitat preference, *Symbiodinium spp.* coupling and in one case, morphology, corroborate these clusters as putative cryptic species. Moreover, the four putative cryptic species are diverged from each other at levels comparable to genetic differentiation at microsatellite loci from *S. caliendrum* (F_{ST}: 0.337 – 0.519). Only one

instance of recent hybridization was inferred between the morphospecies *S. hystrix* and *S. caliendrum*, but none were detected among the putative cryptic species of *S. hystrix*. One rare putative species, only sampled in one region (*Sh_bushy),* displays a distinctive morphology and is almost fixed for private alleles (>82%) at one of the ten loci. Moreover, a phylogeographic analysis of all published *Seriatopora* mitochondrial control region sequences revealed clear affinities among some proximate geographic regions (e.g., GBR and New Caledonia) and one cosmopolitan lineage that occurs in five of eight locations surveyed from Okinawa to the GBR. Together, these findings suggest that species diversity within the genus *Seriatopora* is higher than expected on the GBR, and imply that global diversity may also be higher for the genus, both of which have significant implications for our ability to accurately assess and conserve biodiversity, and infer connectivity patterns.

Using the putative cryptic species identified in Chapter 2 as separate units, I determined and compared levels of population connectivity for the two most abundant putative species, which respectively dominate sheltered (leeward) and exposed (windward) habitats, at three hierarchical spatial scales on the GBR: within reefs (<1 km), between reefs (<10 km), and among regions (~450 km) (Chapter 3). Standard equilibrium population differentiation statistics were combined with Bayesian clustering and spatial autocorrelation analyses, using ten microsatellite loci and individual-level geographic coordinates from seventeen populations. I found consistent patterns of genetic subdivision among reefs and regions, in contrast to previous work, suggesting that widespread cryptic species in *S. hystrix* may account for some of the complex genetic structure previously reported. Significantly, I determined that mean larval dispersal is concentrated within 3 km of source populations for both putative species, indicating that local recovery from disturbances in these putative species will likely be dependent on relatively close proximity of surviving populations. Recolonization

following local extinctions in isolated areas may be possible, but much less certain on immediate time scales.

Within a population, sperm dispersal constitutes the mechanism by which brooding sessile corals interact and mate, and forms the first link in the network of processes that determine species-wide genetic diversity and connectivity patterns, but almost nothing is known about sperm dispersal for any internally fertilizing coral. I conducted a parentage analysis on brooded coral larvae collected from a mapped population to measure the distance of sperm dispersal for the first time in a reef-building coral, and to estimate mating system characteristics of the putative *Seriatopora* species that dominates sheltered habitats (Chapter 4)**.** Furthermore, I define and apply consensus criteria among several replicated methods to maximize accuracy in paternal assignments for a natural population. Thirteen progeny arrays indicated that this putative species produces sexually derived, primarily outcrossed larvae (mean t_m=0.999 ±0.026 SD) in multiple paternity broods (mean r_p=0.119 ±0.052 SD). Self-fertilization was directly detected only at very low frequency for all broods combined (2.8%), but comprised 23% of matings in one brood. Although over 82% of mating occurred between colonies within 10 m of each other (mean sperm dispersal = 5.5 m ±4.37 SD), I found no evidence of inbreeding in the genetic structure of the established population. In particular, more limited dispersal of sperm, compared to greater distances of philopatric larval dispersal, appears to reduce inbreeding among close relatives in this cryptic species.

Given mounting evidence that a diverse range of coral reef organisms exhibit restricted dispersal and potentially widespread cryptic species, protected area networks must be designed with the appropriate criteria in mind, particularly in relation to the size, spacing and inclusion of diverse habitats, to maintain demographic, ecological and evolutionary processes. The results of my research indicate that species diversity may be much higher than previously thought for the genus *Seriatopora,* an abundant

and geographically widespread group of corals. Ecological distributions imply specific habitat preferences for the two dominant putative species, for which the majority of larval dispersal appears to be limited to very local areas (<3 km). However, high population densities and frequent reproductive events throughout the year suggest local resilience and imply that local populations can be successfully preserved. Moreover, the absence of inbreeding, despite relatively small genetic neighborhoods, indicates that mechanisms exist to promote and preserve genetic diversity within populations of these species. Taken together, my results indicate that local conservation efforts will constitute an important strategy for long-term persistence of the genus, and, providing that environmental integrity can be maintained, connectivity processes will continue to support abundant populations of this important group of corals over broad spatial scales.

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Chapter 1.0 General Introduction

1.1 BACKGROUND

Coral reefs display high levels of biodiversity and occur within vast ocean basins without absolute barriers to dispersal. Accordingly, tropical reefs represent a valuable ecosystem for studies of connectivity, combining widespread species and discontinuous habitats with the potential to be extensively linked by individuals and currents over ecological as well as evolutionary timeframes. Despite the pantropical distribution of coral reefs, suitable habitat for active growth of reef-building corals, the keystone group underpinning these ecosystems, is limited to relatively small areas on shallow continental shelves and fringing volcanic islands in warm waters. Consequently, reef habitat has been disproportionately affected by eustatic changes during past geologic events (Porter 1989; Veron *et al.* 1996; Voris 2000), with the most recent cycles of extreme range contractions and expansions in the Indo-Pacific occurring throughout the Pleistocene (Potts 1983, 1984). Thus, many modern reef ecosystems, such as the Great Barrier Reef (GBR) of eastern Australia, developed their existing structure and complexity over remarkably short time periods since the last major glaciations (<10,000 yr; Hopley *et al.* 1983; Carter and Johnson 1986; Chappell and Polach 1991; Jackson 1992; Veron *et al.* 1996) through larval connectivity to populations that persisted in glacial refugia (Davies 1988; Benzie 1991; Doherty *et al.* 1994; Planes *et al.* 2001; van Herwerden and Doherty 2006; van Oppen *et al.* 2011b). Such extensive range expansions are typically examined through an evolutionary perspective, and likely occur gradually over many generations, but there is growing interest in understanding the effective connectivity of populations in ecological time, including the dispersal capacity of individuals within a single generation to the exchanges that occur among populations over several years to decades.

The severe vulnerability of coral reefs to various anthropogenic threats, particularly those related to climate change (Hoegh-Guldberg 1999; Wilkinson 1999; Jackson *et al.* 2001; Knowlton 2001; Hughes *et al.* 2003; Bellwood *et al.* 2004; Knowlton and Jackson 2008; Huang 2012), has emphasized the importance of resilience, or the capacity of an ecosystem, species, or population to resist and/ or recover from disturbances (Gunderson 2000; Thrush *et al.* 2009; Sgrò *et al.* 2011). In order to enhance the resilience of a whole reef system, conservation strategies have focused on designating protected habitats (i.e., Marine Protected Areas (MPA) Lubchenco *et al.* 2003; Botsford *et al.* 2009), often with the intention that interconnected networks of MPAs confer benefits to non-protected areas as well (e.g., spill-over of larvae and adults (Harrison *et al.* 2012b); reservoirs of genetic diversity (van Oppen and Gates 2006; Palumbi 2004; Jones *et al.* 2007; Jones *et al.* 2009). Therefore, connectivity is implicit in management efforts to conserve these ecosystems, yet current knowledge of connectivity among coral populations is limited to relatively few species that show highly variable and inconsistent patterns. In particular, critical information regarding the geographical and temporal scales of dispersal in ecological time is deficient, but required to design appropriate MPA networks (Palumbi 2003; Sale *et al.* 2005; Botsford *et al.* 2009; Gaines *et al.* 2010). Accordingly, this thesis utilizes genetic tools to investigate the spatial scales and processes of connectivity within an abundant group of reef-building corals in order to understand how populations and species are linked through evolutionary and ecological time.

1.2 WHAT IS CONNECTIVITY?

Connectivity is the process by which individuals or populations are joined, a state which can vary in strength and magnitude from complete isolation to direct contact. It is a term that has many different applications in diverse fields, but holds particular significance in studies of population biology, ecology and genetics. The definition of a biological population explicitly includes connectivity amongst its members

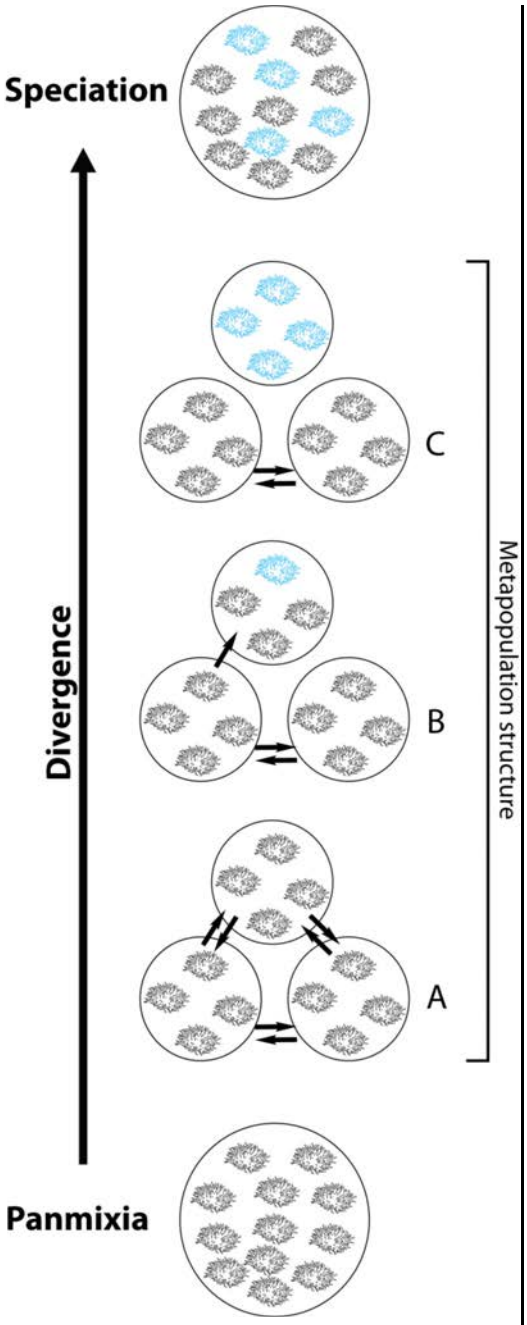


Figure 2.1 A continuum of population structure and divergence. Populations are depicted as circles with coral individuals. At the base, a single panmictic population is shown at various steps of metapopulation substructure and divergence: A) a panmictic metapopulation; B) source/ sink dynamics and genetic changes due to mutation, selection, and/ or drift (in blue); C) population isolation (blue). At the apex, isolation and divergence has led to speciation. Adapted from Waples and Gaggiotti (2006).

(reviewed in Waples and Gaggiotti 2006), yet very few species occur in abundances and space limited enough that all individuals can interact equally with one another (Fig. 1.1, bottom). Almost all species exist in metapopulations, in which semi-isolated subpopulations (i.e., local populations or demes) are linked through dispersal of various life-history forms (e.g., via adults, juveniles, larvae, and/or gametes; Fig. 1.1A–C). Thus, the entire metapopulation network contains a hierarchy of individuals within populations that are associated with one another to various degrees, depending upon the manner in which an organism's biological attributes interact with the environment across space and time. At one extreme, a population may be completely isolated (e.g., spatially, ecologically, or temporally; Fig. 1.1C, blue), although populations of the same species must have been connected at some point in evolutionary time. At the other end of

the spectrum, individuals in two or more seemingly discrete locations may have equal opportunity to interact with individuals at other locations as with members in their own location, thus effectively constituting a single population despite spatial or ecological separation (Fig. 1.1A). Intermediate levels of connectivity include source/ sink dynamics, by which some populations act as sources of emigrants and others as sinks only receiving migrants (Fig. 1.1B), possibly due to reproductive limitations or downstream positions in a passive dispersal system (e.g., wind dispersed pollen; marine larvae). Therefore, population connectivity expresses the extent to which two or more populations are linked through demographic processes, as a continuum between two extremes rather than an exact state (Hellberg *et al.* 2002; Waples and Gaggiotti 2006).

1.2.1 Ecological vs. evolutionary connectivity

Although some ecological interactions occur among members of different populations that do not affect demography (e.g., competition at common foraging grounds), it is the migration of individuals that successfully survive and reproduce that underpins the maintenance and persistence of the metapopulation or species and influences the dynamics of local populations (Hastings and Botsford 2006). Population connectivity resulting from successful reproduction and the exchange of genetic material is commonly and interchangeably referred to as reproductive connectivity, genetic connectivity or gene flow (Slatkin 1985; Waples 1998; Palumbi 2003; van Oppen and Gates 2006; Hedgecock *et al.* 2007; Hellberg 2009; Wang *et al.* 2011). For the purposes of this thesis, I will distinguish ecological and evolutionary connectivity by the temporal scale over which these processes occur. **Ecological connectivity** affects population demography over ecological time frames and is thus relevant to the active management of populations in the present (Palumbi 2003; Jones *et al.* 2009), including the capacity for dispersal within a generation (i.e., the dispersal kernel (Botsford *et al.* 2009)). **Evolutionary connectivity** reflects the long-term effects of ecological

connectivity and gene flow averaged across many generations through evolutionary time, including past range expansions/ contractions and processes of speciation. In turn, the spatial scales of dispersal that occur over ecological versus evolutionary timeframes determine how population connectivity affects demographic and evolutionary processes.

Studies of gene flow may utilize different empirical methods to target particular spatial and temporal scales, but measuring connectivity in some marine species can raise additional challenges that constrain certain techniques. Ideally, ecological studies would track dispersing individuals directly or employ mark/ recapture techniques; however the small, planktonic nature of the larval dispersive stage of most marine taxa has limited the application of such methods (but see Jones *et al.* 1999; Swearer *et al.* 1999; Jones *et al.* 2005; Almany *et al.* 2007). Bio-physical modeling of oceanic circulation coupled with biological data is particularly important for predicting future connectivity patterns (Baums *et al.* 2006; Werner *et al.* 2007; Botsford *et al.* 2009), but currently demands greater basic biological information on most species and is not further considered here. Up until a few years ago, connectivity was most commonly inferred from population genetic structures using a time-averaged evolutionary perspective (Slatkin and Barton 1989; Grosberg 1991; Hellberg 1994; Miller 1997; Bohonak 1999; Barber *et al.* 2000; Taylor and Hellberg 2003; Hedgecock *et al.* 2007; Underwood *et al.* 2007), which does not clearly reveal the scale of dispersal over ecological timeframes, yet recent statistical-genetic advances have produced techniques that can also detect recent migration from similar data (e.g., Rannala and Mountain 1997; Pritchard *et al.* 2000; Wilson and Rannala 2003; Piry *et al.* 2004; Choi and Hey 2011). In particular, genetic methods almost exclusively predominate studies of coral connectivity for both ecological and evolutionary investigations (reviewed in van Oppen and Gates 2006; Jones *et al.* 2009; but see Carlon and Olson 1993; Baums

et al. 2006; Galindo *et al.* 2006; Treml *et al.* 2008), as no methods for direct tracking over long-distances or non-genetic tagging have yet been developed.

1.2.2 Population genetics and measuring connectivity

Species rarely exhibit panmixia (Waples 1998), perfect random mating among all individuals (Fig. 1.1, bottom), and the changes that take place in genotype and allele frequencies due to non-random mating can provide insights into the spatial extent of local populations and the amount of dispersal that connects them (Fig.1.1A-C) (Hellberg 2007). In particular, geographically separate subpopulations experience different ecological conditions and have distinct evolutionary histories that can affect genetic changes in unique ways. Intrapopulation processes (e.g., mutation, selection, genetic drift) determine the genetic changes that take place and the overall amount of variation available (Fig. 1.1B), whereas migration can equalize these differences among subpopulations by spreading new alleles throughout the metapopulation (Slatkin 1987). Therefore, the strength of connectivity among populations will largely determine the magnitude of genetic differentiation that occurs, at one extreme potentially leading to speciation with increased divergence under isolation (Fig. 1.1C and top, blue), or alternatively, apparent panmixia under high gene flow (Fig. 1.1A). Generally, small and isolated populations are expected to experience greater inbreeding among closely related individuals, reduced heterozygosity or genetic variation, and as a result, may be at greater risk of local extinction compared to larger and/ or highly connected populations. Although a surprisingly small proportion of migrant individuals is required for effective panmixa among subpopulations (Slatkin1987), the vast geographic ranges of many marine species result in populations that are not directly connected over ecological time.

To date, genetic connectivity has primarily been estimated indirectly from the allele frequency differences underlying population genetic structure with equilibrium

assumptions (Hedgecock *et al.* 2007; Hellberg 2009). F-statistics, first developed by Wright (Wright 1943; Wright 1949; Wright 1965), describe how genetic variation is partitioned among individuals, populations, and the total metapopulation according to deviations from Hardy-Weinberg Equilibrium (HWE). In particular, F_{ST} approximates the average deviation in allele frequencies between subdivided populations (Wright 1965), reflecting the relative degree of isolation. Several similar metrics have been developed in order to address the criticized sensitivity of F_{ST} to assumption violations in natural populations (Nei 1973; Weir and Cockerham 1984; Nei 1987; Hedrick 2005; Meirmans 2006; Jost 2008), however all such estimators vary in accuracy according to different violations or conditions (reviewed in Excoffier 2008; Meirmans and Hedrick 2011). Furthermore, single statistics of population differentiation reflect a snap shot of connectivity averaged across space and time, from which specific processes and scales are difficult to extract. Isolation-by-distance methods explicitly address how genetic structure varies across space, and with precise estimates of effective density, can provide more exact estimates of dispersal scales that are less sensitive to potentially rare long-distance dispersal events (Rousset 1997; Leblois *et al.* 2004; Pinsky *et al.* 2010). Similarly, the application of spatial autocorrelation analyses (Anselin 1995) with genetic data (Sokal *et al.* 1998) is a powerful method to refine the spatial scales of population structure, particularly for organisms with restricted gene flow over short distances (Smouse and Peakall 1999; Calderon *et al.* 2007; Underwood *et al.* 2007).

Most recently, statistical techniques, collectively referred to as assignment methods, have been developed that can determine the genetic signature of specific subpopulations and detect individuals that have recently migrated to new populations (reviewed in Manel *et al.* 2005). Assignment methods have thus greatly expanded the types of questions we can answer with population genetic data, including detecting structure without a priori assigned populations (Pritchard *et al.* 2000; Falush *et al.*

2003; Guillot *et al.* 2005), specific dispersal events (Rannala and Mountain 1997; Wilson and Rannala 2003; Piry *et al.* 2004), and the admixture of previously distinct populations and hybrid individuals (Pritchard *et al.* 2000; Anderson and Thompson 2002). Finally, advanced methods of relationship inference, including sibship (Ashley *et al.* 2009; Jones and Wang 2010; Almudevar and Andersen 2012) and parentage (Kalinowski *et al.* 2007; Christie 2010; Jones and Wang 2010) analyses, have increased our ability to explore intrapopulation processes like mating structure (Oddou-Muratorio *et al.* 2006; Tani *et al.* 2009), as well direct measurements of dispersal and self-recruitment in marine populations (Jones *et al.* 2005; Harrison *et al.* 2012b). With perhaps the exception of relationship inference, many of these newer techniques are now successfully utilized in coral connectivity studies (van Oppen *et al.* 2008; Bongaerts *et al.* 2010b; Souter *et al.* 2010; Starger *et al.* 2010; Pinzon and Lajeunesse 2011; Van Oppen *et al.* 2011a; Ladner and Palumbi 2012; Maier *et al.* 2012), although the sampling designs typically target larger scale patterns across regional distances (e.g., GBR (van Oppen *et al.* 2011a), Caribbean (Baums *et al.* 2005b), Pacific (Baums *et al.* 2012)). Increasing evidence indicates that many marine populations, including corals (Ayre and Hughes 2004), are surprisingly more closed (i.e., highly self-recruiting) than previously expected from the high dispersal potential of planktonic larvae (Roberts 1997; Mora and Sale 2002), thus there is a critical need for studies conducted at spatial and time scales relevant to ecological processes and hence meaningful for management and restoration initiatives.

1.3 LIFE HISTORY AND CONNECTIVITY IN CORALS

One of the major challenges in interpreting connectivity and genetic structure data for corals is a general lack of biological and ecological information for most study species, especially reproductive characteristics which have extensive implications for genetic processes and patterns. Furthermore, population genetic theory was developed for populations in which mating occurs randomly between separately sexed, diploid

individuals with discreet non-overlapping generations, but most of these assumptions may be violated by many coral species. Reef-building corals exhibit a wide range of life histories (reviewed in Harrison and Wallace 1990; Harrison 2011) and reproductive strategies can vary in frequency (e.g., monthly vs. annually), timing (e.g., summer vs. winter), volume and spatial extent of events (i.e., dispersal distances of gametes and larvae). However, the combination of vast geographic distributions (Veron 2000) and often inaccessible underwater habitats is particularly challenging for comparative studies of coral species. Consequently, the limited knowledge available is concentrated in a few geographic areas (e.g., Caribbean, Red Sea, GBR) and on a few select species (e.g., *Pocillopora damicornis*) or genera (e.g., *Acropora*, *Montastrea*). Investigations of even well studied species sometimes yield inconsistent results that can be difficult to interpret. For example, *P. damicornis* has been reported to either brood asexual or sexually produced planula larvae, and/ or broadcast spawn gametes in different locations throughout its range (Harriott 1983; Richmond and Jokiel 1984; Stoddart and Black 1985; Ward 1992; Tanner 1996; Yeoh and Dai 2010). Such inconsistencies hint that cryptic species may be widespread in coral populations (Knowlton 1993; Ladner 2012; Scmidt-Roach 2012), and if so, also present a significant challenge to resolving genetic structures and understanding connectivity in corals.

Knowledge of the mating systems of corals has grown exponentially in the last few decades, but there are still major gaps that hinder understanding of population connectivity in the group. The majority of coral species are simultaneous hermaphrodites, some are gonochoric, and a few exhibit some variation of sequential hermaphroditism (Fadlallah 1983; Harrison and Wallace 1990; Baird *et al.* 2009). Similarly, most coral species broadcast spawn male and female gametes simultaneously for external fertilization in the water column in annual or biannual mass synchronized spawning events (Baird *et al.* 2009), such as has been documented on

the GBR (Willis *et al.* 1985; Babcock *et al.* 1986), where many congeneric species spawn within a few hours of one another (reviewed in Harrison and Wallace 1990). Fewer, albeit several abundant (e.g., pocilloporids), species brood larvae following internal fertilization from 'spermcast mating' (Bishop and Pemberton 2006), in an alternative strategy with unique implications for gene flow during mating. Where it has been tested, most hermaphroditic species have shown extensive self-incompatibility (Heyward and Babcock 1986; Wallace and Willis 1994; Miller and Babcock 1997; Szmant *et al.* 1997; Willis *et al.* 1997; Hatta *et al.* 1999; Ayre and Miller 2006; Yeoh and Dai 2010; Douek *et al.* 2011), although a few corals have exhibited variable levels of self-fertilization (Heyward and Babcock 1986; Stoddart *et al.* 1988; Brazeau *et al.* 1998; Sherman 2008; Carlon and Lippé 2011) in a mixed-mating strategy similar to that observed in plants (Goodwillie *et al.* 2005). Interestingly, fertilization experiments have shown that several congeneric broadcast spawning *Acropora* species are capable of extensive hybridization, yet individuals are mostly self-incompatible (Willis *et al.* 1997). Moreover, phylogenetic studies of the genus *Acropora* provide evidence for at least occasional introgressive hybridization (Willis *et al.* 1997; Van Oppen *et al.* 2002; Ladner and Palumbi 2012; Palumbi *et al.* 2012), however, the extent to which hybridization occurs naturally in different coral species is still unknown (reviewed in Willis *et al.* 2006). Clearly, the diversity of sexuality (i.e., hermaphroditic/ gonochoric) and reproductive mode (i.e., broadcast spawning vs. brooding) combinations in corals and the extent to which self-fertilization or interspecies hybridization occurs will influence genetic structure in different ways, complicating analysis and interpretation of intraspecific connectivity patterns

In particular, several processes governing fertilization and dispersal in corals may affect the generation and maintenance of genetic diversity within populations, and the amount of connectivity between populations. While broadcast spawning usually occurs on one peak night (per individual) per year, brooding species may release planula

larvae over several days and for multiple months over the brooding season or continuously throughout the year (Atoda 1951; Rinkevich and Loya 1979; Tanner 1996; Villanueva *et al.* 2008). Although the volume of gametes produced in broadcast spawning species certainly exceeds output of most brooders per event, the potentially higher survival rates of mature brooded larvae (Sammarco and Andrews 1989) and dispersal events spread throughout the year might confer less risk of stochastic and catastrophic reproductive failures. Virtually nothing is known about the prior release of sperm in brooding corals, which underlies mating system structure, including when sperm are released, how far they can disperse and how frequently sperm are produced. Nevertheless, brooded corals are expected to have restricted gene flow due to assumed, but untested, limited sperm dispersal (Levitan and Petersen 1995; Yund 2000; Ayre and Miller 2006). At the same time, brooded planulae are generally competent to settle within minutes of release (Atoda 1951; Isomura and Nishihira 2001). Thus, brooding corals are expected to exhibit philopatric recruitment distributions (Harrison and Wallace 1990) and greater population differentiation, which may likewise be exacerbated by increased biparental interbreeding between closely related individuals. In contrast, broadcast spawned larvae require up to 6 days planktonic development, but can remain competent to settle for weeks in the planktonic environment (Graham *et al.* 2008), implying a potential for large dispersal distances. Although different reproductive strategies in corals are commonly interpreted to convey particular abilities for dispersal with general implications for connectivity, most of these assumptions are untested and dispersal scales are still unknown.

A final noteworthy life history trait affecting the connectivity and genetic diversity of coral populations is that, like many plants, corals have the ability to propagate asexually as clonal lineages, and may do so extensively (e.g., Baums *et al.* 2005b). However, the mechanism by which this occurs can vary with species, morphology and habitat. Most species of scleractinian corals grow from a single polyp to a colony of

genetically identical individuals, although genetic anomalies may arise due to somatic mutation (reviewed in van Oppen *et al.* 2011c) and chimera formation (Puill-Stephan *et al.* 2009; Puill-Stephan *et al.* 2012). Branching species, such as many acroporids and pocilloporids, can easily be fragmented by disturbance and may re-establish at relatively short distances from the original colony (Highsmith 1982). Further, at least one species (*Seriatopora hystrix*) has been reported to successfully re-settle following 'polyp bail-out' (Sammarco 1982), in which individual polyps from an adult colony revert to planula-like forms (P. Warner pers. obs.; Fig. 1.2) and may be able to disperse. In addition, *P. damicornis* primarily produces apparently parthenogenic larvae (Ayre *et al.* 1997; Ayre and Miller 2004; Miller and Ayre 2004; Yeoh and Dai 2010; Schmidt-Roach *et al.* 2012). Typically, clonal individuals will be removed from a data set to meet standard model assumptions prior to further population genetic analysis (e.g., Baums *et al.* 2005b; van Oppen *et al.* 2008); however, it is unclear how asexual reproduction specifically influences dispersal distances in species with clonal larvae (Ayre and Miller 2004), or how a dominant asexual strategy affects the future resilience of species with extensive clonal frequencies (Baums *et al.* 2005a).

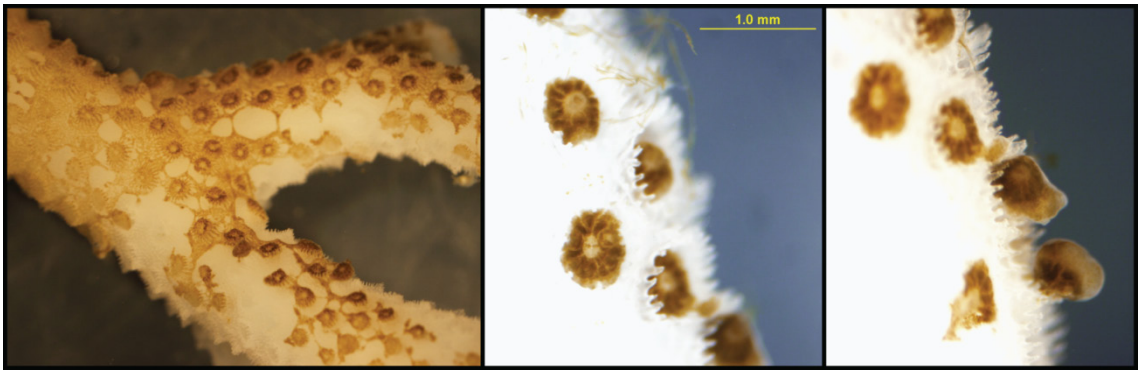


Figure 1.2 Polyp bail-out in the brooding coral, *Seriatopora hystrix*. Condensing of the zooxanthellate tissue can be seen in progression of stages from left to right: 1) contracting of the coenosarc between polyps; 2) isolation of individual polyps within corallites; 3) release of planula-like polyps from the skeletal structure. Photos by P. Warner and B. Willis (December 2008).

All of these complex characteristics interact to impart unique reproductive strategies that will have significant implications for connectivity and genetic structure within populations of different coral species. Frequently, the main reproductive mode (broadcast spawning vs. brooding) is inferred to be the major determinant of dispersal ability and connectivity; however studies have also shown that biologically similar species often exhibit inconsistent population structures. For example, Ayre and Hughes (2004) examined five widely distributed species of scleractinian corals on the GBR, which represent three different modes of reproduction (i.e., broadcast spawning, brooding, and asexual brooding/broadcast spawning). The brooders *S. hystrix* and *Stylophora pistillata* displayed markedly different patterns in gene flow, although both have similarly short larval durations and broad geographic ranges. *Seriatopora hystrix* exhibited extremely low connectivity among locations within the GBR, while *S. pistillata* demonstrated significantly less differentiation, occurring at the opposite end of the connectivity spectrum for the five species investigated. Therefore, caution must be taken when generalizing connectivity patterns based on life history traits for species not explicitly examined. Most importantly, greater effort must be made to gather basic biological and ecological information to integrate with genetic studies. Merging genetic and organismal approaches will enhance information obtained from single-focus studies, increase our understanding of both objectives, and thereby improve our ability to inform conservation and management efforts.

1.4 STUDY OBJECTIVES

The purpose of this work is to resolve the spatial scales over which dispersal occurs in a brooding coral and elucidate the ecological and evolutionary processes that determine genetic connectivity, at scales ranging from local coral colonies to regional populations. In order to accomplish this goal, I have investigated three hierarchical divisions within the genus *Seriatopora* on the Great Barrier Reef with the following specific objectives (Fig. 1.3):

1. <u>Species</u>: To determine if species diversity in the coral genus *Seriatopora* is greater on the Great Barrier Reef than is currently acknowledged based on morphologically-based systematics. Several lines of genetic, ecological and morphological evidence were combined to compare diversity within the morphospecies *S. hystrix* to that found between *S. hystrix* and its only known congener on the GBR, *S. caliendrum*. If present, undetected cryptic species will obscure intraspecies genetic structure data and must be recognized for accurate interpretation of connectivity patterns that effectively inform management strategies.

2. <u>Populations</u>: To determine and compare the degree of population connectivity for two putative species of *S. hystrix* at hierarchical spatial scales. The genetic structure of two putative species was assessed at three spatial levels: within reefs, between reefs, and among regions. Examining population genetic structure at specific and localized spatial distances can reveal the scales at which population connectivity is maintained or decays, with important implications for the design of marine protected area networks.

3. <u>Individuals</u>: To measure the distance of sperm dispersal and estimate mating system characteristics of a seriatoporid coral. A novel parentage analysis was conducted on brooded larvae collected from a mapped population of corals, and paternity assignments used to measure the distance of sperm dispersal. Sperm dispersal underpins the reproductive interactions among sessile brooding individuals, which define a population and affect its genetic structure. Parentage analyses have never been conducted in any scleractinian coral.

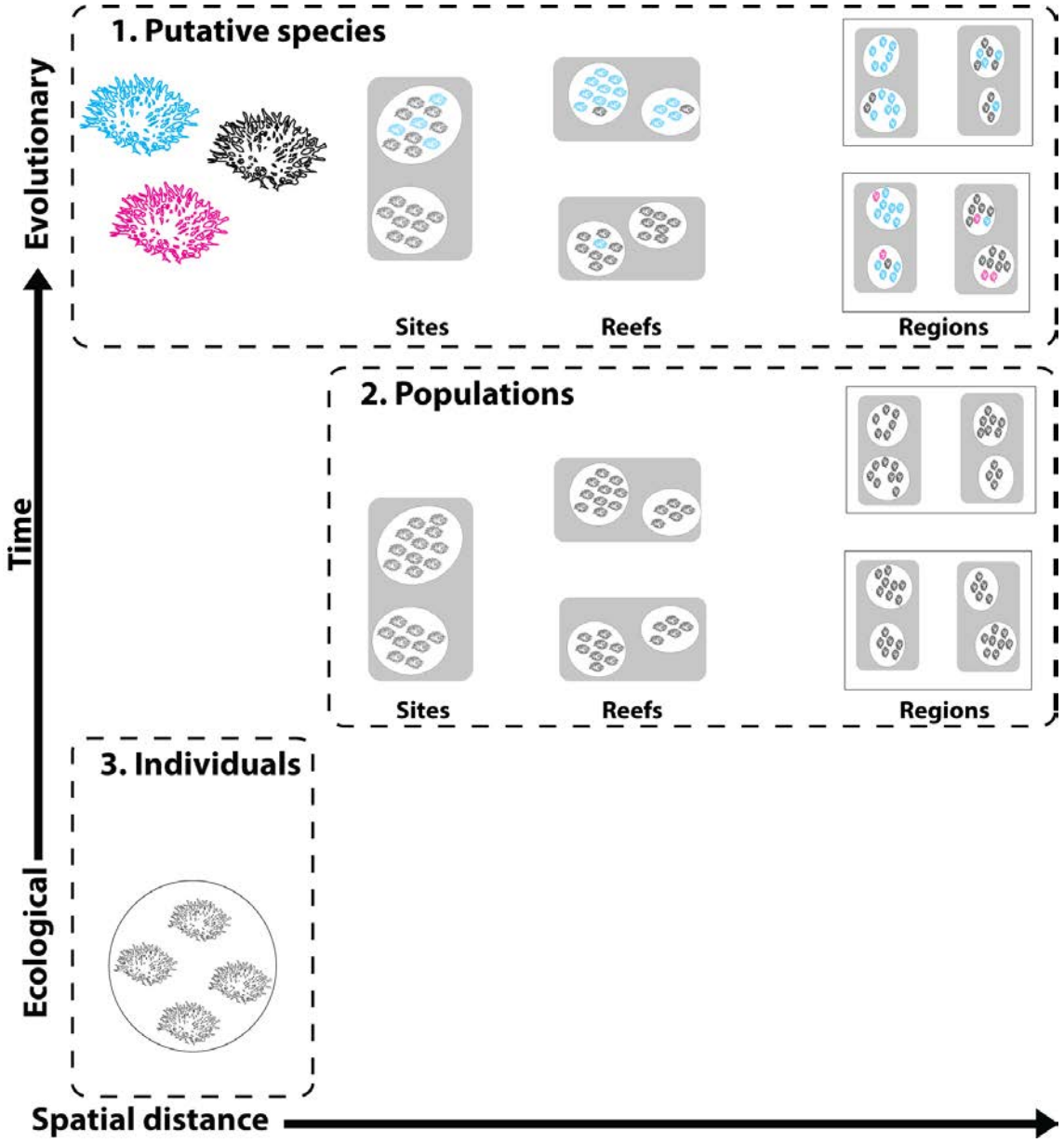


Figure 1.3 A conceptual diagram showing the thesis structure and objectives in terms of understanding ecological and evolutionary genetic connectivity in the coral genus *Seriatopora* across multiple spatial scales. Main objectives were to detect and elucidate: 1) putative cryptic species; 2) interpopulation geographic substructure; 3) mating system and sperm dispersal.

1.5 THESIS STRUCTURE

I was inspired to start this project by the lack of knowledge on sperm dispersal in a widely studied brooding coral species and the need to fill this gap in order to understand the influence of intrapopulation processes on connectivity. Originally, my

plan was to proceed from the smallest spatial scale of reproduction and gene flow among individuals to connectivity among populations at increasing geographic distances. I did not initially include investigations of potential cryptic species in what I thought was a single and easily recognizable coral species. But over the course of my research and sampling, I began to question the long-held assumption of a single species of *S. hystrix* and started a parallel investigation that changed the path of my studies altogether. As my research progressed, it soon became clear that the presence of potential cryptic species in my sample set required the species question to be settled before other studies could proceed.

The following three chapters in this thesis (Chapters 2 – 4) address the objectives as listed above in Section 1.4. In Chapter 2, I developed a hierarchical Bayesian clustering method with microsatellite data to identify putative genetic lineages that occur across two regions of the GBR in both sympatric and allopatric populations. Distinct lineages within the morphospecies *S. hystrix* indicate probable species level divisions and are corroborated with additional evidence from mtDNA, *Symbiodinium* type, habitat association, and morphology. Using phylogenetic analysis, I consider these putative species within the context of all mtDNA diversity reported for the *Seriatopora* genus across the Indo-Pacific. In Chapter 3, I assess the population genetic structure of the two most common putative species detected in my data set, which dominate seriatoporid diversity in two characteristic habitats. Standard population differentiation statistics are combined with Bayesian clustering and spatial autocorrelation analyses to resolve the hierarchical spatial scales at which connectivity is maintained or diminished. Chapter 4 investigates the processes that shape genetic structure within a population of the putative *Seriatopora* species that dominates sheltered, shallow water habitats. I define and apply consensus criteria among several independent and replicated methods of parentage analysis to maximize accuracy in paternal assignments for a natural population. Mating system parameters were

estimated from progeny arrays and explored within the context of the existing spatial-genetic structure of established colonies. Finally, in Chapter 5, I synthesize the findings of the three previous chapters and discuss the relevance of ecological and evolutionary connectivity to understanding resilience in the genus *Seriatopora*, as well as implications for future studies and management.

Chapter 2.0 Sympatric cryptic species in the widespread coral morphospecies, *Seriatopora hystrix,* on the Great Barrier Reef

2.1 INTRODUCTION

Morphologically cryptic species, sometimes called 'sibling species' (Mayr 1942), are a long acknowledged natural reality (Mayr 1948, 1963; Walker 1964; Wake *et al.* 1983; Knowlton 1993), yet since the advent of modern molecular technologies documentation of extensive cryptic diversity has been accumulating in many phyla (Bickford *et al.* 2007). Scleractinian corals are the foundation taxa that create unique and highly diverse coral reef ecosystems; often vast geographic ranges, extensive morphological plasticity, and limited biological knowledge for many species hint that cryptic diversity may be prevalent, thus it is timely to reconsider species within reef-building corals. In particular, the recent proliferation of studies examining the effects of global climate change and disease on corals contains variable results (Page and Willis 2006; Sampayo *et al.* 2008; Guest *et al.* 2012; Stat *et al.* 2012), which potentially reflect inaccurate species identification or insufficient taxonomy. Detecting such inconsistencies and clarifying how conflicting species identification contributes to variable results is crucial if such studies are to be useful for understanding and therefore mitigating the threats of climate change. Similarly, the success of connectivity studies in providing effective information for conservation and management efforts hinges on consistent species recognition in order to resolve patterns of gene flow.

Although morphology remains the prevailing criterion in coral taxonomy, it has long been considered an inadequate basis on which to define a species (Mayr 1963); morphological modification does not necessarily accompany genetic change (Charlesworth *et al.* 1982), nor does morphological distinctiveness signify speciation. In a diverse range of taxonomic groups, many species distinguished by behavioral,

ecological, and/or genetic traits are at least superficially morphologically indistinct (Walker 1964; Vrijenhoek *et al.* 1994; Klautau *et al.* 1999; Dawson and Jacobs 2001; Hayashibara and Shimoike 2002; Baker *et al.* 2003; Murray *et al.* 2008; Ladner and Palumbi 2012). Conversely, allopatric populations of widespread species, such North American cutthroat trout (Allendorf and Leary 1988), may develop distinctive morphological features, but retain complete reproductive compatibility and genetic cohesion (i.e., polytypic species (Mayr 1963)). Certainly, defining species is a contentious and unresolved task (Willis 1990; Luckow 1995; Veron 1995; Sites and Marshall 2003; Agapow *et al.* 2004; de Queiroz 2007; Mallet *et al.* 2007; Amato 2010), yet consistent species delimitation and recognition is essential to advancing our understanding of biological, ecological and evolutionary processes (Dobzhansky 1935; Burma and Mayr 1949; Mayr 1996; Avise and Walker 2000; Sites and Marshall 2003; Sites and Marshall 2004). It is increasingly clear that the same species concept is not applicable to all taxonomic groups, although a general consensus among the different concepts is that species are comprised of genetically cohesive units of populations and individuals that do not appreciably exchange genes with other groups, and thus evolve separately (Good and Wake 1992; de Queiroz 2007; Hey and Pinho 2012). Consequently, some authors have recommended that arguments about species concepts be set aside and instead, we should focus on delimiting species with multiple lines of evidence rather than on defining an all-encompassing concept (Sites and Marshall 2003; Sites and Marshall 2004; de Queiroz 2007). The need to synthesize morphological, molecular, reproductive and ecological data to delineate species boundaries may particularly important in corals, given their apparent propensity to hybridize (Willis *et al.* 2006), which challenges most species concepts, including the biological (Mayr 1942; Dobzhansky 1970), evolutionary (Simpson 1951; Wiley 1978; Mayden 1997) and phylogenetic (Donoghue 1985; Mishler 1985) species concepts.

Accordingly, the coral morphospecies *Seriatopora hystrix* provides an ideal candidate to test whether genetic, morphological and ecological data can be used to delimit a single cohesive unit. The historical record of coral species descriptions includes hundreds of named taxa that have since been synonymized according to presumed intraspecific geographic variation in skeletal characteristics (Veron and Pichon 1976; Veron 1995; Cairns 1999; Veron 2000). The substantial paring of species numbers under the yet prevailing 'morphospecies' concept relies heavily on the assumption of extensive morphological plasticity within coral species, both intracolonial (i.e., variation within in the same colony) and intraspecific (Veron and Pichon 1976), yet the amount attributable to genotypic versus phenotypic variation is still untested for most species (but see Willis 1985; Willis and Ayre 1985; reviewed in Todd 2008). The genus *Seriatopora* is geographically widespread and currently includes six described species (Veron 2000), two of which, *S. hystrix* and *S. caliendrum*, are reported from the Great Barrier Reef (GBR) (Veron and Pichon 1976). Seriatoporid corals are notorious for their morphological plasticity, inadequately defined species boundaries and many synonymized entries in the taxonomic record (Veron and Pichon 1976; Cairns 1999). Like many other coral groups, phylogenetic relationships at the sub-generic level have been poorly studied (but see Flot *et al.* 2008; Chen *et al.* 2008), with limited systematic or taxonomic resolution. In particular, *S. hystrix* has been the subject of a large and diverse body of biological studies (e.g., cellular biology and the bleaching response, reproduction, connectivity, coral reef ecology) in populations from the Red Sea to subtropical Eastern Australia (Potts 1915; Atoda 1951; Ayre and Resing 1986; Hoegh-Guldberg and Smith 1989; Edmunds 2005; Maier *et al.* 2005; Nozawa and Loya 2005; Fan *et al.* 2006; Underwood *et al.* 2007; Chen *et al.* 2008; Flot *et al.* 2008; Sherman 2008; van Oppen *et al.* 2008; Maier *et al.* 2009; Noreen *et al.* 2009; Underwood *et al.* 2009; Bongaerts *et al.* 2010b; Putnam *et al.* 2010; Stella *et al.* 2010; Bongaerts *et al.* 2011; Nir *et al.* 2011; Tchernov *et al.* 2011; van Oppen *et al.* 2011a). By comparison, *S. caliendrum* is rarely studied and "poorly defined" in the taxonomic literature (Veron

and Pichon 1976). *Seriatopora hystrix* is particularly well-studied on the GBR and in Western Australia (WA) waters, where it is an abundant member of diverse habitats and spans large depth ranges from the intertidal to the mesophotic zones (Veron and Pichon 1976). It is well-known for the strong genetic subdivision of its populations (Ayre and Dufty 1994; Ayre and Hughes 2000; Ayre and Hughes 2004; Underwood *et al.* 2007; van Oppen *et al.* 2008; Noreen *et al.* 2009; Underwood *et al.* 2009; Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a). However, recent studies of mitochondrial diversity on the GBR suggest that at least some of this high population differentiation may be attributed to cryptic diversity (Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a).

In this chapter, I use a population genetic approach based on nDNA microsatellites to test for cryptic genetic diversity in sympatric and allopatric populations of the coral *Seriatopora* within and between two regions of the GBR. A variety of molecular markers have proven useful to recognize cryptic taxa in corals (Eytan *et al.* 2009; Forsman *et al.* 2009; Souter 2010; Pinzon and Lajeunesse 2011; Ladner and Palumbi 2012; Nakajima *et al.* 2012; Schmidt-Roach *et al.* 2012; Pinzon *et al.* 2013), however the phylogenetic resolution of mtDNA, frequently utilized in other phyla, is limited by slow rates of evolution (Romano and Palumbi 1997; van Oppen *et al.* 1999; Shearer *et al.* 2002), shared ancestral polymorphisms and ongoing introgressive hybridization in corals (van Oppen *et al.* 2001; van Oppen and Gates 2006; Willis *et al.* 2006; Ladner and Palumbi 2012; Palumbi *et al.* 2012). My objectives were to first conduct a comprehensive phylogenetic analysis of all currently published mtDNA control region sequences for *Seriatopora* to compare the mitochondrial diversity in the genus found on the GBR to that reported for several other regions of the Indo-Pacific. Secondly, I aimed to detect distinct genotypic clusters (Mallet 1995; Hausdorf and Hennig 2010; Ladner and Palumbi 2012) with microsatellite data from GBR populations using hierarchical Bayesian analysis (Rosenberg *et al.* 2002), which can identify groups of individuals that: 1) share greater affiliation across rather than within the same region

(Good and Wake 1992; de Queiroz and Good 1997); and 2) do not exhibit substantial admixture or evidence of "intermediates" among the unique clusters in contact (Mallet 1995). In this way, it is possible to test for genetically cohesive clusters and simultaneously account for potential geographical structure within putative species resulting from intraspecies population processes. Following recognition of several unique clusters found to be consistent across different population genetic analyses, my third objective was to determine if characteristics of mtDNA, habitat preference, host-*Symbiodinium spp.* coupling, and gross morphology further distinguished different clusters of individuals. Finally, I compared clusters based on all criteria to evaluate if multiple lines of evidence provide concordant clusters that better delineate species boundaries in the genus *Seriatopora* than the current morphospecies concept.

2.2 MATERIALS AND METHODS

2.2.1 Study sites and sampling design

Samples of the coral genus *Seriatopora* were collected from five reefs in each of the Palm Islands (central) and Lizard Island (northern) regions of the Great Barrier Reef (GBR) between December 2008 and September 2010 (Fig. 2.1). Sampling targeted the *S. hystrix* morphospecies; however, *S. caliendrum* was also intentionally collected from the two reefs where it was visually identified prior to molecular analysis (NE Orpheus and W. Lizard). Each reef contained two replicate sampling sites, with the exception of W. Lizard, and approximately 50 individuals were sampled from each site. In total, 964 samples were collected, each comprised of a branch 3 – 5 cm long and preserved in 100% ethanol (Table 2.1). Samples were taken approximately 5 m apart, where possible depending on population density, to avoid sampling of clonemates. The sampling design incorporated two different habitats, hereafter referred to as 'sheltered' and 'exposed'. Exposed habitats were on the eastern windward margins of islands, where seriatoporid corals were found only below 7 m in the Palm Islands and 3 – 4 m

at Lizard Island (Table 2.1). Sheltered habitats occur at the western leeward margins of islands where seriatoporid corals were found shallower for both regions (Table 2.1). The West Lizard Island site does not strictly fall into either habitat category; while it is on the western margin of the Lizard Island lagoon, it lies outside of the lagoon proper and corals generally occur deeper (>2 m) than at other sheltered sites of Lizard Island (<4 m). All coral colonies were photographed underwater.

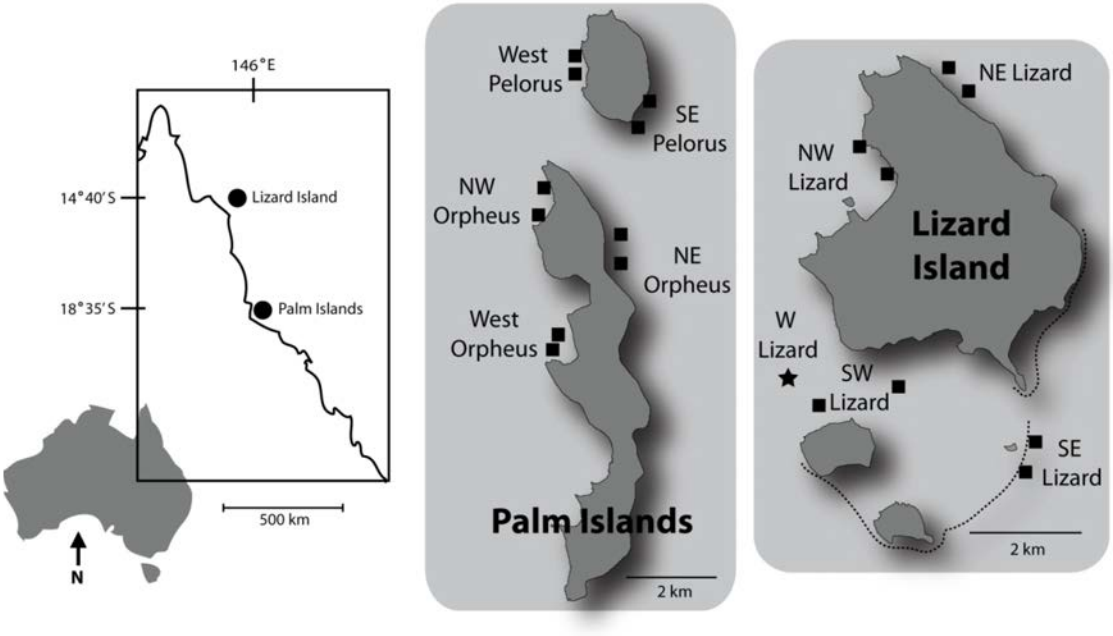


Figure 2.1 Palm Islands and Lizard Island sampling sites and locations on the Great Barrier Reef of Queensland, Australia. West Lizard Island site (indicated by black star) is the only reef that is not replicated and falls outside the normal definition of sheltered (leeward) and exposed (windward) habitats.

DNA was extracted from all samples according to the protocol developed for the black tiger shrimp (Wilson *et al.* 2002), but optimized for coral tissues with the following modifications: Only about 25 mg coral tissue, including skeletal weight, was removed from branch tips and air dried for 10 – 20 minutes. Tissues were placed in 1.5 mL plastic tubes and first equilibrated in 500 uL of extraction buffer. Buffer solution was removed and then 0.75 mL new buffer added. Without any grinding, samples were immediately placed in a 65°C water bath for at least 1 hour and up to 24 hours. All centrifuge steps were completed at room temperature. No RNAse was added. Samples

were centrifuged immediately for 18 minutes following the addition of isopropanol. Pellets were resuspended at 4°C. The same extracts were utilized for both host and symbiont procedures. All 964 samples were amplified with ten microsatellite loci and a mtDNA marker described below, but only a subset of the samples (n = 516) were used for *Symbiodinium* ITS2 type assessment (Table 2.1). The 39 individuals sampled from W. Lizard were also sequenced at the mtDNA control region.

Table 2.1 Description of sample locations and collections for *Seriatopora* spp. from the Palm Islands and Lizard Island, GBR. Code: abbreviated site names; N: total # colonies sampled per location; Msats: collections used for microsatellite analysis; mtDNA: collections surveyed with new mtDNA marker; Sym ITS2: collections used for *Symbiodinium* identification; XX: also sequenced for mtDNA.

Region	Reef	Depth (m)	Site	Code	N	Lat, Long	Msats	mtDNA	Sym ITS2
Palm Islands, Central GBR	West Orpheus	< 7	1	WO1	49	S18° 36.632, E146° 29.195	X	X	X
			2	WO2	50	S18° 36.545, E146° 29.233	X	X	
	NW Orpheus	< 7	1	NWO1	50	S18° 34.801, E146° 28.905	X	X	X
			2	NOW2	50	S18° 34.300, E146° 28.988	X	X	
	West Pelorus	< 7	1	WP1	50	S18° 32.864, E146° 29.274	X	X	X
			2	WP2	50	S18° 33.037, E146° 29.304	X	X	
	NE Orpheus	7 - 12	1	NEO1	89	S18° 35.245, E146° 29.854	X	X	X
			2	NEO2	39	S18° 35.458, E146° 29.884	X	X	X
	SE Pelorus	8 - 15	1	SEP1	50	S18° 33.808, E146° 30.014	X	X	X
			2	SEP2	50	S18° 33.613, E146° 30.109	X	X	
Lizard Island, Northern GBR	SW Lizard	< 4	1	SWL1	50	S14° 41.160, E145° 27.256	X	X	X
			2	SWL2	50	S14° 41.248, E145° 26.606	X	X	
	NW Lizard	< 4	1	NWL1	50	S14° 39.408, E145° 27.034	X	X	X
			2	NWL2	50	S14° 39.649, E145° 27.205	X	X	
	SE Lizard	4 - 8	1	SEL1	50	S14° 41.501, E145° 28.173	X	X	X
			2	SEL2	50	S14° 41.648, E145° 27.965	X	X	
	NE Lizard	3 - 8	1	NEL1	50	S14° 38.953, E145° 27.643	X	X	X
			2	NEL2	48	S14° 39.082, E145° 27.794	X	X	
	West Lizard	2 - 6	1	WL1	39	S14° 41.001, E145° 26.453	X	XX	X

2.2.2 Mitochondrial DNA (mtDNA) characterization of coral host and phylogenetic analysis of the genus *Seriatopora*

Due to an unusually high and obvious morphological diversity of seriatoporids, the W. Lizard site was also used for sequence analysis of the mtDNA putative control and adjacent *atp6* regions using primers F18 (5'–GTCCTTACGTCTTTACACCGAC–3') and R17 (5'–AAAGACCACTCTAAAGCCCGCT–3') (Chen *et al.* 2008). Mitochondrial

sequencing and cryptic diversity was an unplanned aspect of this thesis, and thus only a small subset of samples could logistically be used for this purpose. One µL undiluted template was combined with Mg^{2+} 2.5 mM, dNTP's 200 µM, primers 250 nM each and 0.187 units Bio-X-Act Short DNA polymerase (Bioline, NSW, Australia) in a total volume of 50 µL. Reactions were cycled at 1 x 95 °C (5 min), followed by 30 x [(30 s at 95 °C), (1 min at 58 °C), (1.5 min at 70 °C)] and completed with 10 min at 70 °C. Amplicon size (~1500 bp) was confirmed with 5 µL of product run on a 1% agarose gel and products were sent to Macrogen (South Korea) for both forward and reverse sequencing with the same primers. Sequence chromatograms were imported into Sequencher 4.7 (Gene Codes Corporation), proofread and aligned. Alignments were manually checked and forward and reverse sequences combined into single contigs. Assembled sequences were then imported into MEGA5 (Tamura *et al.* 2011), which was used as the platform for all further sequence arrangements for several different analyses.

All previously published sequences for seriatoporid corals that aligned to the same region of the mitochondrial genome were downloaded from the Genbank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned to the sequences generated in this study (Table 2.2; Chen *et al.* 2008; Flot *et al.* 2008; Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a). Due to the variable sequence lengths obtained in the four other studies that used different primers and amplicons, three different alignments (short = 629 bp, medium = 811 bp, long = 1388 bp) were completed and analyzed to incorporate as much information as possible (Table 2.2). Only sequences from Bongaerts *et al.* (2010b) were not included in any alignment due to the very short lengths (557 bp) and the fact that representative samples had been re-sequenced for longer segments in van Oppen *et al.* (2011a). Instead, haplotype frequencies from Bongaerts (2010b) were recorded from the publication and entered manually into further analyses.

Table 2.2 Summary table of sequences and alignments used in mtDNA phylogenetic analysis. Target Spp.: named species targeted for study; N_{TOT}: total # sequences from the study; Location surveyed: country of collection; N_{LOC}: # sequences from each location; Alignment (bp): sequences included in each of three alignments (Short: 629 bp; Medium: 811 bp; Long; 1388 bp); Sequence length (bp): total length of published sequences including indels. Total at bottom indicates total number of sequences in analysis and number included in each of the three alignments.

Source	Target Spp.	N_{TOT}	Location surveyed	N_{LOC}	Alignment (bp)			Sequence length (bp)	Genbank Accesion No.
					629	811	1388		
P. Warner (unpublished)	All *Seriatopora spp.*	39	Lizard Island, GBR	39	X	X	X	1351	unpublished
van Oppen *et al.* (2011a)	*S. hystrix*	145						1388	HQ878445 – 878586
			W. Australia	104	X	X	X		
			Lizard Island, GBR	41*	X	X	X		
Bongaerts *et al.* (2010b)	*S. hystrix*	336	Lizard Island, GBR	336**				557	HM159623 – 159958
Flot *et al.* (2008)	All *Seriatopora spp.*	51						824	EU622126 – 622227
			New Caledonia	38	X	X			
			Okinawa	4	X	X			
			Phillipines	9	X	X			
Chen *et al.* (2008)	*S. hystrix, S. caliendrum*	1,1	Taiwan	1,1	X	X	X	17,060	EF633600 – 633601
Chen *et al.* (2008)	*S. hystrix, S. caliendrum*	16, 6						629	EF633578 – 633599
			Taiwan	13	X				
			Taiping Is., S. China Sea	4	X				
			Similan Is., Indian Ocean	5	X				
TOTAL SEQUENCES		**595**			**254**	**232**	**179**		

* 41 samples from Bongaerts *et al.* (2010b) were resequenced and presented in van Oppen *et al.* (2011a).

** Due to short fragment legth in Bongaerts *et al.* (2010b), haplotype frequencies reported in publlication were added manually to network analysis.

Unique mitochondrial haplotypes were identified from all three alignments in DnaSP 5.10 (Librado and Rozas 2009) with indels considered. For all downstream analyses, the two large indels present were treated as a single position by manually excluding all base positions except for the first base position at each indel. Two additional single or double base position indels occurred in the long alignment and were retained for analysis, each as an independent position.

Phylogenetic networks reconstruct complicated evolutionary patterns (Bandelt *et al.* 1995; Morrison 2005; Huson and Bryant 2006; Makarenkov *et al.* 2006), such as reticulation, and particularly intraspecific relationships (Legendre 2000; Posada and Crandall 2001) more accurately than trees. Therefore, complementary analyses using both phylogenetic trees and networks are presented. Median-joining networks were created in Network 4.6.1.0 (Flexus Technology Ltd.) for all three alignments. Input files were generated by DnaSP 5.10 (Librado and Rozas 2009) with gaps considered and invariant sites removed. Haplotype frequencies were manually entered into haplotype files in Network. Networks were constructed with epsilon=0, transitions/transversions and the single base indels weighted equally, but the single-base positions representing the two large indels were weighted three times that of other characters. It was found that two haplotypes differed only by the presence or absence of a large 51-base position (bp) indel, which emphasizes the importance of this feature for phylogenetic inference. Each network was generated unrooted and by external rooting based on a related species belonging to the same family, *Pocillopora damicornis*. Final networks were drawn in Network Publisher 1.3.0.0 (Flexus Technology Ltd.).

Several phylogenetic analyses were used to construct trees for each alignment. Initially, trials were run with different indel recoding methods (see Simmons *et al.* 2007 for review); however these alternative methods were ultimately rejected for this study because they could not be readily incorporated into Maximum Likelihood (ML) or Bayesian methods. Consequently, all single-base gaps, including those representing

the large indels, were treated as missing data, which has been shown to be preferable to excluding indels altogether (Simmons *et al.* 2007). Each of the phylogenetic trees was rooted with the two pocilloporid taxa *P. damicornis* (Genbank No. EU400213) and *Stylophora pistillata* (Genbank No. EU400214). Sequences were assessed for the best substitution model by the suite of programs implemented in jModelTest 0.1.1 (Guindon and Gascuel 2003; Posada 2008) using the Akaike Information Criterion (AIC). For the shortest alignment analysis that is presented in the results, jModelTest results indicated that the best fit was a GTR-derived model with rate variation among sites. The actual parameters used in ML models were estimated by a stepwise method implemented in PAUP*4.0b10 (Swofford 2003; Huelsenbeck and Bollback 2008). Phylogenetic trees were constructed by Maximum Parsimony (MP) and ML using PAUP*4.0b10 (Swofford 2003). Bootstrap replicates (1000) were performed under each criterion, and pairwise genetic distances were calculated based on ML distance. Bayesian analyses were conducted in MrBayes v.3.2.1 (Ronquist *et al.* 2012) using the default settings for the Markov Chain Monte Carlo (MCMC) analysis: 4 chains, 10^6 generations (gens) sampled every 500 gens, and a 25% relative burn-in (250,000 gens).

2.2.3 mtDNA marker for the genus *Seriatopora*

To distinguish among major host mitochondrial haplotype groups in the samples collected for this study, the unpublished primers ShMT662F (5'–YTTSAGAGAGTGTGTMSTWAGG–3') and ShMT960R (5'–CAACTGCCTCATCAAKGTT–3') were used to amplify a highly variable ~300 bp region of the *Seriatopora* mitochondrial genome in all samples. This region was previously identified by Chen *et al.* (2008) as the putative control region, between the *atp6* and *nad4* genes, and includes an area of 51-bp tandem repeats that was reported to distinguish between *S. hystrix* and *S. caliendrum* by the number of repeats (4 and 5 copies for *S. caliendrum* and *S. hystrix*, respectively). The same region is included in the 1500 bp segment sequenced for the 39 W. Lizard Island samples, and used in the

above described phylogenetic analyses. Because the amplicon size differs by ~25 bp according to three major mitochondrial haplotypes (Small (S) – 248 bp; Medium (M) – 272 bp; Large (L) – 299 bp) the different products can be easily separated on a high percentage (2-4%) agarose gel. The primers (ShMT662F, ShMT960R) were used to amplify the described region, in all 964 individuals collected in this study, using MyTaq^{TM} DNA Polymerase and 5x MyTaq Buffer (Bioline, NSW, Australia) with primers 250 nM each, 1 µL 1:10 diluted template, and 0.5 units DNA polymerase in 10 µL reactions. Cycling conditions were 1 x 95 °C (1 min), followed by 30 x [(15 s at 95 °C), (30 s at 50 °C), (15 s at 72 °C)] and completed with 10 min at 72 °C. Following PCR amplification, 3 µL of product was run on 3.5% agarose gels at 130 V for at least 120 minutes before being viewed and photographed by the Chemi-Smart imaging system (Vilber Lourmat ChemiSmart 3000) for manual scoring. Haplotypes were scored (Small, Medium, or Large) by comparison to a combination of HyperLadder™ V size marker loads (Bioline, NSW, Australia) and positive controls.

2.2.4 Genetic profiling of algal endosymbionts, genus *Symbiodinium*

For a subset of 516 samples (Table 2.1), the dominant *Symbiodinium* type present was characterized using the internal transcribed spacer (ITS2) region of the nuclear ribosomal DNA (rDNA). Samples were amplified with *Symbiodinium*-specific primers ITSintfor2 (5'–GAATTGCAGAACTCCGTG–3') and ITS2CLAMP (5'–CGCCCG CCGCGCC CCGCGCCCG TCCCGCC GCCCCCGCCC GGGATC CATATGCTTAAG TTCAGCGGGT–3') (GC clamp underlined; Lajeunesse and Trench 2000) and Qiagen Multiplex PCR kit reagents in 30 µL reactions, with 1 µL undiluted DNA extract and 1.2 µL of each 10 µM primer solution according to the cycling conditions described in LaJeunesse (2002). The amplified ITS2 fragments were separated by denaturing gradient gel electrophoresis (DGGE) on an Ingeny System following conditions outlined in (Sampayo 2007), with the following minor modifications: 1) the internal gel gradient used was 30 – 55%; 2) 6 µL of PCR product was loaded; and, 3) gels were run at 100

V for up to 14 h. Gel images were captured using the Chemi-Smart imaging system (Vilber Lourmat ChemiSmart 3000). Representatives of each different profile for each DGGE gel were re-run together so that unique profiles could be identified. Dominant bands from each characteristic profile were excised from the gel and eluted in 50 µL of molecular grade water overnight at 4°C. One µL of elution was used to re-amplify fragments using both GC-clamp and non-GC primers. The GC-clamp products were run a final time on the DGGE system to ensure single band amplification, and then acceptable non-GC products were sent to Macrogen (South Korea) for both forward and reverse sequencing. All sequence chromatograms were imported into Sequencher 4.7 (Gene Codes Corporation), proofread and aligned. Alignments were manually checked, forward and reverse sequences combined and compared to Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) entries using nucleotide BLAST searches.

2.2.5 Microsatellite genotyping of coral hosts

All 964 samples were amplified with the ten fluorescently-labeled microsatellite loci described in Underwood *et al.* (2006) using the Qiagen Multiplex PCR kit (manufacturer's protocol) in 10 µL reactions with 1 µL of 1:10 diluted DNA extract. The ten loci were combined into three multiplex PCRs as follows: Multiplex 1 – Sh4-001, Sh3-003, Sh3-004, Sh3-009; Multiplex 2 – Sh2-002, Sh2-005, Sh4-010; Multiplex 3 – Sh2-006, Sh3-007, Sh3-008. Following amplification, 5 µL of PCR product were purified using Sephadex G-50 columns on 96-well filter plates. Purified products were sent to James Cook University's Genetic Analysis Facility (Townsville, Australia) and separated on the GE Healthcare MegaBace 1000 capillary sequencer with an internal size standard for each sample (ET 400-R; GE Healthcare). Microsatellite electropherograms were imported into the MegaBACE Fragment Profiler Software Version 1.2 (GE Healthcare) to determine the fragment sizes (alleles) present in the samples. All automatic scoring was rigorously checked manually, and samples with ambiguous or missing signals were re-amplified and re-run. Persistent failures were

treated as missing data. Approximately three positive controls per every 92 samples were amplified and separated on every 96-well plate to estimate locus-specific genotyping error rates. No errors were detected for 295 positive controls across all loci.

2.2.6 Delimitation of putative cryptic species

The Bayesian clustering software *structure* v2.3 (Pritchard *et al.* 2000) was used to detect potential cryptic species under the 'genotypic cluster' species definition presented by Mallet (1995). The *structure* analysis does not require *a priori* assignment of individuals into populations and should therefore be ideal for the detection of independent genetic clusters that may be morphologically cryptic. However, given the expectation that both inter- and intra-species subdivisions occur in the dataset, the genetic distance within and between separate genetic clusters is likely to be variable. Clusters form from the most divergent groups first (Rosenberg *et al.* 2002) and are affected by the number of individuals included, thus potentially rare cryptic groups may be harder to detect in a large dataset (Pritchard *et al.* 2000). Therefore, a hierarchical *structure* analysis was employed to separate the most distinct groups that are shared among rather than within geographic regions. Previous work has shown that such an approach allows a full description of genetic structure when hierarchical levels of divergence exist (Rosenberg *et al.* 2002; Garnier *et al.* 2004; Wang *et al.* 2007; Ledoux *et al.* 2010b; Mokhtar-Jamaï *et al.* 2011), as would be expected in a dataset containing multiple species and geographic structure.

All *structure* analyses were conducted using both independent and correlated allele frequency models with admixture (Table 2.3); however, comparisons between model results indicated that genetic differentiation between putative species groups is large and groups were best identified using independent allele frequencies. Therefore, the results presented were all run using independent allele frequencies, except where specifically noted otherwise. Five replicates runs for each K-value were completed for

all analyses. Burn-in period length was 100,000 followed by 500,000 MCMC replications. Raw results and calculations for the ΔK method described in Evanno *et al.* (2005) were obtained using the online software Structure Harvester (Earl and Vonholdt 2012). The ΔK method indicates the "uppermost hierarchical level of structure" (Evanno *et al.* 2005), and therefore was considered a useful metric for distinguishing potential interspecies divisions from intraspecies population structure. Yet, both ΔK and the posterior probability values of K (L(K)) were considered in selecting the appropriate K value to fit the data. Raw results were compiled manually in Microsoft Excel and individual assignment plots were constructed with assignment averages across the five replicate runs. In all cases, the standard error of individual assignment proportions averaged across runs did not exceed 0.2%, demonstrating strong consistency in assignments across replicates.

Table 2.3 Summary of *structure* analyses for 964 *Seriatopora* individuals from 10 GBR reefs conducted with 10 microsatellite loci. N: # individuals; Indep AF: independent allele frequencies model with admixture; Correl AF: correlated allele frequencies model with admixture; Max K: the maximum value of K analyzed; Hierarchy: 1st or 2nd tier in hierarchical analysis.

HIERARCHICAL	N	Indep AF Max K	Correl AF Max K	Hierarchy
All samples	964	10	10	1
Cluster 1	510	7	7	2
Cluster 2	339	6	6	2
Cluster 3	115	5	5	2
INDIVIDUAL REEFS				
NE Orpheus	128	6	6	
SE Pelorus	100	5	5	
W Orpheus	99	4	4	
NW Orpheus	100	4	4	
SW Pelorus	100	3	4	
SE Lizard	100	4	4	
NE Lizard	98	6	6	
W Lizard	39	5	5	
SW Lizard	100	-	3	
NW Lizard	100	-	3	

All samples (n = 964) were combined in an analysis with K ranging from 1 to 10, for which the ΔK method indicated that the uppermost hierarchical value occurred at K=3 (Table 2.3). All individuals were assigned by at least 50% to a single cluster. The sample set was then divided into the three indicated clusters, which corresponded to one large genetic cluster (cluster 1; n=510) and two smaller clusters, all shared across regions (Table 3). All three clusters were run separately in a second tier of analyses up to K-values incorporating the number of reef locations (plus two) represented by the samples in each subdivided dataset (Table 2.3). The K-values (ΔK / L(K)) presented for the second tier of analyses indicate additional groups shared among geographic regions, whereas larger values of K reflect intraspecies (between region) geographic structure (Chapter 3). The combined hierarchical *structure* results were used to designate five genetic clusters, of which all are shared across regions except one rare group only sampled in the Palm Islands.

Secondly, samples collected from each reef were run in ten separate *structure* analyses to assess the occurrence of distinct sympatric genetic clusters without geographic influence (Table 2.3). For each reef, analyses were run up to K-values informed by the likely number of genetic clusters occurring from the hierarchical analysis plus two (Table 2.3). Appropriate K-values were selected based on a combination of ΔK and L(K). For all reefs, all individuals were assigned to a single genetic cluster by at least 94%, except for one individual, and the standard errors of mean assignment across runs did not exceed 0.1%. The distinct clusters found within each reef were cross-referenced back to the hierarchical assignments and, subsequently, all individuals were assigned to one of five genetic clusters for further analysis: *S. caliendrum*, *Sh_exposed*, *Sh_sheltered*, *Sh_large*, *Sh_bushy*. Cluster names reflect distinctive characteristics of ecology, morphology or mitochondrial types that will be fully explained in the results section.

2.2.7 Characterization of *structure* clusters

A neighbor-joining (NJ) tree (Saitou and Nei 1987) of all individuals was constructed in MEGA5 (Tamura *et al.* 2011), using squared codominant genotypic distances computed from the microsatellite data with GenAlEx v6.4 (Peakall and Smouse 2006). A hybrid index was calculated in GenoDive v2.0b20 (Meirmans and van Tienderen 2004) for any individuals showing proportions of admixture of <90% assignment to a single cluster in the reef-specific *structure* analysis according to methods outlined in Buerkle (2005). The hybrid index (h = [0,1]) is a maximum likelihood estimation of the proportion of alleles an individual inherited from one of two putative parental species based on sampled population allele frequencies (Buerkle 2005). Morphological diversity among the five genetic clusters was considered using gross colony morphology observed from the 964 sample photographs matched to genotyped individuals (e.g., branching pattern, branch thickness, branch tip morphology). Host mtDNA types and symbiont ITS2 profiles were likewise matched to genetic clusters.

All further analyses assumed the genetic cluster designations from the *structure* analysis and divided the sample set into populations based on sampling site and cluster (Table 2.4). Four individuals were excluded from population analyses due to low number of individuals per cluster at the sites sampled (two each at WO1 and NWO2). In six instances of rare clusters with low sample numbers, individuals were combined into a single population across the two sites within a reef: *Sh_bushy* at NE Orpheus and SE Pelorus; *Sh_large* at SE Pelorus and NE Lizard; *Sh_sheltered* and *S. caliendrum* at NE Lizard (Table 2.4). Preliminary analyses indicated that between-site population differentiation was not significantly different from zero for these populations. A total of 30 populations were assessed according to these divisions (Table 2.4).

Table 2.4 Summary of the number of individuals assigned to each of five genetic clusters within the genus *Seriatopora* (represented by different colors) at 10 GBR reefs according to *structure* analyses: shelt (yellow): *Sh_sheltered*; exp (blue): *Sh_exposed*; S. cal (red): *S. caliendrum*; large (green): *Sh_large*; bushy (orange): *Sh_bushy*. Black boxes indicate rare clusters that were combined into one population from sites 1 and 2 within a reef. Red type indicates populations in which individuals with matching MLGs were removed prior to analysis. Italicized gray type denotes individuals excluded from population level analysis due to small sample size. Overall, 30 populations were utilized for population level analyses, designated by sample site and genetic cluster.

			Genetic cluster				
Region	**Reef**	**Site**	***shelt***	***exp***	***S. cal***	***large***	***bushy***
Palm Is.	West Orpheus	**WO1**	47	*2*	-	-	-
		WO2	50	-	-	-	-
Palm Is.	Northwest Orpheus	**NWO1**	50	-	-	-	-
		NWO2	48	-	-	*2*	-
Palm Is.	West Pelorus	**WP1**	50	-	-	-	-
		WP2	50	-	-	-	-
Palm Is.	Northeast Orpheus	**NEO1**	-	**39**	48	-	**3**
		NEO2	-	14	21	-	3
Palm Is.	Southeast Pelorus	**SEP1**	-	17	-	19	13
		SEP2	-	40	-	4	**7**
Lizard Is.	Southwest Lizard	**SWL1**	50	-	-	-	-
		SWL2	50	-	-	-	-
Lizard Is.	Northwest Lizard	**NWL1**	50	-	-	-	-
		NWL2	50	-	-	-	-
Lizard Is.	Southeast Lizard	**SEL1**	-	**50**	-	-	-
		SEL2	7	43	-	-	-
Lizard Is.	Northeast Lizard	**NEL1**	1	39	2	8	-
		NEL2	2	27	2	17	-
Lizard Is.	West Lizard	**WL**	4	19	16	-	-
		TOTAL	**509**	**290**	**89**	**50**	**26**

Individuals sharing identical multilocus genotypes (MLG) within populations were identified and the genotype probabilities (GP) were calculated in GenAlEx v6.4 . All but one individual sharing the same MLG was removed from the analysis if the

matching MLG: 1) occurred in individuals within the same sampling site; and 2) had GP < 0.001. Summary descriptive statistics for the marker set were computed and private alleles identified using GenAlEx v6.4 . Weir and Cockerham's inbreeding coefficients (Weir and Cockerham 1984), statistically significant departures from HWE and genotypic linkage disequilibrium (LD) were calculated by the exact test method implemented in GENEPOP v4.1.1 (Raymond and Rousset 1995; Rousset 2008) with default Markov chain parameters. The frequency of null alleles and their effect on pairwise population differentiation were assessed using the software FreeNA (Chapuis and Estoup 2007), which calculates F_{ST} values (Weir 1996) on uncorrected and ENA-corrected data following the method described in Chapuis and Estoup (2007). Null allele frequencies had a minimal effect on F_{ST} values, and all subsequent analyses were conducted with uncorrected data.

A hierarchical AMOVA using the *structure* clusters as the highest level of population subdivision (F_{CT}) was implemented in GenoDive v2.0b20 with 9,999 permutations and standard errors of F-statistics calculated by jackknifing over loci. Values of F_{ST} estimated by the AMOVA approach (Excoffier *et al.* 1992) and G''_{ST} (Meirmans and Hedrick 2011) were also calculated with GenoDive v2.0b20 . G''_{ST} is a standardized version of Nei's G statistic (Nei 1987) that accounts for variable levels of diversity among loci and populations (Hedrick 2005), while correcting for small numbers of populations sampled (Meirmans and Hedrick 2011). Moreover, it is appropriate for comparisons between putative species with potentially different effective population sizes (Meirmans and Hedrick 2011). Significance of pairwise population differentiation was assessed using Fisher's exact method according to the log likelihood ratio (G; Goudet *et al.* 1996) implemented in GENEPOP v4.1.1 with default Markov chain parameters. Statistical significance for all tests was adjusted for multiple comparisons using the B-Y false discovery rate (FDR) (Benjamini and Yekutieli 2001) at α=0.05. The B-Y FDR is an intermediately conservative correction for multiple

comparisons testing, and balances Type I and Type II error rates for ecological genetic studies (Narum 2006).

G"$_{ST}$ was plotted against pairwise geographic distance following a method introduced by Good and Wake (1992) to differentiate between isolation-by-distance patterns within a species and expected geographically independent interspecies divergences (Jackman and Wake 1994; Tilley and Mahoney 1996; de Queiroz and Good 1997; Sites and Marshall 2003). Good and Wake (1992) used the method to test for 'genetically cohesive' species that do not occur in sympatry under the assumption that the regression line of populations within the same putative species should pass through the origin due to increased gene flow with proximity, assuming mutation-migration equilibrium. Conversely, genetically isolated populations of different species will not have significant genetic exchange at any geographic distance and therefore the y-intercepts of such regressions are expected to deviate from zero (Good and Wake 1992; de Queiroz and Good 1997; Sites and Marshall 2003). All within cluster comparisons were plotted separately from between cluster comparisons and regression lines fitted to each data set using Microsoft Excel. Mantel tests were computed in GenAlEx v6.4.

The harmonic mean across loci of Jost's D_{est} (Jost 2008) was calculated in SMOGD v2.6 (Crawford 2010). D_{est} (Jost 2008) assesses population differentiation based on the effective number of alleles rather than heterozygosity. It is best at estimating actual allelic differentiation rather than demographic processes (Meirmans and Hedrick 2011), and should therefore be appropriate for assessing potential interspecific diversity while homogenizing intraspecies differences. GenAlEx v6.4 was used to conduct a principal component analysis (PCA) of D_{est} values with covariance matrices. Finally, phylogenetic analyses were conducted on populations with D_A distances (Nei *et al.* 1983) in Poptree2 (Takezaki *et al.* 2010) from population allele frequencies estimated in GenAlEx v6.4. D_A has been demonstrated to perform better

than other related distance measures used to infer evolutionary relationships with microsatellite loci, particularly in closely related populations (Takezaki and Nei 1996, 2008). Neighbor-joining trees (Saitou and Nei 1987) were constructed with 10,000 bootstraps replicates (Felsenstein 1985).

2.3 RESULTS

2.3.1 Mitochondrial DNA (mtDNA) characterization of coral hosts and phylogenetic analysis of the genus *Seriatopora*

The 39 *Seriatopora* individuals sequenced from W. Lizard yielded an alignment of 1360 bp including indels (unpublished sequences). Prior to further analysis, nine positions that showed ambiguity across several individuals were removed, leaving 1351 bp in the alignment. Thirty-nine alignment positions were parsimony informative (excl. indels), and the number of substitutions between paired sequences ranged from 0 to 38 (excl. indels). The alignment contained one 24-bp indel, which divided the individuals into two major mitochondrial lineages, i.e. those sharing (n=16) versus those not sharing (n=23) the insertion. The latter group consisted of two haplotypes that are distinguished by five substitutions. The three mitochondrial groups detected included previously described *S. hystrix* haplotypes B (n=4) and D3 (n=19) (Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a) (See Table 2.5 for explanation of haplotype names and sources). The third haplotype is similar to sequences from corals previously identified as *S. caliendrum* (Chen *et al.* 2008), and is subsequently referred to as ScA (i.e., *S. caliendrum* clade Australia; Table 2.5). ScA is most closely related to *Seriatopora* individuals in Cluster 3 from Flot *et al.* (2008).

Table 2.5 Summary of nineteen mtDNA control region sequence haplotypes for *Seriatopora* spp. reported in the literature for locations in the Indo-Pacific region. mtDNA CR haplotype: name used in this study; 1st Named/ Published: reference to study in which haplotype sequence was first published or named; Naming explanation: as named in this study (if not previously named); mtDNA clade: haplotype membership in one of two main *Seriatopora* clades named for morphospecies currently recorded on GBR; mtDNA CR amplicon: major haplotype groups according to mtDNA marker amplicon size (See Fig. 2.2): Small (no indel); Medium (24 bp indel); Large (51 bp indel); other studies that have reported the haplotype. Names were given to haplotypes in this study to either continue conventions published previously (red) or to reference the original study from which the sequence was reported (black).

mtDNA CR haplotype	1st Named/ Published	Naming explanation	mtDNA clade	mtDNA CR amplicon	Other reports from published literature
ShC2	Chen *et al.* (2008)	Sh - *S. hystrix*; C - Chen	*S. hystrix*	Small	-
ShC3	Chen *et al.* (2008)	Sh - *S. hystrix*; C - Chen	*S. hystrix*	Small	-
ShF1a	Flot *et al.* (2008)	Sh - *S. hystrix*; F - Flot; 1 - Cluster 1 (Flot)	*S. hystrix*	Small	-
ShF1b	Flot *et al.* (2008)	Sh - *S. hystrix*; F - Flot; 1 - Cluster 1 (Flot)	*S. hystrix*	Small	-
B_{GBR}	Bongaerts *et al.* (2010b)	B - back reef; gbr - GBR	*S. hystrix*	Small	van Oppen et al. (2011a); P. Warner (unpub.)
B_{WA}	van Oppen et al. (2011a)	B - back reef; wa - western australia	*S. hystrix*	Small	-
D1	Bongaerts *et al.* (2010b)	D - deep reef	*S. hystrix*	Small	Flot et al. (2008); van Oppen et al. (2011a)
D2	Bongaerts *et al.* (2010b)	D - deep reef	*S. hystrix*	Small	van Oppen et al. (2011a)
D3	Bongaerts *et al.* (2010b)	D - deep reef	*S. hystrix*	Small	Flot et al. (2008); van Oppen et al. (2011a); P. Warner (unpub.)
SR1	van Oppen *et al.* (2011a)	SR - Scott Reef	*S. hystrix*	Small	-
ShC1	Chen *et al.* (2008)	Sh - *S. hystrix*; C - Chen	*S. hystrix*	Large	-
ShF2	Flot *et al.* (2008)	Sh - *S. hystrix*; F - Flot; 1 - Cluster 2 (Flot)	*S. hystrix*	Large	-
U	Bongaerts *et al.* (2010b)	U - Upper slope	*S. hystrix*	Large	Chen et al. (2008); Flot et al. (2008); van Oppen et al. (2011a)
U_{WA}	van Oppen *et al.* (2011a)	U - Upper slope; wa - Western Australia	*S. hystrix*	Large	-
ScA	P. Warner (unpublished)	Sc - *S. caliendrum*; A - Australia	*S. caliendrum*	Medium	-
ScF3a	Flot *et al.* (2008)	Sc - *S. caliendrum*; F - Flot; 3 - cluster 3 (Flot)	*S. caliendrum*	Medium	-
ScF3b	Flot *et al.* (2008)	Sc - *S. caliendrum*; F - Flot; 3 - cluster 3 (Flot)	*S. caliendrum*	Medium	-
ScF3c	Flot *et al.* (2008)	Sc - *S. caliendrum*; F - Flot; 3 - cluster 3 (Flot)	*S. caliendrum*	Medium	-
ScFC	Chen *et al.* (2008)	Sc - *S. caliendrum*; F - Flot; C - Chen	*S. caliendrum*	Medium	Flot et al. (2008)

When all published *Seriatopora* sequences were included, the short alignment was 629 bp (incl. indels) and contained 254 individuals (Table 2.2). Nineteen mtDNA haplotypes were identified by 34 parsimony informative sites (excl. indels) and substitutions between haplotypes ranged from 1 to 36. All haplotypes names are listed and referenced in Table 2.5. The intermediate length alignment (811 bp incl. indels, 232 taxa) yielded seventeen haplotypes, 36 parsimony informative sites, and substitutions between haplotypes ranged from 1 to 42. The full length alignment of 1388 bp (incl. indels) included 179 taxa and 15 haplotypes. All three alignments included two major indels, one 51-bp tandem repeat and one 24-bp indel downstream of the larger insert.

2.3.2 Three major mitochondrial groups: new mtDNA marker for the genus *Seriatopora*

The new mtDNA primers ShMT662F and ShMT960R aligned to the 1360 bp alignment (W. Lizard sequences) at positions 637 – 658 and 891 – 909 and the whole amplicon corresponded to positions 7174 – 8580 in the complete *Seriatopora* genomes reported by Chen *et al.* (2008). When all published *Seriatopora* sequences were aligned at this region of the genome, the total amplicon size (incl. gaps and primer sequences) was 323 bp (Fig. 2.2). The nineteen unique *Seriatopora* mitochondrial sequences were divided into three major groups by the presence or absence of the two large indels mentioned above. Ten mitochondrial haplotypes were represented by the **Small** amplicon (S-type: 248 bp), which has no insertions: ShF1a, ShF1b, ShC2, ShC3, D1, D2, D3, B_{GBR}, B_{WA}, SR1 (Figs. 2.2, 2.3). Four of these have been described previously from the GBR (Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a), of which two are also reported in this study (D3 and B). The **Large** amplicon (L-type: 299 bp) included four haplotypes (ShF2, ShC1, U-types: U and U_{WA}) that contained a 51-bp tandem repeat insertion absent from the Small and Medium amplicons (Figs. 2.2, 2.3). The **Medium** amplicon (M-type: 272 bp) was distinguished by a 24-bp insertion not present in either the Small or Large amplicons, and included all five reported *S.*

caliendrum sequences (Figs. 2.2, 2.3). This group includes haplotype ScA described in this study, which is the first *S. caliendrum* haplotype reported for the GBR.

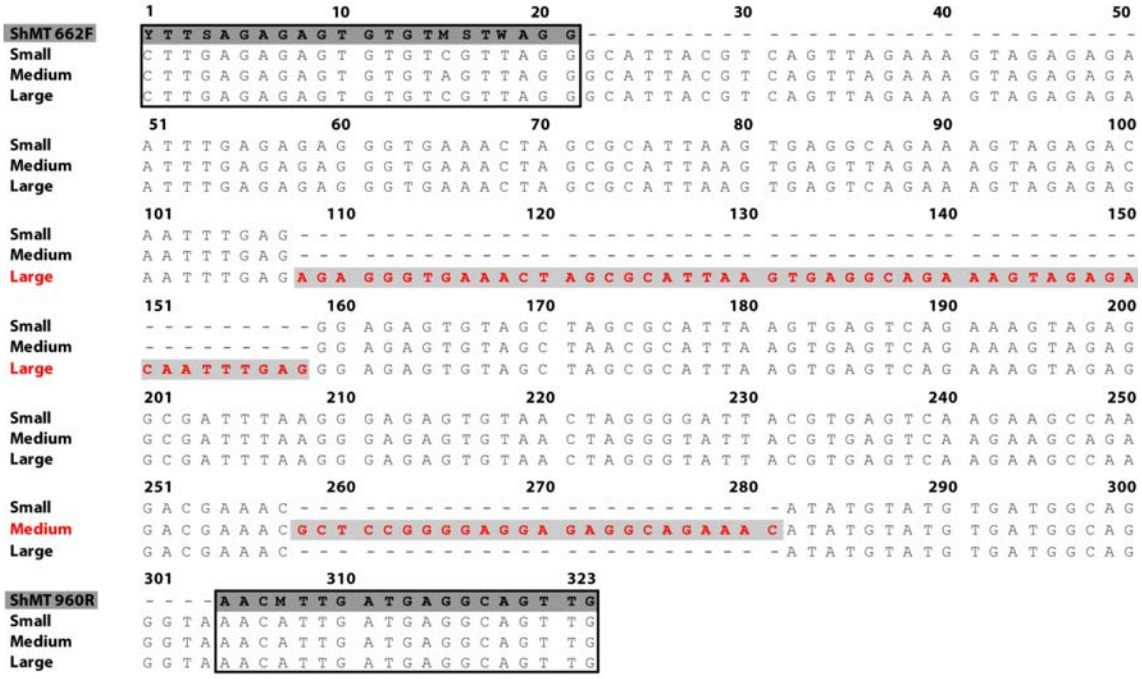
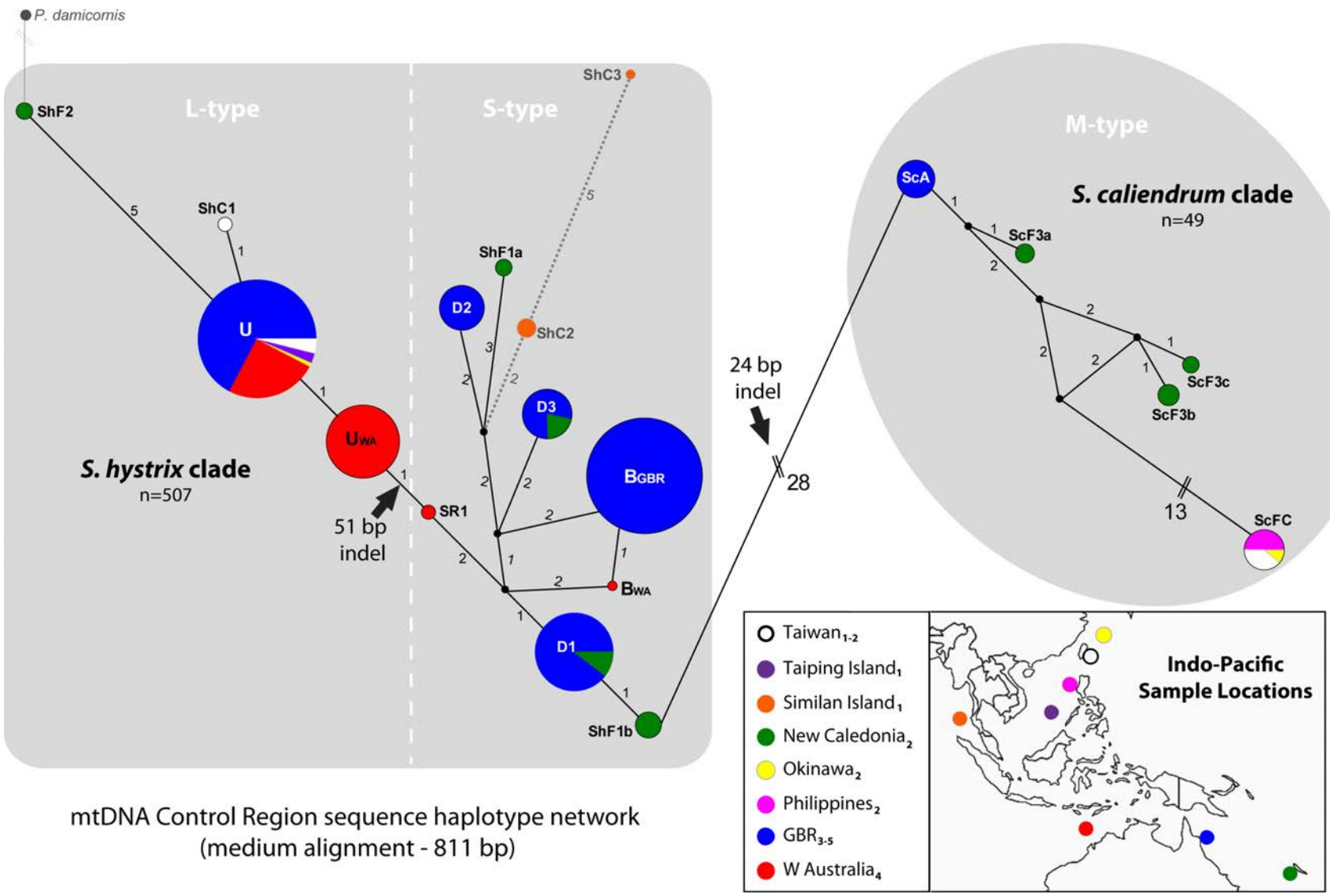


Figure 2.2 Sequence alignment for new mitochondrial primers ShMT662F and ShMT960R for *Seriatopora.* The three amplicon sizes (Small, Medium and Large) represent all of the 19 mitochondrial haplotypes detected for the seriatoporid sequences tested: **1) Small** (248 bp, no indels) detected in 10 taxa – D1, D2, D3, B_{GBR}, B_{WA}, SR1, ShF1a, ShF1b, ShC2, ShC3; **2) Medium** (272 bp, 24 bp indel) in 5 taxa – all *S. caliendrum* clade haplotypes; and **3) Large** (299 bp, 51 bp indel) detected in 4 taxa –U, U_{WA}, ShC1, ShF2. Red type indicates the indels that distinguish types Medium and Large from the Small haplotype. See Table 2.5 for explanation of haplotype names.

2.3.3 mtDNA phylogenetic analysis

Although the fundamental framework of networks was the same for all three alignments, the medium length alignment provided the best overall resolution and is presented here (Fig. 2.3). Both the long and short alignments resulted in networks with additional cycles (reticulations) due to unrepresented haplotypes or limited characters, respectively (not shown). Note that each alignment consisted of a different number of haplotypes and sequence lengths (see Table 2.2). In the network shown (medium alignment), two main clades were separated by 27 point mutations plus the 24-bp

insertion that was characteristic of all *S. caliendrum* sequences. These two clades are therefore subsequently referred to as the *S. hystrix* and *S. caliendrum* clades after the two morphospecies reported from the GBR (Fig. 2.3). The *S. caliendrum* clade appeared to be clearly structured by geography: haplotype ScFC representing northern locations in the Western Pacific (Okinawa, Philippines, Taiwan) was the most divergent, but most closely related to several New Caledonia haplotypes (by 16 substitutions; Fig. 2.3) and slightly more removed from the GBR haplotype. In contrast, the distribution of *S. hystrix* haplotypes in the network generally did not correlate with sampling location. However, the Indian Ocean haplotypes (ShC2 and ShC3) did appear to constitute a relatively divergent and unique branch in the network (orange circles, Fig. 2.3). Clearly, Host U is the most widespread haplotype, reported from both East and West Australia, Taiping Island (S. China Sea), Taiwan, and Okinawa. The three mitochondrial groups (S-, M-, and L-types) represented by the new marker separated clearly within the network (white font, Fig. 2.3); but, SR1 only differs from U_{WA} and the L-type group by the absence of the 51-bp indel, and its rarity (n=2, only reported from Western Australia) does not support it being a direct link between the L-types and all other *Seriatopora* haplotypes. The haplotypes in the *S. hystrix* clade were generally only separated by a few mutations, yet the number of mutations between most distantly related taxa in both clades was the same, 19 for *S. hystrix* (ShF2 and ShC3) and 18 for *S. caliendrum* (ScFC and ScA; Fig. 2.3). Moreover, the *S. hystrix* clade had much greater representation in the dataset, with 507 sequences compared to only 49 for *S. caliendrum*, as most studies to date have targeted *S. hystrix* specifically. Still, substantial phylogenetic structure was suggested in the *S. hystrix* clade by the deep bifurcation between the L-types and most S-types (branching above the main edge, Fig. 2.3).



mtDNA Control Region sequence haplotype network
(medium alignment - 811 bp)

Figure 2.3 mtDNA (811 bp of control region sequence) median-joining network of *Seriatopora* spp. using the medium length alignment (Table 2.2) and samples from Australian and Indo-Pacific regions rooted with outgroup taxon *P. damicornis*. Haplotypes are indicated by colored circles (nodes), with the size of the circles proportional to the frequency of haplotypes reported from the literature and in this study. Colors designate regional sampling locations (shown on map, lower right) and smaller black nodes represent unsampled and/ or ancestral taxa. Branch lengths within clusters (*S. hystrix* and *S. caliendrum*) are proportional to the number of mutations, but sequence divergences >5 substitutions are truncated for visual display. The number of sequence substitutions between nodes is indicated along branches. Haplotype names: 'F' or 'C' denotes unnamed haplotypes published in Flot *et al.* (2008) or Chen *et al.* (2008), respectively; see Table 2.5 for explanation of other names. Similan Island haplotypes (orange) represented only by shorter sequence length (ShC2 and ShC3; Table 2.2) are shown with grey dotted line to denote where these haplotypes were positioned in the short alignment network. White type and white dotted line indicate the major mitochondrial haplotype groups defined in this study: S-type, M-type and L-type. The location of two major indels is indicated with arrows. Location footnotes correspond to references for sequences utilized: 1 – Chen *et al.* (2008); 2 – Flot *et al.* (2008); 3 – Bongaerts *et al.* (2010b); 4 – van Oppen *et al.* (2011a); 5 – P. Warner (unpublished).

The phylogenetic tree incorporating all 19 haplotypes (short alignment) showed a deep divergence between the *S. hystrix* and *S. caliendrum* clades with strong support for the monophyly of the *Seriatopora* genus (100% Bayesian posterior probability (B); 100% bootstrap support from both MP and ML), as well as each of the two main clades (Fig. 2.4). Like the network, the phylogenetic tree demonstrated a clear geographic pattern within the *S. caliendrum* clade, but not the *S. hystrix* clade. There was a bifurcation in the *S. caliendrum* clade between the divergent ScFC haplotype from northern locations and the other four haplotypes reported from New Caledonia and Australia (B: 92%; MP: 83%). Similarly, a deep division occurred in the *S. hystrix* clade between a grouping of the divergent Indian Ocean haplotypes with ShF1a from New Caledonia (B: 90%) and the other haplotypes except for D2 (B: 89%; Fig. 2.4). With the exception of the position of ShF2, the relationships among closely related taxa within the *S. hystrix* clade resembled those in the network (Figs. 2.3, 2.4). Haplotypes ShF1b and D1 formed a strong grouping (B: 96%; MP: 53%) that was closely linked to an equally robust clade containing both U-types (U and U_{WA}), ShC1 and SR1 (B: 98%; MP: 65%; ML: 60%). And, similar to the network, those six taxa formed a clade most closely related to B-types (B_{GBR} and B_{WA}; Figs. 2.3, 2.4). Aside from the separation of

ShF2, the L-types (highlighted in dark gray) formed a clade with the terminal position of SR1, suggesting the loss of the 51-bp indel in a single deletion event. In contrast to the network however, this group of mostly L-types appeared to be most recently diverged rather than basally positioned (Figs. 2.3, 2.4).

The major difference between the two phylogenetic analyses is the way in which the two main clades were connected to each other and rooted. In the tree, the *S. hystrix* and the *S. caliendrum* clades shared a most recent common ancestor through haplotype D2, which has only been reported from the GBR (Fig. 2.4). In the short and long alignment networks (results not shown), the *S. hystrix* and *S. caliendrum* clades also shared an extinct/ unsampled most recent common ancestor most closely related to D2, rather than being connected through ShF1b as shown in the medium alignment network (Fig. 2.3). The network was rooted at the L-type clade in all three alignments; however, rooting of the network did not affect the relationships within or between clades for any alignment (data not shown). Nevertheless, the evolutionary direction implied by the networks suggested that L-types sharing the 51-bp indel form an ancestral state from which the large indel could have been lost once prior to the divergence of the *S. caliendrum* clade from the *S. hystrix* clade (Fig. 2.3). Although the L-types were not similarly implicated as an ancestral lineage in the tree analysis, L-type ShF2 was still close to the ancestral root (Fig. 2.4). Separation of the other L-types from ShF2 in the tree is presumably due to less weight on the shared 51 bp indel compared to the network analyses.

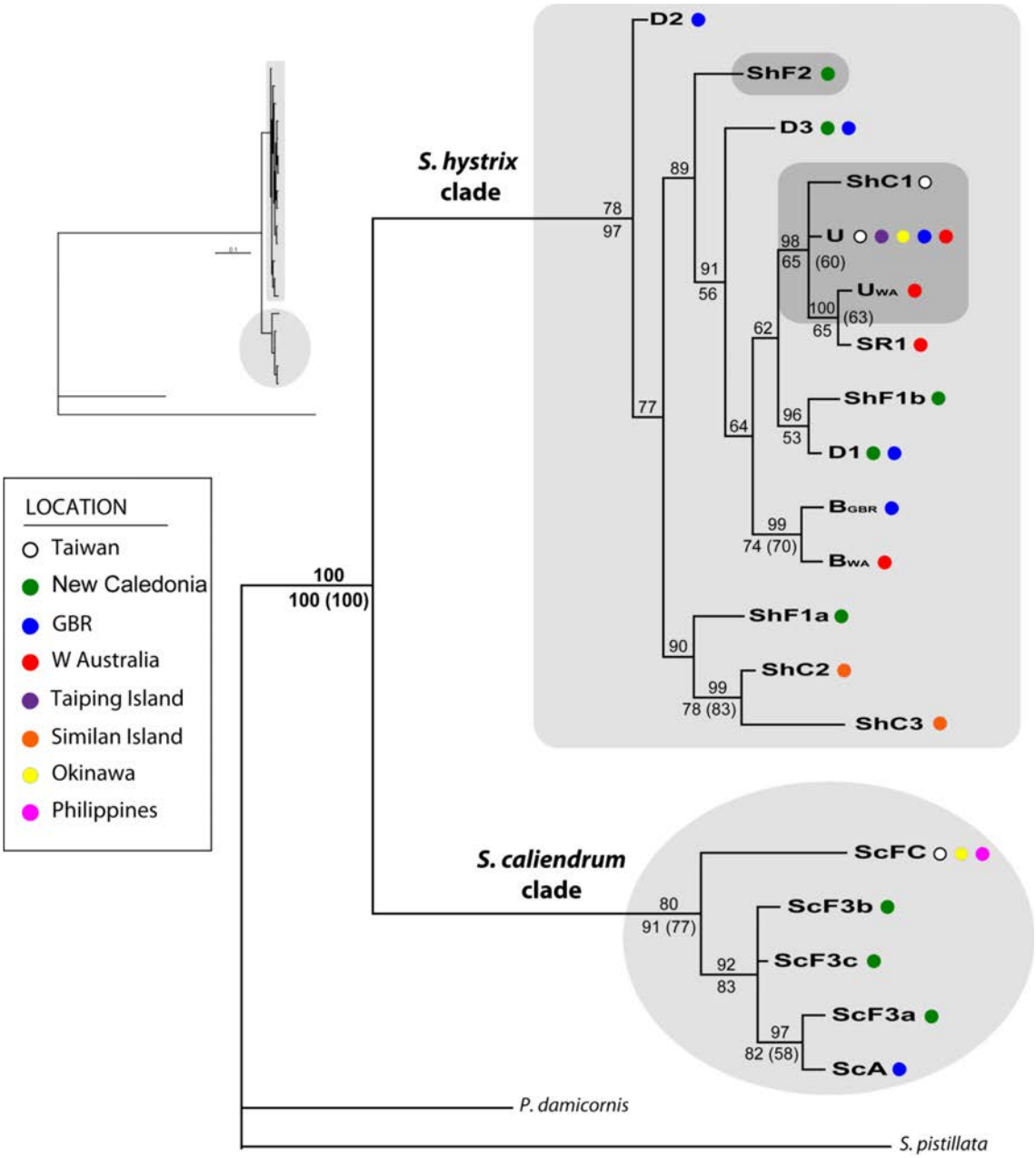


Figure 2.4 Consensus tree constructed by Bayesian phylogenetic analysis with all published *Seriatopora* spp. mitochondrial haplotypes (556 bp control region, short alignment) in MrBayes v3.2.1. Outgroup taxa include pocilloporids *P. damicornis* and *S. pistillata*. Branch support above 50% shown as Bayesian posterior probabilities based on 10^6 MCMC replicates (above branches), and bootstrap values based on 1000 replicates of maximum parsimony (below) and ML (in parentheses below). Colored circles denote regional location of sampled corals (key to left of tree). Mitochondrial haplotypes containing the 51-bp insertion (Large amplicon, L-types) highlighted in dark gray. Light grey rectangle denotes the *S. hystrix* clade haplotypes. Light grey oval denotes the *S. caliendrum* clade haplotypes. Correct scale phylogenetic tree unlabeled in upper left. See Table 2.5 for explanation of haplotype names.

2.3.3 Microsatellite analysis

2.3.3a *Structure* assignment of genetic clusters

All 964 *Seriatopora* individuals were genotyped and included in the *structure* analysis. When all samples were combined, the Evanno ΔK method strongly indicated K=3 as the uppermost hierarchical level of structure (Fig. 2.5A). Only 4.1% of individuals (n=40) were not assigned by at least 90% to a single genetic cluster, and standard errors of mean individual assignment proportions are ≤ 0.2%. At this level of subdivision, the three clusters corresponded to one large cluster dominated by sheltered habitat samples (yellow; n=510), and two smaller clusters containing mostly exposed habitat samples shared across regions (turquoise and dark red; Fig. 2.5A). In the second tier of analyses, the sheltered group (yellow) broke down according to geographic region at K=2, which was indicated by ΔK as the uppermost hierarchy of structure (Fig. 2.5B right, yellow box). The larger exposed cluster (turquoise; n=339) showed multiple levels of subdivision: A strong peak at ΔK=2 combined the darker blue group of the Palm Islands with the mixed region green group (data not shown), however a second smaller peak at ΔK=3 indicated further separation of the green cluster (Fig. 2.5B left, turquoise box). The smaller exposed group (dark red; n=115) split into two clusters, the larger of which was shared across regions (Fig. 2.5B center, dark red box).

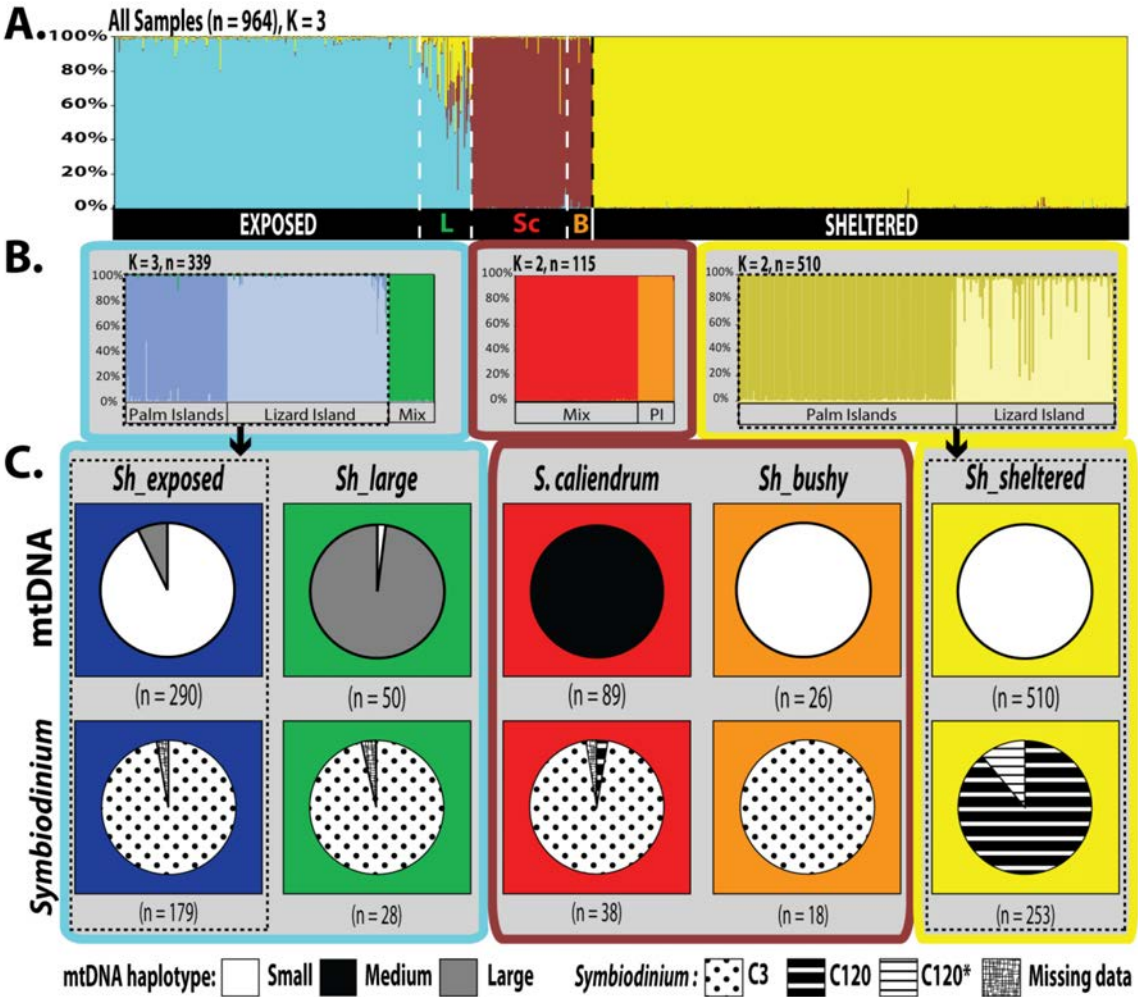


Figure 2.5 Hierarchical *structure* analyses used to designate five *Seriatopora* clusters: **A) 1st tier analysis:** Mean individual assignment plot including all 964 individuals at K=3, five replicates. Samples cluster strongly by habitat: sheltered or exposed. Location of genetic clusters indicated with white dashed lines and color-coded label (see C below). L (green), Sc (red) and B (orange) denote location of individuals in plot that belong to *Sh_large, S. caliendrum* and *Sh_bushy* clusters, respectively. Individuals to the left of L are *Sh_exposed*, to right of B are *Sh_sheltered*. **B) 2nd level of hierarchy** (border colors match original 1st tier clusters): Mean individual assignment plots for the three original clusters, each run separately. K-levels (indicated above plots) selected based on ΔK and L(K). Dashed black line indicates regional-specific clusters inferred to belong to the same genetic cluster. **C) Cross-referenced hierarchical and reef analyses (Fig. 2.6)** indicate five genetic clusters, designated by color and name (See Table 2.6 for naming explanation). Pie diagrams show proportion of major mtDNA type (amplicons: Small, Medium, Large; top row of pie diagrams) and *Symbiodinium* ITS2 type (bottom row) for each cluster. Keys for amplicon size (solid colors) and *Symbiodinium* type (patterns) given below pie diagrams.

In a second series of *structure* analyses, a bottom-up approach was applied to assess the existence of sympatric and distinct genetic clusters within each of the ten reefs sampled (Fig. 2.6; Table 2.3). As expected, the ability of the method to detect distinct clusters was improved by the elimination of geographic influences, with only one of 964 individuals not assigned by at least 90% to a single genetic cluster. Most individual assignments exceeded 95% to a single genetic cluster and seven of the ten reefs contained more than one genetic cluster (Fig. 2.6). Cross-referencing hierarchical and reef-level analyses resulted in the designation of five genetic clusters, all of which occurred in both regions, except one rare group only sampled from the exposed habitat of the Palm Islands (Fig. 2.6 orange cluster). However, the genetic clusters were not evenly distributed or abundant across regions or reefs (Fig. 2.6; Table 2.4). Furthermore, the reef-level results generally showed strong habitat influences on the genetic identity of associated seriatoporids. While sheltered reefs were almost entirely populated by the yellow cluster (99.2% of sheltered samples), the exposed reefs contained the majority of individuals representing the other four genetic clusters (Fig. 2.6). Nevertheless, cross-habitat migrants (i.e., individuals found in the opposite habitat to that in which the genetic cluster is usually found) were detected, but in very low numbers (e.g., 2 blue individuals at W Orpheus, 2 green at NW Orpheus, 3 yellow at SE Lizard). The W. Lizard location was an obvious outlier in terms of genetic makeup (Fig. 2.6), potentially reflecting a dissimilar, specifically deeper, habitat compared to other sheltered reefs.

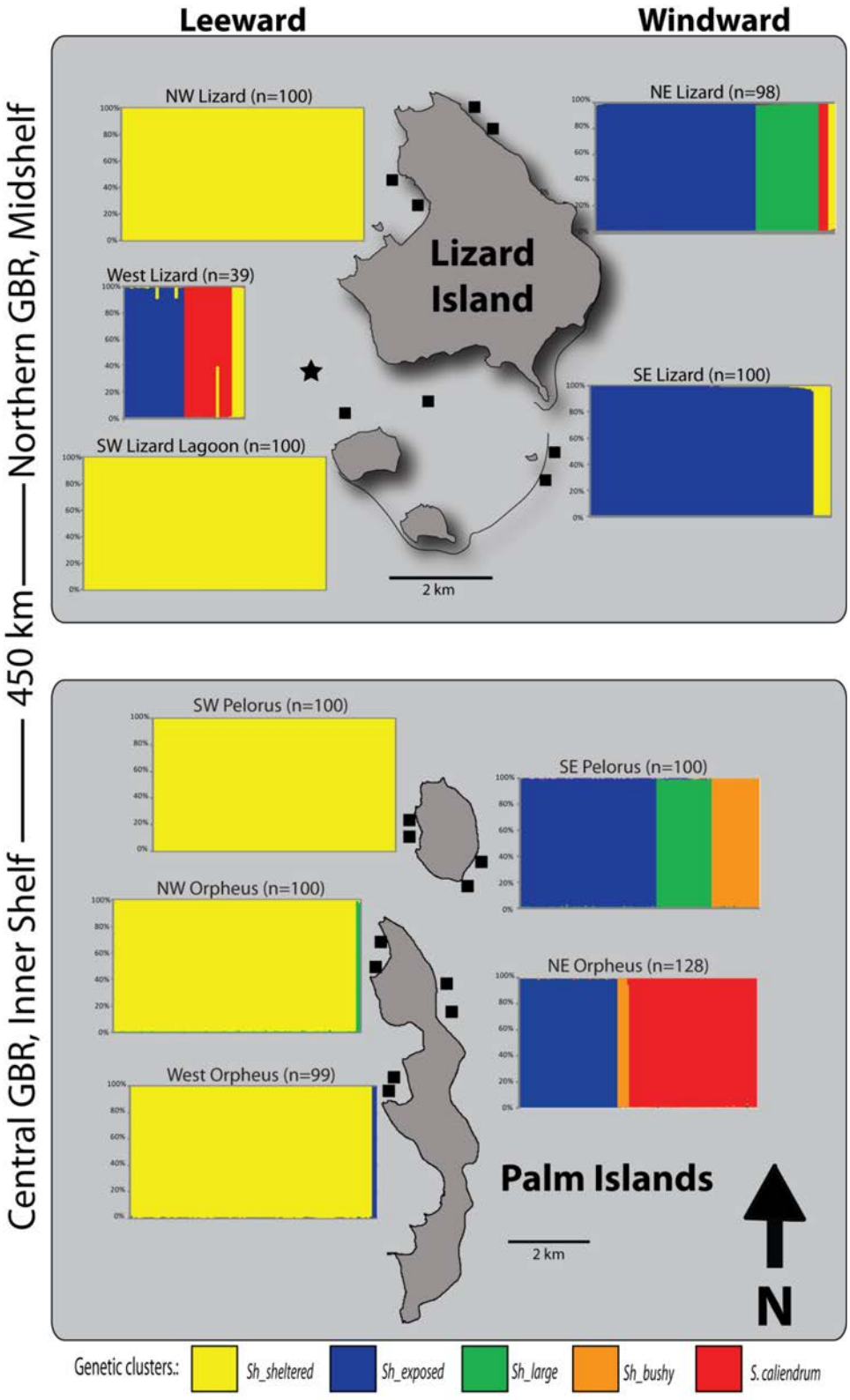


Figure 2.6 Mean individual *structure* assignment plots (5 replicates) for each of ten reefs sampled in the northern and central GBR for *Seriatopora* spp. Black squares denote locations of replicate sites within reefs. Colors match genetic cluster designations in Fig 2.5, key at bottom. K-values selected according to ΔK and L(K), with clusters cross-referenced to hierarchical analysis.

The genetic clusters are represented by the following names and colors based on distinctive characteristics (i.e., habitat, morphology, mtDNA), which will be further explained throughout the chapter: *S. caliendrum* (red), *Sh_exposed* (blue), *Sh_sheltered* (yellow), *Sh_large* (green), *Sh_bushy* (orange) (Figs. 2.5C, 2.6; see Table 2.6 below for naming explanations). Briefly, all individuals originally identified (morphologically at time of sampling) as the ***S. caliendrum* morphospecies** belonged to the red cluster, which was exclusively comprised of **M-type mtDNA (medium amplicon) of the *S. caliendrum* clade** (Figs. 2.5C; 2.3). The other four genetic clusters belong to the *S. hystrix* mtDNA clade, and contained individuals of either S- and/or L-types (small or large amplicon; Table 2.6): **1) *Sh_bushy* (orange)** exhibited a distinct **"bushy" morphology** (explained below in Section 2.3.6). **2) *Sh_sheltered* (yellow)** individuals dominated the **sheltered habitat** (Fig. 2.6). **3) *Sh_exposed* (blue)** individuals were the dominant cluster in **exposed habitats** (Fig. 2.6). **4) *Sh_large* (green)** was a rarer group, only found in significant numbers at one exposed reef in each region (NE Lizard and SE Pelorus; Fig. 2.6), and **almost exclusively comprised of L-type mtDNA (large amplicon)** (Fig. 2.5C). Note that the substantial amount of admixture apparent in the *Sh_large* samples for the first tier of the hierarchical analysis (Fig. 2.5A) was inferred to be a signal of hierarchical differentiation among clusters, and was not replicated in the site-specific analyses (Fig. 2.6). A NJ tree of all individuals using pairwise genotypic distances confirmed the same cluster groupings as the *structure* analysis (Appendix 2.1), including the somewhat stronger geographic separation between regions within the *Sh_exposed* cluster. In contrast, the individuals of other genetic clusters were more evenly mixed within their respective clades (Appendix 2.1).

Table 2.6 Description and naming of five *structure*-assigned genetic clusters of *Seriatopora* detected in corals sampled from the central and northern GBR. Names used in this study and explanation for name based on most distinctive feature of each cluster; color codes used throughout chapter. Individuals fall into major mtDNA control region haplotype groups based on three amplicon sizes: Small (no indel); Medium (24 bp indel); Large (51 bp indel). mtDNA CR sequence haplotypes for putative species members that were sequenced at W. Lizard site. Question mark emphasizes that the majority of individuals were not sequenced and are of uncertain mitochondrial makeup.

***Structure* assigned genetic cluster**	**Naming - distinctive feature**	**Color code**	**mtDNA CR amplicon**	**mtDNA CR haplotype**
S. caliendrum	*S. caliendrum*		Medium	ScA/ ?
Sh_bushy	Sh - S. hystrix; bushy - morphology		Small	-
Sh_exposed	Sh - S. hystrix; exposed - dominant habitat		Small/ Large	D3/ ?
Sh_large	Sh - S. hystrix; large - dominant mtDNA		Large/ Small	-
Sh_sheltered	Sh - S. hystrix; sheltered - dominant habitat		Small	B/ ?

2.3.3b Population level analyses of *structure* clusters

Following the *structure* assignment of the five genetic clusters, the dataset was separated into thirty populations based on cluster designation and sampling site (Table 2.4). Eleven matching multilocus genotypes (MLG) appeared among *Sh_sheltered* populations of the Palm Islands and one matching pair occurred at Lizard Island. As all of these either occurred in different sites and reefs or had genotype probabilities (GP) greater than 0.001, all individuals were retained for further analysis. In contrast, only three matching MLGs were found in two populations of *Sh_exposed*, but only one individual was retained for each pair as all occurred as close neighbors at the same site and had very low GP (Table 2.4). The only other species that exhibited matching MLGs was *Sh_bushy,* with three pairs of colonies. One pair at SE Pelorus did not meet the elimination criteria and both were retained, but one individual each was removed for the two other MLGs (Table 2.4). The genotyping success rate across all individuals and loci exceeded 98% for all four *S. hystrix* clade genetic clusters, but *S. caliendrum* failed to amplify in 31.4% of samples at locus Sh4-010, and 3.4 – 4.5% of samples in three other loci. Estimated null allele frequencies were low (mean 1.5% ± 0.2% SE)

and did not affect the generally large pairwise population differentiation estimates: the mean difference between uncorrected and ENA corrected F_{ST} values was 0.004 (± 0.0002).

The total number of alleles per locus across all individuals ranged from 3 to 22, and mean allelic diversity was 3.29 (± 0.12). Loci polymorphism ranged from 70 – 80% in *Sh_sheltered* populations, while only two of nine *Sh_exposed* populations displayed less than 100% polymorphic loci (Appendix 2.2). *Seriatopora caliendrum* populations exhibited variable loci polymorphism affected by small population sizes, from 60% of loci in NE Lizard (n=4) to 90% at NE Orpheus 1 (Appendix 2.2). Each *Sh_large* population was monomorphic at two loci, but only one was common to both. The two *Sh_bushy* populations displayed low diversity associated with small population size, with 6 and 3 loci fixed for NE Orpheus (n=4) and SE Pelorus (n=19) sites, respectively. Nineteen of the thirty populations were fixed for the same allele at locus Sh3-003 (75% *S. caliendrum* (3), 50% *Sh_bushy* (1), both *Sh_large*, and all 13 *Sh_sheltered*; Appendix 2.2). The one *S. caliendrum* and one *Sh_bushy* populations that were not fixed contained the same allele at 99% and 94.7% frequencies, respectively. In contrast, *Sh_exposed* populations exhibited frequencies at that Sh3-003 allele up to 52.9% in the Palm Islands (SE Pelorus), but none of the five *Sh_exposed* populations at Lizard Island contain the allele at all. A similar pattern occurred at locus Sh3-008 for eighteen non-*Sh_exposed* populations (Appendix 2.2).

Polymorphic loci were largely in HWE for most populations (Appendix 2.2). Only 6.3% of loci displayed significant departures from HWE across all populations. However, 17.5% of loci in *S. caliendrum* and 15% in *Sh_large* populations showed significant heterozygote deficits (Appendix 2.2). For the other three genetic clusters, 95 – 97% of loci met expectations of HWE. In addition, only five locus pairs (0.4%) were found to be in genotypic linkage disequilibrium in four populations (Appendix 2.2). Expected heterozygosity (H_E) ranged from 0 to 0.863 for a single locus in *S.*

caliendrum, but was generally highest in *Sh_exposed* populations (mean H_E = 0.471 ± 0.024) and lowest in *Sh_bushy* populations (mean H_E = 0.257 ± 0.09; p=0.006 for all five clusters compared). The total number of private alleles (PA) for all populations in each genetic cluster was 24 for *S. caliendrum*, 16 for *Sh_exposed*, 8 for *Sh_sheltered*, 4 for *Sh_bushy*, and 2 for *Sh_large*. *Seriatopora caliendrum* exhibited eight PAs at three loci shared between the two regions, with a mean frequency of 16% (± 6%). *Sh_exposed* contained six shared PAs at 5 different loci shared between regions, with mean frequency of 16.6% (± 4.6%). The four PAs found in *Sh_bushy* populations had at a mean frequency of 22% (± 11%), but two of which, when combined, exceeded 82% frequency at locus Sh3-004. Both *Sh_large* (7% frequency) and *Sh_sheltered* (0.2% frequency) populations exhibited only one PA each, shared between the two regions.

A hierarchical AMOVA attributed 35.1% of total genetic variation among the *structure*-defined clusters (F_{CT} = 0.351 ±0.038, p=0). Another 10.7% of variation was apportioned among populations within clusters (F_{SC} = 0.165 ±0.022, p=0), which included the interregional level of spatial-genetic structure. Pairwise population estimates of F_{ST} and G''_{ST} were averaged for each genetic cluster comparison (Table 2.7), and illustrated that standardized versions of population differentiation statistics yielded much higher values of differentiation (~1.7x that of F_{ST}). Nevertheless, the estimates of differentiation were large using either statistic, and the overall pattern of structure within and between genetic clusters was unchanged by different statistics. Within clusters, *Sh_sheltered* (mean G''_{ST} = 0.240 ±0.015) and *S. caliendrum* (mean G''_{ST} = 0.260 ±0.077) generally showed greater similarity among populations than *Sh_exposed* (mean G''_{ST} = 0.353 ±0.035) and *Sh_large* (G''_{ST} = 0.337; Table 2.7A). The greatest separation between clusters occurred between *Sh_exposed* and: *Sh_sheltered* (mean G''_{ST} = 0.801 ±0.003), *Sh_bushy* (mean G''_{ST} = 0.802 ±0.008), and *S. caliendrum* (mean G''_{ST} = 0.804 ±0.01; Table 2.7A). The lowest differentiation

between clusters was between *Sh_large* and all others (G''_{ST} ranged from 0.62 – 0.67), and between *S. caliendrum* and *Sh_bushy* (mean G''_{ST} = 0.658 ±0.018; Table 2.7A). Statistical tests of genotypic differentiation indicated that only 19 of 435 pairwise population comparisons were not significant (Appendix 2.2). Each of the six comparisons between replicate sites for Palm Islands *Sh_sheltered*, *Sh_exposed*, and *S. caliendrum* implied panmictic populations within reefs, but the same pattern was not true for Lizard Island populations. Non-significant values of population differentiation at Lizard Island only occurred between small populations (e.g., NE Lizard *S. caliendrum* or *Sh_sheltered*) and their potential local sources (Appendix 2.2).

Table 2.7 Mean pairwise estimates of population differentiation calculated with ten microsatellites within and between five genetic clusters within the genus *Seriatopora,* based on 30 populations in the central and northern GBR: **A)** G''_{ST} and **B)** F_{ST}. Standard errors are shown to right of average in italicized font. Values in color are the within genetic cluster mean population differentiation estimates averaged for all within and between region comparisons.

A.

S. caliendrum	0.260	*0.077*								
Sh_bushy	0.658	*0.018*	0.083	-						
Sh_large	0.662	*0.023*	0.666	*0.028*	0.337	-				
Sh_exposed	0.804	*0.010*	0.802	*0.008*	0.620	*0.016*	0.353	*0.035*		
Sh_sheltered	0.747	*0.010*	0.787	*0.005*	0.642	*0.007*	0.801	*0.003*	0.240	*0.015*
G''_{ST}	***S. caliendrum***		***Sh_bushy***		***Sh_large***		***Sh_exposed***		***Sh_sheltered***	

B.

S. caliendrum	0.126	*0.029*								
Sh_bushy	0.450	*0.020*	0.061	-						
Sh_large	0.401	*0.017*	0.407	*0.025*	0.190	-				
Sh_exposed	0.470	*0.009*	0.469	*0.010*	0.337	*0.012*	0.184	*0.019*		
Sh_sheltered	0.481	*0.010*	0.519	*0.009*	0.387	*0.006*	0.464	*0.004*	0.155	*0.011*
F_{ST}	***S. caliendrum***		***Sh_bushy***		***Sh_large***		***Sh_exposed***		***Sh_sheltered***	

A PCA of pairwise D_{est} (Jost 2008) demonstrated strong and practically equidistant separation among *Sh_sheltered*, *Sh_exposed* and *S. caliendrum* populations (Fig. 2.7; Appendix 2.3). *Sh_sheltered* and *Sh_exposed* populations were located at the opposite ends of principal component 1 (50.23% of variation), while both opposed *S. caliendrum* along axis 2 (23.6% of variation). Both *Sh_bushy* and *Sh_large*

overlapped the spread of *S. caliendrum* populations along axis 1, however associated closest to *S. caliendrum* or *Sh_sheltered*/ *Sh_exposed* along principal component 2, respectively (Fig. 2.7). Within clusters, populations from different regions tended to separate along axis 2, but not always in the same direction (e.g., compare *Sh_sheltered* with *Sh_exposed*).

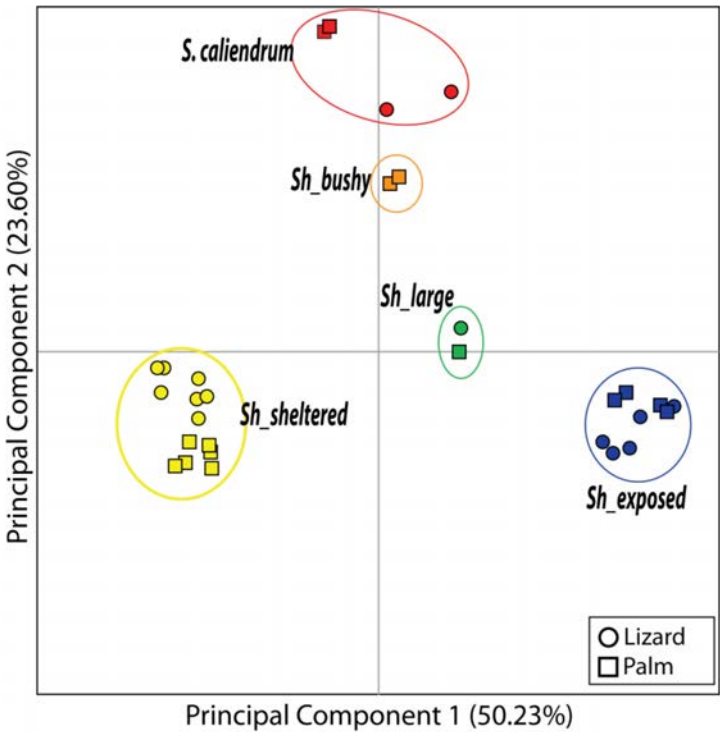
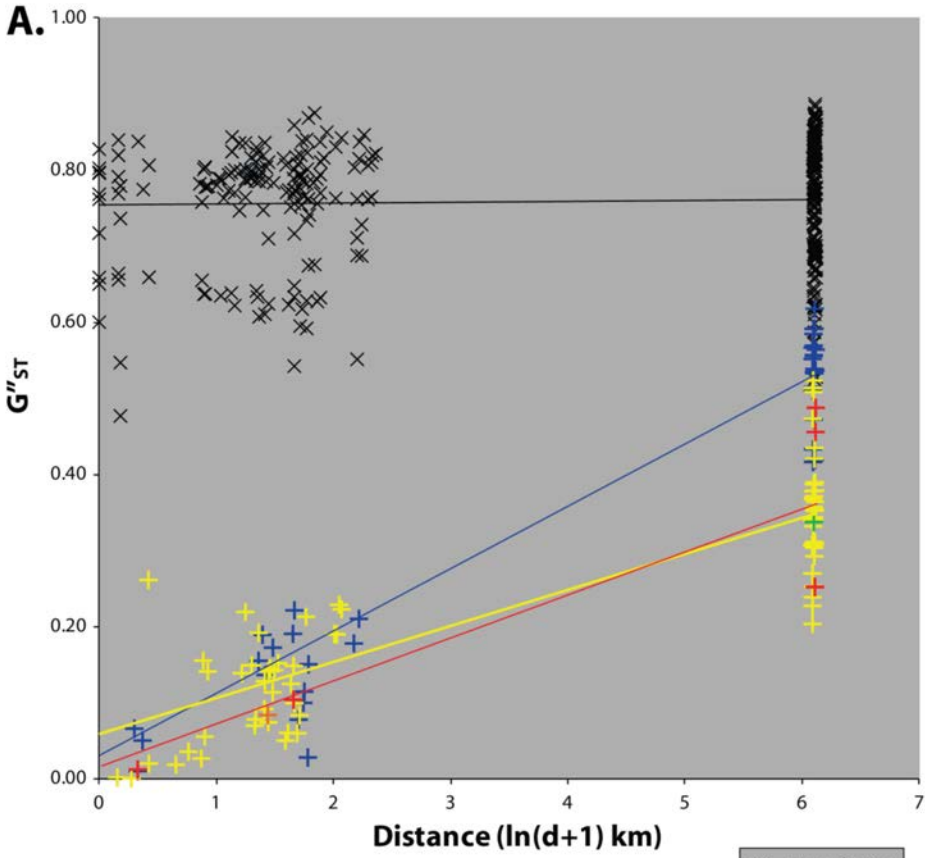


Figure 2.7 Principal component analysis calculated on pairwise D_{est} (Jost 2008) values. Colors indicate *structure*-defined genetic clusters. Shapes denote the region of each population: circle – Lizard Island; square – Palm Islands.

The relationship of pairwise G''_{ST} and geographic distance revealed patterns within and among clusters as expected for putative species based on the premise of Good and Wake (1992) (Fig. 2.8). Between cluster comparisons indicated more or less fixed divergences regardless of geographic separation (y-intercept= 0.734, R^2= 0.002). In contrast within clusters, both *Sh_exposed* (b=0.003 ±0.056; R^2= 0.933, p=0.001) and *S. caliendrum* (b= -0.003 ±0.11; R^2= 0.724, p=0.03) showed strong positive correlations

of genetic separation with distance, and regression lines that intercepted the y-axis very close to the origin (Fig. 2.8B). The y-intercept of the *Sh_sheltered* regression was slightly further from zero (0.052 ±0.067), but genetic differentiation was still highly correlated with geographic distance (R^2= 0.755, p=0.001). The y-intercepts of intraspecies regressions for *S. caliendrum*, *Sh*_exposed, and *Sh_sheltered* were not significantly different from zero (p-values <0.001). Insufficient sample sizes prevented similar tests among *Sh_bushy* and *Sh_large* populations.



B.

Pairwise comparisons	Slope	y-intercept	R^2
within *Sh_sheltered*	0.048	0.052	0.755
within *Sh_exposed*	0.087	0.003	0.933
within *S. caliendrum*	0.060	-0.003	0.724
Between clusters	0.001	0.734	0.002

Figure 2.8 Pairwise genetic distance ($G"_{ST}$) versus geographic distance (ln(1+ km)) for all 30 populations of *Seriatopora* from the northern and central GBR. **A)** Plot of distances for comparisons between genetic clusters (black x) and within clusters (color coded +), and the respective color-coded regression lines. **B)** Summary of regression estimates for slope, y-intercept, and R^2 values.

2.3.4 Comparison of mtDNA and microsatellite phylogenies

2.3.4a mtDNA identity of the *structure*-assigned genetic clusters

Although only a small subset of individuals were sequenced at the mitochondrial control region (W. Lizard site; Table 2.1), those 39 individuals included representatives from three of the five genetic clusters, *S. caliendrum*, *Sh_exposed*, and *Sh_sheltered* (Fig. 2.9). Sixteen individuals sequenced from W. Lizard were most closely related to previously published sequences of *S. caliendrum*, as expected from morphology (Fig. 2.9). All *structure*-assigned *S. caliendrum* individuals contained the 24 bp indel (M-types) characteristic of *S. caliendrum* clade sequences (Fig. 2.3; Table 2.6), although it is unknown whether *S. caliendrum* individuals in other populations had the ScA haplotype found at W. Lizard. Mitochondrial sequences from the four *Sh_sheltered* individuals at W. Lizard matched haplotype B (Figs. 2.9, 2.3), which has only been previously reported in 'back reef' habitats of the northern GBR region (Bongaerts *et al.* 2010b). Moreover, *Sh_sheltered* individuals from all twelve populations were exclusively comprised of S-type mtDNA (small amplicon; Fig. 2.5C). The nineteen *Sh_exposed* individuals sequenced at the mtDNA putative control region matched haplotype D3 (Fig. 2.9), which is an S-type first reported in Bongaerts *et al.* (2010b) in very low abundance at deep sites (~27 m) of the northern GBR. However, *structure* assignments indicated some admixture of L- and S- mtDNA types in *Sh_exposed*; twenty-one L-type individuals were included in the *Sh_exposed* group (7%; Fig. 2.5C). *Sh_large* was composed of L-type (large amplicon) mtDNA, except for a single S-type individual assigned to the group (2%; Fig. 2.5C). Although none were sequenced, all L-type corals are expected to be the same or closely related to mitochondrial sequence haplotype U (Figs. 2.3, 2.4), as it is the most widespread mtDNA haplotype and the only one previously reported from the GBR that contains the 51-bp indel (Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a). *Sh_bushy* corals only had the S-type mtDNA (Small amplicon; Fig. 2.5C).

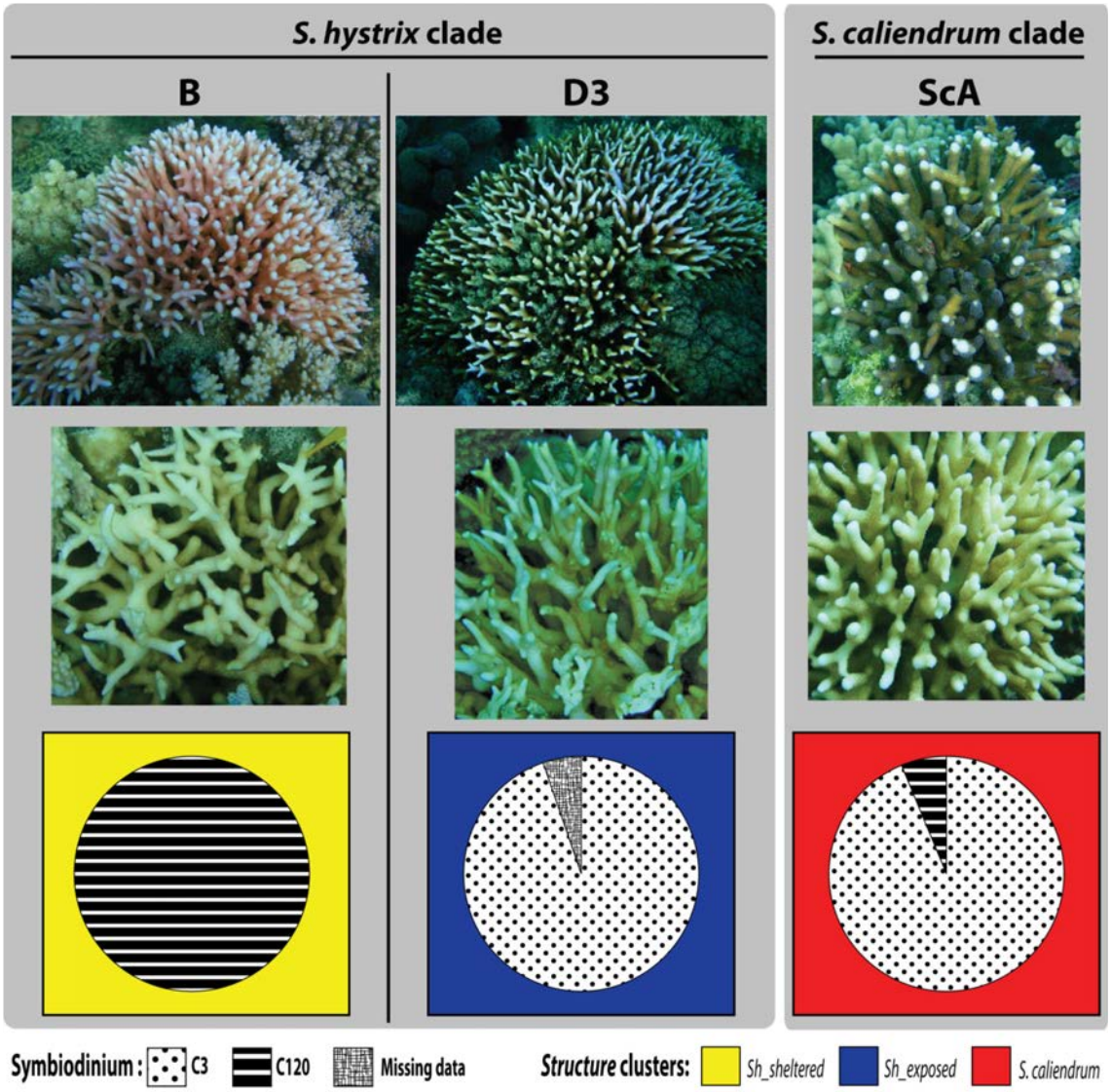


Figure 2.9 Summary of mtDNA sequence haplotypes (B, D3, ScA), colony morphology, branch morphology, *structure*-designated genetic clusters (colored squares; see key at bottom right of figure), and *Symbiodinium* ITS2 type (patterned pie charts; see key at bottom left of figure) for West Lizard Island site.

2.3.4b Microsatellite population-level phylogeny of genetic clusters

The NJ tree of all thirty populations using D_A distance (Nei *et al.* 1983) from microsatellite allele frequencies showed strong support for monophyletic cluster clades and strong interregional separation within clusters (Fig. 2.10; Appendix 2.3). Furthermore, the sympatric occurrence of distinct genetic clusters was emphasized by the spread of sample locations across different clades (Fig. 2.10, shapes).

Sh_sheltered, *Sh_exposed* and *Sh_large* formed a subclade (80% bootstrap support (B)), which did not include *Sh_bushy*. The microsatellite phylogeny suggested that *Sh_bushy* may be a more basal group within the *S. hystrix* clade (Fig. 2.10), based on comparison to the mtDNA phylogeny (Fig. 2.4) and an apparently closer relationship to *S. caliendrum* (Fig. 2.7). Similarly, the strong subclade containing the other three *S. hystrix* groups closely resembled the mtDNA phylogeny and further supported the supposition that *Sh_sheltered* (99% B) corals correspond to mtDNA haplotype B and *Sh_large* (73% B) to haplotype U (Figs. 2.4, 2.10). The potential mtDNA identity of *Sh_exposed* (95% B) might likewise be inferred as D1 based on concordance between microsatellite and mtDNA phylogenies (Figs. 2.4, 2.10). However, given that *Sh_exposed* contained D3 individuals at W. Lizard as well as some L-types (Fig. 2.5C), this group clearly has a more complex mitochondrial composition. In particular, the strong separation among habitats in *Sh_exposed* Lizard Island populations (91% B; Fig. 2.10) might be driven by mitochondrial diversity within *Sh_exposed* among different populations. Notably, a strong separation also occurred among islands (i.e., Pelorus Is. vs. Orpheus Is.) within *Sh_sheltered* Palm Island populations (86% B).

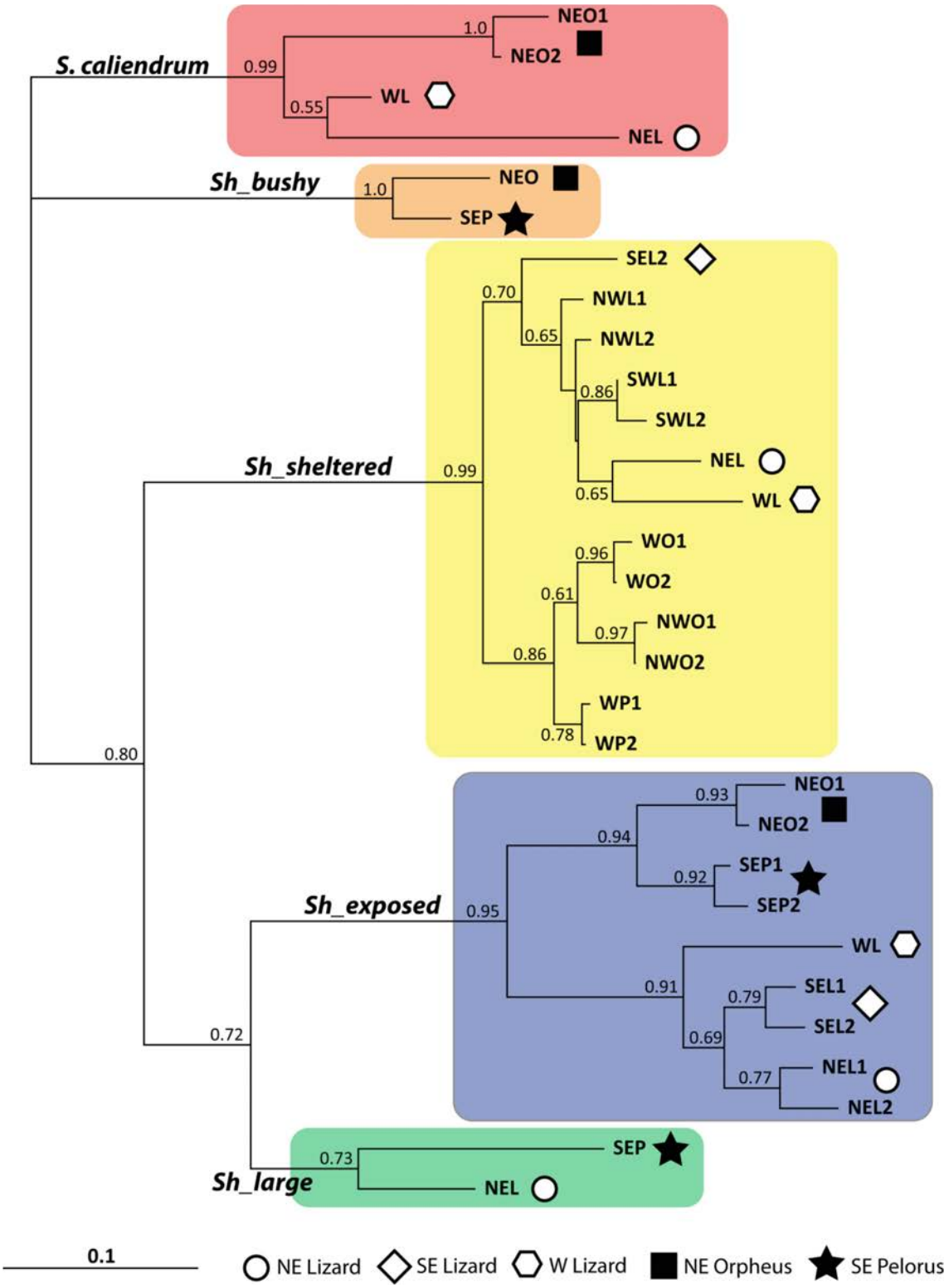


Figure 2.10 NJ tree of all 30 *Seriatopora* populations from the northern and central GBR, calculated on D_A distance (Nei 1983) from microsatellite allele frequencies with 10,000 bootstrap replicates. Five genetic clusters form strong clades, labeled and indicated with color codes. Bootstrap proportions above 50% are provided. Shapes (see key at bottom of figure) denote populations from reefs that contain multiple clusters.

2.3.5 *Symbiodinium*-host coupling of genetic clusters

Patterns of *Symbiodinium*-host coupling were strongly concordant with *structure*-assigned genetic clusters (Fig. 2.5C). A subset of 516 individuals genotyped at the *Symbiodinium* ITS2 region demonstrated the dominant influence of habitat on symbiont type. Regardless of habitat origin, however, *Sh_sheltered* corals exclusively harbored *Symbiodinium* type C120 (Figs. 2.5C, 2.9). About half of the individuals genotyped at the sheltered SW Pelorus sites contained an additional, very similar yet undescribed, co-dominant *Symbiodinium* sequence (one mutation different), referred to in this study as C120* (unpublished) and not detected at any other reef (Fig. 2.5C). The only other coral to contain *Symbiodinium* type C120 was a single *S. caliendrum* individual at W. Lizard (Fig. 2.9). Interestingly, this same individual occurred mixed into the *Sh_sheltered* clade in the NJ tree of all individuals based on pairwise genotypic distances (Appendix 2.1), and admixed between the *Sh_sheltered* (39.7% assignment) and *S. caliendrum* (60.0% assignment) clusters in the *structure* analyses (Fig. 2.6). Moreover, a separate hybrid index calculated for this individual using the same two clusters as parental sources indicated almost identical proportions of cluster contributions (59.6% *S. caliendrum* (CI: 30 – 86%), 40.4% *Sh_sheltered*). All other genetic clusters harbored a dominant symbiont type identified as *Symbiodinium* ITS2 type C3 (Lajeunesse *et al.* 2010). Some additional bands may have been present in DGGE profiles, but either did not reamplify as single bands, suggesting heteroduplexes, or could not be sequenced successfully.

2.3.6 Gross morphological characterization of genetic clusters

Photographs of all individuals were grouped according to the five *structure*-assigned genetic clusters (Fig. 2.11). The representative morphologies presented here demonstrate the strong overlap in macroscopic morphological characteristics amongst clusters, including between the two previously named morphospecies, *S. hystrix* and *S. caliendrum*. Nevertheless, based on the samples analyzed *in situ*, *S. caliendrum*

usually had distinctively thicker, blunt-tipped branches compared to other co-occurring clusters (e.g., NE Orpheus and W. Lizard), and could be indentified where it occurred in numbers at least moderately proportional to other seriatoporids (Figs. 2.9, 2.11). Of the ten reefs sampled, *S. caliendrum* only occurred in significant numbers at two reefs, but when *S. caliendrum* was rarer, the distinctive morphology was not detected at the time of sampling (i.e., NE Lizard. n=4; Fig. 2.6). Furthermore, some colonies from other genetic clusters strongly resembled the typical *S. caliendrum* morphology (Fig. 2.11; e.g., *Sh_sheltered* top photo, *Sh_exposed* bottom photo). The one other distinctive morphology observed besides *S. caliendrum* was that of *Sh_bushy* individuals. The limited sample of *Sh_bushy* (n=26) showed that colonies consistently exhibited many short lateral branchlets along primary vertical branches, which did not appear in other genetic clusters within the same exposed habitat (Figs. 2.12, 2.11). The three most common clusters belonging to the *S. hystrix* morphospecies (*Sh_exposed, Sh_sheltered,* and *Sh_large*) exhibited a similar range of morphologies. Cross-habitat migrants were infrequent (i.e., individuals found in the opposite habitat to that in which the cluster was usually found), and did not provide conclusive evidence of consistent morphological differences between the other three *S. hystrix* clade clusters (Fig. 2.11, top photos in black box). However, morphologies of the different clusters from W. Lizard did suggest slight distinctions between *Sh_sheltered* (B) and *Sh_exposed* (D3) in the same habitat (Fig. 2.9). *Sh_sheltered* colonies appeared to have somewhat thicker branches, whereas *Sh_exposed* branches were somewhat more delicate. *Sh_sheltered* colonies were also much more likely than any other cluster to display pink coloration, which was the dominant color morph at W. Orpheus (data not shown).

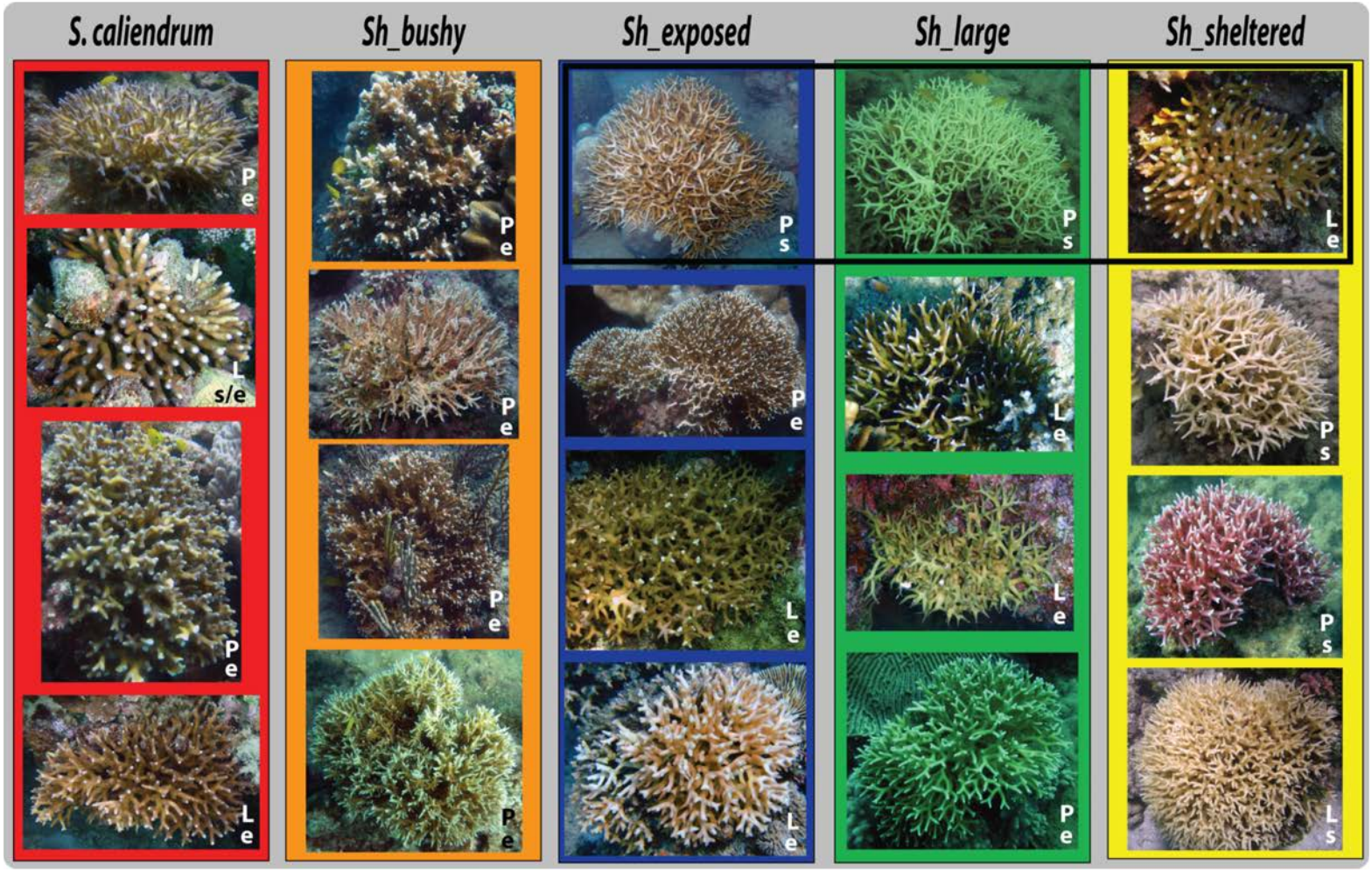


Figure 2.11 Representative colony morphologies for *structure*-assigned genetic clusters. Examples from both regions and both habitats provided, when possible. L: denotes Lizard Island specimens; P: denotes Palm Island specimens; e: denotes exposed habitat; s: denotes sheltered habitat; s/e: denotes specimen from W Lizard site. Black box indicates cross-habitat migrants for the respective species, i.e. individuals found in the opposite habitat to that in which cluster is usually found (*Sh_large* typically found in the exposed habitat).

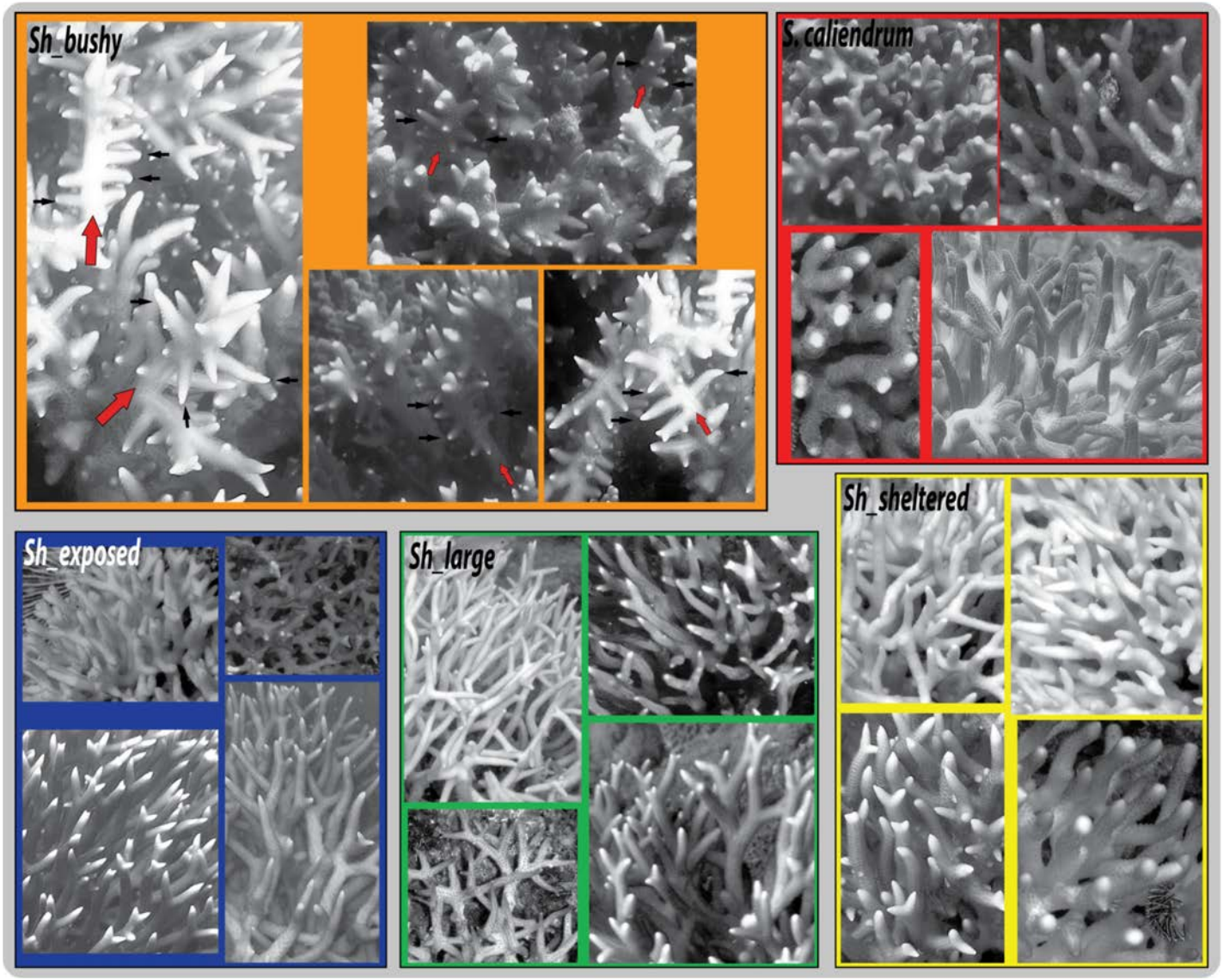


Figure 2.12 Branching morphology of five *Seriatopora* genetic clusters, photos labeled and denoted by background color (See Table 2.6). The distinctive "bushy" morphology of the putative species *Sh_bushy* (orange background) is shown in top left: red arrows indicate primary vertical branch, smaller black arrows denote the short, lateral branchlets. Other clusters branch morphologies vary, but do not typically exhibit "bushy" lateral branclets.

2.4 DISCUSSION

Multiple lines of evidence, including from nuclear and mitochondrial markers, colony morphology, habitat specificity, and *Symbiodinium* associations, were combined to identify five genotypic clusters of *Seriatopora* on the Great Barrier Reef (GBR). Four of these clusters were found in sympatry across two regions of the GBR (northern Lizard and central Palm Island groups) separated by 450 km. An additional rare group was observed only in the Palm Islands (*Sh_bushy*). The *S. caliendrum* cluster represents the previously described morphospecies of the same name. The other four genetic clusters (*Sh_sheltered, Sh_exposed, Sh_large*, and *Sh_bushy*) have been lumped historically into the single morphospecies *S. hystrix*. Of these four clusters, *Sh_bushy* was the only one to display a distinctive morphology at the sites sampled. A deep divergence was detected between two major clades that represent the two morphospecies currently described on the GBR, *S. hystrix* and *S. caliendrum,* based on mitochondrial phylogenies of all published seriatoporid sequences worldwide. nDNA microsatellite data corroborated this division, and, in addition, demonstrated that the level of divergence separating the four *S. hystrix* clusters was similar to that observed between *S. hystrix* and *S. caliendrum*.

2.4.1 Microsatellite clusters likely represent cryptic species

The four microsatellite clusters identified within the current *S. hystrix* morphospecies are likely to represent cryptic species. My hypothesis is based on the strong cohesion demonstrated within genetic clusters across regions of the GBR that maintained discreteness when in direct sympatry with other genetic clusters. Moreover, these four clusters were corroborated with similarities between mtDNA and microsatellite phylogenies, and generally consistent associations found between microsatellite clusters and the four other characters evaluated: amplicon size of the mtDNA marker analyzed, host-symbiont coupling, sheltered vs. exposed habitat preference, and colony morphology (for *Sh_bushy*). Without detailed morphological

data at corallite and skeletal microarchitecture levels, however, it is still unclear how these molecular units correspond to existing morphological species descriptions in this relatively small genus. Nevertheless, comparison of extensive data from Australian studies with information from other regions strongly suggests that there is more species diversity on the GBR within the genus *Seriatopora* than previously reported. To reduce cumbersome terminology, for the remainder of the thesis, I will refer to the four genetic clusters delineated by microsatellite clusters within the current morphospecies *S. hystrix* as cryptic species, but clearly acknowledge that these are putative cryptic species.

2.4.2 mtDNA phylogeny and phylogeography of the genus *Seriatopora*

Mitochondrial phylogenies of my data combined with published data resolve two main clades and nineteen haplotypes of *Seriatopora* across a wide range of geographic locations in the Indo-Pacific. The 556 sequences were collected from studies that either targeted *S. hystrix* (Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a) and *S. caliendrum* (Chen *et al.* 2008), or that sought to represent the full array of morphological diversity in the genus (Flot *et al.* 2008), which currently is comprised of six species globally (Veron 2000). My recovery of two principal clades, a *S. hystrix* and a *S. caliendrum* clade, concurs with previous work on the genus (Chen *et al.* 2008; Flot *et al.* 2008). Moreover, the two large indels that occur in the mtDNA control region separate the genus into three major mitogenomic groups (S, M, L) represented by amplicons of the new mtDNA marker (small, medium, large). Many studies have emphasized the importance of indels in molecular sequence evolution and phylogenetic inference (Lloyd and Calder 1991; Gu and Li 1995; Freudenstein and Chase 2001; Simmons *et al.* 2001; Kawakita *et al.* 2003), albeit not without controversy (Golenberg *et al.* 1993; Lunt *et al.* 2002). In this study, the medium amplicon appeared to consistently occur in corals that conformed to the *S. caliendrum* morphology and form a divergent clade (M-types) from the *S. hystrix* clade. However, the utility of the small and large amplicons

as a diagnostic feature within the *S. hystrix* clade is questionable, given the often stronger divergence found within S- or L-types rather than between the S and L groups in both the mtDNA and microsatellite analysis. Moreover, the apparent admixture of S- and L-types in *Sh_exposed*, but not other microsatellite clusters, further emphasizes that some S- and L-types are more closely related to each other than to other haplotypes within their respective S or L groups. In summary, the new mtDNA marker provided useful additional information when combined with microsatellite analyses and other observed characteristics, but cannot be used on its own to delineate putative species in *S. hystrix*.

Although mitochondrial genes have not been useful for species delineation in some scleractinian genera with very low or no interspecies divergence (reviewed in Shearer *et al.* 2002), mitochondrial diversity in *Seriatopora* indicates promising utility for understanding phylogenetic relationships. The concordance of relationships among most closely related haplotypes in both the network and tree analyses, combined with their relative abundances and geographic distributions, suggests that there are several major sub-lineages, which may correspond to species, within the *Seriatopora hystrix* and *S. caliendrum* clades if haplotypes with sequences differing by a single mutation in the network (Fig. 2.3) and those that occur in pairs in the phylogenetic tree (Fig. 2.4) are merged. Based on these criteria, two to four unique sequences can be merged into each of three distinct phylogenetic units: 1) **U-group** – representing haplotypes ShC1, U, U_{WA}, SR1; 2) **D1-group** – haplotypes ShF1b, D1; and 3) **B-group** – haplotypes B_{GBR}, B_{WA}. Three of the four U-group haplotypes share the 51 bp insertion (i.e., L-types); the fourth S-type SR1, which differs from U_{WA} only by the absence of the indel, presumably lost in a single deletion event, is rare (2 individuals) and has only been reported from Western Australia. Similarly, in Taiwan samples, haplotype ShC1 differs from the main widespread U haplotype by a single point mutation. Most importantly, all of the geographically isolated U-group haplotypes (ShC1 (Taiwan), U_{WA} (W. Australia), SR1 (W. Australia)) co-occur with the widespread U haplotype, and have not been

reported from any location independently. Similarly, the two D1-group haplotypes co-occur at New Caledonia, but whereas D1 is also found on the GBR, ShF1b appears to be geographically isolated. In contrast, although the similar B haplotypes were reported from W. Australia and the GBR, they do not co-occur at either location and B_{WA} was rare (2 individuals) in W. Australia, not conclusively indicating how the two haplotypes are related. Phylogenetic analysis of these groupings in the *S. hystrix* clade indicates that the genetic divergences (0.6% - 1%) between terminal haplotypes (e.g., D2, D3, ShF2) and haplotype groups (i.e., D1, U, B) are substantial when compared to some other scleractinian genera, which have interspecies mitochondrial divergence as low as 0% at the putative control region (van Oppen *et al.* 2002a; Fukami and Knowlton 2005). Moreover, it is noteworthy that the sequence divergence estimates for *Seriatopora* do not fully incorporate information from the large indels. It has been suggested that Robust corals, including the Pocilloporidae, may evolve faster than their Complex counterparts (Fukami and Knowlton 2005), and there is a deep divergence of the Pocilloporidae from other families in the Robust clade (Chen *et al.* 2002). Therefore, evolutionary forces may be acting quite differently on the *Seriatopora* (Chen *et al.* 2008) and conclusions about slow mitochondrial evolution rates drawn from other well-studied and highly divergent genera, such as *Acropora* (van Oppen *et al.* 1999; van Oppen *et al.* 2001), may not be directly comparable.

The distributions of *Seriatopora* mtDNA haplotypes across the locations surveyed (Fig. 2.13C-D) do not obviously correlate to the reported ranges of the six morphospecies currently described for the genus *Seriatopora* (Fig. 2.13A; Veron 2000). Moreover, the only other previous study of *Seriatopora* to explicitly assess and report morphological diversity with genetic analysis found little congruence among morphology and mitochondrial diversity (Flot *et al.* 2008). Although *S. caliendrum* sampled from the GBR (ScA; this study) and New Caledonia (ScF3a/b/c; Flot *et al.* 2008) matched the expected morphological descriptions, specimens in the divergent ScFC haplotype from the Philippines and Okinawa were described as variable and not

fitting consistently under any one existing species description (Flot *et al.* 2008) (Fig. 2.13D). A ScFC individual from Taiwan was fully sequenced at the mitochondrial genome and reported in the literature as *S. caliendrum* (Chen *et al.* 2008); however, given the approximately 2.5% divergence between the northern ScFC haplotype (Philippines, Taiwan and Okinawa) and southern haplotypes (New Caledonia, GBR), more than one geographically limited species in the *S. caliendrum* clade may be likely. In the *S. hystrix* clade, the U-group is perhaps the most notable as it is dominated by the most abundant and widespread haplotype U that is shared across five of the eight locations surveyed (Fig. 2.13C, gray dashed line). The absence of any U-group corals from New Caledonia is surprising considering the extensive sampling conducted by Flot *et al.* (2008), but it is possible that the U-group was missed given the apparent morphological similarity of this group to other seriatoporids (Bongaerts *et al.* 2010b). In contrast to the widespread U haplotype, several divergent haplotypes are more geographically isolated (Indian Ocean (orange), GBR (blue), and New Caledonia (green)) (Fig. 2.13C), and D3 and the D1-group are both shared among the GBR and New Caledonia (turquoise dashed). The connection between W. Australia and the GBR through the B-group (purple) is tenuous given no co-occurrence of B_{GBR} and B_{WA}. As might be expected, *Seriatopora* mitochondrial diversity is highest where is has been most extensively sampled and studied (i.e., the GBR and New Caledonia), yet the genus has been historically restricted to only two species (*S. hystrix* and *S. caliendrum*) at these locations (Fig. 2.13A). Considering the evidence presented in this study for several putative cryptic species within *S. hystrix* on the GBR, which appear to correspond to particular mitochondrial haplotypes, it is likely that further efforts to assess genetic diversity across the reported range of *Seriatopora* will reveal higher species diversity worldwide than is currently recorded.

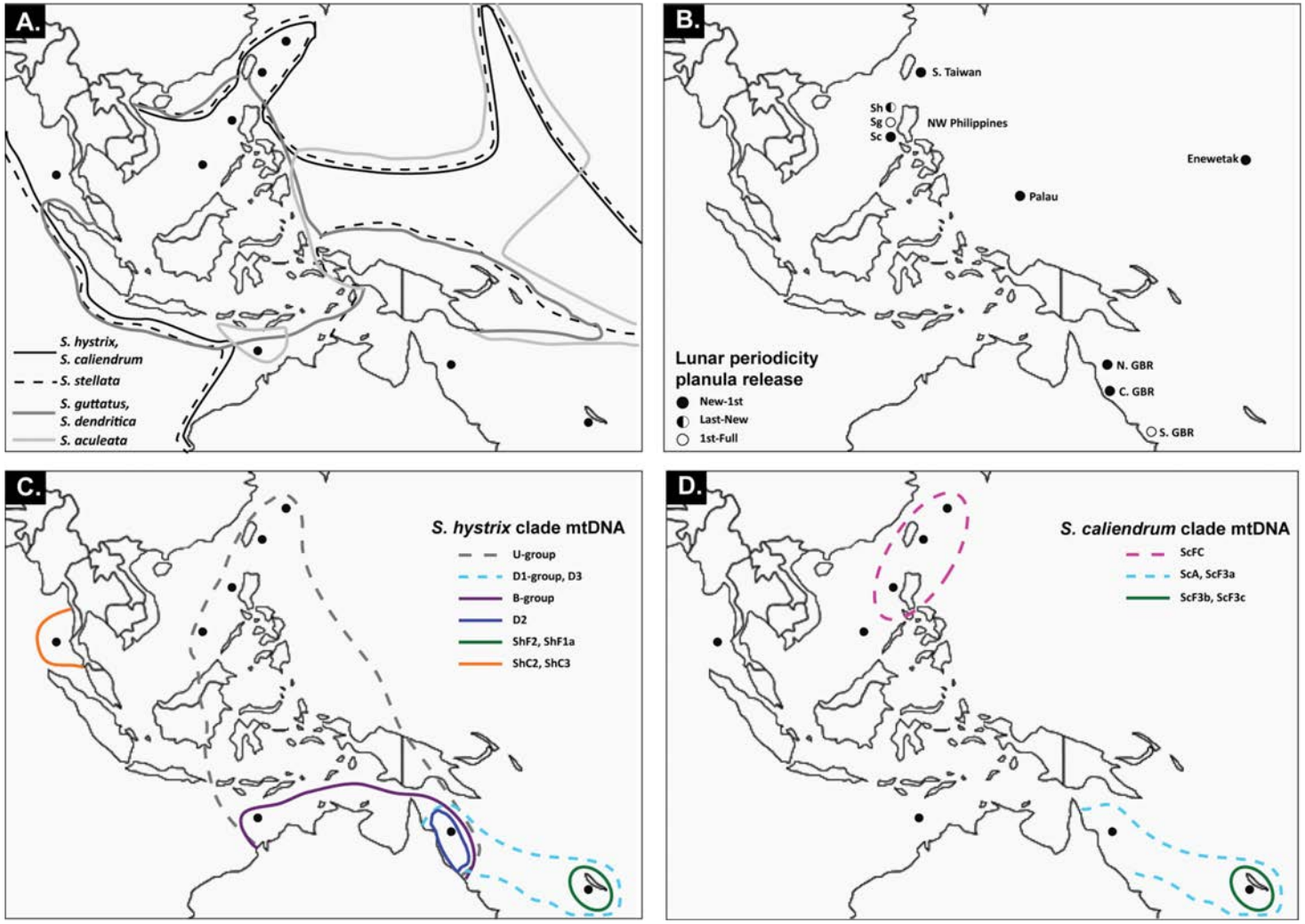


Figure 2.13 Summary of distributions, planula release periodicities, and mtDNA haplotypes for genus *Seriatopora* for the Indo-Pacific. **A)** Species distributions according to Veron (2000). *S. hystrix* and *S. caliendrum* have approximately same distribution and indicated with solid black line, similarly *S. guttatus* and *S. dendritica* both denoted by solid dark gray line. **B)** Lunar periodicities of planula release reported for *Seriatopora* in the literature (see Table 2.8). **C)** Published *S. hystrix* clade mtDNA haplotypes and geographic occurrences used in this study. **D)** *S. caliendrum* clade mtDNA haplotypes used in this study. Colored lines denote shared haplotypes or haplotype groups labeled on figure.

2.4.3 Diagnosing cryptic species in GBR *Seriatopora*

2.4.3a Population genetic analysis

Population genetic analyses from microsatellite data provide conclusive evidence of good 'genetic clusters' in sympatry (Mallet 1995), with the strong cohesion of clusters across spatially separated populations expected within species (Good and Wake 1992). The *structure* analysis clearly revealed sympatric and distinct clusters of all but one of the putative species proposed in this study, and just one case (in ~1000) of recent hybridization between a putative species (*Sh_sheltered*) and *S. caliendrum*. Only *Sh_sheltered* and *Sh_bushy* do not co-occur in the same reef, but very high genetic divergence supports their independent species status. Moreover, the ability to resolve putative species divisions, in spite of intraspecific geographic structure in the hierarchical analysis, emphasizes the reproductive independence of the five clusters over space and time. Population genetic approaches have likewise strengthened cryptic species delimitation in other corals when mtDNA was invariable and/ or inconclusive (Souter 2010; Pinzon and Lajeunesse 2011; Ladner and Palumbi 2012; Nakajima *et al.* 2012), however it is typically difficult to specify the amount of genotypic differentiation required to infer that populations are separate species. To that end, some authors have recommended a threshold of genetic differentiation to delimit species or other taxonomic groups, but usually with mtDNA sequence divergence (Hendry *et al.* 2000; Hebert *et al.* 2003; Lefebure *et al.* 2006; Richards *et al.* 2009). Although such measures are typically not sufficient for higher taxa in isolation (Avise and Walker 2000; Sites and Marshall 2004; Will and Rubinoff 2004), using direct comparisons to an established and described congener, such as *S. caliendrum* in this study, is an ideal approach for delimiting cryptic species with genetic data.

The cryptic species proposed here may at least partially explain the prominent departures from HWE, usually in the form of significant heterozygote deficits at some or

most loci, found in previous population genetic studies of *S. hystrix* throughout the Indo-Pacific, including studies on the GBR, in Western Australia, the Red Sea and the Indonesian Archipelago (Ayre and Dufty 1994; Ayre and Hughes 2000; Ayre and Hughes 2004; Maier *et al.* 2005; Underwood *et al.* 2007; Sherman 2008; Van Oppen *et al.* 2008; Noreen *et al.* 2009; Underwood *et al.* 2009; Starger *et al.* 2010). All of these studies of *S. hystrix* have operated on the assumption of a single recognizable species (Veron and Pichon 1976; Veron 2000), and have almost invariably invoked the possibility of a Wahlund effect (Wahlund 1928) and/ or extensive inbreeding, including selfing (Sherman 2008), to account for high estimated inbreeding coefficients (F_{IS}). In contrast, HWE expectations were met in the two most common putative species proposed in this study, *Sh_sheltered* and *Sh_exposed*, by 96% and 94% of loci respectively, as well as by 95% in *Sh_bushy* despite a small population size. Some of the earlier studies also provide some evidence to support habitat as a strong indicator of genetic identity. For example, both Ayre and Dufty (1994) and van Oppen *et al.* (2008) observed populations to be in HWE at lagoonal sites, which based on my results, may have been populated almost exclusively by the putative species *Sh_sheltered*. In *S. caliendrum* and *Sh_large* populations, more extensive departures from HWE may reflect the low density of conspecific mates and potential for self-fertilization (Chapter 5; Sherman 2008) in these two rarer putative species. However, none of the putative species exhibit genotypic linkage disequilibrium (LD) by more than 0.6% of loci combinations. Like previous studies that took mitochondrial diversity into account (Bongaerts *et al.* 2010b; Van Oppen *et al.* 2011a), the recognition of cryptic species of *S. hystrix* in my study appears to have largely eliminated findings of non-random mating within populations.

Although differentiation among populations within putative cryptic species is significant, consistent patterns of genetic divergence with geographic separation demonstrates that these putative *Seriatopora* species are genetically cohesive.

Inconsistent spatial-genetic correlations in earlier studies of *S. hystrix* on the GBR (Ayre and Dufty 1994; Ayre and Hughes 2000; Ayre and Hughes 2004; van Oppen *et al.* 2008), Western Australia (Underwood *et al.* 2007; Underwood *et al.* 2009) and the Red Sea (Maier *et al.* 2005) yielded inexplicable patterns. For example, Ayre and Hughes (2000) found F_{ST} values within reefs that were almost twice those between reefs separated by 1200 km. Likewise, van Oppen *et al.* (2008) found that some sites within the same reef at Lizard Island were genetically more distinct than others separated by more than 100 kilometers. In contrast, the same study indicated that other relatively large areas (~80 km) of outer reef displayed genetic homogeneity (van Oppen *et al.* 2008). Within the context of widespread cryptic species, these past results imply the uneven occurrence of species across different reef habitats and/ or regions. Although it is also possible that stochastic recruitment events (Connell *et al.* 1997; Hughes *et al.* 1999; Siegel *et al.* 2008; Torda 2012) influence genetic structures heterogeneously (Hellberg *et al.* 2002), the contrast in isolation by distance (IBD) patterns within and among the putative species is compelling evidence that these groups form cohesive genetic units (*sensu* Good and Wake 1992) over vast geographic areas. Populations of the testable putative species (*Sh_sheltered*, *Sh_exposed*, and *S. caliendrum*) within the same region were more closely related than those between regions, presumably due to more frequent exchange of larvae (Chapter 3). Conversely, cross-species comparisons yielded fixed genetic separation and no relationship with spatial distance. Therefore, sympatric populations of different putative species remain distinct without any perceived external barrier to reproduction. In addition, the absence of admixture in the reef specific *structure* analyses suggests that hybridization is rare, and implies that reproductive isolating or recognition mechanisms do exist (Willis 1990; Palumbi 1994; Hayashibara and Shimoike 2002).

Perhaps the most persuasive evidence for the proposed cryptic species lies in the magnitude of genetic differentiation among the different species pairs in direct

sympatry and/ or close proximity. The assignment of 35% of molecular variation among *structure* clusters by AMOVA indicates that these putative species divisions account for an enormous amount of the genetic diversity within the sample set. Moreover, this proportion far exceeds levels determined in other studies of *S. hystrix* using the same microsatellite markers for groups only partitioned by mtDNA and/ or habitat (18% and 11% respectively; Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a), or in microsatellite studies of widespread octocorals from the Mediterranean Sea that also employed hierarchical clustering prior to AMOVA (i.e., 2.7% and 6% of molecular variation explained (Ledoux *et al.* 2010b; Mokhtar-Jamaï *et al.* 2011, respectively). Furthermore, the large numbers of private alleles (PA) shared by individuals within each of the putative species demonstrate their evolutionary distinctiveness. In particular, *S. caliendrum*, *Sh_exposed*, and *Sh_bushy* have several PAs at multiple loci with high frequencies (>15%) and, notably, *Sh_bushy* is almost fixed for PA (>82%) at one of the ten loci. *Seriatopora caliendrum* also fails to amplify in one of the ten loci, signifying probable sequence divergence of primer flanking regions (Primmer *et al.* 1996; Wright *et al.* 2004) and further emphasizing the deep divergence between the *S. caliendrum* and *S. hystrix* clades. Similarly, ascertainment bias at microsatellite loci (i.e., a decline in allelic variability with genetic divergence from the source species (Ellegren *et al.* 1997; Wright *et al.* 2004), originally developed on unknown focal species from the GBR and W. Australia (Underwood *et al.* 2006), may at least partially explain the large discrepancies in heterozygosities and allelic diversity amongst putative *Seriatopora* species, in particular, the two loci that were fixed for almost all populations of *S. caliendrum*, *Sh_bushy, Sh_large* and *Sh_sheltered*, but not populations of *Sh_exposed*. Finally, the degree of separation between putative species pairs in this study is very large (>0.620 G''_{ST}) for all interspecies comparisons, however the approximately equal divergence among taxa within the *S. hystrix* morphospecies versus that between *S. hystrix* and *S. caliendrum* (mean G''_{ST} ~0.720) emphasizes the clear separation of the putative cryptic species.

2.4.3b Combining mtDNA, Symbiodinium, and habitat data

Combined evidence from microsatellite data, *Symbiodinium* type and habitat collectively indicate that at least some of the *structure*-assigned putative species do correspond to specific mtDNA haplotypes discussed above (Section 2.4.2). In particular, *Sh_sheltered* appears to be strongly concordant with mtDNA haplotype B based on exclusive coupling with symbiont type C120, dominance of the sheltered habitat, and clustering with the four B individuals sequenced from the W. Lizard site. These same characteristics of haplotype B concur with previous findings from the northern GBR (Bongaerts *et al.* 2010b). In addition, two other putative species, *Sh_bushy* and *S. caliendrum* are comprised of a single major mitochondrial group, S- and M-type respectively, although the S haplotype is not exclusive to *Sh_bushy*. The *S. caliendrum* species is clearly supported by the concurrence of morphology, microsatellite clusters and mitochondrial sequence data (W. Lizard); although, it is unknown whether Lizard and Palm Island sequences are identical. *Sh_bushy* is the only other putative species with a distinctive morphology. Its rarity in the sample set, occurring only on the deeper exposed sites of the Palm Islands (Bongaerts *et al.* 2010b), and phylogenetic relationship at microsatellite loci, which is divergent from other *S. hystrix* cryptic species and closest to *S.* caliendrum, suggests that *Sh_bushy* may be similar to or the same as haplotype D2. Although Bongaerts *et al.* (2010b) found greater resolution in *Symbiodinium* ITS2 C3 profiles than the general C3 type presented here, including a characteristic type (C3-ff) for deep water D2 corals only found at 27 m, the ITS2-DGGE technique can yield variable interpretations depending on the relative abundances of co-occurring *Symbiodinium* types (Lajeunesse *et al.* 2003; Sampayo 2007; Stat *et al.* 2008; Bongaerts *et al.* 2011). Thus, the general C3 type found in association with *Sh_bushy* in my study does not preclude the possibility that more specific C3 variants were present.

The microsatellite cluster *Sh_large,* which is comprised of the L haplotype except for a single S-type individual, is strongly suspected to belong to the mitochondrial U-group, because the U-group is widespread throughout the Indo-Pacific (Fig. 2.13C) and all L-type samples previously sequenced from the GBR had the U haplotype (Bongaerts *et al.* 2010b; van Oppen *et al.* 2011a). The association of *Sh_large* with *Symbiodinium* type C3 in my study accords with U corals in Western Australia, which associated with a variety of symbiont types over different depths (van Oppen *et al.* 2011a), however Bongaerts *et al.* (2010b) found that U corals exclusively harbored *Symbiodinium* type C120 at two northern GBR outer reef locations across discrete depth habitats. The discrepancy of host-symbiont coupling for L-type corals of the northern GBR in my study compared to Bongaerts *et al.* (2010b) may either reflect the different depths sampled here in comparison to the previous study, or potentially additional mitochondrial diversity within the L-types not previously documented.

Sh_exposed has the most complex mitochondrial makeup, with significant admixture of L-type individuals into the dominant S-type group. The similarity between mtDNA and microsatellite phylogenies does implicate an affiliation between *Sh_exposed* and haplotype D1, which is the dominant component of the 'deep slope' (27 m) surveyed by Bongaerts *et al.* (2010b) and most closely related to U. Still, the more divergent D3 corals sequenced from W. Lizard also clustered in the *Sh_exposed* group. Previously, haplotype D3 was sampled only in very low abundance at 27 m (Bongaerts *et al.* 2010b). However, in this study, I sampled a depth intermediate to the discrete upper slope (~6m) and deep slope (~27m) habitats surveyed in earlier studies (Bongaerts *et al.* 2010b; Van Oppen *et al.* 2011a), capturing an apparent zone of mtDNA type overlap. Moreover, D3 corals sampled from New Caledonia were found at variable, but generally shallower depths (0.6 – 16.2 m; Flot *et al.* 2008), implying that the D3 haplotype might be more abundant in intermediate depth zones. In previous studies, both D1 and D3 corals were found to host symbiont profile C3n-t (Bongaerts *et*

al. 2010b), which can appear almost identical to C3 in DGGE banding profiles depending on product concentrations (Lajeunesse *et al.* 2003; Sampayo 2007; Stat *et al.* 2008). *Sh_large* and *Sh_exposed* are the only two of the putative species that exhibit two major mitochondrial haplotypes (S- and L-types in both cases) and seem to be the most closely related of any species pair. Although there is evidence from *Sh_sheltered* and *S. caliendrum* at West Lizard that hybridization is possible between the *S. hystrix* and *S. caliendrum* morphospecies, there is no indication of recent admixture between *Sh_large* and *Sh_exposed* in the reef specific *structure* analyses. Therefore, more ancient origins of introgression, polymorphism and/ or incomplete lineage sorting are implicated for mtDNA paraphyly among these closely related putative species, such as has been inferred for the genus *Acropora* (Van Oppen *et al.* 2001; Ladner and Palumbi 2012).

2.4.3c Morphology and the need for further operational characters

Discrepancies between molecular and morphological groupings within the genus *Seriatopora* highlight the need for revision of the taxonomic framework for this genus. Veron and Pichon (1976) acknowledged this lack of sufficient distinguishing characters when they first stated that the two morphospecies, *S. hystrix* and *S. caliendrum*, can be separated “on the basis of gross skeletal characters alone” only when the two species co-occur in the same biotope, and more recently when *S. caliendrum* was described as “poorly defined” (Veron 2000). Essentially the single expected distinction is that *S. caliendrum* branches “are usually thicker” compared to those of *S. hystrix* within the same microhabitat (Veron and Pichon 1976). Operationally, it is commonly assumed that branches of *S. hystrix* are tapered, whereas those of *S. caliendrum* are not; however, review of the type specimens (Veron and Pichon 1976) and extensive experience in the field indicate that this assumption does not hold. Importantly, following molecular confirmation, it was found that *S. caliendrum* could generally be distinguished from the four putative cryptic species

within *S. hystrix* based on gross morphological appearance. Briefly, *in situ S. caliendrum* colonies typically have thicker branches and inflated tissues that make branch tips appear untapered, although skeletal morphology may still be tapered when tissues are removed or specimen are observed out of water (P. Warner pers. obs.). Moreover, inflated tissues and extended tentacles may cause *S. caliendrum* live tissues to appear somewhat fuzzy when closely inspected. Still, four *S. caliendrum* specimens were unknowingly collected in this study as part of the *S. hystrix* population at NE Lizard, where *S. caliendrum* existed in low abundance. There is evidence to suggest that misidentification has also occurred across morphospecies boundaries in previous studies of *S. hystrix* on the GBR. For example, the published photograph of *S. hystrix* presented in Ayre and Hughes (2000) (Fig. 1D in Ayre and Hughes (2000)) bears remarkable similarity to the typical *S. caliendrum* morphology (P. Warner pers. obs.). In addition, subsequent investigations of an exceptionally genetically diverse Lizard Island site (West Lizard, = site Lizard 2 in van Oppen *et al.* 2008) yielded high numbers of *S. caliendrum* and one apparent morpho-hybrid, suggesting that the earlier study of *S. hystrix* (van Oppen *et al.* 2008) may have incorporated mixed morphospecies. Clearly, the apparent rarity of *S. caliendrum* in many GBR locations may result in continued multispecies sampling if morphological identification is used as the sole criterion to delineate species in this genus.

The four *S. hystrix* cryptic species proposed in this study cannot be distinguished morphologically based on the current taxonomic description, which explicitly allows for large variability in morphologies. Although the pocilloporid family is widely assumed to comprise highly morphologically plastic species, there is little experimental evidence of phenotypic plasticity confounding described species borders. Anecdotally, shallow-water *S. hystrix* ecotypes displayed some ability to alter growth form when transplanted to deep sites in a study of *Symbiodinium* adaptation (Bongaerts *et al.* 2011). However, the only explicit morphological study conducted on

S. hystrix to date found that one month old recruits adjust growth form according to gravity over a one month period (Meroz *et al.* 2002), and the broader implications of these results have not been tested. On the other hand, the limited morphological analysis conducted for this study detected one obviously unique growth form, that of *Sh_bushy*, which seems to correspond to colonies in Figures 102 – 104 and 113 of Veron and Pichon (1976). This distinct morphology is characterized by short lateral branchlets along the primary vertical and dichotomizing branches, which are not usually apparent in other putative species. Importantly, the *Sh_bushy* corals exhibited this distinctive morphology in the same habitat as other putative species, thus it does not appear to be a general phenotypic response of *Seriatopora* to a particular depth habitat. A recent study of *P. damicornis* found that several distinct genetic groups correspond to morphological variants from Eastern Australia (Schmidt-Roach *et al.* 2012), and closer examination of skeletal morphologies in *Seriatopora* may similarly reveal that additional features exist to distinguish putative cryptic species.

2.4.3d Patterns in reproductive behaviors of *Seriatopora*

Variation in the magnitude of interregional population differentiation within putative cryptic species emphasizes that demographic and genetic processes may influence the putative lineages differently, potentially reflecting underlying disparities in reproduction, larval characteristics or other processes affecting population dynamics. Indeed, strong seasonal variability in recruitment of seriatoporid larvae amongst different habitats (i.e., sheltered vs. exposed) in the Lizard Island and Palm Island regions (Torda 2012) suggest underlying differences in reproduction among putative species. Furthermore, variation in lunar periodicity of planula larvae release among GBR locations in the morphospecies *S. hystrix* (Table 2.8; Fig. 2.13B) potentially reflects dissimilar reproductive behaviors in unevenly occurring putative species. In particular, larval release occurred between the 1st quarter and full moon in southern GBR *S. hystrix* populations (Tanner 1996; Sherman 2008), whereas *Sh_sheltered*

populations in central and northern GBR locations release between the new moon and 1st quarter (Chapter 4). Although reproductive timing is expected to vary according to latitudinal position, potentially explaining these discrepancies, the central/ northern GBR lunar periodicity for *Sh_sheltered* (i.e., peak new moon) is reported for a wide range of latitudes from *S. hystrix* populations in back reef/ lagoonal habitats in the broader Indo-Pacific region (Fig. 2.13B; Table 2.8), demonstrating a strong consistency across geographically widespread populations. Nonsynchronous larval release among putative species implies variation in the timing of gamete development and/ or sperm release, which might be expected to evolve in congeneric species to minimize interspecies hybridization during spermcasting events (i.e., sperm release for brooding organisms (Bishop and Pemberton 2006)). One study found that lunar periodicities of larval release were characteristic for each of three *Seriatopora* morphospecies that co-occur in the Philippines (Table 2.8; Fig. 2.13B; Villanueva *et al.* 2008b). That study reported periodicities that generally do not match reports from the GBR (e.g., *S. caliendrum* matches central/ northern GBR *Sh_sheltered*; Fig. 2.13B); however, as different regional species may be present between the GBR and the Philippines based on the phylogeographic analysis of *Seriatopora* mtDNA presented above (Section 2.4.2), direct comparisons are not justified. Nevertheless, the fact that co-occurring congeneric *Seriatopora* species exhibit distinctive larval release periodicities (Villanueva *et al.* 2008b), suggests that similar patterns may be evident in mixed species assemblages on the GBR. Thus, comparative studies of reproductive behaviors among the *Seriatopora* presented in my study are warranted and may reveal additional characters that distinguish these putative cryptic species.

Table 2.8 Summary of reproductive studies published on *Seriatopora spp*., arranged according to lunar periodicity of larval release. Region: NW – northwest; N – northern; C – central; S – southern. MorphoSpp.: the named target species according to morphological identification. Lunar periodicity indicated by moon quarter phase, as reported in reference by phase or lunar day: black – peak season larval release period; dark grey – low season larval release period; asterisks – biphasic release peaks. Dash (-) denotes when depth or habitat was not reported. References (Ref.): 1 –Villanueva *et al.* (2008b); 2 – P. Warner (unpublished); 3 – Fan *et al.* (2002); 4 –Fan *et al.* (2006); 5 – Stimson (1978); 6 – Atoda (1951); 7 – Fan and Dai (1996); 8 – Tanner (1996); 9 – Sherman (2008).

Region	Latitude	MorphoSpp.	New	1st	Full	Last	Depth (m)	Habitat	Ref.
NW Philippines	16° 21' N	S. hystrix					4-7	-	1
NW Philippines	16° 21' N	S.caliendrum					4-7	-	1
N GBR	14° 41' S	S. hystrix					1-3	lagoon	2
C GBR	18° 34' S	S. hystrix					1-3	fringing back	2
S Taiwan	21° 56' N	S. hystrix					-	flat/ back	3
S Taiwan	21° 56' N	S. hystrix					3-8	fringing	4
Enewetak	11° 30' N	S. hystrix					0-2	lagoon	5
Palau	7° 30' N	S. hystrix					-	lagoon	6
S Taiwan	21° 56' N	S. hystrix					-	-	7
S GBR	23° 30' S	S. hystrix		*	*		5-10	slope	8
S GBR	23° 26' S	S. hystrix					0-2	flat	9
NW Phillipines	16° 21' N	S.guttatus					4-7	-	1

2.4.4 Speciation in sympatric *Seriatopora* populations

Given that extant GBR coral reef communities have been established only in the past 10,000 years following the last glacial maxima (Hopley *et al.* 1983; Carter and Johnson 1986; Chappell and Polach 1991; Jackson 1992), the question remains if these sympatric cryptic species could have evolved rapidly over this timeframe or if they resulted from more ancient speciation events. Previous studies have proposed that divergent selection acting in different habitats could produce speciation in the absence of physical barriers to gene flow, citing correlations of discrete depths with specific host-symbiont coupling in *S. hystrix*, (Bongaerts *et al.* 2010b; Bongaerts *et al.* 2011). Parapatric ecological speciation has been invoked as a mechanism by which

species could evolve without absolute allopatry (Schluter and Rambaut 1996; Lu and Bernatchez 1999; Schluter 2001; Rundle and Nosil 2005; Schluter 2009), and may be particularly relevant in the marine environment (Carlon and Budd 2002; Rocha *et al.* 2005; Duran and Rutzler 2006; Conde-Padin *et al.* 2007). Here, correlations among habitat, *Symbiodinium* type, and mtDNA do support the strong ecological segregation of *Sh_sheltered* versus the other four putative species. However, my results also demonstrate previously undetected flexibility among the presumed mtDNA type U (i.e., *Sh*_large) and *Symbiodinium* types for the northern GBR region and overlapping distributions among putative species within the exposed habitat, in contrast to the tight correlations Bongaerts *et al.* (2010b). Thus, these putative *Seriatopora* species may not be as precisely ecologically specialized or segregated as was previously proposed (Bongaerts *et al.* 2010b), and the mechanism of speciation in these sympatric populations remains unclear.

As an alternative to parapatric ecological speciation, vicariance events during Pleistocene glaciations may have contributed to allopatric speciation in different refugia, followed by secondary contact upon recolonization of the Australian continental shelf. Signatures of intraspecies divergence from past glacio-eustatic events have been inferred from the population genetic structures of other GBR marine taxa, including the coral *Acropora millepora* (van Oppen *et al.* 2011b). Yet, the strong separation observed among these putative species of *Seriatopora* suggests either deeper divergences or faster rates of molecular evolution, evidence of the latter having been found for both the family (Chen *et al.* 2002) and genus (Chen *et al.* 2008). Several reproductive characteristics of the genus may promote rapid genetic divergence and consequent speciation, including young age (size) at sexual maturity (Stimson 1978), highly localized dispersal (Chapter 3), the ability to self-fertilize (Chapter 4), and potentially high rates of mutation (Maier *et al.* 2012). Moreover, the mtDNA phylogeography of this genus indicates that some unique lineages (e.g., haplotypes B, D3) are restricted in

their distributions and may have evolved locally, while the widespread haplotype U is basal to geographically marginal haplotypes in the network analysis (Fig. 2.3). The central-marginal hypothesis for species range distributions and the importance of peripheral populations in speciation, such as *Seriatopora* of the GBR, have a long history in evolutionary theory (Bush 1975; Mayr 1982; Brussard 1984; Garcia-Ramos and Kirkpatrick 1997). In particular, it has been suggested that populations at the margins of species' ranges may be important in reef coral evolution, representing novel habitats that promote survival of offspring from introgressive hybridization (Miller and Ayre 2004; Willis *et al.* 2006; Budd and Pandolfi 2010). A recent morpho-genetic study conducted on the Caribbean *Montastrea annularis* species complex concludes that peripheral locations exhibit higher indices of evolutionary novelty than central range locations due either to lineage splitting (resulting in high morphological diversity) or fusion (low morphological diversity) (Budd and Pandolfi 2010). In summary, although several different speciation processes have likely shaped evolution of the genus *Seriatopora,* it is presently unclear how putative species reflect current species descriptions or worldwide diversity in the genus.

2.4.5 Delimiting cryptic species with hierarchical Bayesian clustering analysis

Undetected cryptic species pose a pervasive problem that can obscure true patterns in a range of biological studies, especially those of genetic connectivity. In this chapter, I developed an approach to initially detect putative cryptic species in the genus *Seriatopora* combining methods for studies of intraspecies hierarchical geographical structure (Rosenberg *et al.* 2002; Garnier *et al.* 2004; Wang *et al.* 2007; Ledoux *et al.* 2010b; Mokhtar-Jamaï *et al.* 2011) with fairly new uses of the *structure* program (Pritchard *et al.* 2000) for delimiting species (Shaffer and Thomson 2007; Bernasconi *et al.* 2010; Hausdorf and Hennig 2010; Ross *et al.* 2010; Leavitt *et al.* 2011; Ladner and Palumbi 2012). In this way, I applied Mallet's (1995) 'genotypic cluster' criterion, in which species represent distinct genetic units, in combination with the principle of Good

and Wake (1992), which states that allopatric populations of a given species maintain genetic cohesiveness across space. If several cryptic species exist, such as was inferred for the genus *Seriatopora*, some putative species are likely to be more closely related to each other than others, and evolutionary relationships may be hierarchical within a phylogenetic group. By separating the most divergent groups first using the ΔK method of Evanno *et al.* (2005), *structure* can better resolve distinct clusters in subsequent analyses among less divergent groups (Rosenberg *et al.* 2002). Moreover, by cross-referencing hierarchical phylogenetic divisions across all geographic locations with sympatric units within a given location, this methodology applies both Mallet's and Good and Wake's criteria to detect cryptic putative species with *structure*. Once those groups are putatively delimited as 'separately evolving metapopulation lineages' (*sensu* de Queiroz 2007), additional lines of evidence and operational criteria (e.g., mtDNA, morphology, ecological characteristics) can then be utilized to corroborate and characterize those units (de Queiroz 1998; Sites and Marshall 2003; de Queiroz 2007). Revealing cryptic species where they occur can significantly improve our understanding of natural organisms and populations.

Additionally, methods of species delimitation that utilize widely available data and familiar analyses are convenient to apply and may be successful across many different taxonomic groups. Of particular importance is that these analyses were conducted with data from microsatellite loci, which continue to be popular markers for genetic studies of connectivity, and one of the most familiar software programs currently employed in population genetic analyses (i.e., *structure*). Although the use of microsatellite markers for phylogenetic inference has been questioned and criticized, most prominently due to the high potential for homoplasious allele states (Estoup *et al.* 2002), methods for delimiting species need not explicitly reveal the evolutionary relationships among taxa or *how* they came to be (Mallet 1995; de Queiroz 1998; Sites and Marshall 2004). Moreover, microsatellites may be especially practical for delineating cryptic congeneric species as primers frequently amplify in closely related

species without further modification (Jarne and Lagoda 1996; Selkoe and Toonen 2006). Even in the most distantly related species of this study, *S. caliendrum*, 70% of individuals amplified at all ten microsatellite loci originally developed for *S. hystrix*. Furthermore, the *structure* program is freely available, can be run on personal computers, and has been extensively tested for various applications (Manel *et al.* 2005). A specific strength of the *structure* method, which may also be advantageous for cryptic species delimitation, is that populations, or in this case putative species, do not need to be designated or known beforehand. Therefore, analyses can be conducted free from the potential biases of a particular observer and/ or the existing taxonomy. Altogether, the methodologies employed in this chapter for delimiting putative species may prove widely effective for studies of other taxa in which cryptic species are suspected.

2.4.6 Conclusion

In summary, the described morphospecies *S. hystrix* contains at least four genotypic clusters on the GBR that are strongly divergent even in sympatry. This study compiles evidence from multiple sources (i.e., genetic, ecological and morphological) to conclude that these genetically distinct groups constitute cryptic species. Given the vast distribution range of *S. hystrix* from the Red Sea to the Central Pacific, these findings are a significant challenge to the continuing assumption of a single cosmopolitan species, and have significant implications for understanding past and future studies in this genus. Current species definitions and identification are inadequate for *Seriatopora* and molecular delimitation is the only existing method to distinguish cryptic species, yet comparisons between studies will remain tenuous without proper taxonomic descriptions that can be consistently applied. Finally, several geographically isolated and divergent *Seriatopora* lineages in the wider Indo-Pacific suggest that species diversity may be much higher than is currently realized and further effort is required to characterize worldwide diversity this abundant coral genus.

Chapter 3.0 Connectivity among populations of two cryptic species of the brooding coral *Seriatopora* is determined by highly localized dispersal

3.1 INTRODUCTION

Demographic connectivity, the linking of populations through the dispersal of individuals (i.e., larvae, recruits, juveniles, or adults), underpins the maintenance of metapopulations over ecological and evolutionary time and drives speciation processes over longer evolutionary timeframes. Characterizing the linkages between populations is essential to understanding the emergence and persistence of metapopulations (Armsworth 2002; Hastings and Botsford 2006; Botsford *et al.* 2009; Jones *et al.* 2009), a collection of subdivided populations (i.e., subpopulations) that potentially exchange individuals or genes. In particular, effective management of biodiversity and ecosystems relies on accurately measured connectivity patterns, including the spatial and temporal scales and the magnitude and direction of linkages, to inform conservation priorities and strategies. For example, demographic links among populations that may be spatially, ecologically, or behaviorally separated, largely determine the effectiveness of marine protected areas and networks (Palumbi 2003; Cowen and Sponaugle 2009; Jones *et al.* 2009). Yet, in the marine environment, where many adult organisms have sedentary or sessile lifestyles, the potential for demographic exchange is limited primarily to the dispersal of planktonic larvae that are very small and difficult to track directly. Consequently, dispersal processes must be indirectly inferred by measures of genetic connectivity representing the effective flow of genes between subpopulations, which reflect the continuum of individuals exchanged across space and time. Specifically, genetic connectivity represents the migration of individuals between populations that successfully recruit, survive and contribute to subsequent generations (i.e., reproductive connectivity). Therefore, the genetic

structure of populations is the outcome of numerous processes and factors affecting the survival and mortality of individuals over many generations, including those operating both pre-recruitment (e.g., reproductive timing, current circulation patterns, larval competency period) and post-recruitment (e.g., local adaptation, ecological and physical disturbances). Within a given species, we might expect that the extent of population connectivity varies across hierarchical spatial scales so that the same processes may not be equally important at local versus regional or global scales. Recently, an increasing number of studies have shown that many coral reef fish species have surprisingly high self-recruitment, despite extended pelagic development periods (Jones *et al.* 1999; Swearer *et al.* 1999; Taylor and Hellberg 2003; Jones *et al.* 2005; Almany *et al.* 2007). These studies suggest that population genetic surveys conducted at small spatial scales may be more likely to capture the processes important to persistence of local populations over ecological time (i.e., ecological connectivity). Such demographic and genetic processes are particularly relevant to local management efforts, as local protection will be expected to convey direct local benefits. Moreover, characterizing and understanding these smaller scale processes will help to elucidate large-scale connectivity patterns observed across a species' range.

Seriatopora hystrix is a widespread and abundant brooding coral species known to occur on reefs from the Red Sea to the Central Pacific (Veron 2000). Unlike most broadcast spawning corals that reproduce over the course of a few days once per year, brooding seriatoporid species can release larvae over several days within a lunar period and over many months to year-round depending upon the geographic location (Atoda 1951; Stimson 1978; Tanner 1996; P. Warner pers. obs.). *Seriatopora hystrix* larvae are competent to settle immediately to within a few hours of release (Atoda 1951; Isomura and Nishihira 2001) and philopatric settlement is expected. However, teleplanic larval dispersal (van Oppen *et al.* 2008; Noreen *et al.* 2009; Underwood *et al.*

2009) and rafting of recruits on marine debris or pumice (Jokiel 1990b, a; Veron 1995) may also be possible. Moreover, long distance dispersal may be facilitated by maternal transmission of algal endosymbionts to larvae (Richmond 1987, 1988), as *Symbiodinium* photosynthesis could provide larvae with the energy necessary to survive and eventually metamorphose. In contrast to confamilial *P. damicornis* (Stoddart 1983; Ayre and Miller 2004; Yeoh and Dai 2010), all genetic assays of *S. hystrix* larvae have inferred strictly sexual production (Ayre and Resing 1986; Sherman 2008; Chapter 5), yet self-fertilization (Sherman 2008; see Chapter 5) and production of asexual propagules through a variety of means (e.g. parthenogenesis, polyp bailout; Sammarco 1982; van Oppen *et al.* 2008) have also been suggested for this species. Altogether, this suite of biological characteristics produces a complex life history with frequent reproductive events that likely influence connectivity patterns on both spatial and temporal scales.

Several extensive population genetic studies of *S. hystrix* on the Great Barrier Reef (GBR) have suggested complicated patterns of low gene flow that have generally been interpreted as resulting from extremely restricted larval dispersal (Ayre and Dufty 1994; Ayre and Hughes 2000; van Oppen *et al.* 2008). Most studies document genetic structure between populations that do not follow isolation-by-distance models, specifically, some populations separated by great distances are more closely related than others in closer proximity (Ayre and Dufty 1994; Ayre and Hughes 2004; van Oppen *et al.* 2008). Similarly, studies from different regions of the GBR report variable differences between populations in separate habitats (Ayre and Dufty 1994; Sherman 2008; Bongaerts *et al.* 2010b). Although these studies have documented extreme levels of genetic divergence (e.g., F_{ST} up to 0.500) and often conclude that individual populations are highly inbred with low genetic diversity (Ayre and Dufty 1994; Ayre and Hughes 2000; Ayre and Hughes 2004; van Oppen *et al.* 2008), analyses did not take into account evidence that several distinct genetic lineages coexist on the GBR, which

likely represent cryptic species (see Chapter 2). Therefore, the seemingly inconsistent patterns found previously for *S. hystrix* may reflect the inclusion of multiple species in sample sets.

In this chapter, my objective is to analyze patterns of genetic connectivity among populations of the two most common putative species identified within the *S. hystrix* morphospecies in Chapter 2: *Sh_sheltered* and *Sh_exposed*. By identifying these putative cryptic species molecularly prior to population genetic analysis, it is hoped that connectivity patterns will be more explicable than those reported in previous population genetic studies of *S. hystrix*. I employ a balanced hierarchical sampling design to assess four spatial scales of population genetic structure within each of the two putative species: 1) between regions (~450 km); 2) among reefs within regions (2 – 10 km); 3) sites within reefs (≤ 1 km); and, 4) individuals within sites (< 500 m). The two putative species are investigated in two regions of the GBR: 1) Palm Islands (Central GBR, inshore) and 2) Lizard Island (Northern GBR, mid-shelf). Recognizing and understanding differences in connectivity patterns that influence the metapopulation persistence and resilience among species is critical to effective management.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

Reefs within two regions of the GBR, the Palm Islands in the Central Region and Lizard Island in the Northern Region (Fig. 3.1), were selected to investigate genetic connectivity among populations of the morphospecies *S. hystrix* across hierarchical spatial scales: Regions (separated by 450 km), Reefs within Regions (2 – 10 km apart), and Sites within Reefs (≦ 1 km apart). Approximately fifty colonies of *Seriatopora* spp. were sampled at each of seventeen sites between December 2008 and November 2009 (Table 3.1; Appendix 3.1). Although all sites occurred on fringing reefs, the sampling design also incorporated two distinct habitats corresponding to the windward

(eastern) and leeward (western) margins of islands, and hereafter referred to as either exposed (windward) or sheltered (leeward). In addition to different wave exposure environments, colonies at sheltered sites generally occurred at shallower depths (1 – 5 m) than at exposed sites (5 – 15 m). Colonies were photographed underwater and branches of 3 – 5 cm were sampled from colonies by hand and preserved in 100% ethanol. A handheld GPS device was synchronized to the digital camera and deployed with a surface float to track the sampling route. The geographic positions of colonies were then extracted from GPS tracks and photographs with the freeware GeoSetter 3.4.16 (©2011, Friedman Schmidt). Latitude and longitude coordinates for each colony sampled in each population were used to calculate pairwise individual geographic distance matrices in GenAlEx v6.4 (Peakall and Smouse 2006). DNA was extracted from all samples according to the protocol developed for the black tiger shrimp (Wilson *et al.* 2002) and optimized for coral tissues (Section 2.2.1).

Analyses were limited to the two most abundant of the four putative cryptic species identified within the morphospecies *S. hystrix* in Chapter 2: *Sh_sheltered* (n=505) and *Sh_exposed* (n=257) (Table 3.1), and the two putative species were analyzed separately. *Sh_sheltered* dominates sheltered habitats on the leeward sides of islands (Fig. 3.1). It exclusively harbors *Symbiodinium* ITS2 type C120 (Chapter 2) and was inferred to be comprised of host mtDNA haplotype B (Bongaerts *et al.* 2010b; Chapter 2). *Sh_exposed* is the dominant putative species found on the exposed windward side of islands (Fig. 3.1). Colonies of *Sh_exposed* harbor *Symbiodinium* ITS2 type C3, or a closely related C3 variant, and have a mixed mtDNA composition (Chapter 2).

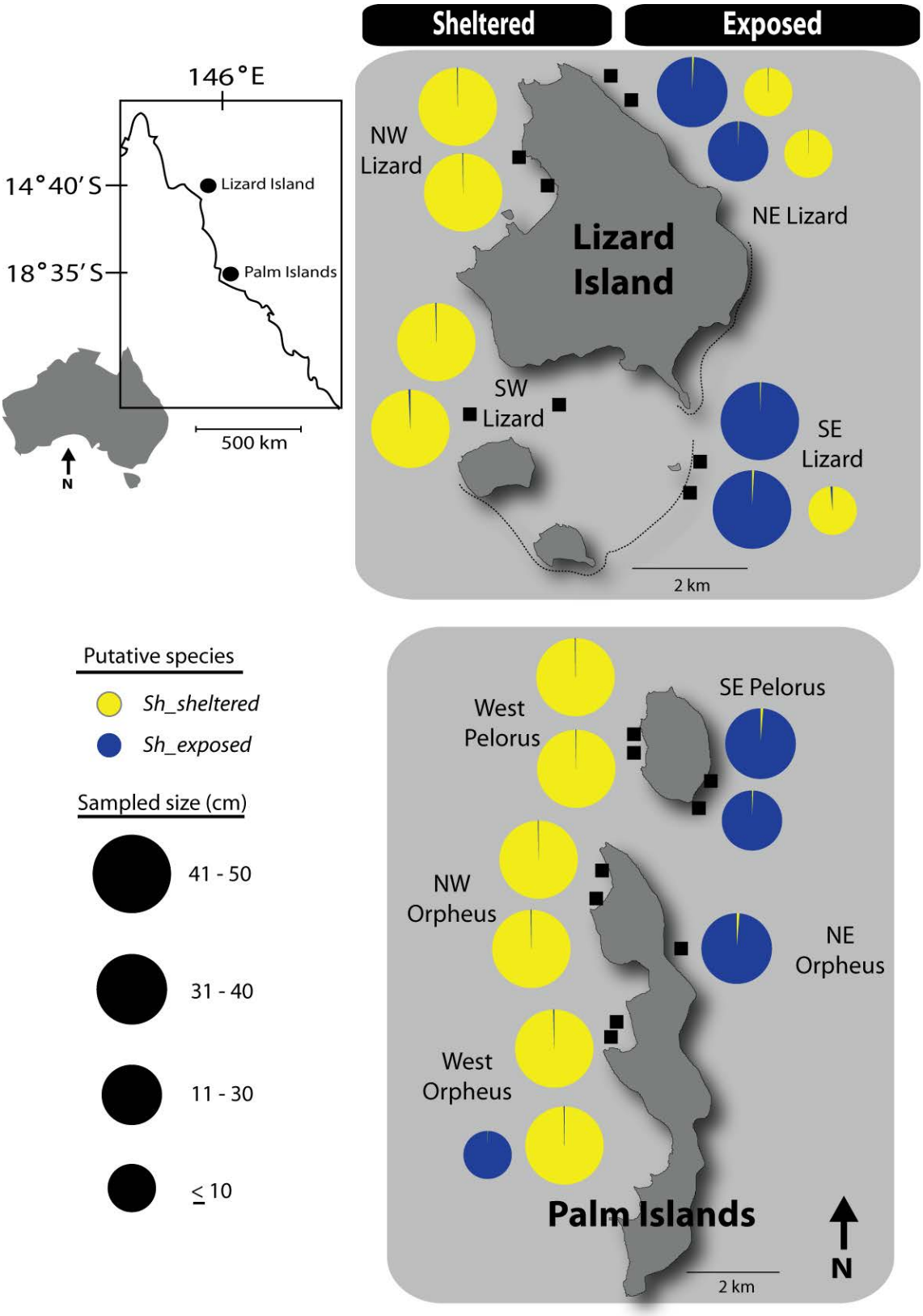


Figure 3.1 Map of sampling sites and Bayesian clustering of all samples (n=762) of the two putative species, *Sh_sheltered* and *Sh_exposed,* at K=2 genetic clusters from *structure* v.2.3 (Pritchard *et al.* 2000). Samples divided into populations by sampling site and putative species. Genetic clusters correspond to putative species and are indicated by color (yellow: *Sh_sheltered*; blue: *Sh_exposed*). Sizes of pie charts indicate the size of population samples (see key in bottom left). Habitat indicated in black bars at top right of figure.

Table 3.1 Sampling locations and sample sizes for two putative cryptic species of *Seriatopora, Sh_sheltered* and *Sh_exposed*, at sites within Palm Islands (upper) and Lizard Island (lower) regions of the GBR. N: total # colonies sampled per site; N Spp.: # individuals per putative cryptic species per location; Pop code: abbreviated population names.

	Reef	Site	Lat, Long	N Total	Species	N Spp.	Pop Code
PALM ISLANDS	West Orpheus	1	S18° 36.632, E146° 29.195	49	*Sh_shelt*	47	WO1
					Sh_exp	2	-
		2	S18° 36.545, E146° 29.233	50	*Sh_shelt*	50	WO2
	NW Orpheus	1	S18° 34.801, E146° 28.905	50	*Sh_shelt*	50	NWO1
		2	S18° 34.300, E146° 28.988	48	*Sh_shelt*	48	NWO2
	West Pelorus	1	S18° 32.864, E146° 29.274	50	*Sh_shelt*	50	WP1
		2	S18° 33.037, E146° 29.304	50	*Sh_shelt*	50	WP2
	SE Pelorus	1	S18° 33.808, E146° 30.014	17	*Sh_exp*	17	SEP1
		2	S18° 33.613, E146° 30.109	40	*Sh_exp*	40	SEP2
	NE Orpheus	1	S18° 35.458, E146° 29.884	39	*Sh_exp*	39	NEO1
LIZARD ISLAND	SW Lizard	1	S14° 41.160, E145° 27.256	50	*Sh_shelt*	50	SWL1
		2	S14° 41.248, E145° 26.606	50	*Sh_shelt*	50	SWL2
	West Lizard	1	S14° 39.408, E145° 27.034	50	*Sh_shelt*	50	NWL1
		2	S14° 39.649, E145° 27.205	50	*Sh_shelt*	50	NWL2
	SE Lizard	1	S14° 41.501, E145° 28.173	50	*Sh_exp*	50	SEL1
		2	S14° 41.648, E145° 27.965	50	*Sh_exp*	43	SEL2
					Sh_shelt	7	-
	NE Lizard	1	S14° 38.953, E145° 27.643	40	*Sh_exp*	39	NEL1
					Sh_shelt	1	-
		2	S14° 39.082, E145° 27.794	29	*Sh_exp*	27	NEL2
					Sh_shelt	2	-

3.2.2 Microsatellite statistical analysis

Populations genetic analyses of microsatellite data described in this chapter are based on the genotypic data presented in Chapter 2. All samples were amplified and genotyped as described in Section 2.2.5, with the ten microsatellite loci from Underwood *et al.* (2006) in three multiplex PCR reactions. Populations were defined by putative species and sampling site. Altogether, there were ten populations of *Sh_sheltered* and seven populations of *Sh*_exposed (Table 3.1). Initially, colonies sharing identical multilocus genotypes (MLG) were identified using GenAlEx v6.4 and genotype probabilities (GP) were calculated. All but one colony sharing the same MLG was eliminated if the matching MLG: 1) occurred within the same sampling site; and 2)

had GP < 0.001. The probabilities of identity by random sexual mating (P_{ID} and $P_{ID(sibs)}$) were also calculated in GenAlEx v6.4 to compare the overall power of the marker set between the two putative species. Summary descriptive statistics of microsatellite loci and pairwise squared genetic distance matrices for individuals (Peakall *et al.* 1995; Smouse and Peakall 1999) were computed in GenAlEx v6.4. Inbreeding coefficients (Weir and Cockerham 1984) and statistical significance of both heterozygote deficits and genotypic linkage disequilibrium (LD) were calculated by the exact test method implemented in GENEPOP v4.1.1 (Raymond and Rousset 1995; Rousset 2008) with default Markov chain parameters. The occurrence of null alleles and their effect on calculations of pairwise population differentiation were assessed using the software FreeNA (Chapuis and Estoup 2007), which calculates F_{ST} values (Weir 1996) on uncorrected and ENA-corrected data following the method described in Chapuis and Estoup (2007). The effect of null allele frequencies on F_{ST} values was negligible and all subsequent analyses were conducted with uncorrected data.

Pairwise population genetic differentiation was assessed by two methods: (1) F_{ST} values were calculated using an AMOVA approach (Excoffier *et al.* 1992) implemented in GenAlEx v6.4; and (2) F'_{ST} (standardized F_{ST} (φ'_{ST}); Meirmans 2006) was calculated in GenoDive v2.0b20 (Meirmans and Van Tienderen 2004). F'_{ST} is an estimate of genetic differentiation based on the AMOVA method (Excoffier *et al.* 1992) and standardized to account for variable intrapopulation diversity (Meirmans 2006) by a method analogous to that outlined in Hedrick (2005). Significance of pairwise population differentiation was assessed by Fisher's exact method according to the log likelihood ratio (G; Goudet *et al.* 1996) and implemented in GENEPOP v4.1.1 with default Markov chain parameters. Statistical significance for all tests was adjusted for multiple comparisons using the B-Y false discovery rate (FDR) (Benjamini and Yekutieli 2001). The B-Y FDR method has been demonstrated to be an intermediately conservative correction for multiple hypotheses testing, and balances Type I and Type

II error rates in ecological genetic studies (Narum 2006). AMOVA analyses were implemented in GenAlEx v6.4 with 9999 permutations on hierarchical arrangements of the data. F_{RT} measures the proportion of variation attributable to the highest level of population subdivision (Peakall 1995), which in this case is geographic region. Principal component analysis (PCA) was conducted in: (1) GenAlEx v6.4 with covariance matrices of pairwise individual genetic distances, F_{ST} and F'_{ST} population values; and (2) GenoDive v2.0b20 on covariance matrices of population allele frequencies with 9999 random permutations (Goudet 1999).

A Mantel test was performed in GenAlEx v6.4 to test for isolation by distance (IBD) (Wright 1943). Geographic distance was transformed by the natural logarithm (ln(d+1)), because of the variable distances sampled within and between regions, and plotted versus $F'_{ST}/ (1-F'_{ST})$ (Rousset 1997). Global multivariate spatial autocorrelation methods were employed with individual pairwise genetic and geographic distances at smaller spatial scales (i.e., within regions and within sites). The methods implemented in GenAlEx v6.4 (Smouse and Peakall 1999; Peakall *et al.* 2003; Double *et al.* 2005; Smouse *et al.* 2008) combine all genetic information from multiple loci in a single analysis to assess the correlation to spatial distances. This analysis generates an autocorrelation coefficient **r** that is closely related to Moran's-I and assesses the genetic similarity between pairs of individuals occurring in geographic distance classes. Statistical significance of **r** was tested with 9999 random permutations and bootstraps. First, under the assumption of no spatial structure, upper and lower bounds of the 95% confidence interval for **r** are calculated through the random shuffling of individuals among the different geographic locations (Peakall and Smouse 2006). Significant spatial structure is inferred if the actual value of **r** falls outside of the 95% confidence belt. Moreover, positive (i.e., restricted dispersal, individuals are more closely related than expected) or negative spatial structure is inferred by a one-tailed test at $\alpha<0.05$ for the upper and lower 95% confidence bounds (Peakall and Smouse 2006). Secondly, a

95% confidence interval is calculated for the observed **r**-value at each distance class with bootstrap resampling. Significant spatial autocorrelation is inferred when the bootstrap confidence interval does not include **r**=0 (Peakall and Smouse 2006). The analyses were conducted on multiple populations with uneven distance classes as needed to account for variable sampling distances in different regions. The resulting correlograms were plotted on even distance scales to ease comparison between analyses.

Three different Bayesian clustering analyses were implemented in *structure* v2.3 (Pritchard *et al.* 2000) under the correlated allele frequencies model (Falush *et al.* 2003) with admixture and no prior information: (1) All individuals and both putative species (n=762; K= 1 – 9); (2) All *Sh_sheltered* individuals (n=505; K= 1 – 12); and (3) *Sh_exposed* individuals (n=257; K= 1 – 10). Each K-value was repeated for five independent runs with burn-in period length of 100,000 followed by 500,000 MCMC replications. Raw results were plotted and assessed using the online software Structure Harvester (Earl and Vonholdt 2012), which also provides calculations for the ΔK method described in Evanno *et al.* (2005) for identifying the uppermost hierarchical level of structure. Both ΔK and the posterior probability values of K were assessed in selecting the appropriate K value to fit the data. Population profiles of assignment results were compiled across replicate runs and pie charts created manually in Microsoft Excel. In all cases, standard errors of mean assignments to a given cluster across replicate runs was <1%, indicating consistency across independent runs.

Finally, GENECLASS2 (Piry *et al.* 2004) was used to identify first generation migrants (i.e., individuals born in a population other than the one from which they were sampled) within all populations of both putative species (analyzed separately) using the 'detect migrants' function. Because there were likely to be many potential source populations that were not sampled, I used the L_h statistic (L_home), which estimates the likelihood of the individual's genotype within the population where it was sampled

(Paetkau *et al.* 2004; Piry *et al.* 2004). Samples sets were analyzed with the Bayesian criterion of Rannala and Mountain (1997) and the Monte Carlo resampling method of Paetkau *et al.* (2004) to determine the critical value of L_h beyond which individuals were inferred to be first generation migrants at an α level of 0.01 with the likelihood distribution calculated from 10,000 simulated individuals per each population. The probability of each individual's genotype was then calculated for every reference population (including home population), using the same computation parameters as used for L_h, to determine the probability that detected immigrants were derived from other populations in the sample set.

3.3 RESULTS

Altogether, 762 colonies of *Seriatopora* spp. from seventeen different sampling sites were genotyped with 10 microsatellite loci. Colonies were assigned to populations defined by site and species according to the classification presented in Chapter 2. A preliminary *structure* analysis of all individuals confirmed that clustering of the two putative species was consistent across regions (Fig. 3.1; K=2). At K=2 (highest hierarchical level of structure indicated by ΔK; data not shown), populations divide according to putative species, not regions, as expected. The maximum assignment to the opposite cluster (i.e., *Sh_sheltered* to *Sh_exposed* and vice versa) was 1.4% for the seven *Sh_sheltered* individuals combined at exposed site SE Lizard 2.

3.3.1 *Sh_sheltered*

3.3.1a Genetic diversity

Among the 505 *Sh_sheltered* colonies genotyped, 12 MLGs were found that were shared between two to four colonies. Eleven of the matching MLGs occurred in the Palm Island samples, and only one MLG occurred at Lizard, where it was shared between two colonies from different reefs. However, all colonies with identical MLG

were retained because they either occurred in different sites or reefs, or had GP > 0.001. Moreover, the probabilities that these repeated MLGs were produced sexually (P_{ID}) were quite large ($2.9x10^{-5}$ to $1.3x10^{-3}$; Table 3.2A). Clonal richness (G/N) reflects the actual number of unique genotypes within the sample set, and was 1 or close to 1 for all populations (Table 3.2A). For population level analyses, the few *Sh_sheltered* individuals at exposed sites of Lizard Island (n=10) were eliminated due to small sample sizes. Altogether, 10 populations were included for *Sh_sheltered* analyses and names are abbreviated as indicated in Table 3.1. Two of the 10 microsatellite loci (Sh3-003 and Sh3-008) were fixed for the same allele in all populations. The remaining eight loci ranged from one to a maximum of seven alleles per locus across all populations (Appendix 2.1). Expected heterozygosity (H_E) was generally higher for Lizard Island populations (Table 3.2A; Appendix 2.1) and the maximum H_E for a single locus was 0.771. Mean H_E for all populations and loci was 0.359 and *Sh_sheltered* met HWE expectations globally (F_{IS} = -0.024, ns; Table 3.2A). Moreover, only 4 individual loci exhibited significant heterozygote deficits in three populations (4% of all locus x population combinations), and all at different loci (Appendix 2.1). When individuals were pooled within regions, Lizard Island populations contained 18 private alleles (PA) compared to 10 found in Palm Island populations. The larger number of PAs at Lizard Island is due to high diversity in the two populations at SW Lizard (Table 3.2A). Mean null allele frequency estimated by the FreeNA analysis across all loci and populations was 0.01 (± 0.002 SE; data not shown), and F_{ST} values corrected for null alleles did not differ substantially from uncorrected F_{ST} values (Table 3.3A).

Table 3.2 Summary genetic diversity statistics for all populations and totals for putative species (bold type): **A) *Sh_sheltered*** and **B) *Sh_exposed***. N: # of colonies genotyped; G: # of unique genotypes; G/N: clonal diversity; P_{ID}: probability of identity; PA: # private alleles; Mean H_O: mean observed heterozygosity across all loci; Mean H_E: mean expected heterozygosity across all loci; Mean F_{IS}: Weir and Cockerham's (1984) inbreeding coefficient calculated across all loci.

A. *Sh_sheltered*

	WO1	WO2	NWO1	NWO2	WP1	WP2	SWL1	SWL2	NWL1	NWL2	Total
N	47	50	50	48	50	50	50	50	50	50	**495**
G	47	50	50	45	49	48	50	50	50	50	**489**
G/N	1	1	1	0.938	0.980	0.960	1	1	1	1	**0.988**
P_{ID}	$1.3x10^{-4}$	$7.7x10^{-5}$	$2.2x10^{-3}$	$1.4x10^{-3}$	$1.3x10^{-3}$	$7.5x10^{-4}$	$4.1x10^{-5}$	$2.9x10^{-5}$	$3.7x10^{-5}$	$2.1x10^{-5}$	**$3.0x10^{-5}$**
P_A	1	2	-	-	-	-	6	5	-	-	**-**
Mean H_O	0.366	0.341	0.277	0.315	0.298	0.319	0.396	0.426	0.419	0.438	**0.365**
Mean H_E	0.372	0.363	0.289	0.303	0.294	0.313	0.407	0.421	0.420	0.431	**0.359**
Mean F_{IS}	0.020	0.070	0.063	-0.038	-0.022	-0.028	0.015	-0.018	-0.007	-0.027	**-0.024**

B. *Sh_exposed*

	SEP1	SEP2	NEO1	SEL1	SEL2	NEL1	NEL2	Total
N	17	40	39	50	43	39	27	**255**
G	17	40	37	49	43	39	27	**252**
G/N	1	1	0.949	0.980	1	1	1	**0.988**
P_{ID}	$1.1x10^{-6}$	$4.0x10^{-7}$	$4.3x10^{-6}$	$3.9x10^{-6}$	$1.5x10^{-5}$	$1.7x10^{-5}$	$1.9x10^{-5}$	**$5.7x10^{-8}$**
P_A	0	1	2	1	-	1	2	**-**
Mean H_O	0.563	0.560	0.457	0.467	0.397	0.431	0.450	**0.475**
Mean H_E	0.523	0.555	0.469	0.478	0.405	0.438	0.425	**0.470**
Mean F_{IS}	-0.086	-0.024	0.023	0.023	-0.006	0.002	-0.066	**-0.019**

Table 3.3 Pairwise uncorrected F_{ST} values (top number in value pairs) (φ_{ST}; Excoffier *et al.* 1992) and ENA null allele corrected F_{ST} (bottom number, italics) (Weir 1996; Chapuis and Estoup 2007): **A) *Sh_sheltered*** and **B) *Sh_exposed***. All pairwise comparisons are highly significant, except those in bold type. Between region comparisons are located within the black bordered box. The within Palm Islands comparisons are located above box and within Lizard Island comparisons to right of box.

A. *Sh_sheltered*

	WO1	WO2	NWO1	NWO2	WP1	WP2	SWL1	SWL2	NWL1
WO2	**0.001** ***0.003***								
NWO1	0.076 *0.076*	0.095 *0.093*							
NWO2	0.065 *0.066*	0.082 *0.083*	**0.013** ***0.013***						
WP1	0.148 *0.147*	0.153 *0.146*	0.107 *0.104*	0.104 *0.103*					
WP2	0.124 *0.125*	0.125 *0.121*	0.100 *0.099*	0.095 *0.095*	**0.000** ***-0.003***				
SWL1	0.220 *0.219*	0.216 *0.213*	0.251 *0.241*	0.248 *0.248*	0.214 *0.214*	0.195 *0.194*			
SWL2	0.185 *0.184*	0.186 *0.185*	0.220 *0.212*	0.215 *0.215*	0.216 *0.216*	0.194 *0.193*	0.011 *0.011*		
NWL1	0.186 *0.180*	0.186 *0.180*	0.237 *0.225*	0.217 *0.214*	0.172 *0.168*	0.150 *0.146*	0.043 *0.042*	0.040 *0.040*	
NWL2	0.173 *0.171*	0.182 *0.179*	0.218 *0.211*	0.193 *0.194*	0.144 *0.143*	0.127 *0.127*	0.082 *0.082*	0.073 *0.073*	0.020 *0.020*

B. *Sh_exposed*

	SEP1	SEP2	NEO1	SEL1	SEL2	NEL1
SEP2	**0.004** ***0.004***					
NEO1	0.095 *0.094*	0.082 *0.082*				
SEL1	0.252 *0.256*	0.246 *0.245*	0.302 *0.302*			
SEL2	0.302 *0.305*	0.292 *0.291*	0.330 *0.328*	0.027 *0.028*		
NEL1	0.280 *0.279*	0.276 *0.272*	0.334 *0.331*	0.060 *0.060*	0.086 *0.086*	
NEL2	0.289 *0.293*	0.279 *0.279*	0.319 *0.320*	0.039 *0.040*	0.056 *0.057*	0.036 *0.037*

3.3.1b Genetic structure

For all locus combinations in all populations (n=450), only three (0.07%) indicated significant LD (Appendix 2.1). A hierarchical AMOVA showed that 15% of the total genetic variation was partitioned among regions (F_{RT}=0.145, df=1, p=0.0001), and a further 6% among populations within regions (F_{SR}=0.069, df=8, p=0.0001). Except for the three within reef comparisons from the Palm Islands, all pairwise F_{ST} values were statistically highly significant (Table 3.3A). F'_{ST} values were approximately 1.5 times higher than F_{ST} values (Tables 3.3A, 3.4A). F'_{ST} for sites within reefs ranged from -0.006 to 0.035, with the highest value occurring between the most distant sites within a reef (1.2 km) at SW Lizard (Table 3.4A). For reefs within regions, F'_{ST} values were 50% higher in the Palm Islands (0.099 – 0.223; Table 3.4A), where average geographic distance between reefs was also greater than at Lizard Island (Fig. 3.2). The mean F'_{ST} between regions was 0.315 (±0.009 SE; Fig. 3.2A), and some lower interregional F'_{ST} values were similar to within region comparisons in the Palms between populations on different islands (Table 3.4A). For example, pairwise comparisons between the northernmost Palm Island sites (WP) and southernmost Lizard sites (SWL), which are separated by 441 km ($F'_{ST} \approx 0.230$), were comparable to those between the northernmost (WP) and southernmost (WO) Palm Island reefs (7 km apart; $F'_{ST} \approx 0.200$; Table 3.4A). These patterns of spatial-genetic structure were also clear when the data were represented by a PCA of population allele frequencies (Fig. 3.3A). Regions were separated along the first axis, which explained 60.8% of variance (p=0), and reefs within regions along the second axis (19.94% of variance explained, p=0.66; Fig. 3.3A). The paired sites within a given reef were very similar in allelic frequencies for the Palm Islands, yet reefs were distinctly separated from each other along principal component 2. In contrast, the Lizard Island sites were more distinct from each other within reefs, but formed a more continuous gradient within the region as a whole (Fig. 3.3A). PCAs constructed with F_{ST} and F'_{ST} showed very similar patterns, although the

percentage variance assigned to the first and second principal components was slightly different (results not shown).

Table 3.4 Standardized pairwise population genetic distance F'_{ST} (φ'_{ST}; Miermans 2006): **A) *Sh_sheltered*** and **B) *Sh_exposed***. All comparisons highly significant for F_{ST} (Table 4.4), except those italicized. Between region comparisons are contained within the black bordered box. The within Palm Islands comparisons are located above box and within Lizard Island comparisons to right of box.

A. *Sh_sheltered*

WO2	*0.000*								
NWO1	0.113	0.137							
NWO2	0.099	0.121	*0.018*						
WP1	0.223	0.224	0.150	0.149					
WP2	0.189	0.185	0.143	0.137	*-0.006*				
SWL1	0.310	0.303	0.367	0.341	0.268	0.237			
SWL2	0.292	0.299	0.342	0.307	0.227	0.202	0.035		
NWL1	0.362	0.348	0.384	0.386	0.331	0.304	0.073	0.142	
NWL2	0.309	0.304	0.342	0.339	0.339	0.307	0.069	0.127	0.019
	WO1	**WO2**	**NWO1**	**NWO2**	**WP1**	**WP2**	**SWL1**	**SWL2**	**NWL1**

B. *Sh_exposed*

SEP2	*0.008*					
NEO1	0.190	0.172				
SEL1	0.509	0.514	0.582			
SEL2	0.557	0.566	0.591	0.048		
NEL1	0.538	0.555	0.618	0.112	0.149	
NEL2	0.557	0.564	0.589	0.074	0.098	0.064
	SEP1	**SEP2**	**NEO1**	**SEL1**	**SEL2**	**NEL1**

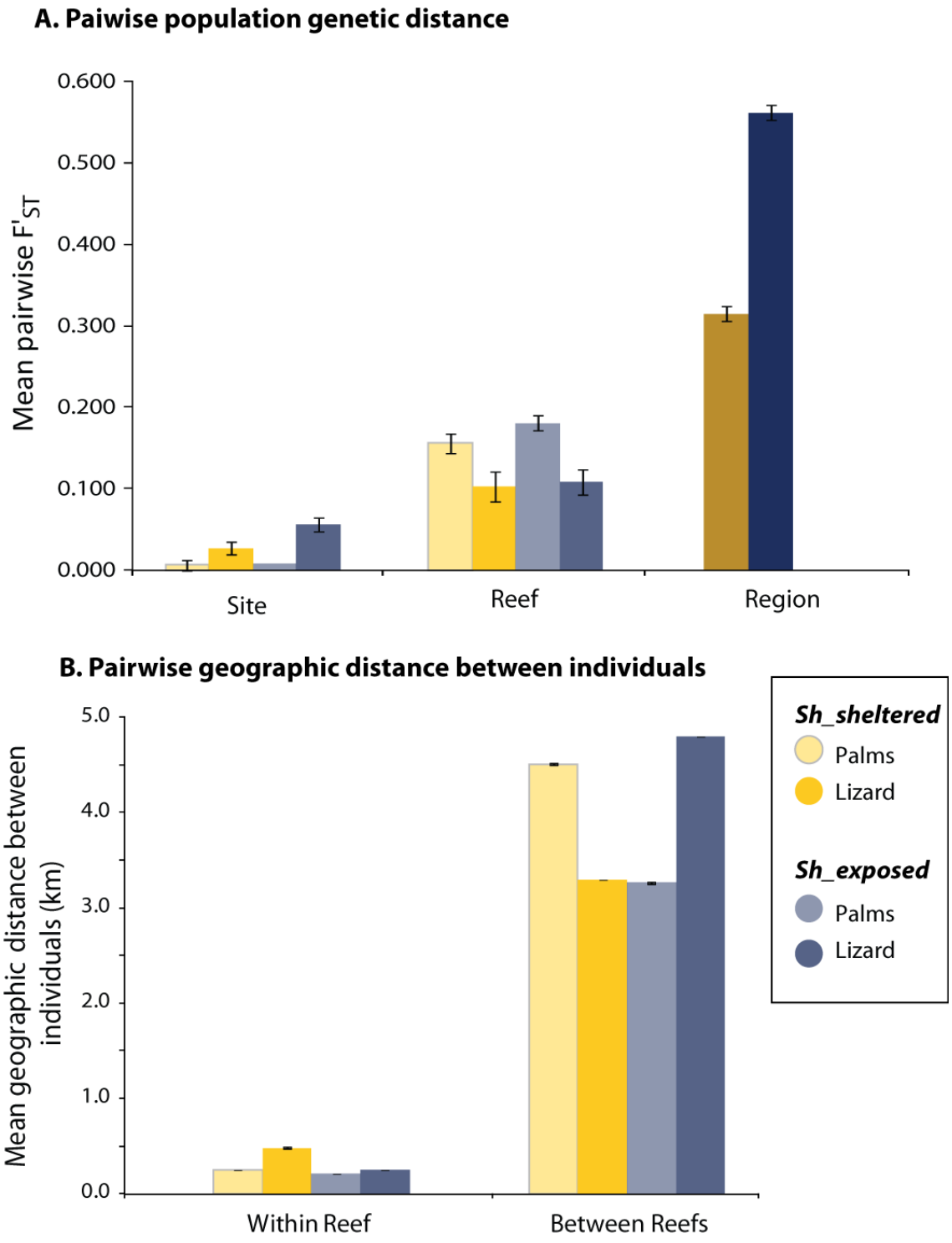
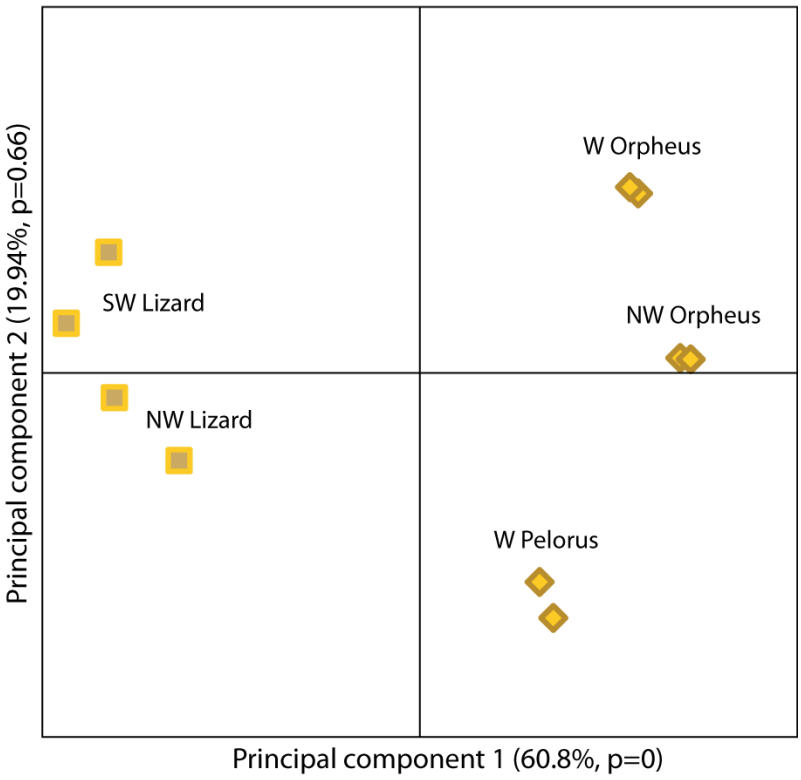


Figure 3.2 Genetic and geographic distances among populations of *Sh_sheltered* and *Sh_exposed*. **A)** Mean standardized pairwise population differentiation (F'_{ST} (±1SE)) for both putative cryptic species at three hierarchical spatial scales (Sites within reefs, Reefs within regions, and between Regions). Colors (legend to lower right) correspond to different putative species and regional groupings: yellows denote *Sh_sheltered* populations; blues denote *Sh_exposed*. **B)** Mean pairwise geographic distance (±1SE) between colonies (km) within reefs (i.e., within site and between site distances) and between reefs.

A. *Sh_sheltered*



B. *Sh_exposed*

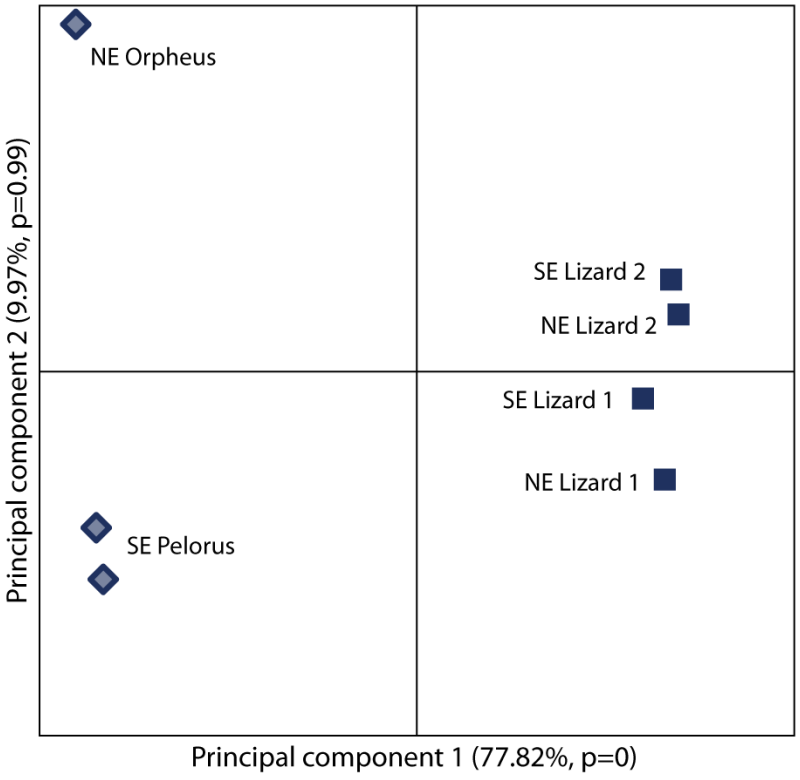


Figure 3.3 Principal component analysis calculated on covariance matrix of allele frequencies for populations of: **A) *Sh_sheltered*** (in yellows)**,** and **B) *Sh_exposed*** (in blue). Percent of variance explained and p-values indicated on axes. Square symbols denote northern Lizard Island region sites; Diamond symbols denote central Palm Islands region sites.

Overall, the data showed a clear and significant pattern of isolation by distance across the spatial gradient (p=0.0002, Fig. 3.4A). The correlation between genetic and geographic distance was strong (R^2=0.831) and positive (m=0.071), although there was a wide range of genetic distance estimates between populations from different regions (Fig. 3.4A). Moreover, the within region genetic structure indicated a strong spatial signal in the spatial autocorrelation analysis conducted on uneven distance classes and pairwise individual genetic distances (Fig. 3.5A). The combined data for both regions resulted in **r** crossing the x-axis at 2.53 km, with significant positive correlation before that point (p=0.0001) and significant negative correlation at greater distances (p=0.0001; Fig. 3.5A). Both regions were characterized by positive spatial-genetic correlation at distances less than 2.5 km, implying that the majority of larval dispersal may be limited to a few kilometers. Further, a negative correlation generally occurred at distances greater than 3 km, signifying that populations separated by more than 3km were less genetically similar than expected under random permutations. Within site spatial autocorrelation analyses conducted for each region revealed that, in both regions, significantly positive spatial structure occurred between individuals for the first distance class, from 0 to 5 m ($p<0.05$), but mostly non-significant correlations occurred from 5 m up to 145 m (Fig. 3.6A). The x-intercept for combined Palm Island sites was 13.8 m and 18.3 m for Lizard Island sites.

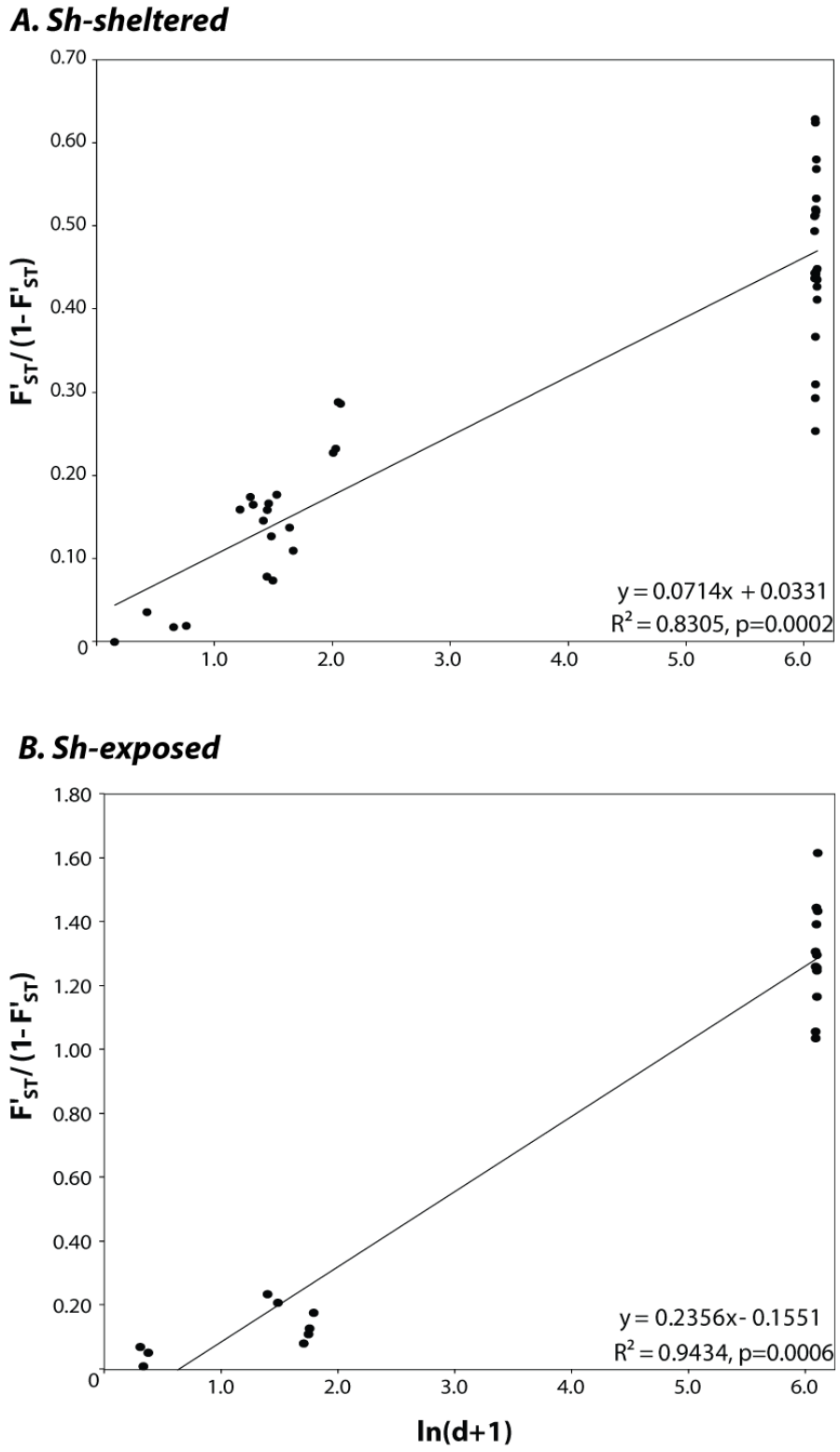


Figure 3.4 Isolation-by-distance regressions for populations of: **A) *Sh_sheltered*, and B) *Sh_exposed*.** Mantel test correlations performed with $F'_{ST}/ (1-F'_{ST})$ against log scaled geographic distance (ln(d+1)) within and between Palm Islands and Lizard Island regions. Regression equations, R^2 values, and p-values displayed on graph.

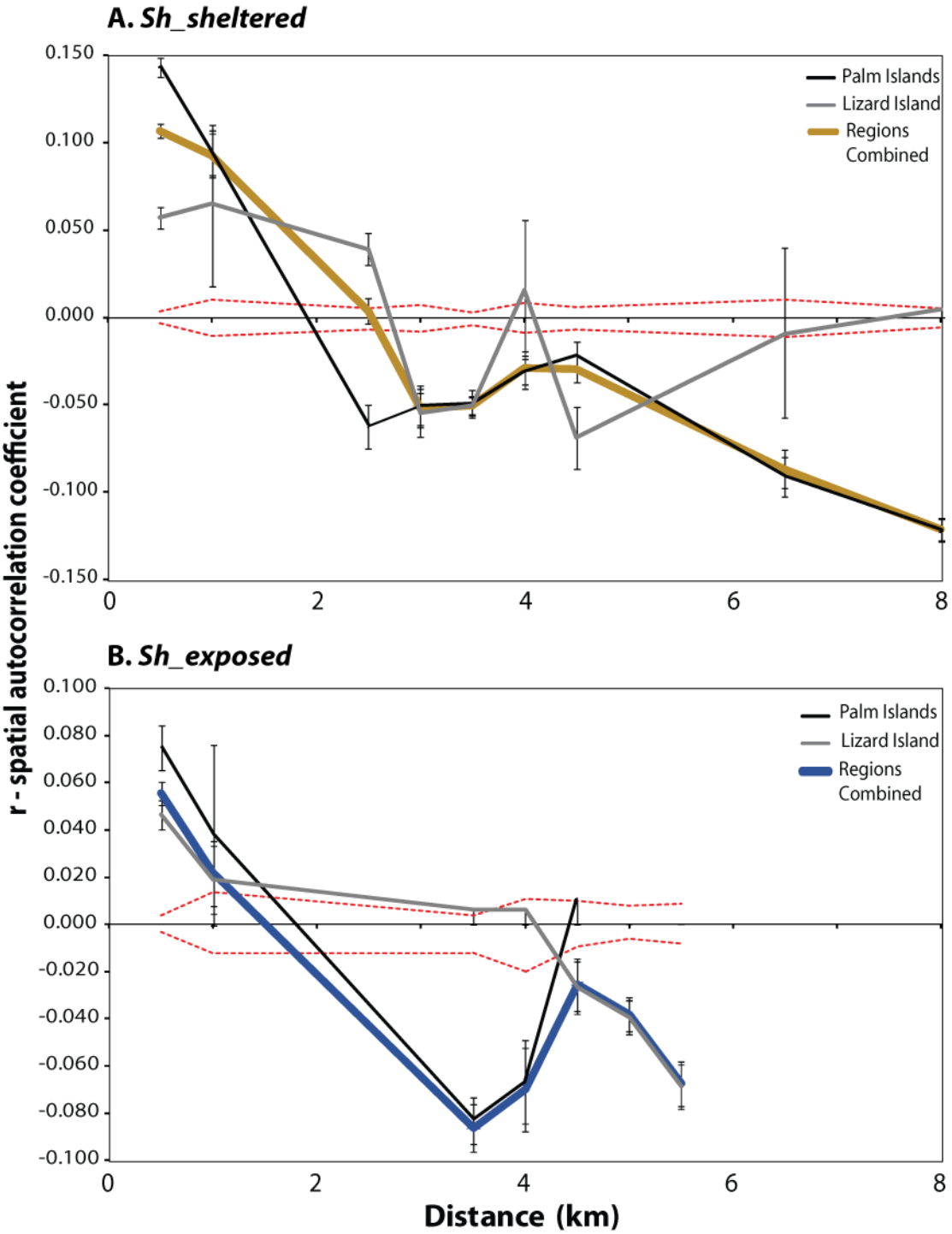


Figure 3.5 Spatial autocorrelation analysis of within region spatial genetic correlation for populations of: **A) *Sh_sheltered,*** with gold line indicating the combined region result; and **B) *Sh_exposed,*** with blue line indicating the combined region result. Dashed red lines indicate 95% confidence interval (CI) about the null hypothesis based on 9999 random permutations for combined regions result (colored lines). Single region results indicated by black (Palms) and gray (Lizard) lines. Error bars indicate upper and lower 95% CI around r, determined by 10,000 bootstrap resamplings at each distance class. Variable categorical distance classes plotted on equal distance scale.

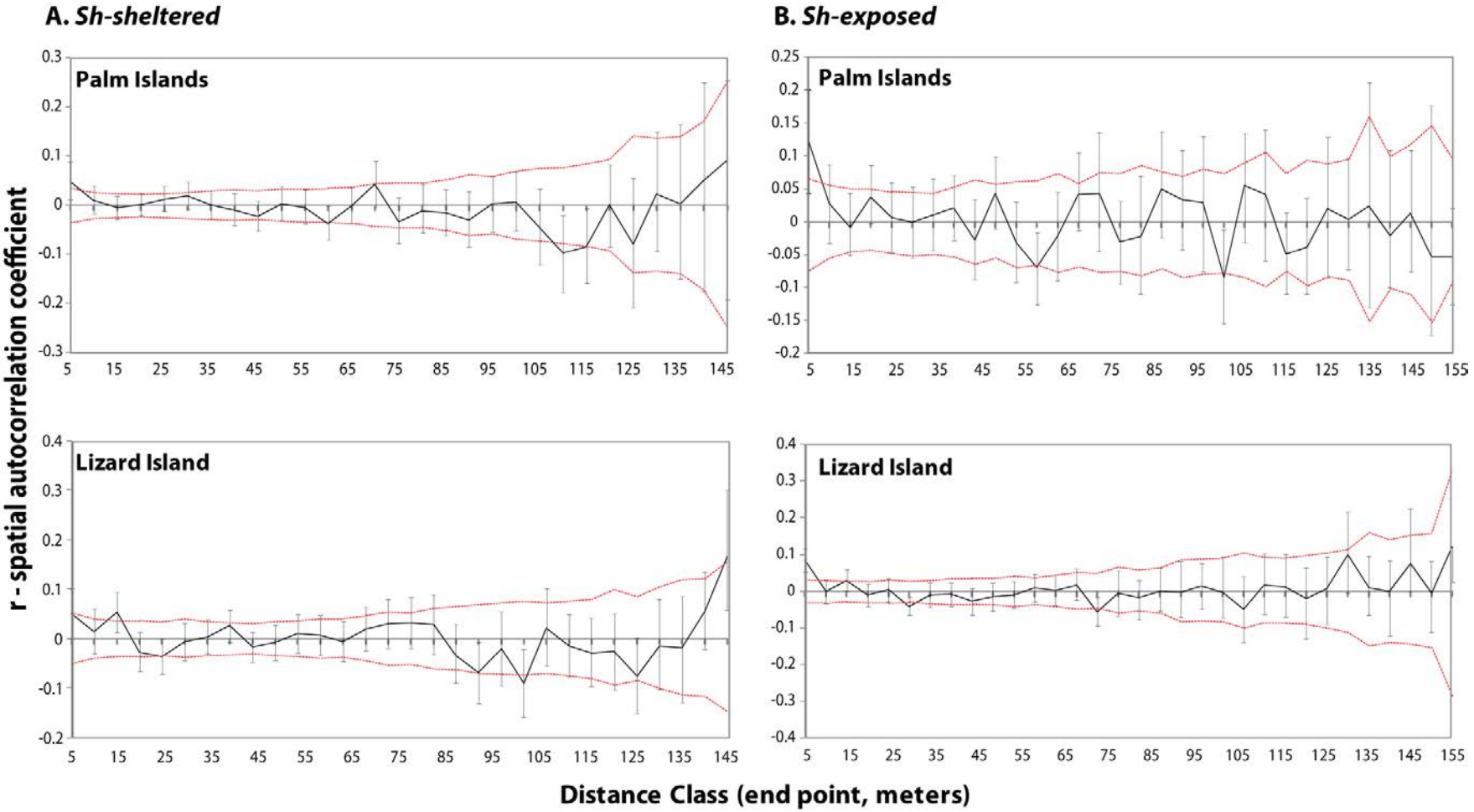


Figure 3.6 Spatial autocorrelation correlograms of within site spatial genetic correlation for each region and species: **A) *Sh_sheltered*** and **B) *Sh_exposed***. Palm Islands analyses are top correlograms and Lizard Island analyses on bottom. Dashed red lines indicate 95% confidence interval (CI) about the null hypothesis based on 9999 random permutations for combined site r (black line). Error bars indicate upper and lower 95% CI around r, determined by 10,000 bootstrap resamplings at each distance class, plotted at the end point in meters.

Bayesian clustering of individuals further supports the strong spatial structuring of populations (Fig. 3.7, left maps, warm colors). The ΔK method (Evanno *et al.* 2005) predictably detects the strongest spatial signal at K=2 (inner pie charts, i.e., charts closest to islands) for the regional scale separation, but with another minor peak in ΔK at K = 5 (outer pie charts) corresponding to reef level structure (Fig. 3.7). Mean L(K) also peaks at K=5 and mean population assignments across five replicate runs show almost no variation (SE≤ 0.2%). Interestingly, the few cross -habitat migrants at the exposed sites of Lizard Island exhibit distinct population profiles, rather than matching the closest source populations sampled (Fig. 3.7). Overall, however, the *structure* results strongly support the patterns detected with other analyses. The assignment profiles of sites within a reef are very similar, while reefs are distinct within and between regions. At K=5, population profiles contain approximately 60 – 70% assignment to a single, unique genetic cluster for each of the five main reefs sampled. Another ~25% (range 15 – 31%) of the profiles is assigned cumulatively to other reefs within the same region, and less than 13% (range 3 – 12.5%) to reefs in the other region. Six individuals were identified in five populations as first generation migrants with the GENECLASS2 analysis, three of which were not assigned to any other population sampled (Table 3.5). One individual from each of two different Palm Island reefs (populations: NW Orpheus 1, W Pelorus 2) was assigned by at least 50% to the same local population at the third most southern reef, W Orpheus 1. The sixth individual from an exposed reef at Lizard Island, NE Lizard, was assigned by similar proportions (>75%) to three of the sheltered sites sampled in that region (Table 3.5).

Table 3.5 Summary of *Sh_sheltered* individuals detected as first generation migrants with the GENECLASS2 analysis using the L_h test statistic at α=0.01. Home population: the population from which the individual was sampled; P(Home): assignment test probability of genotype at its home population; P(Other populations): assignment test probability of genotype in other populations sampled >0.3.

Home population	P(Home)	P(Other populations)			
W Orpheus 1	0	all <0.09			
NW Orpheus 2	0.0013	0.508 (WO1)	0.301 (WO2)		
W Pelorus 1	0.0037	0.537 (WO1)	0.445 (WO2)		
SW Lizard 2	0.0013	all <0.04			
SW Lizard 2	0.0044	all <0.01			
NE Lizard	0.0049	0.818 (SWL1)	0.792 (SWL2)	0.765 (NWL1)	0.301 (NWL2)

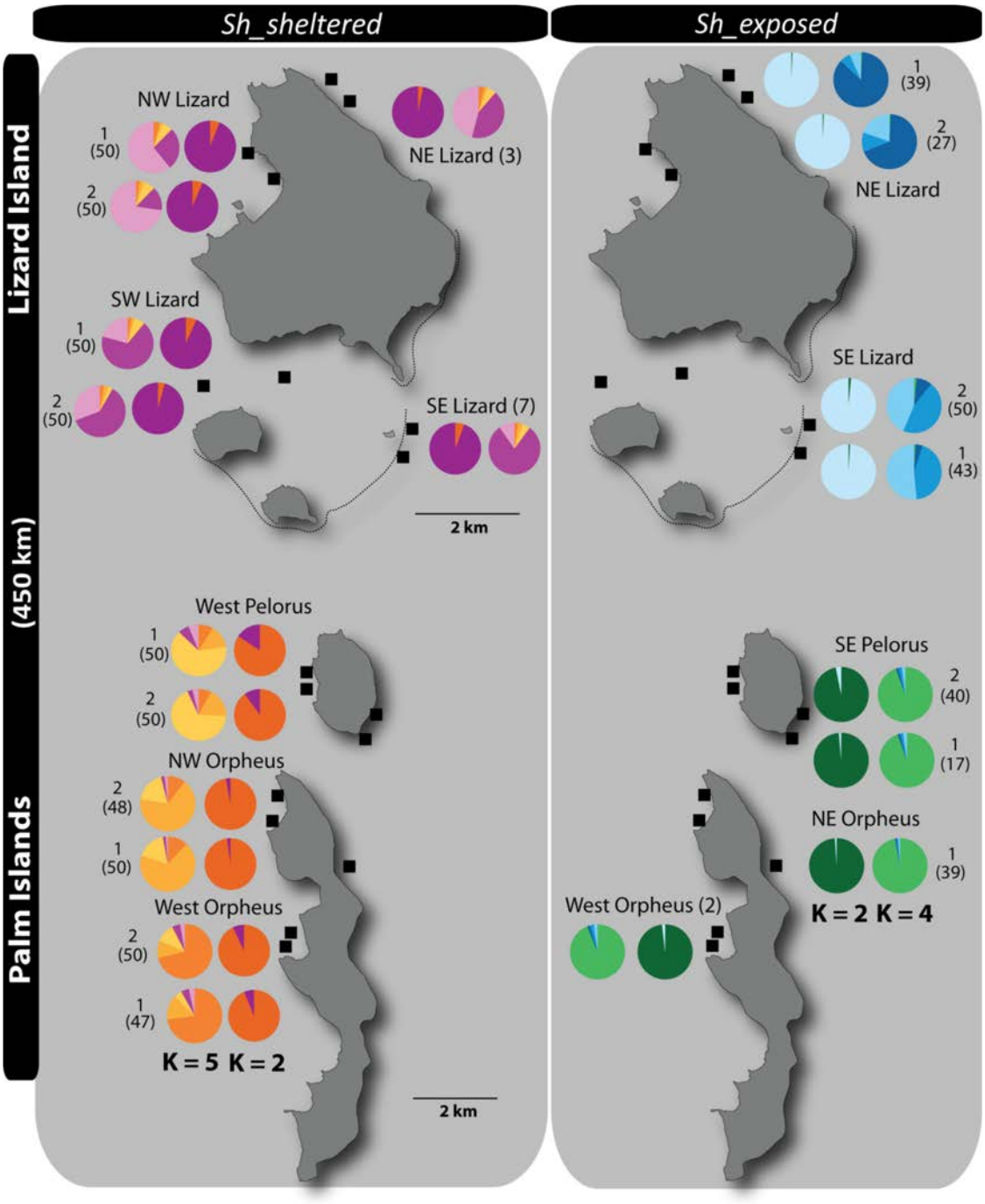


Figure 3.7 Bayesian clustering of two putative *Seriatopora* species**,** *Sh_sheltered* (left maps, n=505 individuals) and *Sh_exposed* populations (right maps, n=257 individuals), for two separate analyses. Results are grouped by sampled populations at the regional scale (K=2; inner pie charts, i.e., those closest to islands) and reef scale (K=5 and K=4, respectively; outer pie charts) using *structure* v.2.3 (Pritchard *et al.* 2000). Site numbers and sample sizes (in parentheses) shown to outside of pie charts.

3.3.2 *Sh_exposed*

3.3.2a Genetic diversity

For the 257 *Sh_exposed* colonies genotyped, only three repeated MLGs were found between three pairs of spatially proximate individuals, two pairs at NEO1 and one at SEL1. All GPs were < 0.001 and P_{ID} for each population was reasonably low ($4.0x10^{-7}$ to $4.2x10^{-5}$; Table 3.2B), implying that the matching MLGs were not produced sexually. Therefore, one of each clone was eliminated from further analyses. The two *Sh_exposed* individuals found on the sheltered side of the Palm Islands (Table 3.1; Fig. 3.1) were only included in the clustering analysis. All loci were polymorphic in all populations, except for Sh4-001 in NEL2 (Appendix 2.1). The number of alleles per locus ranged from 1 to 12 and maximum expected heterozygosity (H_E) was 0.833 (Appendix 2.1). Mean H_E was 0.470 across all populations, and was slightly higher in Palm Island populations (Table 3.2B). Four loci exhibited significant heterozygote deficits in three populations (5.7% of all locus x population combinations; Appendix 2.1), but *Sh_exposed* met HWE expectations globally across all populations and loci (F_{IS} =-0.019, ns; Table 3.2B). The mean estimated frequency of null alleles was 0.012 (±0.0003 SE) across all loci and populations, and the difference between corrected and uncorrected F_{ST} values was negligible (Table 3.3B). Palm Islands *Sh_exposed* contained only 4 private alleles (PA) and three were site specific (Table 3.2B). Lizard Island *Sh_exposed* populations contained eleven PA, six of which occurred at frequencies ≥3.5% up to 16%.

3.3.2b Genetic structure

All pairwise population comparisons (F_{ST}) indicated statistically highly significant structure, except between the two sites at SE Pelorus (Table 3.3B), and F'_{ST} values were almost double that of non-standardized F_{ST} (Tables 3.3B, 3.4B). Even though geographic separation was similar, mean F'_{ST} for sites within reefs was seven times

higher for both Lizard Island reefs (0.056 ±0.008 SE) than for the two SEP sites in the Palm Islands (0.008; Fig. 3.2). In contrast, the level of differentiation among reefs within regions was larger for the Palm Islands (F'_{ST} ~0.180) than Lizard Island, despite smaller geographic distances between reefs (Fig. 3.2; Table 3.4B). Interregional comparisons yield F'_{ST} values that ranged from 0.509 to 0.618 (Table 3.4B). The PCA of population allele frequencies explained 88% of the variance when principal components 1 and 2 were combined (Fig. 3.3B). Axis 1 reflected the clear separation between regions (77.8% of variance, p=0), whereas the second principal component was driven by differentiation among reefs rather than within sites (9.97% of variance, ns). In particular, differentiation between reefs in the Palm Islands was much greater than between reefs in Lizard Island (Fig. 3.3B). PCAs calculated with F_{ST} and F'_{ST} converged on a similar pattern (data not shown). A hierarchical AMOVA partitioned 25% of total genetic variation among regions (F_{RT} = 0.251, df=1, p=0.0001). Another 4% of genetic variation was attributed to populations within the two regions (F_{SR} = 0.056, df=5, p=0.0001). No significant LD existed for the loci tested in these populations.

With both regions combined, populations showed a strong and significant pattern of IBD across all spatial scales (m=0.24, R^2=0.943, p=0.0006; Fig. 3.4B). However, a Mantel test indicated that within region correlations were not significant (p>0.05; data not shown). Using a more powerful test, the overall spatial autocorrelation between individuals appeared to be similar within regions (Fig. 3.5B), despite the difference in sampled spatial distances (Fig. 3.2B; Appendix 3.1). Although the distance classes between 2 and 4 kilometers was empty for Lizard Island (gray line), the important feature was that initial positive spatial-genetic correlation at distances <2 km was followed by negative spatial-genetic correlation at distances greater than 4 km (Fig. 3.5B). The same general pattern was revealed in Palm Island populations, yet the distinction between individuals within 1 km and >1 km was more

striking (Fig. 3.5B, black line). For both regions combined, **r** (blue) indicated significant positive correlation to 1 km ($p<0.001$) with an x-intercept at 1.5 km (Fig. 3.5B). At greater distances (≥ 3.5 km), individuals were significantly less genetically similar than expected ($p=0.0001$). Within sites, analyses for the two regions both showed significantly positive spatial autocorrelation at the first distance class within 5 m ($p\leq0.001$; Fig. 3.6B). Fluctuations in **r** at greater distance classes were seemingly random and mostly non-significant to 160 m. The x-intercept was 10 m and 14 m for Lizard Island and Palm Islands sites, respectively.

Clustering analysis yielded slightly dissimilar patterns (Fig. 3.7, right maps, cool colors) to pairwise population differentiation (F'_{ST} and PCA; Figs. 2 and 3B). At K=2 (inner pie charts), ΔK was maximized (data not shown) and populations separated according to regions (Fig. 3.7). Assignment to their respective dominant clusters was consistently >97% for all populations. However, when the number of clusters was equal to the number of reefs (K=4), there was still no separation among Palm Island reefs or sites (outer pie charts; Fig. 3.7). Instead, the two Lizard Island reefs split assignments among three genetic clusters (blues) and had almost no membership in the Palm Islands cluster (≥1.5%, green). All Palm Islands sites had essentially identical population profiles, including the two individuals that were sampled from WO1 on the sheltered side of Orpheus Island. Each Lizard Island population profile was slightly different for the four sites (Fig. 3.7), reflecting the higher within reef population differentiation compared to the Palm Islands (Fig. 3.2A). The *structure* results did not reflect the strong separation among Palm Island reefs illustrated by the PCA (Fig. 3.3B). Seven individuals were identified in five populations as first generation migrants with the GENECLASS2 analysis, five of which were not assigned to any other population sampled (Table 3.6). Both individuals sampled from a sheltered reef in the Palm Islands (W Orpheus) had highest assignment probabilities to populations from a local exposed reef, SE Pelorus (Table 3.6).

Table 3.6 Summary of *Sh_exposed* individuals detected as first generation migrants with the GENECLASS2 analysis using the L_h test statistic at α=0.01. Home population: the population from which the individual was sampled; P(Home); assignment probability of genotype in its home population; P(Other populations): assignment probability to other populations sampled >0.1. Dash indicates no other population sampled had a greater assignment probability than the home population.

Home population	P(Home)	P(Other populations)	
W Orpheus	0	0.343 (SEP1)	0.177 (SEP2)
W Orpheus	0.0003	0.588 (SEP2)	0.559 (SEP1)
SE Pelorus 2	0.0001	all <0.002	
NE Orpheus	0.007	all <0.04	
NE Orpheus	0.0029	all <0.05	
SE Lizard 1	0.0047	all <0.01	
NE Lizard 1	0.0049	-	

3.3.3 Comparisons between *Sh_sheltered* and *Sh_exposed*

All loci were polymorphic in all *Sh_exposed* populations, except one (NEL2), whereas all *Sh_sheltered* populations were fixed for the same allele in two loci (Appendix 2.1). Lizard Island populations had higher average expected heterozygosities than their Palm Island counterparts for *Sh_sheltered,* with approximately equal population sizes (Table 3.2A). Yet, the opposite interregional trend was found for *Sh*_exposed, despite somewhat larger population sizes at Lizard Island (Table 3.2B). In fact, the Palm Islands region contained both the highest (*Sh_exposed*: SEP2 Mean H_E= 0.555) and lowest (*Sh_sheltered*: NWO1 mean H_E= 0.277) genetic variation of all populations. The limited heterozygosities in Palms *Sh_sheltered* resulted in the highest number of matching MLGs (11) shared across sites and reefs. However, despite generally low mean expected heterozygosities (<0.5) in most populations of both putative species, only eight loci in all 170 evaluated (4.7 %) indicated significant heterozygote deficits. Population differentiation was somewhat dissimilar between putative species and across regions (Figs. 3.2 – 3.4; Table 3.4). In *Sh_sheltered*, patterns in the scale of spatial separation (km) within and between reefs corresponded in magnitude to patterns of population genetic distance (F'_{ST}) for the two regions (Fig.

3.2). In contrast, for *Sh_exposed*, F'_{ST} was much higher among reefs in the Palm Islands (SEP and NEO), despite being separated by smaller geographic distances compared to Lizard Island reefs. At the interregional scale, *Sh_sheltered* populations exhibited differentiation levels (mean F'_{ST} =0.315 ±0.009) approximately half that of *Sh_exposed* populations (mean F'_{ST} =0.562 ±0.009; Fig. 3.2). A comparison of clustering results underscores the variable extent of genetic similarity among putative species and regions (Fig. 3.7). In *Sh_sheltered* populations, profiles reflected fairly equal differentiation with consistent structure across reefs (Fig. 3.7, left maps). In contrast, the large difference among Palms *Sh_exposed* and Lizard *Sh_exposed* populations transcended reef-level separation and suggested almost no mixing across regions for *Sh_exposed* (Fig. 3.7, right maps). Yet, interestingly the PCAs showed strong patterns of regional similarity within the putative species (Fig. 3.3). Specifically, there was a much wider spread in the populations for Palm Islands reefs along axis 2 compared to the more evenly spaced and closely clustered Lizard Island sites. A one-way AMOVA analysis of populations for both putative species (combining both regions) assigned 14% of total genetic variation among *Sh_sheltered* populations (F_{ST}= 0.145; df= 9; p= 0.001) and 20% among *Sh_exposed* populations (F_{ST}= 0.204; df= 6; p= 0.001). The overall trend in spatial autocorrelation analysis was similar in both *Sh_sheltered* and *Sh_exposed* populations within regions, with initially significant positive correlation followed by significant negative correlation (Fig. 3.5). Still, the larger x-intercepts of **r** in *Sh_sheltered* populations implied somewhat greater distances of larval dispersal (combined **r =** 2.5 km) than *Sh_exposed* populations (combined **r =** 1.5 km). Within site analyses of both species indicated that individuals within a 20 meter radius or less were more genetically similar than expected (Fig. 3.6).

3.4 DISCUSSION

For the first time, population genetic connectivity of the abundant coral morphospecies *Seriatopora hystrix* is interpreted in light of previously overlooked putative cryptic species. Here, population genetic structures of the two dominant putative species, *Sh_sheltered* and *Sh_exposed*, showed generally strong differentiation at each of three hierarchical spatial scales ranging from <1 to 450 km on the GBR. Spatial analyses indicated that colonies within a twenty meter radius were more closely related than expected for both putative species, demonstrating a detectable signal of philopatric larval settlement for some (unknown) fraction of offspring. Furthermore, connectivity among populations reduced rapidly beyond two to three kilometers for both *Sh_sheltered* and *Sh_exposed*. Nevertheless, the much larger interregional differentiation among *Sh_exposed* populations suggests that processes shaping genetic connectivity do not operate equally on the two putative species, and possibly that additional unique genetic groups exist within *Sh_exposed*. Although the marked separation between populations raises some concern that demes may be vulnerable to local extinctions following acute disturbance events (e.g., Loya *et al.* 2001; van Woesik *et al.* 2011), the detection of immigrant individuals in several populations of both species and the widespread common occurrence of these prolific corals suggests that many populations will be relatively resilient.

3.4.1 Comparisons of connectivity between putative cryptic species and regions

Detailed population genetic analyses of the two dominant putative cryptic species identified in Chapter 2, demonstrate significantly higher interregional differentiation among populations of *Sh_exposed* compared to *Sh_sheltered* populations, implying that processes governing population connectivity differ between the two putative species and/ or habitats, but may also reflect the more complex mitochondrial makeup found in *Sh_exposed* (see Chapter 2). Briefly, the evidence

presented in Chapter 2 indicated that *Sh_exposed* contained at least two major mitochondrial groups (individuals of both small and medium mtDNA amplicon sizes), and it is unknown how many mtDNA control region sequence haplotypes were present in *Sh_exposed*, as most individuals were not sequenced. Although *Sh_exposed* populations were the *most* closely related to each other across regions (Chapter 2), it is still possible that they are not the same cohesive lineage in both the Palms and Lizard Islands regions, particularly based on clustering results from this chapter, which show almost no mixing across the two regions. In contrast, *Sh_sheltered* corals strongly corresponded to the single mitochondrial haplotype B (exclusively small mtDNA indel; Chapter 2), indicating a single cohesive lineage with consequently greater connectivity seen in shared genetic clusters across regions in this chapter. The depths and habitats sampled for *Sh_sheltered* were consistent between regions in my study, whereas the depths sampled for *Sh_exposed* were substantially shallower at Lizard Island (~5 m) compared to the Palm Islands (~10 m), where no colonies were observed above 8 m. Moreover, the broad, gradually sloping reefs surrounding Lizard Island in both habitats (sheltered and exposed) are substantially different to the steep granitic slope of exposed Palm Island reefs. Previous studies of *S. hystrix* in the northern GBR region indicated that particular mitochondrial haplotypes, which may approximately correspond to putative cryptic species (Chapter 2), are strongly correlated with habitats at specific depths (Bongaerts *et al.* 2010b), thus *Sh_exposed* may contain closely related, but different genetic lineages occurring at dissimilar depths and reef types in the two regions. Further work is required to determine the specific mitochondrial makeup of *Sh_exposed* and to confirm whether *Sh_exposed* corresponds to a single putative species across the Palm Islands and Lizard Island regions, although several other factors may also explain different levels of connectivity among the two putative species.

Differences between habitats (i.e., physical hydrodynamic features) or potential biological dissimilarities among putative species (e.g., reproductive behaviors, larval characteristics) may alternatively underlie the greater isolation of *Sh_exposed* populations compared to *Sh_sheltered*. More extreme features of the exposed habitat (e.g., greater vertical distances, stronger windward surface currents and/ or wave exposure) and more frequent disturbance from major cyclones (van Woesik *et al.* 1991; Harmelin-Vivien 1994), in particular, may partially explain why populations of *Sh_exposed* are demographically distinct with much lower densities than *Sh_sheltered* (P. Warner pers. obs.). Finer scale hydrodynamic effects, such as shorter residence times and stronger diffusion of locally produced larvae in exposed compared to sheltered habitats, may result in smaller population sizes that are more strongly affected by genetic drift, resulting in greater divergence among *Sh_exposed* populations. Reef-scale hydrodynamic models show that reef size and shape have an effect on retention times and physical connectivity (Black 1993; Cetina-Heredia and Connolly 2011), but have generally been conducted on mid-shelf reefs that do not fringe islands and do not take into account different habitats within the reef. Moreover, potentially divergent reproductive ecologies among the putative species, such as variation in larval release timing, frequency, duration and fecundity, may confer different levels of connectivity among habitats and regions (Chapter 2). A study of co-occurring congeneric mussels on the California coast found that phenological differences in reproduction combined with seasonal changes in surfaces currents resulted in distinct patterns of intraspecies larval dispersal (Carson *et al.* 2010). Similar studies of the processes governing dispersal, which integrate reproductive behaviors with the fine-scale physical effects of habitat features and broad-scale oceanographic patterns (e.g., Baums *et al.* 2006), are warranted to elucidate the underlying differences in interregional connectivity between these two putative cryptic species.

Interestingly, the two putative species share some parallel patterns within regions, implying that common local conditions may similarly influence the connectivity

of different species. For example, apparent panmixia occurs over greater areas within reefs in the Palm Island group (i.e., all between site comparisons) for both putative species, whereas Lizard Island populations are significantly differentiated at all spatial scales. In contrast, greater genetic separation between reefs in the Palm Islands compared to Lizard Island appears to be strongly driven by divergence among populations on Orpheus and Pelorus Islands, which are separated by a channel approximately 750 – 1000 m wide and 14 m deep. A recent study of the broadcast spawning coral *Acropora millepora* also found significant differentiation in exposed populations across the same channel, but attributed the differentiation to variable disturbance histories (van Oppen *et al.* 2011b). Consistent with the notion that differentiation between Orpheus and Pelorus reefs in the Palm Island group reflects a physical barrier, higher connectivity among populations at different Lizard Island reefs may result from the more continuous habitat with no comparable deep water barriers that might act as sharp breaks in local larval dispersal.

Broad-scale larval dispersal models for reef-associated fish have predicted low connectivity between the northern and central regions of the GBR, as well as dissimilar local patterns within regions (James *et al.* 2002; Bode *et al.* 2006), however fine-scale hydrodynamic models of highly localized areas are generally limited on the GBR, and do not exist for the reefs sampled here. The complex reef matrix and broad continental shelf of the GBR yield complicated patterns of current circulation influenced by winds, tides, oceanic current inflow, and seasonally high freshwater runoff from summer flooding (Wolanski and Pickard 1985; Brinkman *et al.* 2002; Luick *et al.* 2007). Large-scale circulation studies of the GBR lagoon indicate seasonal and regional variability in passive particle movement and residence times (Luick *et al.* 2007; Choukroun *et al.* 2010), which may be particularly relevant to the connectivity of species with year-round and prolonged larval release periods, such as brooding pocilloporid corals. Model particles released in the Palm Islands region under summer conditions were shown to

reach the Lizard Island region within 30 days, whereas winter releases were retained within the Palm Islands region for the same amount of time before moving mostly southward (Luick *et al.* 2007). However, in direct contrast, simulations of fish larval dispersal have concluded an almost exclusively southward dispersal trajectory for summer spawning seasons from 14° to 19°S latitudes, inclusive of the Lizard and Palm Islands regions (Bode *et al.* 2006). The discrete sampling of two distant regions in my study does not suggest a particular direction of larval dispersal in these two putative coral species, and connectivity between these two regions most likely occurs through intermediate stepping-stone reefs (Hellberg 1995; Crandall *et al.* 2012). Thus, additional studies are needed that incorporate intermediate distances to those sampled here in order to determine broad-scale gene flow in these corals along the Great Barrier Reef.

3.4.2 Local scales of disconnectivity

My findings emphasize that populations of putative species within *Seriatopora hystrix* are strongly differentiated at relatively small spatial scales compared to many marine species (Miller 1997; Benzie 1999; Rhodes *et al.* 2003; Pinsky *et al.* 2010), including some other reef-building corals (Ayre and Hughes 2004; Nishikawa 2008; Underwood *et al.* 2009; Souter *et al.* 2010; van Oppen *et al.* 2011b). Although increasingly coral reef organisms are found to exhibit significant population structuring and surprisingly high proportions of self-recruitment (Jones *et al.* 1999; Barber *et al.* 2000; Taylor and Hellberg 2003; Jones *et al.* 2005; Almany *et al.* 2007; Harrison *et al.* 2012b), contrasting historical assumptions of open marine populations (Palumbi 2003; Cowen *et al.* 2007; Jones *et al.* 2009), the genetic structures of *S. hystrix* have largely suggested demographic independence among populations over ecological timescales (Ayre and Hughes 2004; Underwood *et al.* 2009). For example, while a larval tagging study of two coral reef fish showed that recruitment to natal reefs was 60% for species that have an obligate planktonic stage of up to 38 days (Almany *et al.* 2007), 40% of

recruits were externally derived, still indicating strong connectivity among reefs. Conversely, the magnitude of population differentiation revealed in the two brooding *Seriatopora* species is unusually high over short distances. In both *Sh_sheltered* and *Sh_exposed*, significant population differentiation occurs within reefs (<500 m) that is similar to that found over 100s km in other coral species, including brooders and broadcast spawners (Ayre and Hughes 2004; Starger *et al.* 2010; van Oppen *et al.* 2011b). High self-recruitment and presumed local retention in *Seriatopora* should maintain local populations with a strong stock-recruitment relationship while conditions remain relatively stable (Hastings and Botsford 2006; Botsford *et al.* 2009), however concern arises that demographically isolated populations will be more vulnerable under increasing disturbances (Wilkinson 1999; Pandolfi *et al.* 2003; Hoegh-Guldberg *et al.* 2007; Hughes *et al.* 2010).

Most striking is the short distances over which relatively large genetic subdivision accumulates for these species within regions (<10 km), and which is strongly supported by population differentiation estimates, clustering, and spatial autocorrelation. A similar spatial-genetic analysis of *S. hystrix* in Western Australia showed that populations were constrained by larval dispersal within 20 km (Underwood *et al.* 2009), however the results of my study indicate a strong genetic break at distances of ~3 km within regions, beyond which genetic differentiation does not increase at the same rate in *Sh_sheltered*. A review of dispersal distances for marine organisms found that invertebrates exhibit a wide range of dispersal capabilities from tens of meters to hundreds of kilometers (Kinlan and Gaines 2003), and mean dispersal of 2 – 3 km for these putative species is average. The dispersal distance inferred for *Seriatopora* represents the majority of successful larval recruitment in these species (Sammarco *et al.* 1989), and strong dilution of larval concentrations beyond this distance with increasingly lower probabilities of survival and reaching a suitable habitat for settlement (Cowen *et al.* 2000). High local recruitment in these *Seriatopora*

species reflects the philopatric settlement of immediately competent brooded larvae, which can metamorphose and settle within minutes to hours of release and without any obvious cues or particular settlement surface requirements (P. Warner pers. obs.). The obvious advantage of immediate settlement is avoidance of high planktonic mortality and assured suitable habitat (Vance 1973; Sebens 1983; Grosberg 1987), which may be especially important to a coral with maternally transmitted obligate photosymbionts, *Symbiodinium*, that provide the majority of the coral's energetic needs (Muscatine and Cernichiari 1969; Muscatine and Porter 1977). The different putative species of *Seriatopora* harbor distinct and highly specific types of *Symbiodinium* (Chapter 2) that are coevolved with their host species and adapted to particular depth habitats (Bongaerts *et al.* 2011). Furthermore, these associations with particular symbiont types are relatively inflexible (Bongaerts *et al.* 2011; Fabina *et al.* 2012), unlike many broadcast spawning corals that derive symbiont populations from the environment during settlement and may be able to take up different types under different conditions (e.g., Abrego *et al.* 2012). Thus, the evolution of philopatry and short dispersal distances in these brooding corals may be favored to maintain strong host-symbiont adaptation to local conditions, and is important for self-sustaining local populations.

A small degree of migration between reefs on ecological timescales is indicated by the detection of several first generation immigrants in populations of both putative species. Evidence from other population genetic surveys of *Seriatopora* (van Oppen *et al.* 2008; Noreen *et al.* 2009; Underwood *et al.* 2009; Starger *et al.* 2010) and inference from coral larval biology (Richmond 1987; Isomura and Nishihira 2001; Graham *et al.* 2008) suggest that immediate settlement of most larvae does not preclude the longer distance dispersal of others (Sammarco *et al.* 1989; Kinlan *et al.* 2005). In laboratory settings, although 60% of *S. hystrix* larvae settled within 24 hours, approximately 30% of larvae remained swimming for up to four days before finally settling (Atoda 1951; Isomura and Nishihira 2001). Moreover, a single colony can produce several thousand

larvae released over days to weeks in a single reproductive cycle (i.e., month; P. Warner pers. obs.), incorporating different tidal conditions that combined with the range of larval behaviors possibly acts to retain or disperse planulae in a diversified bet-hedging strategy (Seger and Brockman 1987; Philippi and Seger 1989; Hopper *et al.* 2003; Krug 2009). Since *Seriatopora* larvae contain *Symbiodinium*, pelagic survival may be prolonged by the ability to derive photosynthetic energy while in the planktonic environment, insinuating extended larval competency that has been demonstrated by confamilial *Pocillopora damicornis* with successful metamorphosis after 103 days of larval duration (Richmond 1987). Under the high reproductive capacity of large equilibrium populations in which colony densities of seriatoporids can reach 0.5 m^{-2} on sheltered reefs (Chapter 4), the disproportionately small numbers of immigrants would be difficult to detect and self-recruitment is overwhelmingly the most prominent process. Nevertheless, even rare longer distance dispersal allows colonization of new territory (Noreen *et al.* 2009; Starger *et al.* 2010), which may be enhanced by the ability to self-fertilize (Chapter 4; Sherman 2008) when conspecifics are not present, and is important for population recovery following acute local disturbances (Underwood *et al.* 2007).

The fine-scale spatial analysis within sites identified small neighborhoods of high genetic similarity for both *Seriatopora* species (<20 m), likely caused by philopatric settlement of larvae, and also detected in Western Australian populations of *S. hystrix* (Underwood *et al.* 2007). Colonies separated by greater distances show random associations of genetic relatedness, implying that larval dispersal and mixing within sites and often reefs (i.e., Palm Islands region), largely results in effectively panmictic populations with no evidence of inbreeding. Similar fine-scale spatial-genetic structure is seen in plants with low or absent self-fertilization, implying that low seed dispersal, rather than limited pollen dispersal distances, causes such patterns (Loiselle *et al.* 1995). The apparent absence of inbreeding in these corals, contrary to many previous

genetic assessments of *S. hystrix* (reviewed in Chapter 2), and several private alleles found among populations indicate that mating systems in these corals maintain and even promote genetic diversity (Chapter 4; Noreen *et al.* 2009). Reasons for the substantially higher allelic richness and expected heterozygosities in *Sh_exposed* compared to *Sh_sheltered* populations are unclear, although microsatellite ascertainment bias is expected to be a factor (See Section 2.4.3a). In summary, despite highly localized recruitment in these putative *Seriatopora* species, intrapopulation processes maintain genetic diversity and combined with frequent reproductive events may enhance the ability of populations to rapidly adapt to changing conditions.

3.4.3 Implications for conservation and management

Despite concerns that strong subdivision in *S. hystrix* and apparently low genetic diversity jeopardizes ecological persistence (Ayre and Hughes 2004), identifying cryptic species in my data combined with the prolific reproductive strategies of these putative species suggest that many populations should be relatively resilient. While some studies have concluded that *S. hystrix* is particularly sensitive to heat- and light-induced bleaching (Marshall and Baird 2000; Loya *et al.* 2001; Stimson *et al.* 2002; van Woesik *et al.* 2011), other populations have demonstrated high potential for recovery and recolonization, providing evidence of resilience to such disturbances through metapopulation connectivity (Underwood *et al.* 2007; Noreen *et al.* 2009; Starger *et al.* 2010; van Oppen *et al.* 2011a). Shallow-water *S. hystrix* remains locally extinct at Sesoko Island, Okinawa fourteen years after the 1998 coral bleaching event (Loya *et al.* 2001; van Woesik *et al.* 2011), despite surviving colonies found locally in the mesophotic zone below ~35 m (Sinniger *et al.* 2012). Conversely, *S. hystrix* populations in Western Australia have recovered after catastrophic mortality following a 1998 bleaching event (Underwood *et al.* 2007; van Oppen *et al.* 2011a), and populations in the Indonesian Archipelago have completely recolonized new substrata

with normal levels of genetic diversity in 150 years since the volcanic eruption of Krakatau (Starger *et al.* 2010). Certainly, several factors may influence the probability of recovery in a particular location, yet evidence of potentially widespread cryptic species in this genus may also explain local discrepancies in resilience (Chapter 2). Recognizing such cryptic species clarifies our understanding of connectivity and demonstrates that these corals follow patterns that can be predicted and therefore utilized in management. Instead of a cosmopolitan generalist species, *S. hystrix* may be comprised of many regionally and/ or ecologically isolated cryptic species that are adapted to particular habitats and conditions. Such specialization and cryptic diversity have profound implications for conservation, and emphasize the need to account for different critical habitats and ecological processes in management planning.

Although *Seriatopora* corals typically do not produce large colonies that contribute to major reef accretion in the way that corals like the massive *Porites* do, they do contribute to the complexity of reef structure and constitute significant habitat for small coral reef organisms. A recent study of coral-associated epifauna found that *S. hystrix* harbored three times the abundance and 2.5 times the species richness compared to acroporid corals (Stella *et al.* 2010), highlighting the importance of maintaining seriatoporid populations for overall reef diversity. The apparently high reproductive capacity of *Seriatopora* and their low mean dispersal distance suggest that most populations will be self-maintained at local reef-scales (< 5 km), with strong stock-recruitment relationships (Armsworth 2002; Strathmann *et al.* 2002). Direct local benefits from protecting such populations underscore the effectiveness of marine protected areas (Halpern and Warner 2003), and promote worldwide efforts to conserve even small areas of reef habitat. At larger scales, recruitment from external sources is harder to predict, but populations will likely be maintained if sufficient stepping-stones exist.

Chapter 4.0 Parentage analysis, mating system and sperm dispersal in the brooding coral *Seriatopora*

4.1 INTRODUCTION

The exchange of gametes among individuals represents the first link in the network of associations that determine species-wide connectivity patterns. In sessile organisms, such as plants and reef-building corals, opportunities for gene flow mostly occur during mating or subsequent seedling/ larval dispersal, and the distance over which reproduction occurs determines the number of individuals involved and defines the local population, or deme (Waples and Gaggiotti 2006; Hartl and Clark 2007; Hedrick 2011). The spatial extent of gamete and offspring dispersal also determines the degree of inbreeding within and genetic structure among populations of a given species (Jain 1976; Hamrick 1982; Dick *et al.* 2008). Simultaneous hermaphroditism, a common mating system in sessile organisms, may give rise to complex interactions among reproductive characteristics, influencing the degree of selfing vs. outcrossing (i.e., mixed mating; reviewed in Goodwillie *et al.* 2005; Jarne and Auld 2006), correlated-paternity (Ritland 1989, 2002) among maternal siblings or vice versa (i.e. multiple matings/ multiple paternity; (Johnson and Yund 2007; Tani *et al.* 2009)), and variation in the extent of biparental inbreeding among close relatives (Ledoux *et al.* 2010a; Li *et al.* 2012). Knowledge of reproductive behaviors and mating system characteristics is essential not only to fully understand the genetic connectivity of a species at a given time, but also to predict how populations and species will respond under different climate change or disturbance scenarios (Hoegh-Guldberg 1999; Aitken *et al.* 2008). While plant research has long recognized the importance of pollen flow and mating system characteristics on genetic connectivity (Bawa 1974; Palmer *et al.* 1988; Ellstrand *et al.* 1989; Burczyk *et al.* 1996; Chase *et al.* 1996; Ruckelshaus 1996; Goodell *et al.* 1997; Streiff *et al.* 1999; Hardy *et al.* 2004; Latouche-Halle *et al.* 2004;

Bittencourt and Sebbenn 2007; Garcia *et al.* 2007; Ismail *et al.* 2012; Ottewell *et al.* 2012), comparable investigations in corals remain remarkably limited (but see Heyward and Babcock 1986; Oliver and Babcock 1992; Knowlton *et al.* 1997; Miller and Babcock 1997; Szmant *et al.* 1997; Willis *et al.* 1997), particularly under natural conditions (but see Ayre and Miller 2006; Sherman 2008; Yeoh and Dai 2010; Carlon and Lippé 2011; Douek *et al.* 2011).

Scleractinian corals exhibit two main modes of reproduction that differ in their implications for population connectivity: 1) broadcast spawning, which is widespread in many aquatic organisms; and 2) brooding, also known as 'spermcasting' (Pemberton *et al.* 2003b). Species that broadcast spawn release both eggs and sperm for external fertilization followed by obligate pelagic larval development for several days before reaching settlement competency. More similar to pollen flow in plants, brooding species release sperm, but retain eggs for internal fertilization. Subsequently, brooded larvae are released in various states of advanced development (Harrison and Wallace 1990), and may be able to settle immediately (P. Warner pers. obs.), which has prompted expectations of consequently shorter mean dispersal distances and philopatric behavior relative to broadcast spawned larvae (Harrison 2011). Yet unlike broadcast spawned larvae, which mostly derive their photosynthetic endosymbionts environmentally upon settlement, brooded larvae frequently obtain their *Symbiodinium* by vertical transmission from the maternal colony (Baird *et al.* 2009). Thus, association with their energetic partners in the planktonic environment suggests that brooded larvae may also be able to survive for long periods of time and disperse widely (Richmond 1988). Both modes of reproduction may include gonochorism (i.e., dioecious) or hermaphroditism, however the majority of coral species are broadcast spawning hermaphrodites (Baird *et al.* 2009). Nevertheless, several abundant and widespread corals brood larvae, most notably many species of the well-studied coral family Pocilloporidae (Baird *et al.* 2009). Yet, almost nothing is known about sperm

dispersal for any brooding coral, including all processes that occur from the time the spermatozoa mature and are released to the subsequent release of mature larvae (e.g., sperm dispersal, sperm acquisition, fertilization, embryogenesis).

Genetic parentage analysis is increasingly used to investigate mating systems and pollen dispersal in natural plant populations (Streiff *et al.* 1999; Robledo-Arnuncio and Gil 2004; Oddou-Muratorio *et al.* 2006; Bittencourt and Sebbenn 2008; Pluess *et al.* 2009), however almost no comparable studies exist for corals (but see Lasker *et al.* 2008; Yeoh and Dai 2010). Brooding species can be excellent subjects for parentage studies, as offspring can be collected in large broods of half-siblings for which the maternal parent is known (Jones *et al.* 2010). Accordingly, *Seriatopora hystrix* is a simultaneous hermaphroditic pocilloporid coral that has been shown to release sexually produced brooded larvae (Ayre and Resing 1986; Sherman 2008), although population genetic studies have indicated that asexual propagules might also exist (van Oppen *et al.* 2008). One study conducted on the Southern Great Barrier Reef (GBR) found that *S. hystrix* colonies were self-compatible and that rates of self-fertilization were approximately 50% in significantly inbred populations (Sherman 2008). However, evidence presented in this thesis demonstrates that the single morphospecies *S. hystrix* is likely comprised of several cryptic species on the GBR, which may have dissimilar reproductive strategies (Chapter 2). In this study, I use a novel parentage analysis on brooded larvae of the putative species *Sh_sheltered* (Chapters 2, 3) to estimate sperm dispersal for the first time in any brooding coral species. Moreover, I develop a consensus method for maximizing accuracy in paternity assignments for a natural population in which no prior information was available. Finally, I estimate mating system parameters and analyze the spatial structure of the established population of coral colonies in order to better understand how intrapopulation processes affect genetic connectivity at larger spatial scales.

4.2 MATERIALS AND METHODS

4.2.1 Field mapping and sampling

A 16 m^2 study site was marked semi-permanently with stakes on the reef flat of the Lizard Island lagoon (Fig. 4.1) in May 2009. All colonies of *Seriatopora* within the area were numbered and tagged. Samples of 2 – 3 cm were collected by hand for each colony and preserved in 100% ethanol for preliminary genetic analysis. Prior to the new moon in November 2009, all colonies of *Seriatopora* at the study site (i.e., the standing population) were mapped by x, y coordinates, resampled and their perpendicular diameters and heights measured. To incorporate some colonies at greater distances, two 16 m transects were run parallel to the study site at 5 and 10 meters on either side perpendicular to the reef edge. All seriatoporids within 1 m of transects were similarly mapped, measured and sampled with reference to the study site.

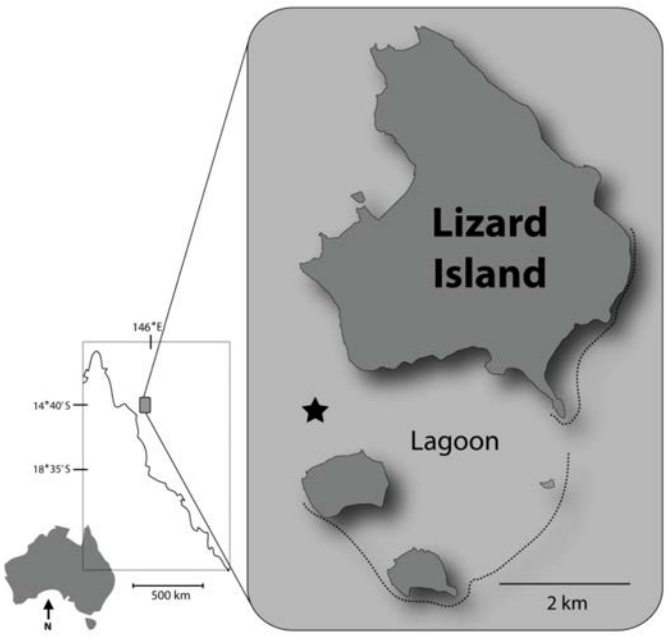


Figure 4.1 Study site (star) of mapped *Sh_sheltered* colonies at Lizard Island lagoon. The inset shows the location of Lizard Island relative to the coast of Queensland, Australia.

4.2.2 Larval collection

Following preliminary genotyping, 18 colonies were selected for larval collection based on genetic identity, size and central position within the study site. Whole specimens or fragments of maternal colonies were removed from the reef one day before the November new moon (15 Nov. 2009) and isolated in individual flow-through aquaria at Lizard Island Research Station. Aquaria were located outside and shaded. Seawater was filtered to 1 µm to eliminate possible contamination from external larval sources and supplied to aquaria at ambient temperature. The outflow of each aquarium was directed through a larval trap of 200 µm plankton mesh. Each morning larval traps were examined and the number of larvae present were counted, collected and stored in individual vials of 100% ethanol. Colonies were monitored daily for two weeks, and larvae stored separately for each day and maternal colony. After the two week collection period, all live colonies were returned to the reef.

4.2.3 Colony and larval genotyping

Except for one colony, which released only 26 larvae over the two weeks, at least 30 and up to 73 larvae were genotyped for twelve maternal broods. To spread the genetic sample evenly over time, larvae from each colony were subsampled across all days of release according to the proportion of larvae released per day versus the total number released for each mother. For each larva, DNA was extracted in 35 µL of Gloor and Engels' buffer (10 mM Tris-Cl pH 9.0, 1 mM EDTA, 25 mM NaCl, 200 ug/ml Proteinase K), incubated at 37 °C for 30 min followed by 95 °C for 2 min to deactivate the enzyme (Gloor and Engels 1992). DNA was extracted from all mapped colonies according to the protocol developed for the black tiger shrimp (Wilson *et al.* 2002) and optimized for coral tissues (Section 2.2.1). All individuals were amplified with ten microsatellite loci (Underwood *et al.* 2006) in 10 µL reactions with 1 µL of DNA extract (colonies diluted 1:10, larvae undiluted). The ten loci were combined into three

multiplex PCRs, as detailed in Chapter 2. Five µL of purified PCR product was separated on the GE Healthcare MegaBace 1000 capillary sequencer at James Cook University's Genetic Analysis Facility (Townsville, Australia). The MegaBACE Fragment Profiler Software Version 1.2 (GE Healthcare) was utilized to determine the fragment sizes (alleles) present in microsatellite electropherograms. I manually verified all genotype scores, and samples with ambiguous or missing signals were re-amplified and re-run. Approximately three positive controls were amplified and separated on every 96-well plate to estimate locus-specific genotyping error rates. All individuals (colonies and larvae) were also amplified with the mtDNA primers (ShMT662F, ShMT960R) following the protocol described in Chapter 2. Three µL of product was run on 3.5% agarose gels at 130 V for at least 120 minutes with HyperLadder™ V size marker loads (Bioline, NSW, Australia) and positive controls. Final gels were photographed by the Chemi-Smart imaging system (Vilber Lourmat ChemiSmart 3000) and manually scored as Small, Medium, or Large amplicons (Chapter 2). All individuals (colonies and larvae) contained the small sized mitochondrial amplicon (S-types), and are assumed to belong to the *Sh_sheltered* putative species based on habitat and genetic similarity to other lagoonal populations of Lizard Island (Chapter 2).

4.2.4 Genetic statistical analysis

Individuals (colonies and larvae) sharing identical multilocus genotypes (MLG) were identified and the individual genotypic probabilities (GP) calculated with GenAlEx v6.4 (Peakall and Smouse 2006). All colonies sharing the same MLG had GP <0.001 and occurred in close spatial proximity, and were inferred to be asexual clone mates. Therefore, only one individual of paired clone mates was included in subsequent genetic analyses. Summary descriptive statistics, probabilities of exclusion (P_{ex}) for the marker set, pairwise squared genotypic distances, pairwise relatedness (2x [Lynch and Ritland 1999] so that relatedness is indicated on a scale of -1 to 1, rather than -0.5 to 0.5) and pairwise geographic distances between individuals were computed in

GenAlEx v6.4. The P_{ex} indicates the effectiveness of marker loci to exclude unrelated individuals from the parentage of a given offspring, and presented here as the probability of excluding an unrelated individual as father given the known mother (Jamieson and Taylor 1997). Deviations from HWE were assessed in Cervus 3.0 (Kalinowski *et al.* 2007) using a chi-square test with a Bonferroni correction, and verified by the exact test method implemented in GENEPOP v4.1.1 (Raymond and Rousset 1995; Rousset 2008) with default Markov chain parameters. Linkage disequilibrium was assessed with GENEPOP v4.1.1 and significance adjusted with a Bonferroni correction. Null allele frequencies were estimated in Cervus 3.0 and locus-specific genotyping error rates estimated by a combination of positive controls and mother-offspring mismatches. Allele frequencies were calculated in Cervus 3.0 using established colonies only, excluding larvae. For the colonies sampled from the study site, a global multivariate spatial autocorrelation was implemented in GenAlEx v6.4 (Smouse and Peakall 1999; Peakall *et al.* 2003; Double *et al.* 2005; Smouse *et al.* 2008) to assess the correlation of pairwise squared genetic distance to spatial distance (Chapter 3). Correlograms were created by GenAlEx v6.4 and statistical significance of **r** was tested with 9999 random permutations and bootstraps. The analysis was conducted with uneven distance classes to account for variable pairwise distances between colonies at increments of: 1) 0.5 m for distances up to 10 m, 2) 1 m up to 20 m, and 3) 2 m up to the last distance class of 38 m.

4.2.5 Mating system and parentage analyses

Several different analyses were employed to assess mating system parameters, sibling groups and paternity in the larval broods, as well as the standing population (i.e., the colonies mapped and sampled for this study). MLTR v3.2 (Ritland 2002) was used to estimate the following mating system parameters averaged across all broods, as well as for each individual brood: 1) multilocus (t_m) and single locus (t_s) outcrossing rates; 2) the correlation of paternity (r_p), which is the fraction of siblings

within a brood that share the same father (i.e. full siblings); and 3) an estimate of biparental inbreeding (mating among close relatives) based on the difference between single and multilocus outcrossing rates ($t_m - t_s$). See Ritland (2002) for explanation of how parameters are estimated. MLTR was developed for plants with mixed mating systems that can both outcross and self-fertilize, and can have large offspring arrays with multiple paternity. Organisms with such mixed mating systems are expected to self-fertilize at a rate **s**, and the rate of outcrossing is the remaining fraction of fertilizations (t = 1-s). MLTR analyses were conducted with known mothers using the Newton-Raphson method on all loci, 1000 bootstraps, separately estimated sperm/ egg gene frequencies, inference of the most likely parent, and (microsatellite) bin size= 0. The probability that individual larvae were produced by selfing was also calculated.

For the paternity analysis of known maternal broods, I based individual assignments on the consensus of two common programs: Cervus 3.0 (Kalinowski *et al.* 2007) and COLONY 2.0 (Jones and Wang 2010). Both programs use a likelihood framework and incorporate genotyping errors, however they use different methods to assign paternity. Cervus 3.0 employs categorical allocation, based on Mendelian transition probabilities (Marshall *et al.* 1998), to select the most likely father for each offspring (i.e., larva) when a number of candidates cannot be excluded. Candidate fathers with the highest likelihood-odds ratio (LOD) score are assigned to a larva, and statistical confidence is estimated by a critical value of Δ (the difference in LOD between the highest and second highest scores of candidate fathers) determined from simulations (Marshall *et al.* 1998). COLONY 2.0 simultaneously performs sibship reconstruction and paternity assignment with a group-based approach that considers the likelihood over the entire pedigree rather than pairwise sibling or parent-offspring dyads (Jones and Wang 2010), but also concurrently performs independent pairwise analyses similar to Cervus. Under the assumption of HWE, linkage equilibrium and Mendelian segregation, COLONY 2.0 employs the simulated annealing technique

(Kirkpatrick and Vecchi 1983) to find the best configuration of sibship and associated parentage without searching every possible arrangement (Wang 2004). Although the several versions of Cervus have been used extensively for parentage analysis (Pemberton 2008; Jones *et al.* 2010), recent studies have concluded that the joint sibship reconstruction methods employed by COLONY 2.0 generally result in higher accuracy with greater power from sibling groups (Wang 2004; Wang 2007; Ashley *et al.* 2008; Wang and Santure 2009; Walling *et al.* 2010; Harrison *et al.* 2012a). Nevertheless, preliminary investigations indicated that different runs of COLONY 2.0 yielded some variable assignments and/ or posterior probabilities (Wang and Santure 2009). Moreover, as the goal of these analyses was to measure sperm dispersal distance, thus accurate paternal assignments are essential, I applied strict confidence categories based on consensus among several runs within and among different methods (Fig. 4.2). Combining results from independent runs within (Wang and Santure 2009) and between (Coltman *et al.* 2005; Herbinger *et al.* 2006; Sheikh *et al.* 2008a; Sheikh *et al.* 2008b; Walling *et al.* 2010) different methods has been suggested as a robust means to reach a common solution in the face of uncertainty.

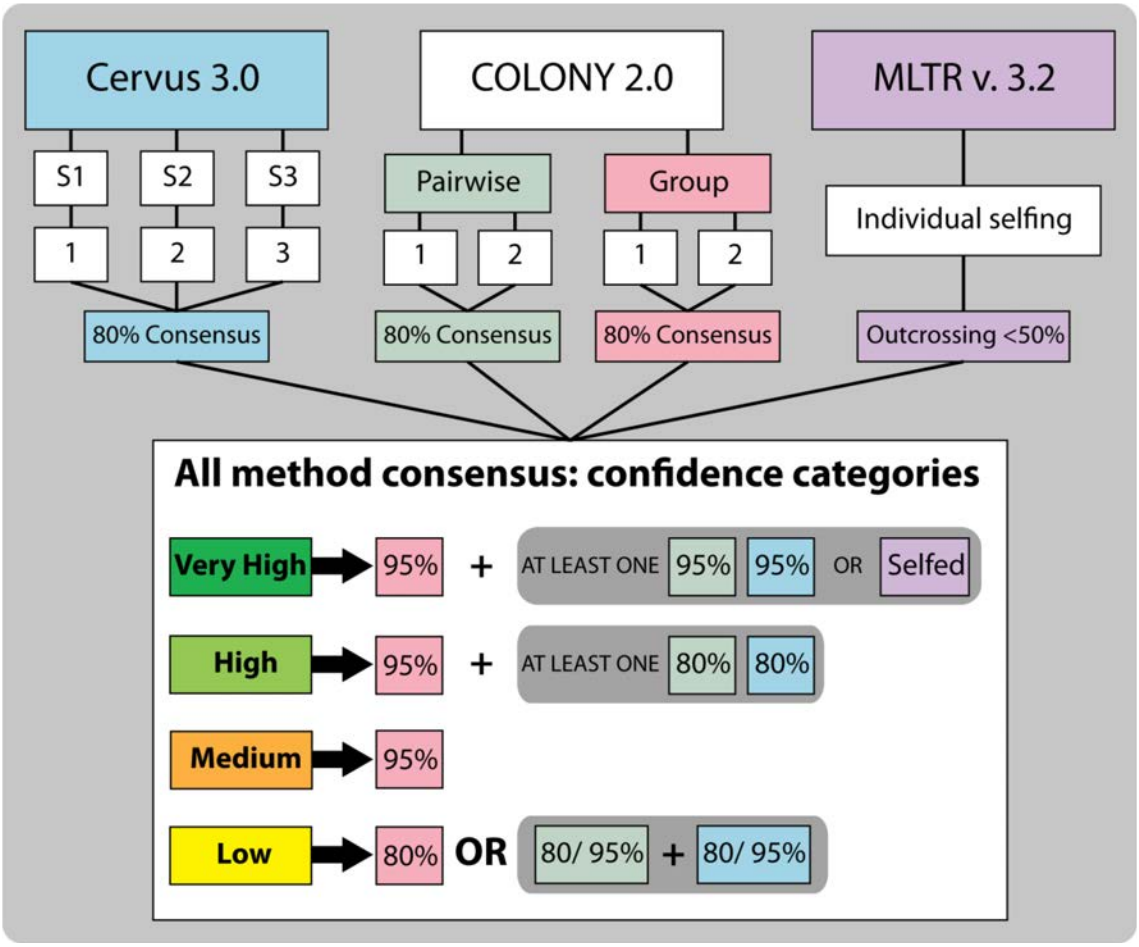


Figure 4.2 Four consensus confidence categories for paternity assignments defined for this study: Very high, High, Medium, and Low. Three software programs were utilized in these analyses: Cervus 3.0 (Kalinowski *et al.* 2007), COLONY 2.0 (Jones and Wang 2010), and MLTR v. 3.2 (Ritland 2002). The Cervus analysis (aqua) was conducted with three replicate runs on three separate data simulations (S1-3). The COLONY analysis included two methods (Pairwise (green) and Group/ Full-likelihood (rose)) per each of two replicate runs for each maternal brood. MLTR (purple) was run once with 1000 bootstrap replications. For Cervus and COLONY only those assignments with at least 80% confidence and common to all replicate runs per method were retained for further analysis. MLTR was only used to infer the paternity of larvae that resulted from self-fertilizations, and only those individuals estimated to have been derived by less than 50% outcrossing were retained. All retained paternity assignments from the first level of consensus were separated according to the four confidence categories depicted on the diagram: **Very high (dark green):** 95% confidence COLONY-group AND at least one assignment of 95% confidence from either pairwise method or MLTR selfed; **High (light green**): 95% confidence COLONY-group AND at least one assignment of 80% confidence from either pairwise method; **Medium (orange):** 95% confidence COLONY-group only; **Low (yellow**): 80% confidence COLONY-group OR both pairwise methods with at least 80% confidence. Pairwise assignments found for only one method were eliminated.

With both approaches, maternal genotypes were known and entered into the analyses. Based on the area of reef surveyed, the estimated distance over which sperm can disperse estimated from longevity expectations, and the observed density of the population, all paternity analyses were run with the assumption that 90% of potential fathers were sampled. The proportion of candidates sampled can have a significant effect on the success of parentage analysis (Marshall *et al.* 1998; Nielsen *et al.* 2001; Wilson and Ferguson 2002; Jones and Ardren 2003; Koch *et al.* 2008; Harrison *et al.* 2012a), and the effects of this assumption on the results of this study and possible violations will be fully discussed. However, to minimize Type I errors (i.e., false positive assignments), only colonies sampled from the study site that were at least 8 cm or greater mean diameter were included as candidate fathers for the following reasons. First, under the likelihood statistical approach, non-excluded full siblings of offspring can generate higher likelihood values for paternity assignments than actual parent-offspring relationships (Thompson 1976; Meagher and Thompson 1987). Secondly, with overlapping generations and a high expectation for philopatric larval settlement, the smallest size class of colonies sampled may be the recent progeny of other corals in the study site and therefore have the highest likelihood of shared parentage with larvae assayed (i.e., full or half siblings of larvae). Thirdly, the limited information available indicates that *S. hystrix* colonies do not release larvae at sizes < 8cm mean diameter (Stimson 1978). Although it is still possible that smaller colonies could be protandric hermaphrodites with mature spermaries (Harrison and Wallace 1990), their contribution is likely a small proportion of the available sperm pool with consequently fewer paternities. Therefore, it was judged that a slight potential loss in the proportion of candidate fathers sampled due to elimination under the size criteria was preferable than spurious paternity assignments to possible full siblings included in the candidate father pool.

One of the primary disadvantages to the COLONY method is the computational time requirements, which are strongly influenced by the number of offspring (Wang 2004). Therefore, for the COLONY 2.0 analyses, the larval sample set was divided into maternal broods and each half-sib assay was run individually with two independent replicate runs from different starting number seeds. Input parameters for each run were: both sexes polygamous and monoecious (i.e., hermaphroditic), allowing inbreeding, long runs of the full likelihood model, very high likelihood precision, the sibship complexity prior, and no updating of allele frequencies. Each run included explicit marker error rates, null allele frequencies, allele frequencies calculated from the standing population, 90% sampled candidate fathers and known maternal sibships (half-sib arrays). Moreover, any larvae with matching MLG within broods were assumed to be full siblings and therefore input as paternal sib-groups. Following analyses, all results were compiled and only assignments found in common to both replicate runs with at least 80% confidence were considered for either the pairwise or group approach (Fig. 4.2). The Cervus 3.0 analysis was run three times with three different data simulations that were used to calculate the strict (95%) and relaxed (80%) confidence levels for paternity assignments. All simulations were conducted on allele frequencies calculated from the standing population with 10,000 offspring, 90% sampled candidate fathers, and the error rate was 1%. Only assignments with at least 80% confidence in all three Cervus runs were considered under subsequent consensus criteria (Fig. 4.2). Finally, all the accepted assignments from Cervus and COLONY, as well as individual selfing estimates from MLTR, were combined into the following four categories of confidence, based on consensus and no conflicting assignments between methods (Fig. 4.2): 1) **Very high** – group COLONY 95% posterior paternity probability + at least one pairwise (COLONY and/or Cervus) 95% confidence assignment or inferred selfing from MLTR; 2) **High** – group COLONY 95% posterior paternity probability + at least one pairwise 80% confidence assignment; 3) **Medium** – group COLONY 95% posterior paternity probability, but no matching pairwise assignment; 4) **Low** – group

COLONY 80% posterior paternity probability or pairwise consensus (both COLONY and Cervus) 80/ 95% confidence assignments. Based on the resulting paternity assignments, various estimates of sperm dispersal distances and larval shipship were calculated manually in Microsoft Excel.

4.2.6 Sibship and parentage analysis of population size cohorts

In addition to the larval analyses, I conducted an exploratory investigation of relatedness among colonies sampled from the study site. These results are intended primarily to estimate the extent of relatives within the population, which might adversely affect a paternity analysis. Based on size classes, which are considered to be generally analogous to age classes in coral populations, sibship reconstruction was implemented in COLONY 2.0 to assess the potential for full and half-siblings within different cohorts. Colonies were divided into four size groups based on mean diameters: <8 cm, 8 – <14 cm, 14 – <20 cm, >20 cm. The total size of clones was utilized, i.e., both clonemates added together. Each size cohort was analyzed with a single run in COLONY 2.0 using no candidate parents and the following input parameters: both sexes polygamous and monoecious, allowing inbreeding, long runs of the full likelihood model, medium likelihood precision, the sibship complexity prior, updating of allele frequencies from the sample with explicit marker error rates and null allele frequencies. Similarly, analyses were conducted to explore whether colonies in the smallest “pre-reproductive” size class (<8 cm) could have full sibling relationships with larval broods, and/ or be assigned parentage to other larger colonies sampled from the study site. In one analysis, all larvae (n=495) and colonies < 8cm (n=32) were assessed in a single run with larval broods in maternal half-sibships, but without known maternal genotypes or any sampled candidate parents. The same input parameters were used as for other larval runs, except that length of run and likelihood precision were set to medium. Another analyses performed a joint sibship and parentage analysis on the small colonies only (n=32) with medium likelihood precision, long run length and using the

remaining sampled colonies as candidate parents, assuming that only 50% of the potential parents were sampled.

4.3 RESULTS

4.3.1 Mapped study site and population size structure

In total, 149 *Seriatopora* colonies were mapped, measured and sampled from the study site (Fig. 4.3). Based on the 125 colonies recorded within the central 256m^2 area, the population density was calculated as approximately one colony per 2 m^2 of reef. Twenty-four colonies were surveyed from the four transects outside of the central mapped area. The mean pairwise spatial distance between colonies within the total surveyed area was 9.53 m (±0.06 SE; median= 8.42 m; range: 0 – 36.54 m), whereas the mean distance between mother colonies and all other colonies (i.e., candidate fathers) was only 7.32 m (±0.11 SE; median= 6.16 m; max: 22.42 m). The size distribution of the population was skewed towards smaller colonies, with the greatest number of colonies within the 8 – <14 cm mean diameter size class (n=61) and 66% of all colonies under 14 cm mean diameter (Figs. 4.3, 4.4). Thirty-two colonies were <8 cm mean diameter and therefore eliminated from the candidate pool of fathers, because they were below maturity size (Section 4.2.5). The largest colony measured was 31.5 cm mean diameter and the smallest was 0.5 cm. The average size of the twelve mother colonies was 17.04 cm (±1.12 SE). Considering the 125 colonies within the central square (256 m^2) and the size of each colony (in cm^2 perpendicular diameters), *Seriatopora* colonies made up approximately 0.77% of the reef benthos.

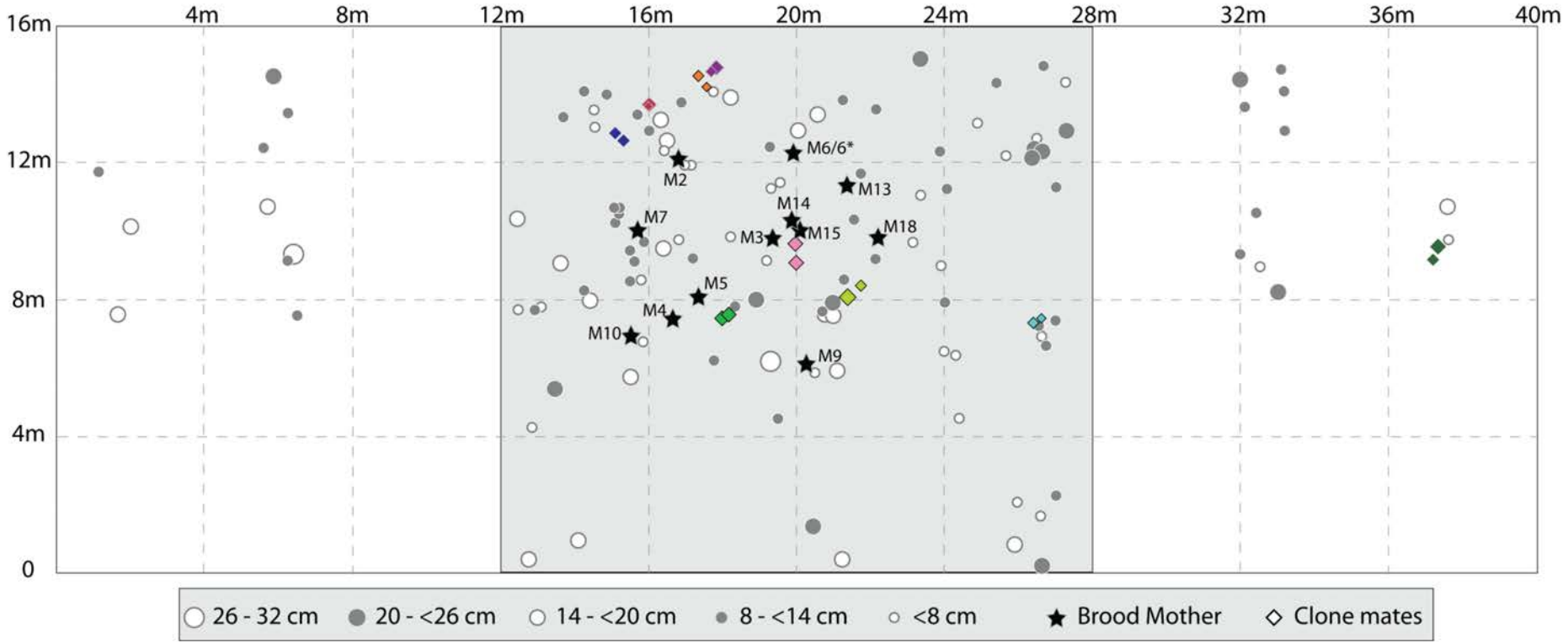


Figure 4.3 The study area at Lizard Island lagoon showing position and size of all mapped *Sh_sheltered* colonies. The main area in which all colonies were sampled is 16 m x 16 m (gray central square). Outside of this area, two 2-meter belt transects were run parallel on either side at 5m and 10m within which all colonies were similarly mapped and sampled. Colony sizes are depicted according to five size classes as defined in the key underneath the map. Clone mates, individuals with matching genotypes, are indicated by matching colored diamonds. The position of each of twelve mother colonies is shown by black stars, and labeled by IDs matching Table 4.2. The reef edge is at the high end of the y-axis (i.e., 16 m).

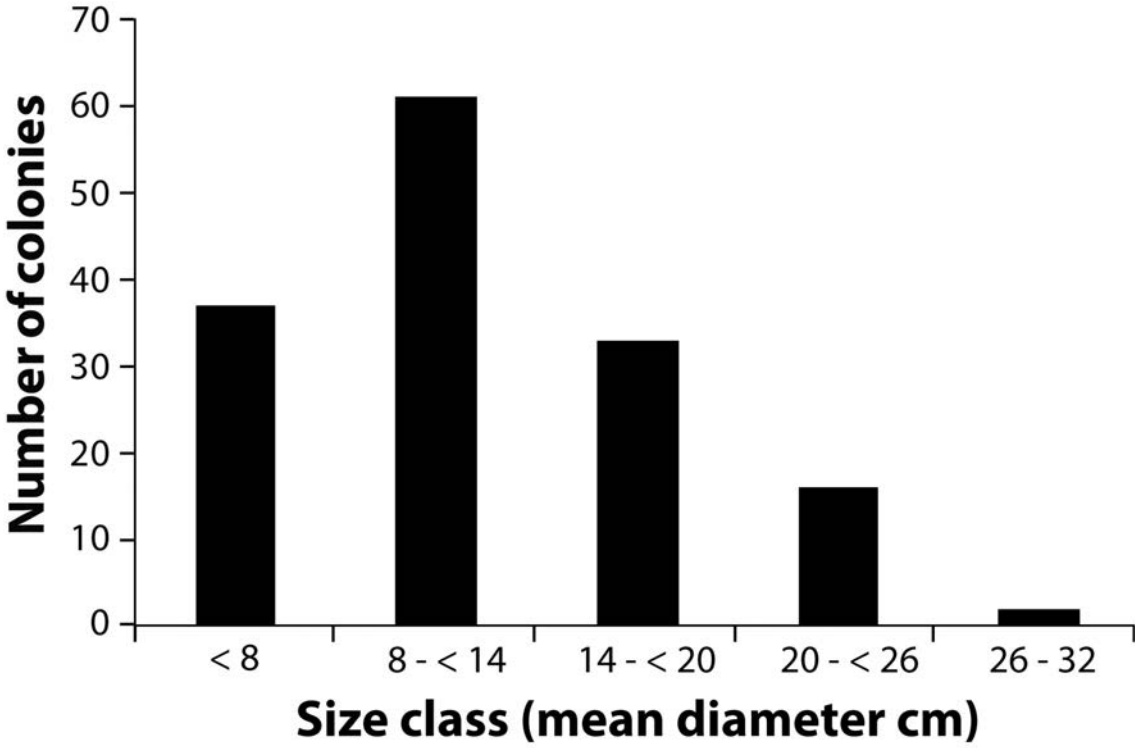


Figure 4.4 Size structure distribution of 149 *Sh_sheltered* colonies sampled at the Lizard Island lagoon site. Each colony was measured by perpendicular diameters in centimeters.

4.3.2 Larval release

Of the 18 colonies that were collected and monitored, six did not release any larvae over the observation period from the last quarter moon (9 November 2009) to three days after the first quarter moon (27 November 2009). Two colonies died during the experiment following complete 'polyp bailout' (*sensu* (Sammarco 1982); see Fig 1.2), but one of those colonies (M3) also released larvae prior to bailout. Larval release began one day prior to the new moon, but was not synchronous among all colonies. Several colonies did not start releasing until five days after the new moon, and one colony started releasing seven days after the new moon. Once larval release began, colonies generally released larvae every morning over several days (4 – 8) and many were still releasing when the experiment was terminated. A total of 2454 larvae were collected from the twelve colonies that did release and the number of larvae per colony

ranged from 26 to 429. In some cases, only fragments of the maternal colony were collected for larval sampling, and it is likely that some larvae escaped traps and were not collected; therefore these numbers do not reflect total outputs for each colony.

4.3.3 Genetic diversity and microsatellite marker panel

A total of 644 individuals (149 colonies and 495 larvae) were genotyped at ten microsatellite loci with 99.9% complete genotype data. The experimental error from positive controls was zero for all loci, however five larvae mismatched their maternal genotypes at a single locus (0.1%). One of these mismatches was detected prior to paternity analysis as a probable *de novo* mutation event or genotyping error and manually corrected. The other four mismatches potentially occurred as a result of null alleles, but a null allele was only detected by the population analysis at one (Sh3-004) of two affected loci (Table 4.1). The error rate for the other locus (Sh3-009) was adjusted appropriately. Two loci were monomorphic for all individuals and a third had just two alleles, of which one was present in only a single colony (Table 4.1). Therefore, all paternity analyses were conducted on seven polymorphic markers with mean expected heterozygosity of 0.578 (range 0.449 – 0.737) and 5.14 alleles/ locus. All loci met expectations of Hardy-Weinberg equilibrium and linkage equilibrium, except locus Sh2-006, which showed significant heterozygote excess. Null alleles were detected at four loci at low frequencies (<0.007; Table 4.1). The combined probability of exclusion for the marker set was 0.77, indicating a 23% chance of not excluding an unrelated individual from paternity of a given larva. The Cervus 3.0 analysis further indicated that power to exclude paternity in this population was limited. When all possible fathers were inferred, a total of 4401 putative fathers yielded positive LOD scores (~9 fathers per each larva) and 92% (4035) did not mismatch their prospective progeny at any loci.

Table 4.1 Summary statistics for ten microsatellite loci. N: number of colonies genotyped from standing population; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; P_{ex}: probability of exclusion given the known maternal genotype; F_{IS}: Weir and Cockerham's (1984) inbreeding coefficient; Null: frequency of null alleles; Error: frequency of genotype error. Three loci (italicized) were monomorphic and eliminated from the subsequent analyses. The mean values (bold) indicate the statisitcs average across the seven polymorphic loci, except the mean P_{ex} represents the additive estimate for all loci. Red bold type indicates significant heterozygote excess at α = 0.05. All other loci meet HWE expectations.

	Sh4-001	**Sh2-002**	*Sh3-003*	**Sh3-004**	*Sh2-005*	**Sh2-006**	**Sh3-007**	*Sh3-008*	**Sh3-009**	**Sh4-010**	**Mean**
N	141	141	*141*	141	*141*	141	141	*141*	141	141	**141**
N_A	3	8	*1*	4	*2*	3	4	*1*	5	9	**5.14**
H_O	0.582	0.752	*0.000*	0.496	*0.007*	0.582	0.652	*0.000*	0.553	0.511	**0.590**
H_E	0.630	0.737	*0.000*	0.449	*0.007*	0.497	0.629	*0.000*	0.571	0.534	**0.578**
P_{ex}	0.20	0.34	*0.00*	0.10	*0.00*	0.12	0.20	*0.00*	0.17	0.16	**0.77***
F_{IS}	0.081	-0.016	-	-0.102	-	**-0.167**	-0.033	-	0.034	0.047	**-0.023**
Null	0.0061	0.0033	-	0.0068	-	-	0.0029	-	-	-	**-**
Error	-	-	-	-	-	-	-	-	0.004	-	**-**

4.3.4 Clones, chimeras and spatial-genetic structure

Of the 149 colonies genotyped, nine genotypes were identical between nine pairs of colonies (94% genotypic diversity; Fig. 4.3). The paired clone mates were located a maximum of 0.56 m from one another (mean= 0.32m ±0.15 SD) and all had genotype probabilities <0.0001. In one case, field observations identified one of these paired clone mates as a result of partial mortality from a single large colony prior to genetic confirmation. Following removal of replicated genotypes, 140 unique multilocus genotypes remained in the surveyed population for subsequent analysis. However, when the larval broods were compared to maternal genotypes, 52% of larvae (i.e. 38 larvae) from one large brood (M6, n=73 larvae) mismatched the sampled maternal genotype at 1 – 2 loci. Many of the mismatched larvae were homozygous or heterozygous at locus Sh4-001 for two alleles not present in the maternal genotype, and homozygous for non-maternal alleles at locus Sh3-007. This large number of mismatches could not be attributed to either null alleles or genotyping errors (Table 4.1), but all 38 mismatched larvae could be explained by a single maternal genotype different from that of their sampled mother (M6). The larval mismatches were also checked against other maternal colonies to confirm that a sampling/ labeling error was not at fault. Therefore, it was inferred that this particular mother colony was a chimera comprised of at least two genotypes and the brood split accordingly into M6 and M6*. The missing maternal genotype inferred by both MLTR v.3.2 and COLONY 2.0 was identical between the two programs and added into the candidate parent population for a total of 141 unique genotypes (Table 4.1). The inferred genotype (M6*) differed from the sampled colony (M6) at four loci, and contained three unique alleles. The presence of one chimera amongst the twelve maternal colonies suggests a frequency of cryptic genetic diversity of at least 8% in the population. One other chimera was identified during mapping and opportunistically sampled. The chimera contained two color morphs (pink and cream) within its 13 cm mean diameter, but was completely fused

with no discernible rejection reaction. Genotyping of tissues from the two different color morphs confirmed two distinct genotypes that differed at six loci and six unique alleles.

The spatial autocorrelation analysis of mapped colonies did not reveal any significant spatial-genetic structure within the study site (Fig. 4.5), indicating that the population is effectively panmictic over the spatial scale studied. Correlation coefficients were statistically significant (α=0.05) for a few distance classes, however these appear as seemingly random and slight deviations from expectations at distances greater than 5.5m.

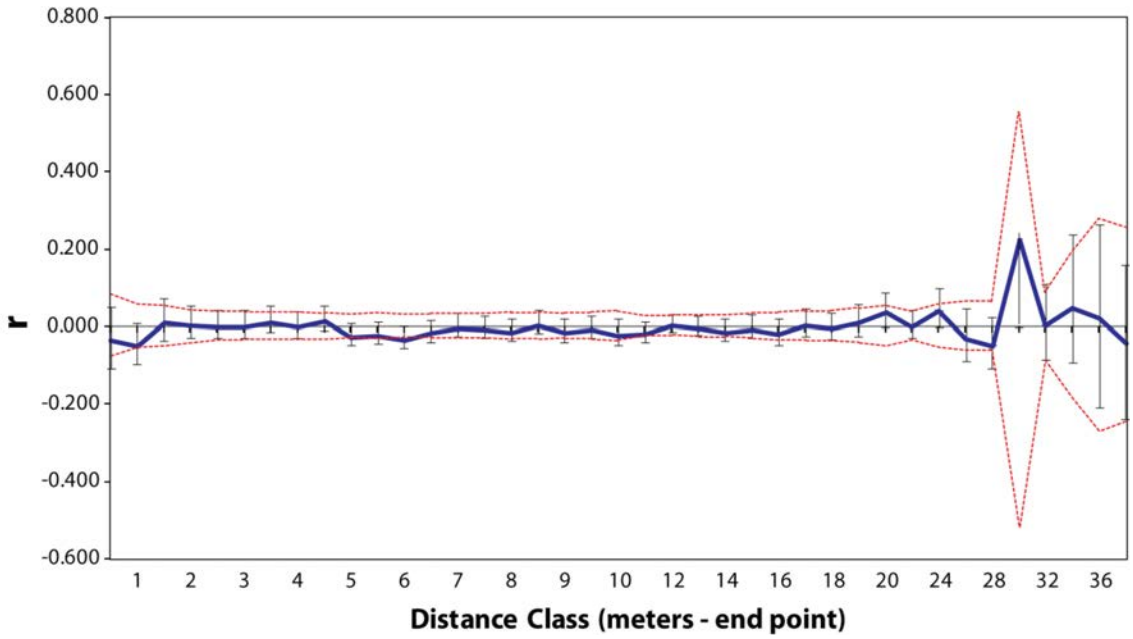


Figure 4.5 Spatial autocorrelation analysis conducted with pairwise codominant genotypic distances and geographic distances amongst all *Sh_sheltered* colonies sampled from the study area at Lizard Island lagoon. Dashed red lines indicate 95% confidence interval (CI) about the null hypothesis based on 9999 random permutations for genetic correlation coefficient r (blue line). Error bars indicate upper and lower 95% CI around r determined by 10,000 bootstraps at each distance class, plotted at the end point in meters. Distance classes are uneven, starting with every 1 m for the first 10 meters, every 2 m up to 20 m, and every 4 m up to 38 m.

4.3.5 Mating system analysis

Overall, the data gave no indication of parthenogenic production of larvae. Only two larvae (0.4%), each in a separate brood, had a multilocus genotype (MLG) identical to that of the maternal colony, however both of these genotypes also had probabilities (GP) >0.004, suggesting that they may also have been produced sexually. Moreover, three other larvae matched MLG of non-maternal colonies sampled from the site. Among the 495 larvae analyzed (N), there were only 457 unique genotypes (G) and within-brood genotypic diversity (G/N) ranged from 0.61 in brood M4 to 1 in six broods (Table 4.2). Most of the matching multilocus genotypes (MLG) occurred within the same broods, although seven MLG were shared between larvae in different broods. Twelve larvae (2.4%) in seven broods contained alleles at 5 loci that were not found in any of the adults sampled (Table 4.2).

The consensus paternity analysis assigned 2.8% of all larvae (n=14) to self-fertilization, but selfing was not detected at all in nine of the thirteen broods. Selfed larvae were detected in the remaining four broods, in which the proportion of selfed larvae ranged from 2% to 23.3% (Table 4.2). For all broods combined, the maximum amount of selfing detected by any one analysis was 3.6% (18 larvae) by MLTR v.3.2, and the results from both the consensus paternity and the MLTR analysis indicated that the broods were almost exclusively outcrossed (mean t_m=0.999 ±0.026 SD; Table 4.2). Furthermore, the MLTR analysis indicated that mating among close relatives (biparental inbreeding) occurred, but was limited. Only four broods had single locus outcrossing rates (t_s) that were greater than multilocus outcrossing rates (t_m), where the difference indicates the amount of apparent selfing that is, in fact, attributable to biparental inbreeding (Table 4.2, italicized). The parental inbreeding coefficient estimated from progeny arrays in MLTR v.3.2 was small (F= 0.052 ±0.09 SD, ns), yet slightly larger than mean F_{IS}= -0.023 (±0.026, ns) calculated from the adult genotypes for the whole sample set.

Table 4.2 Mating system parameters estimated for each larval brood (M#) and the average across all broods (All Larvae) for thirteen *Sh_sheltered* colonies from the Lizard Island lagoon. Where multiple methods were used, estimates are given for each analysis. PA Con: the consensus results from all parentage analyses (See Fig. 4.2); MLTR: results obtained from MLTR v.3.2 (Ritland 2002); Colony-group: mean best configuration results from two replicate full-likelihood runs in COLONY 2.0 (Jones and Wang 2010). Each estimate is provided with a standard deviation (SD) when applicable, except for the proportion of selfed larvae which includes the number of selfed larvae (n). Larvae with unsampled alleles exhibited genotypes with one allele each that was not detected in the standing population of colonies sampled from the study site.

	Analysis	M 2	M 3	M 4	M 5	M 6	M 6*	M 7	M 9	M 10	M 13	M 14	M 15	M 18	All Larvae
Larvae genotyped (N)		30	26	54	59	35	38	30	51	30	31	30	51	30	**495**
Unique genotypes (G)		30	25	33	56	35	38	30	50	29	31	29	48	30	**457**
Genotypic diversity (G/N)		1.00	0.96	0.61	0.95	1.00	1.00	1.00	0.98	0.97	1.00	0.97	0.94	1.00	**0.92**
Larvae with unsampled alleles		1	-	-	3	-	-	-	3	-	1	1	2	1	**12**
Proportion selfed larvae (n)	**PA Con**	0	0	0	0	0	0.105 (4)	0.067 (2)	0.020 (1)	0	0	0.233 (7)	0	0	**0.028 (14)**
Outcrossing rate t (SD)	**MLTR**	1.200 (0.574)	1.200 (0.576)	1.200 (0.574)	1.200 (0.574)	1.008 (0.481)	0.918 (0.437)	0.970 (0.459)	0.995 (0.477)	1.200 (0.569)	1.009 (0.480)	0.753 (0.363)	1.014 (0.487)	1.200 (0.572)	**0.999 (0.026)**
	PA Con	1	1	1	1	1	0.895	0.933	0.980	1	1	0.767	1	1	**0.972**
Biparental inbreeding ($t_m - t_s$)	**MLTR**	0.130 (0.073)	0.085 (0.055)	0.261 (0.120)	0.059 (0.061)	*-0.055 (0.041)*	0.086 (0.048)	0.045 (0.029)	*-0.100 (0.046)*	0.226 (0.111)	0.035 (0.031)	*-0.136 (0.074)*	*-0.040 (0.040)*	0.074 (0.052)	***-0.028 (0.030)***
Correlation of paternity, r_p (SD) proportion of larvae sharing the same father	**MLTR**	0.025 (0.017)	0.070 (0.041)	0.429 (0.195)	0.051 (0.029)	0.245 (0.117)	0.015 (0.010)	0.027 (0.019)	0.016 (0.017)	-0.072 (0.028)	-0.010 (0.008)	-0.023 (0.026)	0.102 (0.048)	0.037 (0.021)	**0.119 (0.052)**
	Colony - group	0.259 (0.366)	0.75 (0.027)	0.630 (0)	0.759 (0.013)	0 (0)	0.684 (0.074)	0.567 (0.094)	0.771 (0.029)	0.667 (0)	0.283 (0.354)	0.603 (0.219)	0.837 (0.029)	0 (0)	**0.524 (0.306)**
Proportion unsampled fathers (SD)	**Colony - group**	0.246 (0.056)	0.702 (0.185)	0.225 (0.035)	0.706 (0.089)	0.171 (0)	0.379 (0.030)	0.191 (0.013)	0.858 (0.082)	0.600 (0.141)	0.266 (0.230)	0.268 (0.025)	0.802 (0.123)	0.328 (0.024)	**0.442 (0.259)**

The correlation of paternity, or the proportion of larvae sharing the same father within a given brood (i.e., full sibs), was calculated manually from the group approach in COLONY V. 2.0 best configuration and generally indicated much higher numbers of full siblings than estimated from the MLTR analysis (Table 4.2). The mean MLTR estimate for correlated paternity was 0.119 (±0.052 SD), signifying that almost 90% of larvae within broods had different fathers. In contrast, the COLONY results were more variable, ranging from all larvae sired from different fathers in two broods to approximately 84% of larvae sharing the same father in brood M15 (Table 4.2). The proportion of unsampled fathers was similarly calculated from the best configuration COLONY results and indicated that as many as 44% (±0.25% SD) of fathers were not sampled.

4.3.6 Paternity analysis and sperm dispersal

The paternity analysis was ultimately conducted with 109 candidate fathers after the removal of clonal replicates and colonies <8 cm mean diameter, and the addition of the inferred genotype for mother M6*. Twenty-two colonies (20% of candidate fathers) were included from the transects outside the central 16x16 m square, and 80% of candidate fathers (n=87) were located within the central area (Figure 4.6). Considering all four consensus confidence categories, 26**%** of larvae (n=129) were assigned to 57 fathers from the candidates sampled (Table 4.3). The majority of assignments (91%) were to candidate fathers within the central square, but 9% of the total paternity assignments were attributed to seven fathers outside the central square (Figure 4.6). Many colonies in close proximity to mothers were not assigned paternity to any of the larvae analyzed. Eleven of the thirteen mothers were also assigned paternity, but two of these were implicated only in self-fertilization. Subsequent exploration of the fathers versus non-fathers as genetic subpopulations yielded no significant differentiation or distinctions in genetic diversity between the two groups. Yet, mean pairwise genetic relatedness (2x [Lynch and Ritland 1999]) between mothers and inferred fathers (mean r = -0.124 ±0.029 SE) was less than the average

among all colonies within the study area (mean r = -0.007 ±0.002; two tailed t-test, $p < 0.0001$), emphasizing that biparental inbreeding appears to be limited. Approximately 25% of assignments (n=34) met the criteria for the highest confidence category (Very High), which assigned paternity to twenty fathers (35% of fathers assigned; Table 4.3, Fig. 4.6). The smallest category of high confidence added only seven additional fathers for the 21 larvae assigned, however together, almost 50% of the assigned fathers (n=27) met the criteria for at least one assignment in the two highest confidence categories. Thirteen assigned fathers (23%) and 31% of assignments met only the lowest criteria for confidence.

Table 4.3 Summary consensus results of paternity analysis of *Sh_sheltered* broods from Lizard Island and sperm dispersal estimates for all four confidence categories (see Fig. 4.2) and all assignments combined. The number of larvae assigned for each category is listed along with the number of fathers assigned per each category, the cumulative number of new fathers added with each category, and the number of selfed larvae inferred. Sperm dispersal estimates (mean, median, minimum and maximum) are listed in meters with standard deviations (SD).

Paternity Assignment Confidence Category	Larvae Assigned	Fathers Assigned/ Category	Fathers Added/ Category	Selfed	Sperm dispersal (m)			
					Mean (SD)	Median	MIN	MAX
Very High	34	20	20	12	5.59 (3.81)	5.01	0.94	15.08
High	21	11	7	0	3.33 (4.17)	0.94	0.77	15.23
Medium	34	24	17	2	6.26 (4.57)	4.90	0.73	17.07
Low	40	26	13	0	6.01 (4.39)	5.16	0.45	13.90
All Assignments	**129**	**81**	**57**	**14**	**5.50 (4.37)**	**4.35**	**0.45**	**17.07**

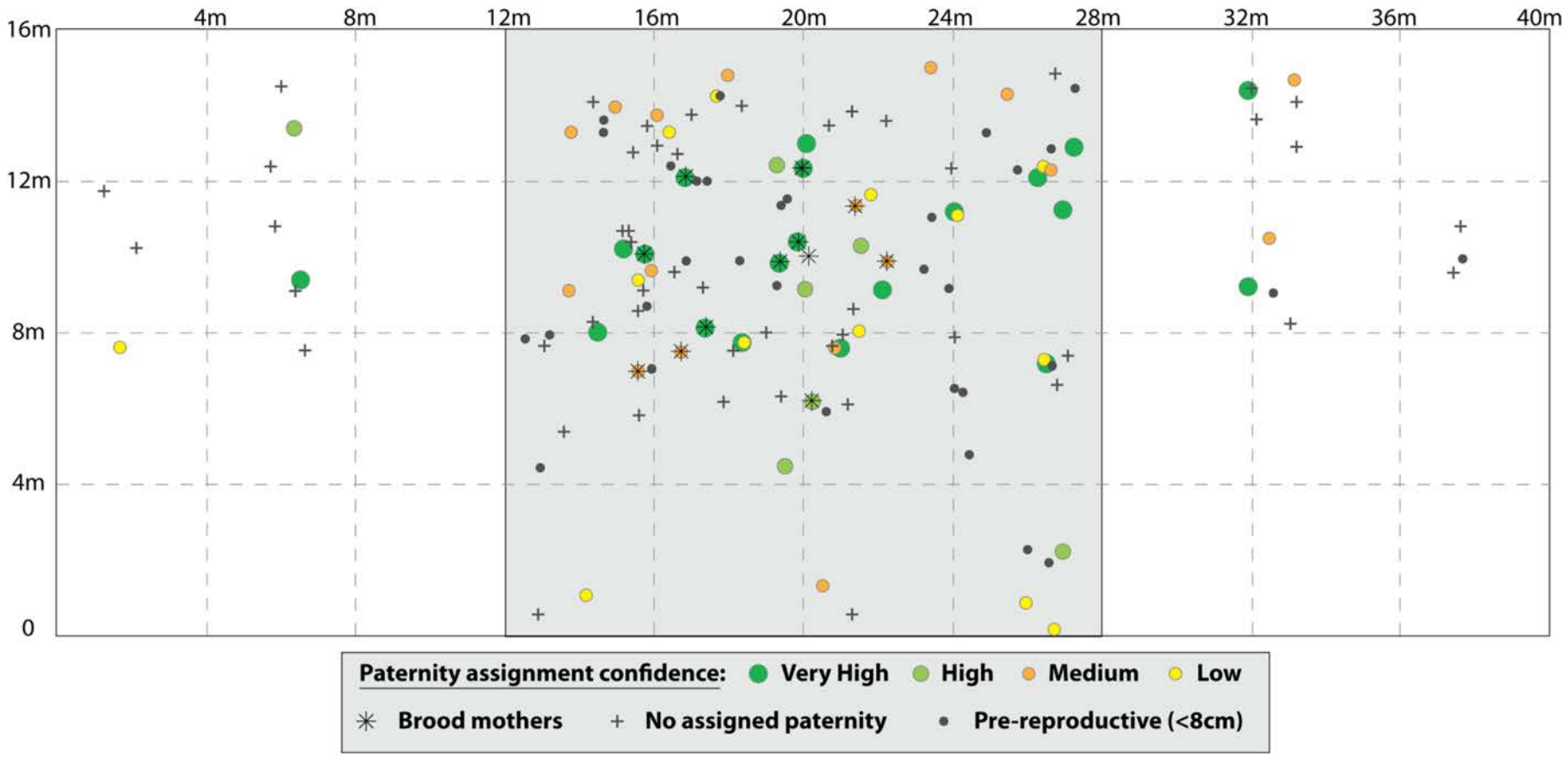


Figure 4.6 Mapped paternity analysis results for thirteen *Sh_sheltered* larval broods from Lizard Island. Results are compiled for all 129 paternity assignments according to consensus confidence categories (key below). Assigned fathers (57) are indicated by color and size for the highest confidence category obtained by larvae assigned to that father. Brood mothers overlaid on paternity assignments with large asterisk. Non-assigned candidate fathers indicated by (+). Colonies excluded as candidates based on size < 8 cm are depicted with small grey circle.

Including all 129 assignments, the largest number of larvae attributed to a single father was 18 from the same brood, M4 (Fig. 4.7 upper). The next highest number of larvae assigned to a single father was eight, but from five different broods. In total, half of the fathers (n=29) sired at least two larvae each and 78% of all larvae assigned. The other 28 fathers were each assigned paternity to only one larva. No relationship existed between size of the father and the number of paternity assignments. The father of the 18 M4 larvae was also mother M5, located 0.94 m from M4. This unusually high input from a single sperm source most likely explains the high number of repeated multilocus genotypes within brood M4 (n=10; Table 4.2), of which four are also shared with larvae in brood M5. However, there was no indication of any reciprocal exchange of sperm from M4 to M5 from the parentage analysis. On the other hand, both broods were assigned paternity from two other shared candidate fathers (Fig. 4.7 upper). Similarly, the chimera M6/6* had larvae assigned paternity from four sources in common to both broods and included fertilization of an M6 egg with M6*sperm (Fig. 4.7 lower). Brood M6* was also assigned four self-fertilizations, but no cross-fertilizations from M6 sperm. Overall, there was no obvious pattern or direction of sperm flow within the study area (Figs. 4.7, 4.8). In most cases, sperm appeared to disperse longer distances across (long axis) through the most densely populated areas of the study site, rather than perpendicular (short axis) to the reef.

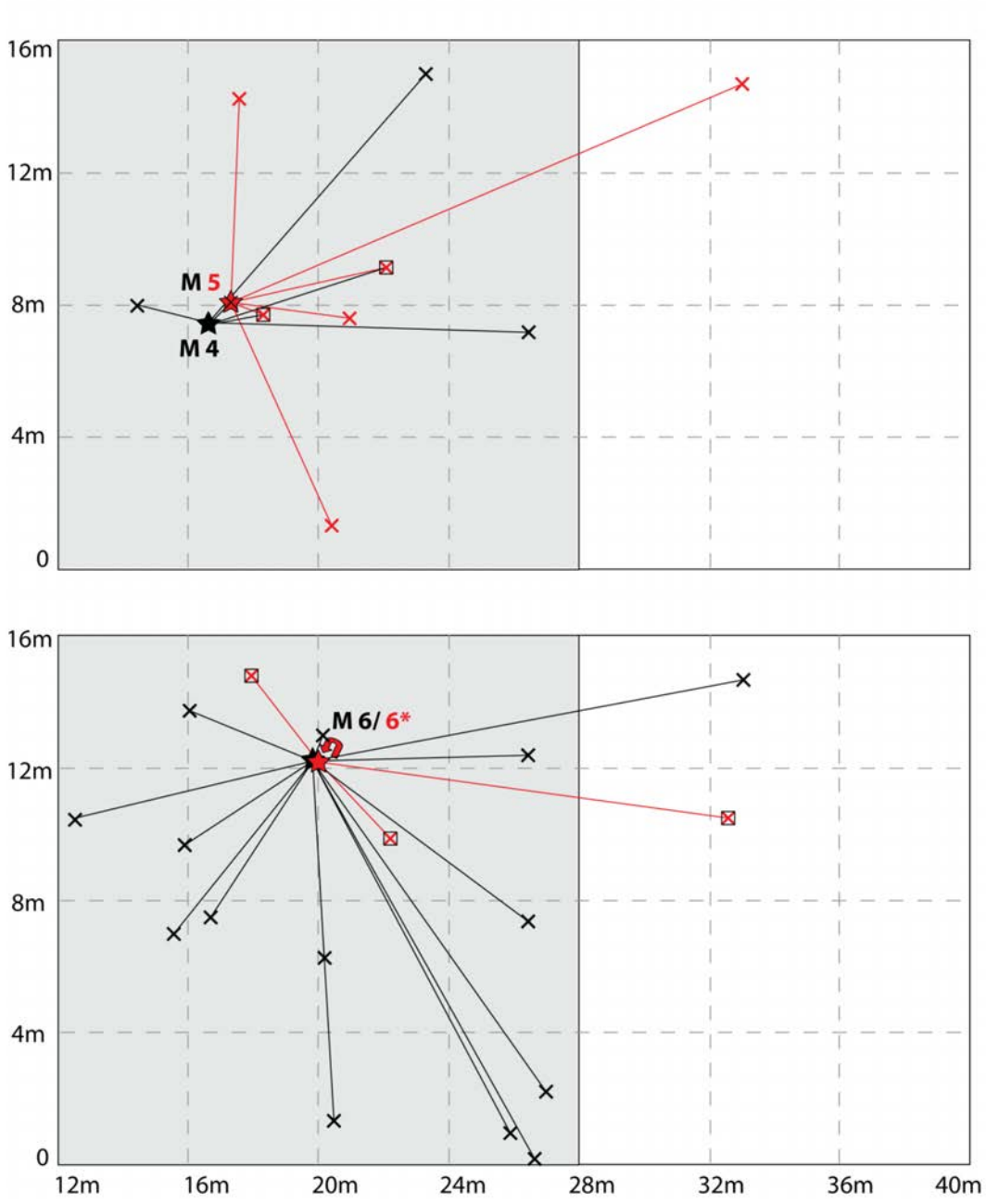


Figure 4.7 Mapped sperm dispersal and paternity analysis for four larval broods of *Sh_sheltered* from Lizard Island. **Top:** Broods M4 and M5; **Bottom:** Chimera brood M6/ M6*. The gray square represents the central study square in which all colonies were mapped (Figs. 4.3 and 4.6). The maps include all fathers assigned to broods, but are truncated where no fathers were assigned outside the central square. Different broods are depicted in either black or red. Mothers are indicated by stars, and assigned fathers by (x). If the same father was assigned to both broods, then that father is designated by a black square with red (x). Mothers also assigned paternity are overlaid by (x). Selfing is represented by a u-shaped arrow.

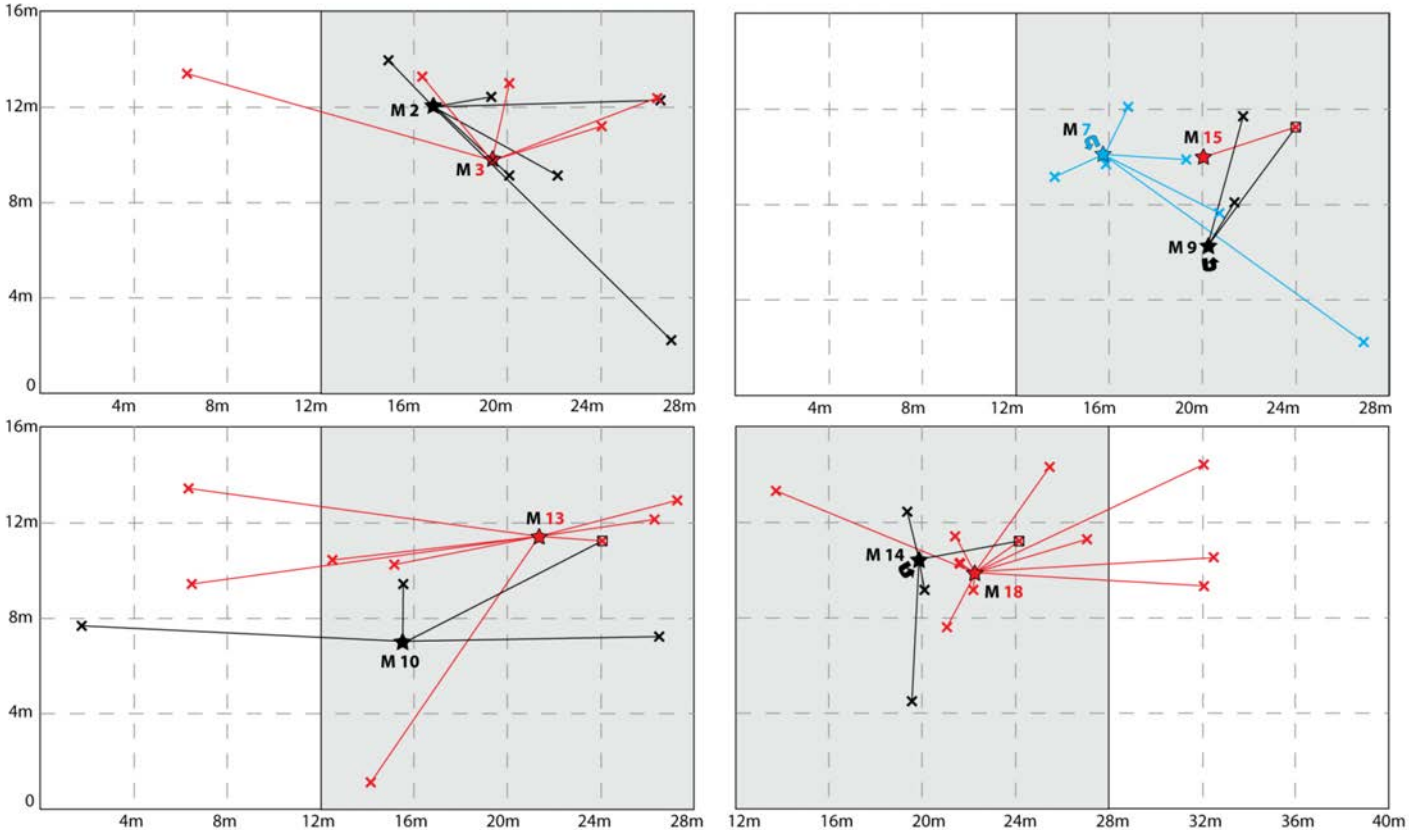


Figure 4.8 Mapped sperm dispersal and paternity analysis for nine larval broods of *Sh_sheltered* from Lizard Island. The gray square represents the central study square in which all colonies were mapped (Figs. 4.3 and 4.6). The maps include all fathers assigned to broods, but are truncated where no fathers were assigned outside the central square. Different broods are depicted in either black or red. Mothers are indicated by stars, and assigned fathers by (x). If the same father was assigned to both broods, then that father is designated by a black square with red (x). Mothers also assigned paternity are overlaid by (x). Selfing is represented by a u-shaped arrow. The upper right map includes three broods with the third being depicted in light blue. The number of each brood is indicated. The position of the central gray square and x-axis labels should be utilized to orient these maps to Figs. 4.3 and 4.6.

Excluding selfed larvae, the mean sperm dispersal distance was 5.5 m (±4.37 SD) from all 129 assignments, ranging from 0.45 m to a maximum of 17.07 m (Table 4.3). Dispersal distances from the medium and low confidence assignments were somewhat greater than those of the highest confidence categories, but not statistically different. There was a clear difference in distributions when sperm dispersal distances were compared to pairwise distances between mothers and all other colonies within the study site (Fig. 4.9). Over 60% of assigned sperm dispersal occurred within 5 m of maternal colonies, whereas only 38% of potential fathers occurred within the same area. Over 90% of sperm dispersal events in the highest confidence categories occurred within 10 m, but the lower confidence categories extended the dispersal tail, with 23% of dispersal over distances larger than 10 m. Although there is some appearance of a bimodal second peak in sperm dispersal for both confidence groupings (Fig. 4.9), this pattern is most likely driven by the discrete distances sampled outside the central study square.

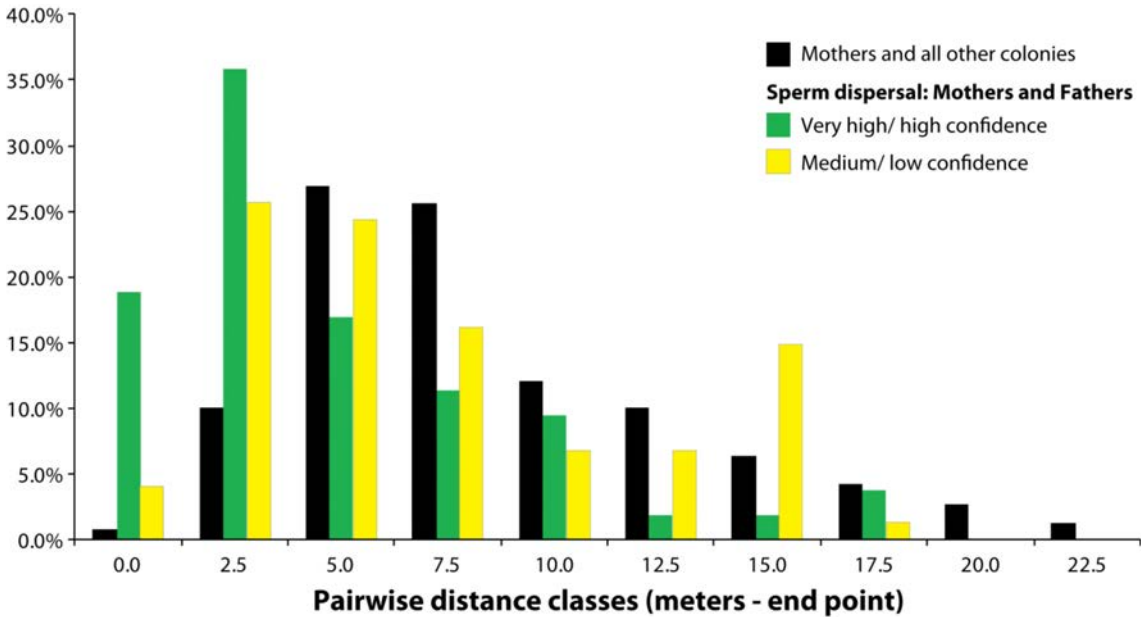


Figure 4.9 Frequency histogram of sperm dispersal distances inferred from paternity assignments for *Sh_sheltered* colonies at Lizard Island, compared to pairwise distances among mothers and all potential fathers in the study area (1866 pairwise distances). Paternity assignments are combined into two groups: **Very High + High** confidence assignments (Green, n=55) and **Medium + Low** confidence assignments (Yellow, n=74). Distance size classes are inclusive of the endpoint. See Fig. 4.2 for explanation of confidence categories.

4.3.7 Sibship analysis of population size classes

Sibling analyses of cohorts corresponding to four size classes of established colonies revealed small frequencies of full siblings within the colonies sampled from the study area (Table 4.4). The results demonstrate that genetic similarity among members of the population is such that full sibship was inferred for at least 3.57% of pairings (i.e., 93 pairings) across all cohorts. There was no indication that individuals of the two chimeras identified were fused full siblings. The overall evidence of selfing in the adult population was three times higher (9.2%) than in the larval population assayed (2.8%; Table 4.2). Interestingly, the 14 – <20 cm size class contained the largest number of full siblings, the lowest distance between putative siblings and the highest rate of inferred selfed colonies (Table 4.4). This cohort included 10 of 13 mother colonies, of which four are included in the same full sibling family. Two other mothers in this size class were also inferred to be derived from selfing. Mean distance between full siblings (9.26 m ± 5.76 SD) was almost twice that of sperm dispersal distances (5.5 m ± 4.37; Tables 4.3, 4.4), but closely mirrored the spread of pairwise distances among all colonies within cohorts (Table 4.4).

Table 4.4 Summary results of sibship analyses for each colony size class (cm) and combined for all colonies within the mapped study area: N: number of individuals; Selfed: number of individuals inferred to be derived from self-fertilization; Proportion of selfed individuals; Full Sibs: number of pairs inferred to be full siblings. Distance estimates include the means and standard deviations, minimum and maximum, alternately for inferred paired full siblings and all colonies within each size cohort.

						Full Sib Pairwise Distance (m)			All Pairwise Distance (m)		
Size Class (mean diameter)	N	Selfed	% Selfed	Full Sibs	% FullSibs (# pairs)	Mean (SD)	MIN	MAX	Mean (SD)	MIN	MAX
<8 cm	32	2	6.25%	20	4.03% (496)	9.16 (5.52)	2.36	20.58	8.41 (4.69)	0.17	25.39
8- <14 cm	53	5	9.43%	27	1.96% (1378)	10.00 (5.67)	0.30	26.23	9.71 (5.93)	0.17	32.14
14- <20 cm	31	5	16.13%	29	6.67% (435)	7.98 (5.68)	0.45	21.19	9.17 (6.04)	0.23	36.09
20 - 32 cm	25	1	4.00%	17	5.69% (299)	10.40 (6.37)	0.19	26.14	10.68 (5.97)	0.19	31.89
All	**141**	**13**	**9.22%**	**93**	**3.57% (2608)**	**9.26 (5.76)**	**0.19**	**26.23**	**9.53 (5.87)**	**0.17**	**32.14**

The additional analyses of the “pre-reproductive” size class (<8 cm mean diameter) confirmed that, if included in the pool of candidate parents, a high likelihood of shared parentage with larval broods could adversely influence paternity assignments of larvae. When all larvae and colonies <8 cm were run together without candidate parent genotypes, only one of the 32 colonies was not inferred to be related to any of the thirteen larval broods by half or full sibship. Twenty-seven small colonies (84%) were assigned full sibship to larvae, and four shared only a single parent (i.e., half-sibs). Although the same sibling relationships were not upheld in the parentage analysis of the small colonies without larvae, a few parental assignments and full sibships did overlap. Over 70% of small colonies (23) were assigned at least one parent from the sampled candidates despite the starting assumption that 50% of the candidate parents were unsampled for both sexes.

4.3.8 Parentage analysis in a non-ideal population: methods and assumptions

In this analysis, relationship inference was complicated and/ or hindered by low genetic diversity in the few loci that were analyzed, as well as by the complexities of a mating system that included hermaphroditism, polygamy, a potential for high inbreeding (selfing and biparental) and overlapping generations. Conversely, parentage analysis was facilitated by known mothers, moderately sized half-sibling progeny arrays, complete genotype data and a paternal sample size that was assumed to represent a high proportion of candidate males. Moreover, null allele frequencies were low and the few mismatched single-locus genotypes among offspring and mothers suggests that error and/ or mutation rates were similarly low. Several studies have highlighted the strong influence of the proportion of sampled fathers on the success of parentage analysis (Marshall *et al.* 1998; Nielsen *et al.* 2001; Wilson and Ferguson 2002; Oddou-Muratorio *et al.* 2003; Koch *et al.* 2008). In an extensive analysis of simulated data, Harrison *et al.* (2012a) recently demonstrated that the accuracy of the full-likelihood method of COLONY increased with the proportion of sampled adults, particularly for lower diversity populations. In preliminary analyses using Cervus 3.0, the effect of lowering the proportion of fathers sampled resulted in decreased assignments with this data set, but did not change the identity of the “most-likely” father. Without considering sibling relationships, the pairwise Cervus method revealed that 93% of larvae could be sired by the sampled candidates based on Mendelian inheritance (data not shown). In contrast, the COLONY 2.0 results attributed almost half of larval paternity to unsampled fathers, despite initial settings specifying that 90% of fathers had been sampled. The full-likelihood approach appeared to preferentially group larvae into fullsib cohorts assigned to inferred unsampled paternal genotypes rather than increasing the number of fathers assigned to a brood. Consequently, high correlated paternities (52% shared fathers, r_p = 0.524) calculated for the COLONY

results contradict the MLTR v.3.2 estimate that only 12% of larvae within broods were sired by the same males (r_p = 0.119; Table 4.2). Therefore, knowledge of the study organism and inference from other studies are required to interpret incongruous results from different parentage analysis methods,

The discrepancy in correlated paternity estimates between methods is difficult to reconcile without any prior information regarding the mating system of *Sh_sheltered*, and because the assumption that 90% of candidate fathers were sampled was largely based on sperm studies in other species, further discussion of this assumption is warranted. In broadcast spawning corals, experiments have shown that the sperm of some species can remain viable for up to eight hours after spawning (Willis *et al.* 1997), though fertilization rates are low at sperm densities <1000 sperm/ ml (Oliver and Babcock 1992). Although dilution occurs following broadcast spawning, the synchronous nature of spawning combined with the buoyant nature of eggs means that fertilization in such species occurs in an essentially two-dimensional layer at the sea surface (reviewed in Harrison and Wallace 1990), in obvious contrast to the three-dimensional dispersal of sperm among internally fertilizing colonies attached to the substrate. Some general differences in spermcasting systems (reviewed in Bishop and Pemberton 2006), may confer greater sperm dispersal distances compared to broadcast spawning organisms, including greater sperm longevity (Manriquez *et al.* 2001; Johnson and Yund 2004) and fertilization under lower sperm densities (Pemberton *et al.* 2003a). Similarly, a surface egg-brooding gorgonian has demonstrated successful fertilization under low sperm densities and over extended time periods (Lasker 2006). Nevertheless, studies of the brooding ascidian *B. schlosseri* have shown that fertilization decreases with distance from the sperm source (Grosberg 1987, 1991; Yund and Mccartney 1994; Yund *et al.* 2007), and effective sperm dispersal distances are expected to diminish under high population densities as sperm intercept high abundances of available eggs at greater proximity (Yund and

Mccartney 1994; Levitan and Petersen 1995). A paternity analysis of the gorgonian *Pseudopterogorgia elisabethae* found that mean sperm dispersal was 5.2 m (±3.1 SD; max= 11.8 m) under similar adult densities and reef area to this study (Lasker *et al.* 2008). Given the high density of the *Sh_sheltered* population studied here (0.5 m^{-1}), the assumption of 90% of potential fathers sampled within the study area seems reasonable, where the mean distance between maternal colonies and potential sires was 7.32 m (±4.56 SD; max= 22.42 m). The 90% proportion reflects the expectation that most sperm will be limited to well within the area covered by the survey, but allows for a small percentage of undetected and unsampled genetic diversity (e.g., chimeras, somatic mutations, or otherwise unobserved colonies), as well as immigrant sperm. The findings of inferred chimeras that were both undetected during sampling (i.e., mothers M6/ M6*) and observed *in situ* as different color morphs verified that such cryptic diversity exists in this population. Moreover, 2% of larvae contained one allele not found in the adult population, which is presumably derived from local cryptic diversity and/or longer distance sperm dispersal. While both lines of evidence confirm that 100% of potential sires were not sampled, neither suggest frequencies of unsampled gene flow that would greatly exceed 10%.

If the assumption that 90% of fathers were sampled is correct, then the results of unsampled fathers and high correlated paternities from COLONY must be rejected. The accuracy of the full-likelihood group method to infer parentage is enhanced by full sibling arrays (Wang and Santure 2009), however it is unclear whether the algorithm is written to intentionally maximize full sibships. Other pedigree and parentage inference programs do explicitly aim to estimate the minimum number of potential fathers to explain a given progeny array (i.e., parsimony; Jones *et al.* 2010), for example GERUD2.0 (Jones 2005) and KINALYZER (Ashley *et al.* 2009). In the same way, COLONY seems to be more constrained by the goal of finding full sibships than by the original input condition of 90% of fathers sampled. For the present study, this

propensity of COLONY would lower Type I errors of false positive assignments among several possible sires, albeit at the expense of increased Type II errors of not assigning paternity to the true sampled father. Under the limited power of this data set, halfsib broods likely contain many individuals that could be assigned to full sibships based on Mendelian segregation, yet highly correlated paternity under spermcast mating in a continuous and dense population of outcrossed larvae may not be expected. In particular, high frequencies of large fullsib families would most likely require dense sperm input from a single source in close proximity that would have been sampled, such as the eighteen M4 larvae sired by M5 at less than 1 m distance (Fig. 4.7 top). Sperm from greater distances would likely consist of more dilute (Denny and Shibata 1989; Levitan *et al.* 1992), highly mixed pools from multiple source colonies resulting in a greater number of fathers per brood, similar to patterns observed in many wind-pollinated plants (El-Kassaby and Jaquish 1996; Robledo-Arnuncio *et al.* 2004; Bittencourt and Sebbenn 2007; Bittencourt and Sebbenn 2008). Using MLTR, others have found low to moderate correlated paternities for the brooding corals *S. hystrix* (r_p = 0.34; Sherman 2008) and *Acropora palifera* (r_p = 0.14 and 0.39; Ayre and Miller 2006) that are more similar to those estimated here by the same program. In summary, the MLTR estimate of high multiple paternity is supported by inference from other studies with similar biological and/ or demographic characteristics and consequently the inference of a high proportion of unsampled fathers indicated by COLONY 2.0 is rejected.

4.4 DISCUSSION

This study demonstrates that the putative cryptic coral species *Sh_sheltered* produces sexually derived, primarily outcrossed larvae in multiple paternity broods at Lizard Island. Self-fertilization was inferred, but occurred at very low frequency in the total larval assay (<3%). The majority of mating occurred between colonies located within 10 m of one another, however only 25% of larvae were assigned to a father from

the pool of candidates sampled. The limited power of the genetic markers used in this study suggests that the low rate of paternity assignment is likely caused by high numbers of non-excluded potential fathers (i.e., Type II errors), rather than a large contribution from fathers outside the study area. Sibling analysis of colony size classes and genetic relatedness among parents indicated that, on average, philopatric larval dispersal distance (inferred from the distances separating putative full siblings) within the natal reef is greater than sperm dispersal distance, thus reducing the potential for inbreeding at least among full siblings. Fine-scale spatial autocorrelation and population inbreeding estimates similarly revealed no evidence of inbreeding in the established population, though there was some indication of low levels of selfed individuals (<10%) from the size class sibling analysis. Although the different methods provided somewhat dissimilar results for some parameters (e.g., correlated paternity and proportion of fathers sampled), which appear to be driven by features that differ characteristically among software programs, the consensus approach adopted here provides an effective starting point for the exploration of unstudied natural mating systems.

4.4.1 Larval release and sperm dispersal of *Sh_sheltered*

The timing of larval release following the new moon found for *Sh_sheltered* matches the periodicity reported in most previous studies of *S. hystrix* from similar habitats (reviewed in Chapter 2), but notably contrasts with observations from the Southern GBR (Tanner 1996; Sherman 2008). Variation in timing of larval release among mothers indicates that reproduction is not synchronous in the population as a whole, and may suggest that sperm release is also asynchronous. However, observations of tighter synchrony in larval release from *Sh_sheltered* populations in the Central GBR (P. Warner pers. obs.) imply that the degree of synchrony among individuals may vary among populations, locations and/or time. An extended period of larval release over several days is common in pocilloporid corals (Tanner 1996; Fan *et*

al. 2002; Fan *et al.* 2006), but it is unknown whether sperm release and/ or fertilization parallels such patterns. Synchronous spawning increases gamete concentration and consequently fertilization success in broadcasting species (Babcock *et al.* 1986; Oliver and Babcock 1992), but the direct advantages of population-wide synchronous spermcasting are less clear for the sperm donor. Unlike filter feeders, such as ascidians (Pemberton *et al.* 2003a; Phillippi *et al.* 2004) and sponges (Ryland and Bishop 1993; Maldonado and Riesgo 2008) that are capable of sampling large volumes of water to extract sperm, corals are heterotrophic suspension feeders, and no structures have been identified that either deliver sperm en masse (i.e., spermatophores), concentrate spermatozoa from the water column or store sperm once they are captured. Hence, sperm limitation may be an issue in suspension feeding species (Yund 2000; Bishop and Pemberton 2006), such as corals, and explain why the volume of spermaries significantly exceeds that of eggs in brooding corals (reviewed in Harrison and Wallace 1990). Such significant energy investment in sperm production promotes outcrossing in brooding corals (Szmant 1986) and highlights the important role of sperm dispersal in this group (see Section 4.4.2).

In the Lizard Island population of *Sh_sheltered* studied here, sperm dispersal in outcrossed fertilizations occurred over distances less than 20 m. Although the maximum possible distance of detectable sperm dispersal under this sampling design was 22.5 m, over 80% of sperm dispersal occurred within 10 m. One limitation of my data is that dispersal estimates are based on only the 25% of paternity assignments that met strict consensus criteria, thus it is unknown how these results reflect the full range of sperm dispersal. A relatively low return rate of assignments in parentage analysis for natural populations is not uncommon (e.g., Lasker *et al.* 2008; Harrison *et al.* 2012b), and may be due to incomplete sampling and/ or failure to assign when the true parent has been sampled as a result of low marker power (i.e., Type II error, false negative; Oddou-Muratorio *et al.* 2003; Harrison *et al.* 2012a). In similar studies of

plants, high proportions of unassigned progeny are also common when there is a large input from external pollen sources, particularly for wind pollinated trees (Burczyk *et al.* 1996; Dow and Ashley 1998; Streiff *et al.* 1999; Bittencourt and Sebbenn 2008). However, two arguments can be made against the likelihood that a large fraction of fertilizations resulted from immigrant sperm: 1) the limited paternity assignments generated from this analysis are most likely due to inadequate discriminatory power of the genetic marker panel and Type II errors (Section 4.3.8); and 2) the distribution of sperm dispersal distances suggests that assignments are not random, but clearly skewed towards short distances, with a conspicuous absence of assignments to colonies within the two largest distance classes. Moreover, the mean sperm dispersal of 5.5 m is very similar to estimates for a surface egg-brooding gorgonian (5.2 m; Lasker *et al.* 2008), although the maximum distance found here (17 m) is about 50% greater. Estimates of sperm dispersal for other spermcasting marine invertebrates (Grosberg 1987; Yund 1990; Grosberg 1991; Pemberton *et al.* 2003b) and a red alga (Engel *et al.* 1999) are typically much less than 5 m on the order of centimeters, but these are mostly organisms with much smaller body sizes and presumably lower sperm output. Conversely, one study reported sperm dispersal in excess of 100 m for the brooding ascidian, *Botryllus schlosseri* (Yund *et al.* 2007), which has extremely long-lived spermatozoa and can successfully fertilize at very dilute concentrations (Johnson and Yund 2004). Similarly, in *Acanthaster planci*, a large broadcast spawning starfish with notoriously high sperm investment, fertilization has been detected at 100 m from the source individual (Babcock *et al.* 1994). In these brooding corals, reproductive studies documenting prolific larval output (Tanner 1996; Fan *et al.* 2002; Fan *et al.* 2006; Villanueva *et al.* 2008b) and the abundance of seriatoporids on many reefs indicate that they are successful with the comparatively intermediate sperm dispersal distances found here.

The only persuasive evidence that sperm may be dispersing farther than measured was the 2% of larvae containing an allele that was undetected in the standing population of colonies sampled. Given the limited frequency of these alien alleles, it is equally plausible that they represent genotyping errors, *de novo* mutations, and/ or fertilizations from unsampled genotypes within the study area. The detection of two chimeras, fusions of genetically distinct individuals, in the population of colonies demonstrates cryptic genetic diversity is present, but the frequency of such entities is uncertain. A recent study of *S. hystrix* estimated that 17% colonies exhibit genetic heterogeneity, although these instances were mainly inferred to be mosaicisms as a result of somatic mutations (Maier *et al.* 2012). In corals, somatic mutations can theoretically be transmitted to offspring, as germ cells are continuously differentiated from somatic stem cells (van Oppen *et al.* 2011c). High rates of somatic mutations would unquestionably reduce the success of parentage analysis in the same way as genotyping errors. Nevertheless, the low frequency of maternal-offspring mismatches in the data set does not implicate somatic mutations as a major issue here. The presence of chimeras within the study area would not change sperm dispersal estimates or increase the maximum distances inferred, however a high frequency of chimeras would increase the proportion of unsampled candidate fathers and reduce the number of paternity assignments (Section 4.3.8).

This study provides the first evidence for a reef-building coral that chimeras not only persist in natural populations, but that both genotypes can successfully reproduce and contribute approximately equally to larval broods. The formation and persistence of chimeras has been widely documented in nature (e.g., 5% in a broadcast spawning coral (Puill-Stephan *et al.* 2009); <3% in *S. hystrix* (Maier *et al.* 2012)), and has been well studied for the spermcasting ascidian *B. schlosseri* (reviewed in Rinkevich 2005), but evidence that the dominant genotype can hijack reproductive machinery in a cellar slime mold (Buss 1982) has raised questions about the nature of the association. In

one of the two chimeras analyzed (mother M6/ M6*), 48% of the larval brood (M6) was attributed to one of the genotypes present within the mother and 52% to the other genotype (M6*). In the second chimera identified (non-mother), both partners were assigned paternity to eleven larvae combined (8 and 3 larvae respectively), which is the second highest number of assignments to any candidate father. The two unique genotypes of both chimeras were not inferred to be closely related from the sibship analyses, representing neither full nor half-sibs, which is contrary to a study of alleorecognition maturation in juveniles of the coral *Acropora millepora* (Puill-Stephan *et al.* 2012). Further work is required to assess whether these partnerships confer any physiological advantages that may confer greater resilience under environmental challenges, but such research is outside the scope of this thesis.

4.4.2 The mating system of *Sh_sheltered*

This work confirms that the putative species *Sh_sheltered* produces sexually derived larvae, in contrast to the primarily clonal larvae produced by another extensively studied pocilloporid, *Pocillopora damicornis* (Ayre *et al.* 1997; Ayre and Miller 2004; Miller and Ayre 2004; Yeoh and Dai 2010; Schmidt-Roach *et al.* 2012). In combination with earlier studies of the morphospecies *S. hystrix* on the GBR (Ayre and Resing 1986; Sherman 2008), it appears that sexual reproduction may be a general characteristic of the seriatoporids. Furthermore, the proportion of clones in the standing population of colonies sampled was low and most likely derived from fragmentation of established colonies. In the absence of asexual production of planulae, polyp bail-out (*sensu* Sammarco 1982) has been suggested to explain the dispersal of matching genotypes across distances of 10s-100s km (van Oppen *et al.* 2008). With the seven microsatellite markers used here, my results demonstrate that apparent clones can be readily derived from sexual reproduction at moderate frequencies (~8%), including identical 7-locus larval genotypes within and between broods and cross-generational matches between larvae and potentially unrelated adults. However, it is still unclear

whether identical 7-locus larval genotypes within the same brood represent instances of polyembryony or independent fertilization events that could not be detected with these markers. Histological observations indicate that most polyps contain a single larva, although occasionally two and rarely three larvae have been observed (P. Warner pers. obs.). Furthermore, population genetic surveys indicate similar signals of sexually derived matching genotypes in other populations of *Sh_sheltered* (Chapter 3). The successful resettlement of polyps of *S. hystrix* following polyp bail-out has only been demonstrated at very low frequencies (≤ 5%) in strictly controlled laboratory settings (Sammarco 1982). Moreover, observations of polyp bail-out in the laboratory reveal that the resultant propagules are essentially dormant, more than likely resettling in the immediate vicinity, if at all (P. Warner pers. obs.). Therefore, it is possible that seemingly clonal genotypes found among distant locations represent long distance dispersal of sexually produced larvae that appear to be genetically identical based on the low resolving power of the markers used, particularly from populations with generally low genetic diversity and common genotypes.

Self-fertilization events were rarely detected in the total larval array (2.8% of 495 larvae) with any of the three software utilized (i.e., Cervus, COLONY, MLTR), and were completely absent from nine of thirteen broods. Although brooding is relatively uncommon in the Scleractinia, hermaphroditism is the predominant form of sexuality (Baird *et al.* 2009). As a result, many authors have postulated that selfing may be an important process in coral populations, and particularly for brooding corals that would otherwise require independent sperm dispersal (Veron 1995; Carlon 1999). Specifically, self-fertilization is often promoted as an explanation for the high inbreeding coefficients estimated and widespread heterozygote deficiencies found in brooding coral populations (Carlon 1999; Ayre and Hughes 2000; Underwood *et al.* 2007; Sherman 2008; Maier *et al.* 2009). Here, most broods were almost exclusively outcrossed (mean t_m= 0.999 under assumed equilibrium population conditions),

suggesting that this species prioritizes cross-fertilization. These outcrossing estimates are significantly higher and less variable than those of *S. hystrix* from the Southern GBR (t_m= 0.53 ±0.2 SD; Sherman 2008), and it is unclear whether this inconsistency reflects different strategies in distinct putative species (Chapter 2), population-specific conditions, or variation in experimental methods. In contrast to this study, the Southern GBR *S. hystrix* populations also exhibited higher clonality among the adults surveyed and significant departures from HWE at two-thirds of allozyme loci (Sherman 2008). Direct evidence indicates that self-fertilization is either minimal or absent in most hermaphroditic corals (Heyward and Babcock 1986; Wallace and Willis 1994; Miller and Babcock 1997; Szmant *et al.* 1997; Willis *et al.* 1997; Hatta *et al.* 1999; Willis *et al.* 2006; Yeoh and Dai 2010; Douek *et al.* 2011); but see (Heyward and Babcock 1986; Stoddart *et al.* 1988; Brazeau *et al.* 1998). Moreover, it has been proposed that imperfect self-incompatibility may be a general condition in spermcasting marine invertebrates and has been observed in several other taxa (Bishop and Pemberton 2006).

Nevertheless, these results do implicate some role for self-fertilization in *Sh_sheltered* populations, as the frequency of selfed larvae within four of the thirteen broods varied from 2% (M9) to 23% (M14). Furthermore, slightly higher inferred selfing rates in the colonies sampled compared to larvae hint that the frequency of self-fertilization may vary over time according to local environmental or demographic conditions (Ellstrand *et al.* 1978; El-Kassaby *et al.* 1993), or that selfed larvae may be better adapted to survival following settlement in the natal environment (Antonovics 1968; Schmitt and Gamble 1990; Jarne and Charlesworth 1993). Philopatry has likewise been proposed as a means to enhance and maintain local adaptation (Balkau and Feldman 1973; Grosberg 1987; Wiener and Feldman 1993), and is expected to be a dominant process in structuring seriatoporid populations as a result of precocious settlement competency (Atoda 1951; Isomura and Nishihira 2001; Chapter 3). The

most accepted advantage to self-fertilization, however, is reproductive assurance (Antonovics 1968; Lloyd 1979, 1992; Jarne and Auld 2006), with the ability to self-fertilize being retained as an effective strategy of persistence following colonization of new territory (Baker 1955; Stebbins 1957) or last resort under outcrossed sperm limitation (Levitan and Petersen 1995; Yund 2000). Maintaining such a mixed mating strategy of selfing and outcrossing is particularly common in plants (reviewed in Goodwillie *et al.* 2005), and may be an important factor in how *Seriatopora* spp. invade and then successfully colonize new locations (e.g., Starger *et al.* 2010). Several aspects of seriatoporid larval biology and reproductive behavior also imply that larval dispersal might be bimodal, incorporating both philopatric and teleplanic strategies (Chapter 3). Moreover, the low genetic diversity of *Sh_sheltered* populations, which otherwise meet HWE expectations (Chapter 3), may reflect signatures of past population bottlenecks, as an alternative to microsatellite ascertainment bias in a non-focal species (Section 2.4.3a). Higher frequencies of selfing during foundation events or following disturbances in an existing population will increase homozygosity as an extreme form of inbreeding (Wright 1921; Jain 1976). Yet as the population size and density increases, outcrossing may be increasingly favored to reduce potential inbreeding depression and facilitate the success of long distance dispersal (Holsinger 1986; Charlesworth and Charlesworth 1987; Johnson and Gaines 1990).

Here, multiple paternity broods (Ellstrand and Marshall 1986; Marshall and Ellstrand 1986; Yasui 1998, 2001; Johnson and Yund 2007) and low estimates of biparental inbreeding, under assumed equilibrium conditions at high population density, implicate a strategy to enhance genetic diversity by maximizing cross-fertilization. Moreover, there was no evidence of heterozygote deficiencies in the adult population structure, despite the inference of a moderate proportion of individuals derived from self-fertilization (<10%) in the sibling analysis. In particular, these results suggest that shorter sperm dispersal relative to inferred larval dispersal distances among putative

full siblings may minimize inbreeding between close relatives. Populations are expected to retain larvae locally for predominant self-recruitment, however larval dispersal within the natal reef appears to be greater than the dispersal of sperm, resulting in apparent panmixia up to 1 km in many populations (Chapter 3). If the reverse pattern were true, that sperm dispersal was equal to or exceeded larval dispersal, then inbreeding would be expected to increase with more frequent consanguineous mating among siblings and parents (Jackson 1986; Grosberg 1987), which is found in some wind pollinated trees and can result in pronounced inbreeding depression (Isagi *et al.* 2007). Another possibility is that gametic incompatibility exists amongst closely related individuals, and further work is required to fully understand the mechanisms that limit inbreeding in *Sh_sheltered* populations. Although a decrease in fitness is not a universal consequence to inbreeding (Antonovics 1968) and even the opposite extreme of outbreeding depression is sometimes documented (Grosberg 1987), outcrossing promotes the generation of new genetic diversity, which theoretically enhances the resilience of a population by providing the genetic diversity upon which selection operates. Thus, for *Seriatopora* populations, high rates of outcrossing, limited biparental inbreeding and frequent reproductive events should facilitate adaptation and resilience under changing environmental conditions.

4.4.3 Implications for future studies

The original motivation for this study was to measure sperm dispersal distances for the first time in a brooding coral. To that end, I applied a consensus criterion among several methods to minimize Type I errors and drawbacks of particular methods at the expense of decreasing overall assignments (i.e., Type II errors). Others have suggested such an approach to decrease uncertainty in relationship inference (Coltman *et al.* 2005; Sheikh *et al.* 2008a; Sheikh *et al.* 2008b; Wang and Santure 2009; Walling *et al.* 2010). Here, the consensus method provided an effective starting point for evaluating genealogical relationships in a natural population for which almost

no prior information existed. Further work is required to improve these analyses with new marker development and optimization of statistical methods for the complexities of coral population structure. Most importantly, comparable analyses must be conducted with simulated data of known pedigrees to explicitly test accuracy and error rates for the current data set, and such work is currently underway. Although relationship inference and parentage analysis, in particular, can provide valuable information about natural systems, which can have important applications for conservation and management, wild populations and certain organisms of interest will frequently not meet the desired qualities for such analyses despite concerted effort to optimize sampling design. Moreover, the specific behaviors of different methods must be considered within the context of the intended subject and objectives, not just in terms of model populations and circumstances for universal application (Oddou-Muratorio *et al.* 2003). In conclusion, the use of several methods and consensus criteria should be considered in future studies as a means to maximize certainty for relationship inference in non-ideal populations.

Chapter 5.0 General discussion

5.1 ECOLOGICAL AND EVOLUTIONARY CONNECTIVITY IN THE GENUS *SERIATOPORA*

In this thesis, I present important and novel results regarding inter- and intra-specific connectivity patterns for the currently recognized morphospecies *Seriatopora hystrix,* which correspond to three key findings. First, I determined that *S. hystrix* consists of at least four putative cryptic species on the Great Barrier Reef (GBR) (Chapter 2). The significant diversification found within *Seriatopora* in a small region (i.e., the central and northern portions of the GBR) relative to the reported Indo-Pacific range of *S. hystrix*, implies that species diversity within the genus may also be higher globally than is currently recognized. Second, in contrast to previous reports for the single morphospecies *S. hystrix*, patterns of genetic connectivity within two putative species (*Sh_sheltered* and *Sh_exposed*) were generally consistent across hierarchical spatial scales, highlighting infrequent long-distance dispersal. Similar to earlier studies of *S. hystrix*, populations of both putative species were still strongly subdivided (Chapter 3). For both *Sh_sheltered* and *Sh_exposed*, mean larval dispersal was locally concentrated within 3 km, thus ecological connectivity may be drastically reduced by minor habitat discontinuities within the same island group. Third, sperm dispersal in the putative species *Sh_sheltered* was not detected beyond 20 m from source colonies. Apparent low incidence of self-fertilization in the whole larval assay and greater average larval compared to sperm dispersal minimize inbreeding within populations (Chapter 4). Reproductive strategies that maximize outcrossing indicate that this putative species is able to generate and maintain local genetic diversity. Although the capacity of these putative species to tolerate disturbances or adapt to climate change remains unclear, high self-recruitment and probable local retention, in combination with reproductive strategies that maintain genetic diversity, imply that populations are self-sustaining and resilient at local scales.

5.1.1 Summary of major findings

The results of this thesis significantly advance our understanding of levels of ecological and evolutionary connectivity and the processes underpinning them in the abundant coral genus *Seriatopora*. The major findings of this research include:

- **Chapter 2: Cryptic species in the reef-building coral genus *Seriatopora***
    1. Four putative cryptic species were identified in populations of the morphospecies *S. hystrix* on the GBR based on genetic cohesion of nDNA microsatellites and corroborated with characteristic mtDNA haplotypes, habitat preference, *Symbiodinium* spp. coupling, and in one case, morphology. These putative species cannot be fully discriminated by mtDNA alone.
    2. A phylogeographic examination of all published *Seriatopora* mtDNA revealed that most lineages are geographically constrained but that one is widespread across several locations throughout the Indo-Pacific.
    3. A hierarchical approach to a Bayesian model-based clustering analysis of microsatellite data proved a successful method for delimiting putative cryptic species that occur sympatrically and allopatrically.
    4. Results presented in this chapter suggest that complicated genetic patterns reported in earlier studies of *S. hystrix* likely reflect mixed-species sampling, including interspecific in addition to intraspecific processes.
- **Chapter 3: Genetic connectivity across hierarchical spatial scales of two abundant, putative species of *Seriatopora* on the GBR**
    1. Spatial autocorrelation analyses reveal higher relatedness among individuals within 15 m of each other for the dominant exposed habitat species (*Sh_exposed*) and within 20 m for the dominant sheltered species (*Sh-sheltered*), indicating philopatric larval dispersal, however inbreeding was not detected in population genetic structures.

2. Populations are significantly differentiated at all spatial scales (<1 km to 450 km) for both putative species, although several comparisons at the within-reef level indicate panmixia between sites separated by less than 1 km within reefs containing continuous habitat.
3. Within regions, the majority of larval dispersal is concentrated within ~2.5 km for *Sh_sheltered* and within ~1.5 km for *Sh_exposed*.
4. Stronger connectivity among *Sh_sheltered* populations (F'_{ST}=0.315) compared to *Sh_exposed* populations (F'_{ST}=0.562) between regions separated by 450 km, suggest that dissimilar biological characteristics of the putative species, including higher levels of mitochondrial diversity within *Sh_exposed*, and/or physical features of their respective habitats may have different implications for connectivity patterns.

- **<u>Chapter 4</u>: Parentage analysis and sperm dispersal in a natural population of a brooding coral**
    1. The abundant putative species *Sh_sheltered* produces sexually derived, primarily outcrossed larvae with limited biparental inbreeding in multiple paternity broods, although the moderate extent of selfing detected in a few broods suggests that the ability to self-fertilize may be variable among colonies or under different conditions.
    2. While the maximum sperm dispersal distance was 17 m, over 80% of detected sperm dispersal events occurred within 10 m.
    3. Greater mean spatial distance between full siblings (9.26 m) compared to mean distance of sperm dispersal (5.5 m) is suggestive that on average larval dispersal is greater than sperm dispersal, and may be one way that biparental inbreeding is reduced.
    4. Using multiple and replicated methods of parentage analysis, a consensus approach maximized accuracy in paternal assignments in order to measure unknown mating system properties in this natural population.

5.1.2 Future studies in the genus *Seriatopora*

This work emphasizes the critical need to assess and characterize the extent of genetic diversity and the existence of putative cryptic species throughout the range of the genus *Seriatopora*. In order to understand and compare the implications of past and future studies, it is imperative that researchers have the tools to recognize and target single species within a given study. The hierarchical clustering methods I used to detect putative cryptic species within GBR *Seriatopora* populations will be valuable to explore the existence of putative species within *Seriatopora* in other geographic regions. While requiring further verification, corresponding unique mitochondrial haplotypes may prove effective markers to identify putative cryptic species molecularly. However, in-field methods of identification are required for many biological and ecological studies. Consequently, further work is required to document geographic ranges and, in particular, to characterize putative species with additional operational criteria (e.g., habitat, morphology, reproductive characteristics).

In terms of understanding connectivity within putative species of *Seriatopora* on the GBR, my work has focused on the putative species *Sh_sheltered*, elucidating processes occurring at scales ranging from local intrapopulation levels (Chapter 4) to regional patterns (Chapter 3), as well as broad-scale biogeographic distribution (Chapter 2). Similar approaches should be followed for other putative species within the genus in order to fully understand the ecological and evolutionary processes operating on these corals. Other key areas requiring further research include information on the genetic connectivity of obligate photosymbionts and timing of reproductive processes, which when integrated with molecular and ecological data on the host coral, will provide the most comprehensive base from which to understand connectivity in *Seriatopora*. Below, I outline research I have done in each of these areas, which will be analyzed and published following submission of my thesis.

- **Connectivity among populations of vertically transmitted *Symbiodinium* spp. within the putative species *Sh_sheltered* and *Sh_exposed*:** Microsatellite genotyping of *Symbiodinium* spp. for a subset of the coral populations presented in this thesis will reveal the amount of genetic diversity within populations of photosymbionts and help clarify the extent to which *Symbiodinium* is exchanged among individuals and populations of the coral host. The symbiotic relationship between the coral host and *Symbiodinium* is a key determinant of coral resilience under changing climate, yet is poorly understood for corals with vertical transmission, such as *Seriatopora*.
- **Reproductive ecology of the putative species *Sh_sheltered*:** A year-long monthly monitoring of a central GBR population, and observations of larval release for populations in both the central and northern GBR, will provide detailed information regarding the amount and frequency of reproductive activity. Knowledge of the quantity, frequency and timing of larval release will help improve bio-physical connectivity models and enhance understanding of the biological and ecological processes underlying genetic connectivity.
- **Timing of gametogenesis, sperm release and planula development in *Sh_sheltered*:** Histological examination of tissues collected from twenty colonies every other day for an entire lunar cycle during the peak reproductive season may reveal the timing of sperm release and subsequent development of planulae for a central GBR population. Further investigations of sperm dispersal and fertilization biology in brooding corals hinge on our ability to determine when these processes occur.

5.2 OPTIMIZING STATISTICAL-GENETIC METHODOLOGIES FOR NATURAL POPULATIONS

Genetic tools and statistical methods have unquestionably increased our understanding of biology, ecology and evolution, such as has been presented in this thesis for a group of reef-building corals, yet can also provide misleading information when applied to natural organisms and ecosystems that do not meet model assumptions. The genetic code presents a seemingly universal scale on which to measure and compare the diversity of life; however, the theoretical framework for doing so still relies heavily on inference from model organisms and simple scenarios, which may not accurately reflect the complexities of natural systems. Although molecular techniques are frequently integrated as ready-made tools into studies of a diverse range of taxa, statistical-genetic methodologies may need extensive optimization for the particular study subject (e.g., the genus *Seriatopora*) and the question of interest (e.g., putative cryptic species; Chapter 2). Some taxa continue to challenge more standard approaches, despite demonstrated utility in many other groups, and may violate model assumptions intrinsically (e.g., coral populations with overlapping generations in parentage analysis; Chapter 4). While every effort should be made to optimize sampling designs and utilize models robust to violations, thoughtful design of methodologies (e.g., a consensus criterion for parentage analysis) applied to non-model species can provide valuable exploratory information with currently available genetic methods (e.g., sperm dispersal estimates; Chapter 4). Importantly, the limitations of the analyses need to be recognized and the assumptions acknowledged.

Parentage analysis demands fairly stringent criteria under idealized scenarios, which are not met in many natural populations (Pemberton 2008; Jones *et al.* 2010; Harrison *et al.* 2012a); yet the information that can be gained from successful parentage assignments may greatly expand our understanding of the biological and ecological processes operating in species, particularly when no prior information exists.

The direct estimates of sperm dispersal presented in Chapter 4 highlight how using a consensus approach, which maximized accuracy in paternity assignments at the expense of the number of offspring assigned, can further studies of brooding corals, although the utility of this approach for future studies depends on the question of interest and spatial scale of examination. For example, the timing of sperm release is unknown for most brooding coral species and directly tracking sperm in the marine environment is yet unfeasible. Therefore, any accurate assignments advance our knowledge of this critical reproductive process that affects genetic diversity and connectivity in reef-building corals, and help to inform the appropriate design of future studies. In contrast, investigations of broader scale phenomena, such as larval dispersal (Harrison *et al.* 2012b), may require a minimum quantity of assignments to infer relevant patterns, but may also be less sensitive to a small proportion of assignment errors (Oddou-Muratorio *et al.* 2003). Under either situation, the system of interest (e.g., species, population, mating system, genetic markers) and method of parentage analysis should also be evaluated for accuracy with simulated data if a known pedigree is unavailable (e.g., Harrison *et al.* 2012a), as would be the case for most marine taxa including corals. Nevertheless, defining and applying consensus criteria (Sheikh *et al.* 2008b), as presented in Chapter 4, can maximize certainty in parentage assignments and should be an effective strategy for other studies where gathering primary information and accuracy is paramount, but non-ideal circumstances prevail.

5.3 UNDERSTANDING RESILIENCE IN THE GENUS *SERIATOPORA*

By resolving the extent of connectivity among individuals, populations and putative species in the genus *Seriatopora*, this thesis elucidates ecological and evolutionary processes that underpin the stability and persistence of these entities through time. However, resilience has two components: the ability to resist or survive

disturbance or environmental change, and the capacity to recover (Gunderson 2000). While most commonly resilience has been described at the ecosystem level (Thrush *et al.* 2009), these two components of resilience also have different implications for the hierarchical divisions within a taxonomic group (i.e., individuals, populations, species, genera). Therefore, resilience may have many interpretations depending on the biological units and time frames (e.g., one disturbance event, one generation or 1000s of generations) of interest. Currently, there is grave concern that corals and the reef ecosystems that rely on them may not survive the next few decades of global climate change and predicted increases in seawater temperatures (Hughes *et al.* 2003; Hoegh-Guldberg *et al.* 2007), particularly as they are already in a weakened state from more localized anthropogenic pressures (e.g., poor water quality and sedimentation, overfishing, ship groundings; Jackson *et al.* 2001; Pandolfi *et al.* 2003). Ultimately, species persistence will be determined by the continuing ability of populations to adapt to changing conditions (i.e., evolutionary resilience Sgrò *et al.* 2011) and the survival of existing individuals and populations in the interim (i.e., ecological resilience). My work highlights some of the genetic processes underlying resilience in corals of the genus *Seriatopora* and, in combination with other current knowledge for the genus, sheds some light on both the long and short-term future of this abundant group of reef-building corals.

Ecological resilience at the level of the coral colony (i.e., the individual) is determined by the capacity to survive biological (e.g., disease, predation) and physical disturbances (e.g., cyclone damage; freshwater runoff; elevated temperatures). To date, very few studies have directly assessed the physiological capabilities of seriatoporid corals to respond to stress (but see Edmunds 2005; Villanueva *et al.* 2008a; Putnam *et al.* 2010). Observations of bleaching (i.e., the loss of *Symbiodinium* and/ or photosynthetic pigments) in some natural populations have led to the conclusion that *Seriatopora* may be especially sensitive to heat stress (Marshall and

Baird 2000; Loya *et al.* 2001), thus vulnerable to expected increases in seawater temperature as a result of global climate change. However, extensive field observations throughout my thesis work indicate that *Seriatopora* is normally strikingly pale in all seasons, particularly in shallow water populations (see Fig. 2.9). Furthermore, previous conclusions about the severe susceptibility of *S. hystrix* to bleaching, were based on a very small sample size (n=11) from the Palm Islands of the GBR, in which 73% of colonies were categorized as severely bleached, but without reference observations of normal conditions (Marshall and Baird 2000). In contrast, an experimental study of thermally-induced bleaching found that *S. hystrix* survived for six weeks of exposure to 32 °C (elevated from 26 °C). Given the potential for widespread cryptic species in this genus (Chapter 2), it is unclear whether the few reports of natural bleaching reflect a general vulnerability of *Seriatopora* or more specific responses of distinct putative species responding to localized conditions. At the same time, rapid sexual maturity (Stimson 1978), intensive maternal effort to brood larvae and frequent reproductive events throughout the year (Atoda 1951; Stimson 1978; P. Warner unpublished data) demonstrate an extensive investment in reproduction. Therefore, while it is currently unclear if *Seriatopora* is sensitive or resilient to environmental perturbation at the individual level, high reproductive effort may represent a physiological trade-off to other attributes that confer greater ecological resilience (e.g., thermo-tolerance, large body size).

At the population level, both ecological and evolutionary resilience depend on population maintenance through continued successful recruitment from individuals within the population (i.e., local retention = self-sustaining), whereas successful recruitment from other demes (i.e., connectivity) reflects resiliency of the metapopulation or species as a whole (Hastings and Botsford 2006; Botsford *et al.* 2009). In *Seriatopora*, high reproductive output of immediately competent larvae, strongly structured populations over short spatial distances (Chapter 3) and often high

abundance (Chapters 4), indicate high local retention, suggesting that local populations are self-sustaining in the present. For long-term persistence under changing conditions (e.g., climate change), the population must have the capacity to adapt (population resilience) (Lynch and Lande 1993; Lande and Shannon 1996) or receive immigrants that have novel adaptive alleles (metapopulation resilience) (Davis and Shaw 2001; Shirley and Sibly 2001; Morjan and Rieseberg 2004). Although the potentially extensive longevity and clonal lineages of coral colonies may impede rapid adaptation in some species (Potts 1984; Hughes *et al.* 2003; Hoegh-Guldberg *et al.* 2007), the reproductive strategies of "weedy" *Seriatopora*, which promote frequent sexual reproduction and short generation times that can generate the genetic diversity necessary for selection (Chapters 3, 4), provide some hope that these corals may be able to evolve relatively quickly. Thus, although random genetic processes (Harding and Mcnamara 2002) and environmental disturbances will likely result in some local extinction (e.g., Loya *et al.* 2001; van Woesik *et al.* 2011), there is some promising evidence that self-sustaining local populations of *Seriatopora* may contain the ability to survive global climate change, even without extensive ecological connectivity.

A variety of mechanisms promoting and maintaining genetic diversity enhance the capacity of populations of *Seriatopora* to adapt to changing conditions. In particular, very low levels of inbreeding (Chapters 3, 4), evidence that the mating system of *Sh_sheltered* promotes outcrossing (Chapter 4; but see Sherman 2008), and limited asexual reproduction (Chapters 2 – 4; Sherman 2008; Van Oppen *et al.* 2008; Maier *et al.* 2009; Van Oppen *et al.* 2011a) all enhance genetic diversity and imply an evolutionary strategy that is not heavily reliant on environmental stasis, in contrast to highly clonal species (e.g. *P. damicornis* (Yeoh and Dai 2010)*; Acropora palmata* (Baums *et al.* 2005a)). Moreover, similarity among sperm dispersal estimates (<20 m; Chapter 4) and genetic neighborhoods detected in two putative species (*Sh_exposed* (15 m) and *Sh_sheltered* (20 m); Chapter 3) suggest that effective population sizes and

the number of individuals with the potential to directly mate with each other could be surprisingly large for a sessile organism. For example, in *Sh_sheltered* populations, where population density of mature colonies (>8 cm mean diameter) may be as high as 0.34/m^2 (Chapter 4), a 20 m radius for sperm dispersal may incorporate as many as 427 mature colonies, of which two-thirds (285) might be reproductively active over peak months, based on my work on Lizard Island (Chapter 4) and Palm Island populations (P. Warner unpublished data). Thus, a single colony may have the opportunity to mate with tens, if not hundreds, of individuals within a reproductive event, as supported by low proportions of correlated paternities within broods (Chapter 4), promoting extensive genetic mixing that might be similarly enhanced by changing environmental conditions throughout multiple events during the year (e.g., small-scale tidal currents, seasonal wind conditions). In comparison to broadcast-spawning species, in which individuals typically reproduce once per year, spreading events throughout the year can protect against massive reproductive failures due to inclement weather and provides multiple opportunities to generate new genetic material for immediate selection under variable conditions. Furthermore, apparent panmixia in *Seriatopora* over approximately 1 km in continuous habitats (e.g., semi-enclosed bays; Chapter 3), indicates that local larval dispersal sufficiently negates the potentially constraining effects of shorter sperm dispersal (Chapter 4). In summary, the evolutionary strategy of *Seriatopora* relies on rapid and frequent reproduction, which combined with mechanisms that maximize outcrossing, may facilitate population resilience over ecological time-frames.

Ultimately, population recovery following local extinctions, resilience of the metapopulation, and species persistence all require some degree of connectivity among subpopulations (Rieseberg and Burke 2001; Hastings and Botsford 2006; Botsford *et al.* 2009; Jones *et al.* 2009), thus the big question is *how much dispersal is enough?* Clearly, the relative abundance of this widespread coral group indicates evolutionary success under natural conditions; however, the concern is that more

frequent and severe disturbances, as a result of global climate change, might unbalance the normal functioning of the metapopulation, which by ecological definitions includes dynamic processes of local extinctions followed by recolonization (Hanski 1998; Harding and Mcnamara 2002). To date, reports of local extinctions in *Seriatopora* populations lasting a decade (Loya *et al.* 2001; Van Woesik *et al.* 2011) are at least balanced by evidence of population recovery over relatively short time periods in other locations (Underwood *et al.* 2007; Noreen *et al.* 2009; Starger *et al.* 2010). Moreover, the detection of selfing in a few broods of *Sh_sheltered* (Chapter 4) and further evidence from Southern GBR populations (Sherman 2008) suggest a variable ability to self-fertilize that may prevent reproductive failure and facilitate population recovery, if at least some individuals survive a given disturbance. Similarly, regular larval dispersal up to 2.5 km for one putative species (*Sh_sheltered*) implies that connectivity among regional populations (e.g., within the Palm Islands or Lizard Island groups; Chapter 3) may occur frequently enough to effectively repopulate after spatially localized disturbances in which some proximate populations would be expected to survive (e.g., cyclones, crown-of-thorns outbreak). Some evidence for longer distance dispersal over ecological time (e.g., Chapter 3; van Oppen *et al.* 2008; Noreen *et al.* 2009; Underwood *et al.* 2009)v and the presence of photosymbionts in larvae, which may facilitate extended pelagic larval durations (Richmond 1988), indicate that infrequent long-distance dispersal can be successful and may facilitate population recovery or new colonizations. More extensive studies are needed across broader spatial scales with intermediate distances to assess how connectivity within putative species of *Seriatopora* might be aided by stepping-stone migration (Hellberg 1995; Crandall *et al.* 2012), which could not be resolved by the discrete regions sampled for this thesis. Nevertheless, recognition of putative cryptic species has at least initially indicated that connectivity within species is more consistent across spatial scales and likely greater than could be previously inferred for the morphospecies *S. hystrix*.

Finally, greater genetic diversity implied by additional putative species in *S. hystrix* (Chapter 2) suggests that the genus as a whole may be more resilient to environmental change than might be expected under a single species. Differences in genetic patterns and connectivity among the putative species emphasize that underlying biological characteristics are also unique, and thus some putative species may already be better adapted to specific conditions and habitats (Chapters 2, 3), which could be conducive to survival through climate change. At the same time, the specific habitat preferences, and potentially requirements, demonstrated by putative species on the GBR (Chapter 2; Bongaerts *et al.* 2010b), cast some doubt that refuge populations at mesophotic depths can effectively preserve shallow water populations of presumably different putative species, as has been suggested (Bongaerts *et al.* 2010a). On the GBR, the apparent association of putative species of *Seriatopora* with specific maternally transmitted *Symbiodinium* types (Chapter 2), which have exhibited differential physiological performance and habitat partitioning (Bongaerts *et al.* 2010b; Bongaerts *et al.* 2011), imply adaptation to specific environmental conditions (Bongaerts *et al.* 2011) and may promote greater environmental tolerance through coevolutionary mechanisms, as recently suggested for massive *Porites* (Putnam *et al.* 2012). On the other hand, the generality of host-symbiont specificity in *Seriatopora* remains uncertain, and one mitochondrial lineage specifically, haplotype U which may correspond to the putative species *Sh_large* (Chapter 2), has clearly demonstrated the ability to associate with several different *Symbiodinium* types in Western Australia (Van Oppen *et al.* 2011a) and the GBR (Chapter 2; Bongaerts *et al.* 2010b). Interestingly, the same mitochondrial lineage is widespread across several locations of the Indo-Pacific (Chapter 2), and dominated all depths sampled (range 0 – 43 m; van Oppen *et al.* 2011a) in Western Australia following recovery from a “catastrophic” bleaching event ten years earlier (Underwood *et al.* 2007), suggesting that flexibility in symbiont associations and a generalist strategy is a viable alternative as well. What remains abundantly clear is that the genus *Seriatopora* contains a diversity of life-history

strategies and potentially widespread occurrence of cryptic species that likely have similar variability in ecological and evolutionary resilience. Further effort to document the geographic ranges and characterize the biology and ecology of putative cryptic species is essential to fully understand how those unique putative species can respond under a changing climate. Overall, results presented in this thesis, combined with evidence from other studies, suggest that *Seriatopora* will continue to be a successful genus over the next few decades.

5.4 CONCLUDING REMARKS AND IMPLICATIONS FOR MANAGEMENT

In conclusion, *Seriatopora* populations have good potential for ecological resilience at local scales, which is particularly desirable for conserving species in marine protected areas, provided that environmental integrity and habitat quality can be maintained. Local efforts to limit direct anthropogenic threats and conservation of even small areas of habitat can preserve viable populations, whose reproductive efforts could bolster populations in proximate non-protected areas as well. High fecundity and frequent sexual reproduction combined with mechanisms that maintain genetic diversity should facilitate adaptation to changing conditions, and at the same time, potential variability in selfing rates may protect against reproductive failures in low density populations affected by disturbance. Of particular importance, however, is that management plans need to consider cryptic diversity and the association of putative species with specific reef habitats in order to maintain essential ecological and evolutionary processes when designing protected area networks. Given opportunities afforded by protection, these widespread coral populations should continue to support sufficient connectivity across broader spatial scales to maintain both ecological and evolutionary resilience.

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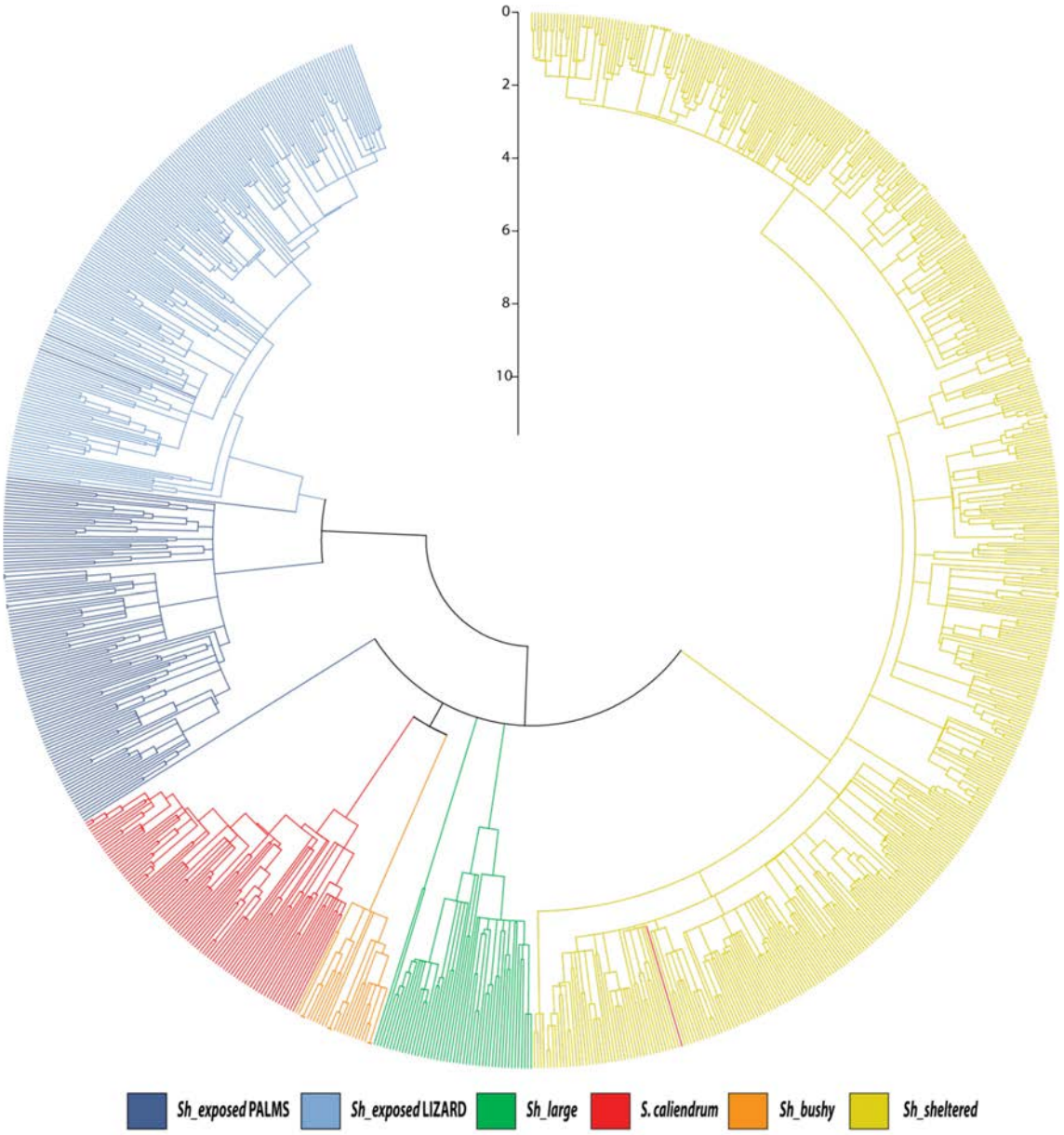
Appendix 2.1 NJ tree of all colonies of *Seriatopora* (n=964) sampled at sites in the Palm Islands and Lizard Island, using squared genotypic distances from ten microsatellite loci. Colors designate *structure*-assigned species (see key at bottom of figure). *Sh_exposed* is additionally separated according to region: dark blue – Palm Islands; light blue – Lizard Island.



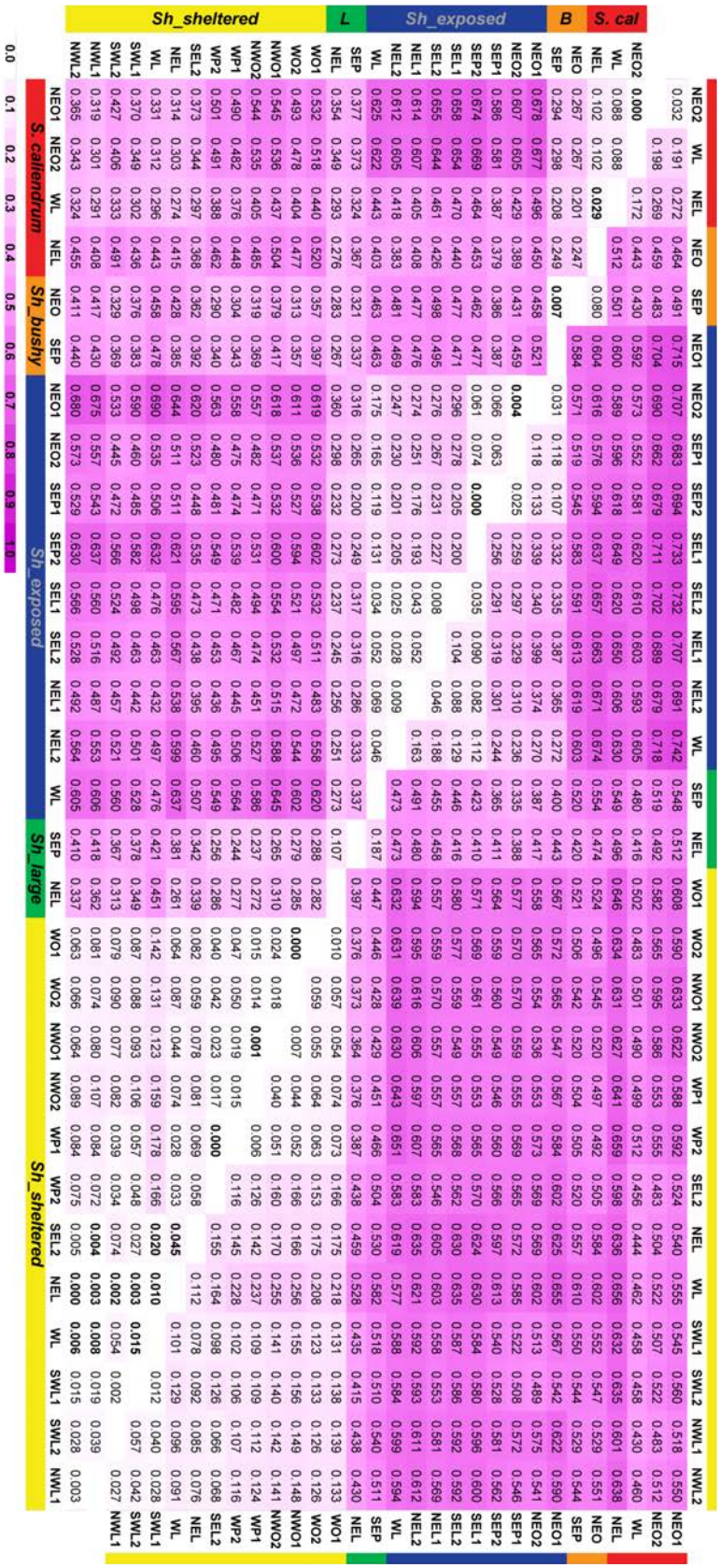
Appendix 2.2 Descriptive statistics for 10 microsatellite loci in all 30 populations of 5 *structure*-assigned putative species within the genus *Seriatopora* at northern and central GBR sites. N: # of samples; N_A: # of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: inbreeding coefficient (Weir and Cockerham 1984). Bold red type indicates statistically significant (α=0.05) heterozygote deficiencies at specific loci (red numbers), or populations averaged across all loci (global deficiencies; red species/site names). Bold black type denotes statistically significant heterozygote excesses. Paired loci in significant genotypic linkage disequilibrium indicated by asterisks.

			Sh4-001	Sh2-002	Sh3-003	Sh3-004	Sh2-005	Sh2-006	Sh3-007	Sh3-008	Sh3-009	Sh4-010
S. caliendrum	*S. caliendrum* NE Orpheus 1	N	45	47	48	48	48	46	48	48	48	39
		N_A	4	5	2	2	10	4	1	3	6	4
		H_O	0.311	0.362	0.021	0.104	0.917	0.630	0.000	0.208	0.792	0.077
		H_E	0.275	0.517	0.021	0.099	0.855	0.528	0.000	0.223	0.744	0.122
		F_{IS}	-0.121	**0.310**	-	-0.044	-0.061	-0.184	-	**0.077**	-0.0534	**0.382**
	S. caliendrum NE Orpheus 2	N	21	19	21	21	17	21	21	21	21	16
		N_A	5	3	1	2	9	3	1	2	5	4
		H_O	0.381	0.474	0.000	0.095	0.765	0.286	0.000	0.095	0.714	0.375
		H_E	0.424	0.521	0.000	0.091	0.863	0.316	0.000	0.091	0.728	0.322
		F_{IS}	0.126*	0.117*	-	-0.026	**0.144**	0.121	-	-0.026	0.043	-0.132
	S. caliendrum NE Lizard	N	4	4	4	4	4	4	4	4	4	1
		N_A	2	4	1	2	2	2	1	1	2	1
		H_O	0.250	0.250	0.000	0.250	0.750	0.500	0.000	0.000	0.250	0.000
		H_E	0.219	0.656	0.000	0.219	0.469	0.500	0.000	0.000	0.219	0.000
		F_{IS}	-	**0.700**	-	-	-0.500	0.143	-	-	-	-
	S. caliendrum West Lizard	N	16	16	16	16	16	16	16	16	16	5
		N_A	1	6	1	2	9	3	2	1	3	3
		H_O	0.000	0.688	0.000	0.313	0.813	0.750	0.000	0.000	0.438	0.400
		H_E	0.000	0.816	0.000	0.342	0.854	0.549	0.117	0.000	0.420	0.580
		F_{IS}	-	**0.189**	-	0.118	0.080	-0.338	**1.000**	-	-0.010	0.407
Sh_bushy	*Sh_bushy* NE Orpheus	N	5	5	5	5	5	5	5	5	5	5
		N_A	1	1	1	4	1	2	1	1	3	4
		H_O	0.000	0.000	0.000	0.400	0.000	0.000	0.000	0.000	0.600	0.800
		H_E	0.000	0.000	0.000	0.640	0.000	0.320	0.000	0.000	0.620	0.700
		F_{IS}	-	-	-	0.467	-	1.000	-	-	0.143	-0.032
	Sh_bushy SE Pelorus	N	19	19	19	19	19	19	19	19	19	19
		N_A	2	5	2	4	1	6	1	1	3	7
		H_O	0.105	0.368	0.105	0.632	0.000	0.368	0.000	0.000	0.526	0.789
		H_E	0.100	0.364	0.100	0.569	0.000	0.504	0.000	0.000	0.532	0.684
		F_{IS}	-0.029	0.016	-0.029	-0.083	-	**0.294**	-	-	0.037	-0.127
Sh_large	*Sh_large* SE Pelorus	N	23	23	23	23	23	23	23	23	23	23
		N_A	3	6	1	6	4	4	3	1	5	2
		H_O	0.348	0.696	0.000	0.696	0.522	0.435	0.435	0.000	0.652	0.261
		H_E	0.474	0.654	0.000	0.643	0.495	0.366	0.411	0.000	0.642	0.340
		F_{IS}	0.286	-0.041	-	-0.060	-0.031	-0.167	-0.035	-	0.006	0.254
	Sh_large NE Lizard	N	25	25	25	25	25	25	24	25	25	24
		N_A	3	6	1	6	3	1	4	2	4	5
		H_O	0.480	0.920	0.000	0.720	0.320	0.000	0.583	0.040	0.360	0.625
		H_E	0.633	0.783	0.000	0.716	0.381	0.000	0.603	0.039	0.682	0.652
		F_{IS}	**0.261**	-0.155	-	**0.015**	0.180	-	0.054	-	**0.488**	0.063

			Sh4-001	Sh2-002	Sh3-003	Sh3-004	Sh2-005	Sh2-006	Sh3-007	Sh3-008	Sh3-009	Sh4-010
Sh_exposed	*Sh_exposed* NE Orpheus 1	N	37	37	37	37	37	37	37	37	37	37
		N_A	6	8	3	6	5	2	2	2	4	3
		H_O	0.378	0.838	0.432	0.676	0.676	0.027	0.351	0.459	0.649	0.081
		H_E	0.374	0.802	0.508	0.714	0.682	0.027	0.447	0.499	0.555	0.079
		F_{IS}	0.003	-0.031	**0.163**	0.067	0.023	-	0.228	0.092	-0.156	-0.019
	Sh_exposed NE Orpheus 2	N	13	14	13	14	14	14	14	14	14	14
		N_A	6	7	3	6	4	1	3	2	4	2
		H_O	0.769	0.857	0.538	0.714	0.714	0.000	0.571	0.357	0.500	0.214
		H_E	0.624	0.776	0.615	0.653	0.689	0.000	0.518	0.436	0.564	0.191
		F_{IS}	-0.194	-0.069	0.164	-0.057	0.000	-	-0.067	0.217	0.150	-0.083
	Sh_exposed SE Pelorus 1	N	17	17	17	16	17	17	17	17	17	17
		N_A	3	8	3	4	4	3	3	2	6	4
		H_O	0.294	0.647	0.706	0.688	0.706	0.235	0.353	0.588	0.765	0.647
		H_E	0.299	0.720	0.602	0.697	0.593	0.213	0.304	0.484	0.734	0.581
		F_{IS}	0.048	0.131	-0.143	0.046	-0.160	-0.076	-0.129	-0.185	-0.012	-0.083
	Sh_exposed SE Pelorus 2	N	40	40	40	40	40	40	40	40	40	40
		N_A	3	10	3	6	4	4	3	2	6	5
		H_O	0.475	0.625	0.500	0.800	0.725	0.325	0.425	0.450	0.650	0.625
		H_E	0.449	0.747	0.570	0.686	0.623	0.289	0.381	0.439	0.739	0.630
		F_{IS}	-0.045	**0.176**	0.136	-0.154	**-0.151**	-0.112	-0.103	-0.013	0.133	0.020
	Sh_exposed SE Lizard 1	N	49	49	49	48	49	49	49	49	47	49
		N_A	4	12	2	4	7	4	3	3	8	3
		H_O	0.286	0.735	0.184	0.563	0.796	0.122	0.408	0.469	0.681	0.429
		H_E	0.269	0.714	0.167	0.590	0.726	0.154	0.541	0.452	0.751	0.414
		F_{IS}	-0.052	-0.019	-0.091	0.057	-0.087	0.216	**0.255**	-0.029	**0.104**	-0.025
	Sh_exposed SE Lizard 2	N	43	43	43	43	43	43	43	43	42	43
		N_A	3	11	2	4	4	3	3	3	8	2
		H_O	0.186	0.814	0.093	0.605	0.674	0.163	0.442	0.186	0.714	0.093
		H_E	0.190	0.833	0.089	0.582	0.649	0.152	0.528	0.171	0.765	0.089
		F_{IS}	0.030	0.034	-0.037	-0.027	-0.027	-0.058	0.174	-0.079	0.0787	-0.037
	Sh_exposed NE Lizard 1	N	39	39	39	39	39	39	39	39	39	39
		N_A	2	8	2	5	5	3	4	3	6	4
		H_O	0.103	0.744	0.128	0.692	0.513	0.256	0.436	0.410	0.538	0.487
		H_E	0.097	0.680	0.120	0.632	0.534	0.264	0.420	0.418	0.699	0.512
		F_{IS}	-0.041	-0.080	-0.056	-0.082	0.052	0.042	-0.024	0.032	0.242	0.061
	Sh_exposed NE Lizard 2	N	27	26	27	27	27	27	27	27	27	27
		N_A	1	9	2	4	5	3	3	2	6	3
		H_O	0.000	0.577	0.370	0.778	0.778	0.333	0.481	0.148	0.704	0.333
		H_E	0.000	0.570	0.302	0.658	0.699	0.341	0.499	0.137	0.726	0.313
		F_{IS}	-	0.008	-0.209	-0.164	-0.094	0.041	0.055	-0.061	0.05	-0.047
	Sh_exposed West Lizard	N	19	19	19	18	18	18	18	19	18	19
		N_A	3	7	2	5	6	2	3	3	5	3
		H_O	0.211	0.789	0.263	0.722	0.722	0.111	0.278	0.368	0.611	0.263
		H_E	0.194	0.823	0.229	0.736	0.705	0.105	0.248	0.467	0.640	0.309
		F_{IS}	-0.059	0.067	-0.125	0.047	0.005	-0.030*	-0.090*	0.236	0.074	0.174

			Sh4-001	Sh2-002	Sh3-003	Sh3-004	Sh2-005	Sh2-006	Sh3-007	Sh3-008	Sh3-009	Sh4-010
Sh_sheltered	*Sh_sheltered* W Orpheus 1	N	47	47	47	47	47	47	47	47	47	47
		N_A	3	6	1	2	3	5	2	1	1	6
		H_O	0.723	0.745	0.000	0.298	0.277	0.745	0.404	0.000	0.000	0.468
		H_E	0.639	0.738	0.000	0.254	0.359	0.719	0.442	0.000	0.000	0.569
		F_{IS}	-0.122	0.002	-	-0.165	0.240	-0.026	0.096	-	-	0.187
	Sh_sheltered W Orpheus 2	N	49	50	49	48	50	50	50	50	49	50
		N_A	3	5	1	3	3	4	2	1	1	6
		H_O	0.735	0.680	0.000	0.292	0.340	0.640	0.220	0.000	0.000	0.500
		H_E	0.665	0.695	0.000	0.308	0.315	0.695	0.428	0.000	0.000	0.520
		F_{IS}	-0.095	0.031	-	0.065*	-0.071	0.090	**0.493****	-	-	0.049*
	Sh_sheltered NW Orpheus 1	N	50	50	50	49	50	50	50	50	50	50
		N_A	3	4	1	2	3	3	2	1	2	5
		H_O	0.300	0.580	0.000	0.429	0.080	0.400	0.460	0.000	0.060	0.460
		H_E	0.311	0.565	0.000	0.459	0.078	0.507	0.476	0.000	0.095	0.398
		F_{IS}	0.047	-0.017	-	0.077	-0.021	0.220	0.043	-	0.377	-0.145
	Sh_sheltered NW Orpheus 2	N	48	48	48	48	48	48	48	48	48	48
		N_A	3	4	1	2	3	5	2	1	2	5
		H_O	0.313	0.604	0.000	0.396	0.188	0.583	0.438	0.000	0.083	0.542
		H_E	0.321	0.548	0.000	0.385	0.174	0.507	0.451	0.000	0.080	0.562
		F_{IS}	0.038	-0.093	-	-0.017	-0.066	-0.141	0.041	-	-0.033	0.047
	Sh_sheltered W Pelorus 1	N	50	50	50	50	50	50	50	50	50	50
		N_A	3	3	1	2	2	6	2	1	2	6
		H_O	0.460	0.400	0.000	0.620	0.040	0.520	0.340	0.000	0.060	0.540
		H_E	0.525	0.439	0.000	0.495	0.039	0.546	0.308	0.000	0.058	0.524
		F_{IS}	0.134	0.100	-	-0.243	-0.010	0.057	-0.095	-	-0.021	-0.020
	Sh_sheltered W Pelorus 2	N	50	50	50	50	50	49	50	50	50	50
		N_A	3	3	1	2	1	6	2	1	2	5
		H_O	0.480	0.560	0.000	0.500	0.000	0.653	0.380	0.000	0.080	0.540
		H_E	0.563	0.492	0.000	0.484	0.000	0.566	0.354	0.000	0.077	0.596
		F_{IS}	0.158	-0.129	-	-0.023	-	**-0.143**	-0.063	-	-0.032	0.103
	Sh_sheltered SW Lizard 1	N	50	50	50	50	50	50	50	50	50	49
		N_A	3	5	1	3	2	3	2	1	4	5
		H_O	0.540	0.840	0.000	0.580	0.200	0.560	0.500	0.000	0.500	0.469
		H_E	0.561	0.771	0.000	0.515	0.180	0.640	0.466	0.000	0.509	0.560
		F_{IS}	0.047	-0.079	-	-0.116	-0.101	0.135	-0.062	-	0.029	0.172
	Sh_sheltered SW Lizard 2	N	50	50	50	50	50	50	50	50	50	50
		N_A	3	5	1	3	2	3	2	1	4	5
		H_O	0.520	0.740	0.000	0.620	0.220	0.640	0.380	0.000	0.560	0.700
		H_E	0.551	0.746	0.000	0.634	0.196	0.635	0.375	0.000	0.580	0.588
		F_{IS}	0.067	0.018	-	0.032	-0.114	0.003	-0.003	-	0.045	-0.181
	Sh_sheltered NW Lizard 1	N	50	50	50	50	50	50	50	49	50	50
		N_A	3	6	1	2	4	6	3	1	5	7
		H_O	0.520	0.600	0.000	0.380	0.060	0.700	0.560	0.000	0.640	0.500
		H_E	0.652	0.662	0.000	0.375	0.059	0.683	0.516	0.000	0.615	0.504
		F_{IS}	**0.212**	**0.103**	-	-0.003	-0.010	-0.015	-0.075	-	-0.030	0.018
	Sh_sheltered NW Lizard 2	N	50	50	50	50	50	50	50	50	50	50
		N_A	3	6	1	3	3	4	3	1	6	7
		H_O	0.600	0.700	0.000	0.500	0.100	0.620	0.540	0.000	0.600	0.600
		H_E	0.622	0.705	0.000	0.486	0.096	0.633	0.479	0.000	0.620	0.575
		F_{IS}	0.045	0.017*	-	-0.020	-0.029	0.030	-0.118*	-	**0.043**	-0.034
	Sh_sheltered West Lizard	N	3	4	4	2	4	4	4	4	3	4
		N_A	2	3	1	2	2	2	2	1	3	3
		H_O	0.333	0.750	0.000	0.500	0.250	0.250	0.250	0.000	0.667	0.500
		H_E	0.278	0.531	0.000	0.375	0.219	0.219	0.469	0.000	0.667	0.406
		F_{IS}	-	-0.286	-	-	-	-	0.571	-	0.2	-0.091
	Sh_sheltered SE Lizard 2	N	7	7	7	7	7	7	7	7	7	7
		N_A	3	2	1	2	2	4	3	1	3	3
		H_O	0.571	0.286	0.000	0.571	0.286	0.714	0.714	0.000	0.714	0.714
		H_E	0.622	0.408	0.000	0.408	0.245	0.684	0.500	0.000	0.571	0.602
		F_{IS}	0.158	0.368	-	-0.333	-0.091	0.032	-0.364	-	-0.177	-0.111
	Sh_sheltered NE Lizard	N	3	3	3	3	3	3	3	3	3	3
		N_A	3	4	1	2	1	2	2	1	2	2
		H_O	0.333	0.667	0.000	0.333	0.000	0.667	0.333	0.000	0.667	0.333
		H_E	0.500	0.667	0.000	0.278	0.000	0.444	0.278	0.000	0.444	0.278
		F_{IS}	0.500	0.200	-	-	-	-0.333	-	-	-0.333	-

Appendix 2.3 Pairwise genetic distance between all thirty populations of five putative *Seriatopora* species from the central and northern GBR, using ten microsatellite loci. Jost's D_{est} (Jost 2008) lower left triangle; D_A (Nei *et al.* 1983) upper right triangle. Populations divided by putative species (color coded). Background color for distance values indicate magnitude of genetic distance on scale of 0 to 1 (shown in bottom left). Bold values indicate non-significant (α=0.05) pairwise genotypic differentiation.



Appendix 3.1 Pairwise geographic distance matrix (km) between all sites sampled in the Palm Islands and Lizard Island regions of the Great Barrier Reef. All between region distances contained within the black bordered box. Palm Islands pairwise distances located above black box. Lizard Island pairwise distances are to right of black box.

	WO1	WO2	NWO1	NWO2	WP1	WP2	SEP1	SEP2	NEO1	SWL1	SWL2	NWL1	NWL2	SEL1	SEL2	NEL1	NEL2
WO2	0.2																
NWO1	3.4	3.3															
NWO2	4.3	4.2	0.9														
WP1	7.0	6.8	3.6	2.7													
WP2	6.6	6.5	3.3	2.4	0.3												
SEP1	5.4	5.2	2.7	2.0	2.2	1.9											
SEP2	5.8	5.6	3.0	2.3	2.0	1.8	0.4										
NEO1	2.5	2.3	2.1	2.7	4.9	4.6	3.1	3.4									
SWL1	447.8	447.7	444.4	443.6	441.1	441.4	443.1	442.8	446.0								
SWL2	448.0	447.8	444.6	443.7	441.3	441.6	443.3	443.0	446.2	1.2							
NWL1	451.1	450.9	447.7	446.8	444.4	444.7	446.4	446.1	449.3	3.3	3.5						
NWL2	450.6	450.4	447.2	446.3	443.8	444.2	445.9	445.5	448.7	2.8	3.1	0.5					
SEL1	446.8	446.7	443.4	442.6	440.1	440.4	442.1	441.8	445.0	1.7	2.8	4.3	3.8				
SEL2	446.7	446.5	443.3	442.4	439.9	440.3	442.0	441.6	444.8	1.5	2.5	4.4	3.9	0.5			
NEL1	451.6	451.5	448.2	447.4	444.9	445.2	446.9	446.6	449.8	4.1	4.6	1.4	1.5	4.8	5.0		
NEL2	451.3	451.2	447.9	447.1	444.6	444.9	446.6	446.3	449.5	3.9	4.5	1.5	1.5	4.5	4.7	0.4	