Phylogeography and evolution of the crown-of-thorns starfish *Acanthaster planci*

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Summary

Understanding the processes that lead to diversification and speciation in the marine realm is one of the central questions of marine biogeography, especially considering the high dispersal potential of many marine invertebrates with long-lived planktonic larvae. Molecular tools allow gaining insights into the mechanisms that shape biodiversity and drive speciation. By investigating the phylogeography of the crown-of-thorns starfish *Acanthaster planci* with the most complete sample coverage to date, this study aimed to (1) increase our understanding of the processes driving diversification in the marine realm and (2) gain more insight into an organism that still amounts for a large proportion of the disturbance to coral reefs today due to its devastating population outbreaks.

By first exploring the phylogeography of the crown-of-thorns starfish throughout its entire Indo-Pacific range with a mitochondrial marker that is commonly used to discriminate species, we showed that, unlike what was previously thought, *A. planci* was not a single widespread species but instead formed a species complex, constituted of four highly differentiated lineages with restricted distributions, located in the Pacific, the Red Sea, the Northern and the Southern Indian Ocean.
Using a mitochondrial marker (the putative control region) with a higher resolution than the allozymes used in previous studies, we were then able to establish that the Pacific sister-species not only had a high dispersal potential, but also achieved it, as evidenced by the signature of ongoing gene flow between areas that were isolated in the past, and by the high levels of connectivity, even among distant populations.
Past and present surface circulation patterns in conjunction with ocean primary productivity were identified as key processes in shaping the genetic structure between and within both Indian Ocean sister-species, and the strong contrasts between them indicated they are now on different evolutionary trajectories.
To understand more about the evolution of the crown-of-thorns starfish species complex, we then reviewed the information available on other Indo-Pacific or-

ganisms and on the biology of the different crown-of-thorns starfish sister-species. Past sea level changes, land bridges, circulation patterns and cold-water upwelling played an important role in shaping the genetic structure of this species complex. But the strong population fluctuations that the crown-of-thorns starfish is infamous for could have further contributed to the formation of the four species. Differences in the biology and ecology of the four sister-species were also uncovered, although a lack of comparative data strongly limited the interpretability of these findings, but nonetheless indicated that the sister-species are not only on different evolutionary trajectories but have also diverged in some aspects of their biology.

Although the crown-of-thorns starfish is arguably one of the most researched marine organisms to date, by performing a thorough phylogeographic study including this organism's entire distribution and markers with different resolutions, we were able to uncover new and unexpected aspects of crown-of-thorns starfish biology, as well as determine future research directions that will hopefully allow increasing our understanding of this threat to coral reefs. Moreover, our extensive dataset also revealed some new phylogeographic patterns in comparatively under-researched areas of the Indo-Pacific. This highlighted the importance of integrating a species' entire range in phylogeographic studies in order to obtain a complete picture of its evolutionary history. These results stimulate similar investigations in other coral reef associated organisms, to find out whether they share these biogeographic patterns, or whether these are a special feature of the crown-of-thorns starfish species complex.

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Introduction

Diversification and evolution in the marine realm

The sheer scale of the world's oceans presents a challenge to understanding the processes that shape biodiversity patterns in the marine realm. The larvae of many marine organisms can drift for weeks in the plankton (Palumbi 1997). Given the speed of oceanic currents, this potentially allows these larvae to cover hundreds or even thousands of kilometres in a single generation (Hellberg 2009), and species with such high dispersal potential often have ranges spreading over thousands of kilometres (Palumbi 1997). This dispersal potential could be expected to limit the opportunities for diversification and speciation. Yet species richness is often very high in marine environments, especially in coral reef ecosystems of the Indo-Pacific region (Roberts et al. 2002). Understanding what processes lead to speciation in the marine realm has thus become a central question in marine biogeography. The advent of molecular tools opened a new window in this perspective, by providing the means to uncover cryptic speciation, estimate divergence times and speciation rates, and investigate the genetic structure within species (Palumbi 1997).

Phylogeography

As a subdiscipline of biogeography, the field of phylogeography is "concerned with the principles and processes governing the geographic distribution of genealogical lineages, especially those within and among closely related species" (Avise 2000). It lies at the junction between microevolutionary (population genetics) and macroevolutionary disciplines (phylogenetics, palaeontology). Within the framework of phylogeography, coalescent theory as applied to the mathematical and statistical properties of phylogenies allows estimating past population parameters, such as historical bottlenecks, changes in population size over time, divergence dates and the location of past refugia (Avise 2000; Hewitt 2004). Usually, mitochondrial DNA (mtDNA) markers are used to reconstruct phylogenies, and the geographical relationships among or within the lineages serve to deduce what historical patterns and processes shaped the contemporary distribu-

tions (Avise 2000; Hewitt 2004). The mitochondrial genome of most Metazoa is a circular, double-stranded DNA molecule (Fig. 0.1). The attractiveness of mtDNA fragments as markers for phylogeographic studies has several reasons: (1) the high abundance of mitochondria relative to nuclei in animal tissues means they are more readily amplified, even from degraded tissue; (2) the higher mutation rates of most mitochondrial genes (rRNA and protein-coding) compared to nuclear-encoded genes make them more appropriate for studies at the intra- and shallow interspecific level; (3) the mitochondrial genome's maternal inheritance mode, which leads to more complete lineage sorting than found in recombining nuclear markers due to the four-fold smaller effective population size, means that the signature of past vicariant events is often stronger in mtDNA than nuclear phylogenies; and (4) the higher amount of estimated mutation rates available for mtDNA enable obtaining approximations of divergence times and relating them

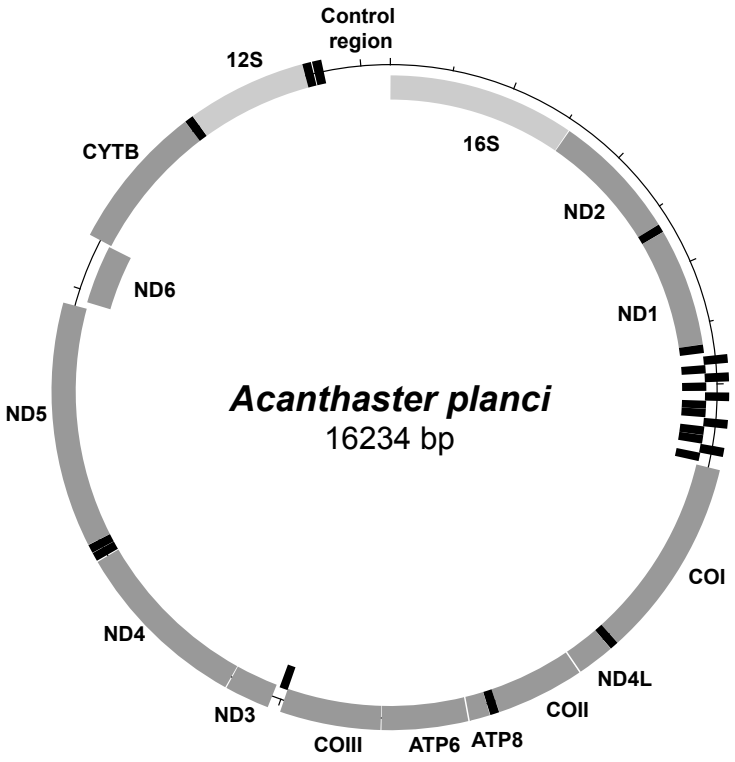

Figure 0.1. Organisation of the mitochondrial genome of *Acanthaster planci* (Yasuda et al. 2006). Protein coding genes are in dark grey, ribosomal RNA genes in light grey, transfer RNA genes in black and the putative control region is indicated (figure made by Oliver Voigt).

to potential vicariant events (Zink and Barrowclough 2008).
In the Indo-Pacific, phylogeographic and population genetic studies have identified some of the factors that play a key role in shaping the genetic structure of marine organisms, such as for example the repeated sea levels lowstands (up to 120 m below present levels) that during Pliocene and Pleistocene glacial cycles imposed a nearly complete land barrier between the Pacific and Indian Oceans (Voris 2000), thus leading to a strong genetic differentiation in many organisms either side of this barrier (e.g. McMillan and Palumbi 1995; Williams and Benzie 1998; Duda and Palumbi 1999; Nelson et al. 2000; Lessios et al. 2001; Bay et al. 2004; Crandall et al. 2008; Meyer 2003).
Most of these studies have however focussed on the Pacific Ocean and the Indo-Australian Archipelago, and only little is known about other areas of the Indo-Pacific such as the Indian Ocean and the Red Sea. Yet to obtain a thorough understanding of the evolutionary history of individual species, samples from their entire range should ideally be included, to avoid biases in the interpretation of the results (Benzie 2000). Unfortunately, due to the scale of the Indo-Pacific, extensive datasets are hard to come by, as they require intensive sampling efforts across a potentially very large area. This is particularly problematic for many marine invertebrates with bipartite life histories consisting of a benthic adult and a pelagic larva that can lead to widespread ranges. The focal organism of this study, the crown-of-thorns starfish *Acanthaster planci* (Fig. 0.2) comprises such a widespread range, occurring from the Red Sea to the eastern Pacific.

The crown-of thorns starfish

The corallivorous crown-of-thorns starfish *Acanthaster planci* (Linnaeus, 1758) is arguably one of the most researched marine invertebrates, mainly because of the threat its devastating population outbreaks have posed to coral reefs of the Indo-Pacific over the last 50 years (Fig. 0.3; Birkeland and Lucas 1990; Fabricius et al. 2010), and the urge to understand more about this organism in order to adequately manage it (Sapp 1999).

Population outbreaks

The first account of population outbreaks of the crown-of-thorns starfish came from Japan in 1957 and the Great Barrier Reef in 1962 (Endean and Chesher

Figure 0.2. Crown-of-thorns starfish from Fiji (photo credit: Nina Yasuda, 2005).

1973). Usually found in low numbers on reefs (Moran 1986), during outbreaks extremely high population densities have been reported (up to several hundreds of thousands of starfish per reef; Birkeland and Lucas 1990). Since these first outbreaks, reports estimating that up to 90% of the living cover of hard coral of reefs had been consumed by outbreaking populations have been common (Endean 1973; Chesher 1969; Birkeland and Lucas 1990), and the effects of such mortality on the coral reef community is found to extend through several trophic levels (Birkeland and Lucas 1990). The scale of the devastation inflicted to coral reefs by crown-of-thorns starfish outbreaks has served to focus a considerable amount of scientific attention on this organism, which was the subject of nearly a thousand publications until 1990 (Birkeland and Lucas 1990) and several major reviews (e.g. Moran 1986; Birkeland and Lucas 1990). A number of research committees were dedicated to investigating the "*Acanthaster* phenomenon", particularly in the western Pacific, where not only the first but also the most destructive outbreaks occurred (Birkeland and Lucas 1990). This research allowed uncovering a substantial amount of knowledge on the biology and ecology of this organism,

Figure 0.3. Crown-of-thorns starfish outbreak in Miyako, Japan (photo credit: Kenji Kajiwara, 2005).

although many aspects still remain unclear, especially in relation to the causes of outbreaks. Key findings of this research relevant to the present study as well as the most important gaps in our current understanding of this phenomenon will shortly be presented below.

Taxonomy

The crown-of-thorns starfish *Acanthaster planci* (Echinodermata: Asteroidea) belongs to the monogeneric family Acanthasteridae. This group can in short be described as "large starfish with many short arms and numerous large, dorsal spines, articulated on prominences from small basal ossicles" (Madsen 1955). *A. planci* has been known for a long time. First described by Rumphius (1705) as "a starfish covered in sharp spines that was best left alone, due to the excruciating pain caused by wounds from its spines", and later by Plancus and Gualteri in 1743 (Vine 1973), it was finally named by Linnaeus in 1758. Because of the high

morphological variability between specimens and some communication issues, for a while confusion surrounded the number of recognised species in the genus *Acanthaster* and their potential names (Birkeland and Lucas 1990). After reviewing the available information, Madsen (1955) finally recognised three species in this genus, *A. planci*, *A. brevispinus* and *A. ellisii*. By obtaining further samples and non-morphological evidence, the specific status of the omnivorous soft-bottom inhabitant *A. brevispinus* was confirmed (Lucas and Nash 1985; Nishida and Lucas 1988), whereas genetic data refuted the specific status of the eastern Pacific *A. ellisii* (Nishida and Lucas 1988), found to be synonymous to *A. planci*. There are thus currently two recognised species in the genus *Acanthaster*, the corallivore *A. planci* and the omnivore *A. brevispinus*. The present study focuses on the crown-of-thorns starfish *A. planci*.

Morphology

The crown-of-thorns starfish is quite unusual and conspicuous (Fig. 0.2). Its name is derived from the numerous toxic spines that cover its aboral surface, usually between 40 and 50mm in length. Unlike other coral reef asteroids, the crown-of-thorns starfish has a pliable and prehensile body, supported by a loose skeletal matrix (Birkeland and Lucas 1990). There is considerable morphological variation between individuals. Normally ranging from 25-35cm in diameter, crown-of-thorns starfish can occasionally grow over 70cm, and possess between seven and 23 arms (Birkeland and Lucas 1990). The number of anuses has been found to range from one to six, and the number of madreporites from three to 16 (Moran 1986). Crown-of-thorns starfish from the Pacific Ocean are variable in colour but cryptic: they are usually gray-green to gray-purple, often with reddish papulae (respiratory and excretory organs that project through pores in the epidermis of the aboral surface of the starfish; Birkeland and Lucas 1990). Individuals found in the northern Indian Ocean on the other hand tend to have a very different colour, sometimes referred to as 'electric blue' (Birkeland and Lucas 1990).

Feeding behaviour

The crown-of-thorns starfish is a specialist corallivore that feeds extra-orally by everting its stomach and wrapping it around the surface of its prey. The stomach then secretes digestive enzymes that break down the coral tissue and allow it to

be reabsorbed (Moran 1986). The white calcium carbonate skeleton that remains after the coral colony has been stripped of its living tissue is often referred to as a 'feeding scar', soon to be overgrown by algae (Birkeland and Lucas 1990). The crown-of-thorns starfish is thought to be a highly efficient predator, having an advantage over other corallivorous asteroids due to both its flattened disk-shape that allows a greater stomach area to bodymass ratio and its pliable and prehensile morphology, enabling it to feed on corals that more heavily ossified asteroids are unable to, such as branching acroporids (Birkeland and Lucas 1990).

Life history

Due to the technical difficulties involved in identifying, locating and/or tagging larvae, juveniles and adults in the field, the life history of the crown-of-thorns starfish has been deduced mostly from studies conducted in the laboratory, and much uncertainty remains at each of these life stages (Birkeland and Lucas 1990). The crown-of-thorns starfish is dioecious and is only known to reproduce sexually, unlike some other coral reef asteroids (e.g. *Linckia laevigata*; Yamaguchi 1975). It is highly fecund, and large mature females may produce as many as 60 million oocytes per spawning season (Moran 1986). Planktonic larvae are produced by external fertilisation during synchronised spawning events that are thought to occur during the warmer months of the year, at least at higher latitudes (Birkeland and Lucas 1990). Once fertilised, the zygote develops into a larvae that goes through the typical asteroid bipannaria and brachiolaria developmental stages while feeding on phytoplankton (>1μm; Okaji et al. 1997), before settling onto the reef and metamorphosing into a five-armed juvenile (Yamaguchi 1973; Lucas 1973). Based on laboratory studies, the pelagic larval duration is thought to last from three to four weeks in normal conditions (Yamaguchi 1973), and can be extended to about seven weeks in marginal food regimes as found in oceanic conditions (Lucas 1982), although the occurrence of a facultative teleplanic larva remains to be confirmed (Birkeland and Lucas 1990). The larvae display negative geotactic behaviour, i.e. after hatching they swim to the surface and remain there until the late brachiolaria stage (Yamaguchi 1973). Ocean surface currents therefore have an important impact on their dispersal. Following metamorphosis, the highly cryptic juveniles consume coralline algae for the next six months, growing to about 8mm in diameter before shifting their diet to corals. During the next two

years, the starfish undergoes a period of rapid growth before reaching sexual maturity (Birkeland and Lucas 1990). The longevity of the crown-of-thorns starfish remains debated, mostly due to the difficulty involved in implanting long-term tags in individuals in the field or identifying a reliable aging method (Birkeland and Lucas 1990; Souter et al. 1997). In artificial laboratory conditions, they survived from five to more than seven years (Birkeland and Lucas 1990).

Causes of outbreaks

As pointed out by Birkeland and Lucas (1990), the major problem in coping with the "*Acanthaster* phenomenon" is that we still do not have an adequate understanding of this organism, despite all the research performed on it. Both the reasons why the crown-of-thorns starfish differs from other coral reef organisms and the causes of outbreaks remain unclear (Birkeland and Lucas 1990). The causes of primary outbreaks (as opposed to secondary outbreaks which are the consequence of the large number of gametes produced by other outbreaking populations) are still debated, and the importance of anthropogenic impacts in initiating these outbreaks also remains unresolved (Fabricius et al. 2010). The currently prevailing hypotheses on the causes of outbreaks are the 'predator removal hypothesis' (Birkeland and Lucas 1990; Sweatman 2008), which suggests that more juveniles survive to maturity due to the removal of fish predators through human exploitation, and the 'larval starvation hypothesis' (Birkeland and Lucas 1990; Fabricius et al. 2010; Brodie et al. 2005; Houk et al. 2007), that argues that nutrient-limited survival of larvae controls population outbreaks. Under most scenarios, increased larval survival is thought to play a key role in the onset of outbreaks. Yet, as mentioned before, larvae are extremely difficult to locate and distinguish in the field, and dispersal patterns can therefore not be studied directly (Moran 1986). Genetic methods that allow uncovering the connectivity between populations can provide indirect information on dispersal, and as a result were soon used to understand more about this organism.

Genetic studies on the crown-of-thorns starfish

As uncovering the connectivity between crown-of-thorns starfish populations is a key aspect of correlating any external factor to the cause and spread of outbreaks, when molecular tools became available to obtain indirect measures of gene flow

between populations, they were applied to the crown-of-thorns starfish, generating several datasets spanning different parts of its range (Benzie and Stoddart 1992a, b; Benzie and Wakeford 1997; Benzie 1999; Nash et al. 1988; Katoh and Hashimoto 2003; Nishida and Lucas 1988; Yasuda et al. 2009; Gérard et al. 2008). The main findings of these studies in relation to spatial genetic structure were (1) the existence of a strong genetic differentiation between the Indian and the Pacific Oceans (Benzie 1999); (2) low levels of differentiation among Pacific populations, even over large distances, which were interpreted as indicative of a high dispersal propensity (Benzie and Stoddart 1992a; Benzie 1999; Nash et al. 1988; Nishida and Lucas 1988; Yasuda et al. 2009); (3) but also the presence of a few isolated regions (reviewed in Benzie 2000; Yasuda et al. 2009).
Most of this research was performed with allozymes, which have a relatively limited resolution, can potentially be subjected to selection, and may not always have reached equilibrium between drift and migration over the range investigated (Hellberg 2007; Williams and Benzie 1997). Indeed, the conclusions drawn from allozyme data can be misleading, as an absence of genetic structure might not necessarily reflect high gene flow and a high dispersal propensity in the investigated organism (Williams and Benzie 1997). Two more recent molecular studies used markers with a higher resolution (microsatellites; Yasuda et al. 2009; and mtDNA; Gérard et al. 2008), but focused mostly on the western Pacific and a few populations from the southwestern Indian Ocean. They confirmed the existence of the genetic break between the Pacific and Indian Oceans, and the microsatellite study uncovered high gene flow between populations as well as some structure, mostly concordant with present-day current systems (Yasuda et al. 2009).

Aims of this study

By performing a phylogeographic study with the highest geographic sample coverage to date for a coral reef organism in the Indo-Pacific, the crown-of-thorns starfish, the aim of this study was to (1) increase our understanding of the processes driving diversification in the marine realm, especially in areas that have been poorly researched up to now, and (2) gain more insight into the distribution, dispersal and evolutionary history of an organism that still amounts for a large proportion of the disturbance to coral reefs today.

We included samples stemming from populations covering the crown-of-thorns starfish's entire distribution range, and used two mtDNA markers with different mutation rates: a fragment of the cytochrome oxidase I (COI) gene (Fig. 0.1), commonly used to investigate evolutionary relationships among closely related species, and the faster evolving putative control region (Fig. 0.1), suitable for exploring the genetic structure at the population level (Avise et al. 1987; Féral 2002).

This allowed us to explore the nature of genetic breaks in the crown-of-thorns starfish across the Indo-Pacific (**chapter 1**) and the detailed genetic structure of this organism in the Pacific and Indian Oceans, as well as the mechanisms that shaped this structure (**chapters 2 and 3**). We then investigated to what extent our findings corroborated known patterns and processes operating in the Indo-Pacific, and whether they revealed new, unexpected aspects of Indo-Pacific biogeography (**chapter 4**). Finally, we examined what new insights our findings could provide on the crown-of-thorns starfish itself (**chapter 4**).

1. A threat to coral reefs multiplied? Four species of crown-of-thorns starfish

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1. A threat to coral reefs multiplied? Four species of crown-of-thorns starfish

Abstract

In the face of ever-increasing threats to coral reef ecosystems, it is essential to understand the impact of natural predators in order to devise appropriate management strategies. Destructive population explosions of the crown-of-thorns starfish (COTS) *Acanthaster planci* have devastated coral reefs throughout the Indo-Pacific for decades. But despite extensive research, the causes of outbreaks are still unclear. An important consideration in this research is that *A. planci* has been regarded as a single taxonomic entity. Using molecular data from its entire distribution, we find that *A. planci* is in fact a species complex. This discovery has important consequences for future coral reef research, and might prove critical for successful reef conservation management.

1.1. Introduction

Coral reefs, the most species-rich marine ecosystems, are subjected to growing anthropogenic pressure, limiting their resilience to natural threats such as corallivorous predators (Bellwood et al. 2004). Among those, the crown-of-thorns starfish (COTS) *Acanthaster planci* is infamous for its dramatic population explosions (called outbreaks) that have devastated coral reefs throughout the Indo-Pacific for decades, making it a major management issue (Birkeland and Lucas 1990). But despite extensive research into COTS biology, the causes of outbreaks are still not clear. They probably involve a variable set of interacting natural and anthropogenic factors that lead to increased recruitment (Engelhardt and Lassig 1997). An important consideration in both COTS research and management is that *A. planci* has been regarded as a single species throughout its distribution, and therefore the same ecological and behavioural traits are assumed worldwide. *A. planci*'s long-lived pelagic larva – surviving from 3 to 4 weeks in normal condi-

tions (Yamaguchi 1973), to about seven weeks in marginal food regimes as found in oceanic conditions (Lucas 1982) – would be expected to promote genetic homogeneity. But this species appears to be highly structured (Benzie 1999), in line with other recent studies of widespread marine invertebrates (e.g. Becker et al. 2007). Using sequences of the mitochondrial Cytochrome Oxidase subunit I gene (COI) from samples covering its entire distribution, we show that *A. planci* consists of four deeply diverged clades that form a pan-Indo-Pacific species complex (as identified by DNA taxonomy; Vogler and Monaghan 2007).

1.2. Methods

DNA was extracted using a Qiagen MagAttract 96 DNA Plant Core Kit from 237 *A. planci* and two *A. brevispinus* tissue samples, collected by SCUBA and snorkel from 1987 to 2008 (Tab. S1.1). A fragment of the Cytochrome Oxidase subunit I gene (COI), corresponding to the "barcoding" fragment, was amplified and sequenced with the following primers: COTS_COI_F4734 5'-GCCTGAGCAGGAATGGTTGGAAC-3' and COTS_COI_R5433 5'-CGTGGGATATCATTCCAAATCCTGG-3'. Sequences were assembled using CODONCODE ALIGNER (http://www.codoncode. com/aligner), and the 632 bp remaining after quality-based end-clipping were aligned in SEAVIEW (Galtier et al. 1996) with *Patiria pectinifera* (accession number: D16378), as an outgroup. All sequences were deposited in EMBL (accession numbers: FM174472-174675, FM177190-177203, FM202070-202090).

Genetic distances between and within clades were calculated with MEGA4 (Tamura et al. 2007) using the Kimura 2-parameter model of sequence evolution (K2P), to enable comparisons with other asteroid datasets (Waters et al. 2004). There are no fossil data or geological calibration points available to date the separation between the four clades, so divergence times were approximated by applying the most accurate COI divergence rates available for echinoderms to the K2P distances (2.9-4.5%.Myr^{-1}; Lessios 2008). To test the 95% connectivity limit as a species threshold (Hart and Sunday 2007), a parsimony haplotype network was built using TCS 1.2.1 (Clement et al. 2000). A neighbor-joining (NJ) analysis and NJ bootstrap analysis (1000 replicates) were carried out in PAUP*4.0b10 (Swofford 2003). After inferring the best-fit nucleotide evolution model using the Akaike Information Criterion as implemented in MODELTEST 3.7 (Posada and

Crandall 1998), we estimated the maximum likelihood tree under a GTR+Γ+I model in PHYML 2.4.4, including 1000 bootstrap replicates (Guindon and Gascuel 2003).

We used a method separating species diversification from coalescent processes in a phylogenetic tree by comparing two models describing the likelihood of branching patterns (Pons et al. 2006). The null model assumes that the entire sample derives from a single population undergoing a single coalescent process, whereas the general mixed Yule coalescent (GMYC) model classifies the observed branching time intervals into two categories, as either the result of inter- or intraspecific processes of lineage sorting. A log-likelihood ratio test is then used to assess which model provides a better fit. The GMYC model additionally integrates scaling parameters for both the diversification (p_{k+1}) and coalescent (p_j) processes, which allow departures from strict assumptions of constant population size and rates of cladogenesis. The models were fitted using an R script provided by T. Barraclough (Pons et al. 2006) to an ultrametric tree obtained by non-parametric rate smoothing of the NJ tree (Sanderson 1997), as implemented in the R package APE (Paradis et al. 2004).

1.3. Results

Evidence for species status of the clades comes, first, from the extent of the genetic distances between them. These ranged from 8.8 to 10.6% (as opposed to <0.7% within clades), equivalent to the distances between sibling species in other starfish (Waters et al. 2004). According to these distances, the four clades are estimated to have diverged between 1.95 and 3.65 Mya. Second, the COI haplotypes grouped into four disconnected statistical parsimony networks at the 95% connection limit (Fig. 1.1c), suggested as a species delimitation threshold (Hart and Sunday 2007). Third, using a method that differentiates interspecific (i) from intraspecific (ii) diversification processes through a phylogenetic approach (Pons et al. 2006), we identified the same four clusters, corresponding to the putative sibling species (Fig. 1.1a). Indeed, the GMYC model, which assumes a steep increase in branching rates from (i) to (ii) at a threshold T, was preferred over the null model of uniform branching rates ($LogL_{GMYC}$=432.6, $2\Delta L$=31.1, χ^2 test, d.f.=3, p<0.001; Fig. 1.1b). Both the scaling parameters for the diversification (p_{k+1}=-0.27) and the coalescent (p_j=0.04) processes were smaller than 1.

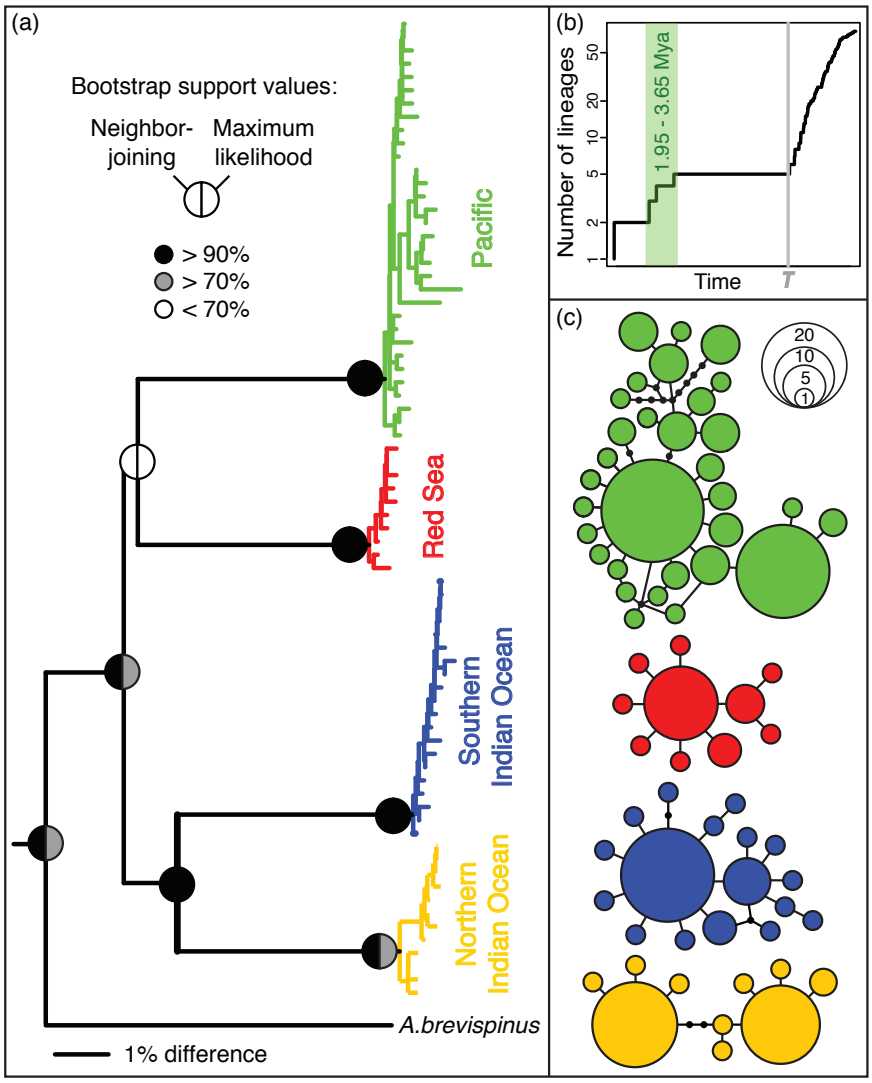

Figure 1.1. Pan-Indo-Pacific *Acanthaster* species complex (a) *Acanthaster* COI neighbor-joining (NJ) tree, rooted with *Patiria pectinifera* (not shown) [General Mixed Yule Coalescent (GMYC) clusters in colour, bootstrap support values for both the NJ and maximum likelihood analyses depicted on main nodes only]. (b) Lineages-through-time plot [based on the ultrametric tree obtained by non-parametric rate smoothing of the phylogeny depicted in (a); grey line is branching rate threshold *T*; green shaded area highlights the timing of the diversification events, which at a COI divergence rate of 2.9-4.5%.Myr^{-1} (Lessios 2008) corresponds to the Pliocene-early Pleistocene (between 1.95 and 3.65 Mya)]. (c) Four disconnected statistical parsimony networks at the 95% connectivity limit, corresponding to the putative species, same colours as in (a).

Figure 1.2. Geographic distribution of COI haplotypes from the four putative COTS species, pie charts indicate relative frequency of each species per sampling location, colours are the same as in figure 1.1.

1.4. Discussion

We find that *Acanthaster planci* consists of four strongly differentiated and highly supported mitochondrial clades, from the Red Sea, the Pacific (Pac), the Northern (NIO), and the Southern Indian Ocean (SIO) (Fig. 1.1a and 1.2), that together form a species complex. Although cryptic speciation is a widespread phenomenon in the marine realm, this finding is quite surprising for an organism as extensively studied as *A. planci* over the last decades.

Assuming a COI divergence rate of 2.9-4.5%.Myr^{-1} (Lessios 2008), the four clades are estimated to have diverged in the Pliocene-early Pleistocene (1.95 to 3.65 Mya). The speciation process was probably driven by sea level changes (Pillans et al. 1998), isolating populations between major oceans (e.g., Pac vs. NIO; Voris 2000). Additionally, restricted circulation patterns could have reduced larval interchange between populations (e.g., SIO vs. NIO; Pollock 1993). Furthermore, the strong patterns of regional differentiation may have been enhanced by ecological differences among lineages (Reid et al. 2006). The populations of all four sibling species appear to be expanding, as supported by both the GMYC scaling parameter for the coalescent process (p_j<1; Pons *et al.* 2006), and the overall star shape of each species' haplotype network (Fig. 1.1c; Avise 2000).

Our discovery of four highly differentiated clades in one of the world's most destructive coral predators has significant conservation implications. Identifying cryptic speciation is essential to adequately study and contain species that require managing (Bickford et al. 2007). Although the status of *A. planci* is relatively

poorly documented from the Indian Ocean and the Red Sea, outbreaks there do not appear to be as massive and widespread as in the Pacific (Zann 2000), suggesting that outbreak patterns might vary between the different sibling species. Up to now however, the overwhelming majority of COTS research has been performed in the Pacific. Failure to recognise the existence of the sibling species could have contributed to a lack of understanding of the processes that lead to outbreaks in the different COTS lineages, by extrapolating results obtained from the Pacific studies to *A. planci*'s entire distribution for both research and management purposes.

Future research will be required to investigate whether the life history, behavioural patterns, and/or ecological requirements that may affect the outbreak dynamics of these four independent evolutionary COTS lineages have sufficiently diverged as to necessitate lineage-specific management. This could prove to be crucial for the design of appropriate management strategies to minimise the impact of future catastrophic COTS outbreaks in different regions of the world.

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2. Phylogeography of the Pacific crown-of-thorns starfish, a high dispersal coral predator

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2. Phylogeography of the Pacific crown-of-thorns starfish, a high dispersal coral predator

Abstract

Previous studies of the population genetic structure of the corallivorous crown-of-thorns starfish (COTS) *Acanthaster planci* in the Pacific Ocean showed high levels of gene flow which were assumed to reflect a high dispersal potential. However, most of this research was either performed with markers that have a limited resolution, or with a restricted sampling scheme, which can both lead to biases in the interpretation from molecular data. In this study, we investigated the phylogeography of the Pacific crown-of-thorns starfish with a mitochondrial marker (control region) and the most complete geographic coverage to date. We uncovered high levels of gene flow as well as genetic structure (Φ_{ST}=0.198), which revealed a complex history of range restrictions and expansions, most likely due to changes in topography and oceanography associated with sea-level changes. Both the signature of ongoing gene flow between past isolated areas as well as the high levels of connectivity, even among distant populations, confirmed the high dispersal potential of the crown-of-thorns starfish in the Pacific Ocean is often achieved.

2.1. Introduction

The corallivorous crown-of-thorns starfish (COTS) *Acanthaster planci* is a marine invertebrate mostly infamous for the threat its population outbreaks have posed to Indo-Pacific coral reefs over the last 50 years (Birkeland and Lucas 1990). Although outbreaks still amount for a large proportion of the disturbance to Indo-Pacific reefs today (Fabricius et al. 2010), their causes remain debated, as well as appropriate monitoring strategies to predict their occurrence and management plans to reduce their impact (Birkeland and Lucas 1990; Houk et al. 2007; Sweatman 2008; Fabricius et al. 2010).

COTS have been found to be a species complex constituted of four highly differentiated lineages with restricted distributions, located in the Pacific, the Red Sea, the Northern and the Southern Indian Ocean (Vogler et al. 2008). Of the four sibling species, the Pacific one has by far been the most intensively studied, mostly due to the location of the first reported outbreaks (Japan in 1957 and the Great Barrier Reef in 1962; Endean and Chesher 1973) and the large amounts of funding invested in research on the biology of COTS and the causes of outbreaks in these areas (Sapp 1999). Outbreaks are also believed to be more intense and of a larger scale in the Pacific species than in the three others, although the latter have been comparatively under-researched (Zann 2000).

As uncovering the connectivity between COTS populations is a key aspect of correlating any external factor to the cause and spread of outbreaks, when molecular tools became available to obtain indirect measures of gene flow between populations, they were soon applied to COTS, detecting low levels of differentiation among Pacific populations, even over large distances, which were interpreted as indicative of a high dispersal propensity (Benzie and Stoddart 1992a; Benzie 1999; Nash et al. 1988; Nishida and Lucas 1988). Nonetheless, a few isolated regions were also detected (Hawaii, western Australia, and Lord Howe Island; reviewed in Benzie 2000). All of this research was performed with allozymes, which have a relatively limited resolution, can potentially be subjected to selection, and may not always have reached equilibrium between drift and migration over the range investigated. Indeed, as highlighted by Williams and Benzie (1997) for the starfish *Linckia laevigata*, the conclusions drawn from allozyme data can be misleading, as an absence of genetic structure might not necessarily reflect high gene flow and a high dispersal propensity in the investigated organism. A more recent COTS study that focused on the western Pacific and a few populations from the Indian Ocean using microsatellites uncovered high gene flow between populations as well as some structure in relation to peripheral populations (Moorea), mostly concordant with present-day current systems (Yasuda et al. 2009).

The aim of this study was to investigate the genetic structure of Pacific COTS with a mitochondrial DNA marker (the highly variable putative control region) and the most complete sample coverage to date, to (1) find out whether the high levels of gene flow identified with allozymes truly reflect a high dispersal capacity; and (2) gain more insight into past restrictions to gene flow, which can help

identify the type of barriers that played an important role in shaping COTS' genetic structure.

2.2. Materials and Methods

2.2.1. Sampling and sequencing

A total of 673 COTS samples were collected by SCUBA and snorkel from 45 different sites between 1987 and 2008 (Tab. S2.1). The tissue sampled included pyloric caeca (Benzie 1999) and tube feet, and was stored as soon as possible after collection, either at -80°C for the pyloric caeca (Benzie 1999), or in ethanol (>80%) or DMSO buffer (Seutin et al. 1991) for the tube feet. DNA was extracted from the pyloric caeca using a MagAttract 96 DNA Plant Core Kit (Qiagen) according to the manufacturer's manual DNA purification protocol, with the following preliminary steps: the tissue was manually ground in a 1.5ml Eppendorf tube after freezing in liquid nitrogen, then incubated at 35°C for an hour in RLT lysis buffer (Qiagen), vortexed at full speed for 20s, and centrifuged at 8000 x g for 5min. DNA was extracted from tube feet using a DNeasy Tissue Kit (Qiagen) according to the manufacturer's protocol.

A DNA fragment containing the putative mitochondrial control region (CoReg) and the 5' end of the adjacent 16S rRNA gene was amplified with the following primers, designed using the available mitochondrial genome (Yasuda et al. 2006): COTS-CoReg-F15635 5'-CAAAAGCTGACGGGTAAGCAA-3' and COTS-CoReg-R114 5'-TAAGGAAGTTTGCGACCTCGAT-3'. DNA sequencing was performed using the PCR reverse primer, and the following internal forward primer: COTS-CoReg-seqPac-F15735 5'-TTAGCTGGGTATAAG-3'. Sequences were assembled using CODONCODE ALIGNER (http://www.codoncode.com/ aligner) and aligned in SEAVIEW v4.2 (Galtier et al. 1996) using the built-in MUSCLE software (Edgar 2004).

2.2.2. Genetic structure and diversity

Unless stated otherwise, all the following analyses were run in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010).

To investigate the genetic structure of COTS in the Pacific Ocean, we first estimated a minimum spanning tree based on pairwise differences and re-drew it with ADOBE ILLUSTRATOR. To assess the robustness of the signal in the minimum

spanning tree, we also constructed a split graph in SPLITS-TREE v4.11.3 using the NeighborNet method (Huson and Bryant 2006), which allows detecting incongruences in the signal and alternative phylogenetic histories.
We then estimated the overall Φ_{ST} value without any *a priori* structure (10'000 replicates). To understand how this structure was partitioned among and within regional groups of populations, we ran a series of analyses of molecular variance (AMOVA) with different regional groupings of populations, based on geography and published regional provinces (Marine Ecoregions of the World; Spalding et al. 2007), to determine which combination explained the highest amount of genetic variance over the whole dataset (locus-by-locus AMOVA, 50'000 replicates). This first series of tests revealed that 35 of the populations belonged to a single West Pacific group (Tab. S2.2a). To further investigate the genetic structure at a smaller scale, we ran a second series of AMOVA tests within this group. To visualise the genetic relationship between indviduals from the different regional groups and subgroups, we plotted them onto the minimum spanning tree.
To further explore how COTS' genetic diversity is distributed throughout the Pacific, we then calculated standard measures of genetic diversity (haplotype frequencies, haplotype diversity h and nucleotide diversity π) for each population as well as for the regional groups and subgroups we previously identified with the AMOVAs.

2.2.3. Gene flow

In order to gain an appreciation of gene flow between populations in the Pacific, we first calculated pairwise Φ_{ST}s and the corrected average number of pairwise differences between all populations (50'000 random replicates, standard Bonferroni correction for multiple tests), as well as between the groups and the subgroups identified by the AMOVAs. We then used a Mantel test (100'000 permutations) to determine whether genetic (pairwise Φ_{ST} values) and geographic distances were correlated over the Pacific as a whole as well as within the largest groups. All these analyses were also performed in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010).

2.2.4. Demographic history

We investigated the demographic history of the Pacific COTS using statistics that

have the ability to detect signatures of recent population expansions. Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989) were calculated using ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010; 50'000 replicates), and Ramos-Onzins' R_2 (Ramos-Onsins and Rozas 2002) was estimated using the R package PEGAS v0.3-2 (Paradis 2010; 10'000 replicates). D has been found to perform rather poorly except for very large population sizes, and we therefore only used this test at the group and subgroup level, whereas F_S and R_2 have in most cases proved to be the most powerful tests for detecting population growth, and were therefore also used at the population level (Ramos-Onsins and Rozas 2002).

2.3. Results

The 673 control region sequences of approximately 530bp yielded 443 unique haplotypes. We detected high levels of genetic diversity (h=0.997, π=0.026), and substantial amounts of genetic structure, both at an oceanic and regional scale. The overall Φ_{ST} without *a priori* grouping was 0.198 (p<0.001) and that of the regional West Pacific group 0.107 (p<0.001). The hypervariable mitochondrial control region thus appeared to be an appropriate marker to investigate the genetic diversity and structure of the crown-of-thorns starfish, both at an oceanic and regional scale.

2.3.1. Divergence patterns

The minimum spanning tree (MST) revealed the existence of four MST-groups separated by at least 18 mutation steps (Fig. 2.1a), which were also reflected in the split graph (Fig. 2.1b), although not in the same branching order. We mostly focus on discussion of the minimum spanning tree, due to the high number of haplotypes and the more direct visual appreciation of detail gained from the minimum spanning tree. However, interpretations included consideration of the split graph to prevent erroneous interpretations that did not take account of ambiguities in the data. The largest MST-group, MST-group A, contained individuals from most populations (Fig. 2.1c) and was strongly star-shaped, suggesting a recent population expansion (Avise 2000). The three remaining MST-groups on the other hand were quite restricted: MST-group B was limited to individuals from Johnston Atoll, MST-group C contained individuals from southern Pacific populations and MST-group D individuals from Vanuatu and Kingman (Fig. 2.1).

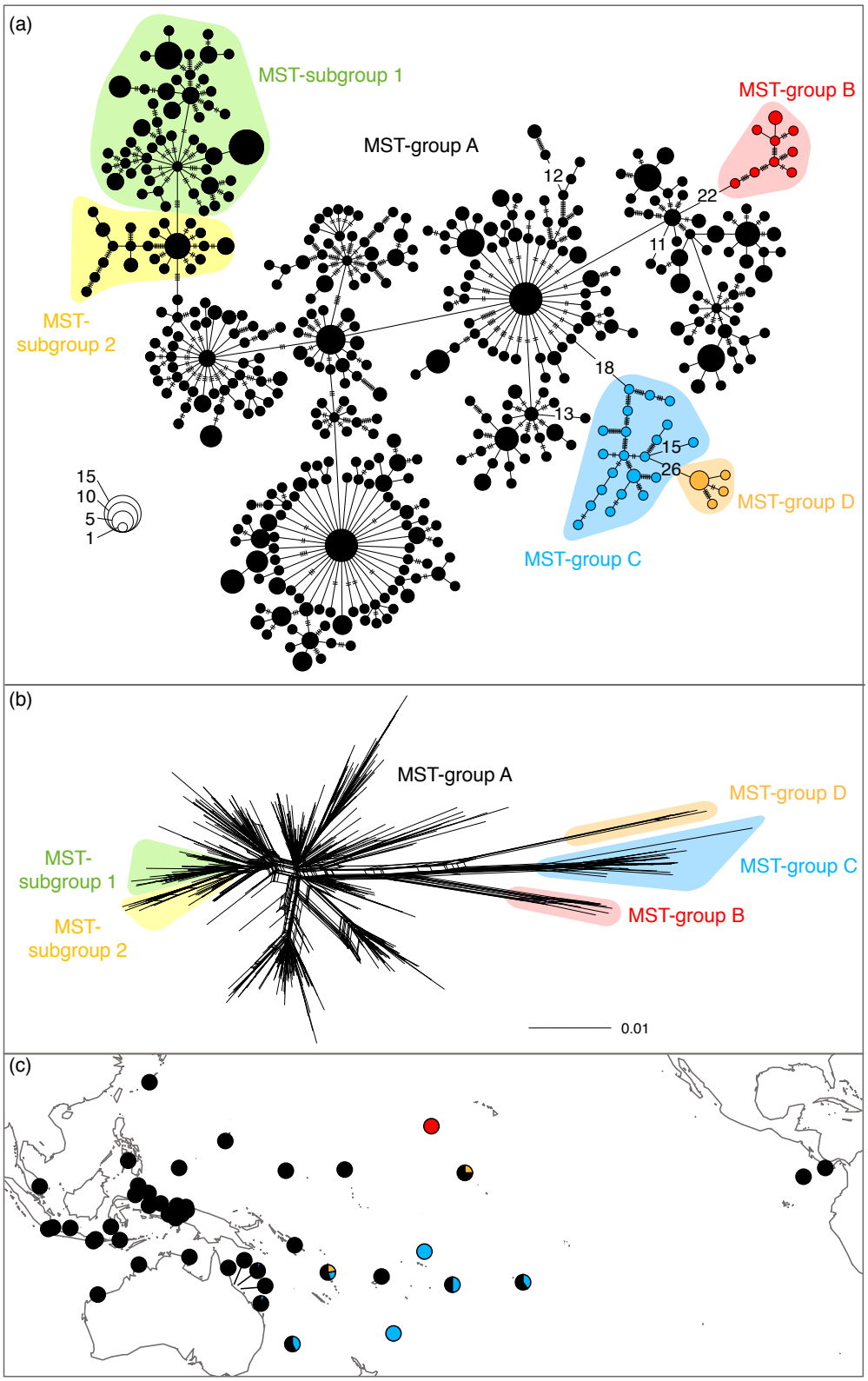
2.3.2. Oceanic scale

The first series of AMOVAs at the level of the entire Pacific showed that the regional combination of populations that explained most of the genetic variance (Φ_{CT}=0.265, p<0.001; Tab. S2.2a) was composed of four groups: a large West Pacific group (35 populations), a central Pacific islands group (7 populations), a group containing only Johnston Atoll, and an East Pacific group (populations) (Fig. 2.2, Tab. 2.1, Tab. S2.1).

Johnston Atoll appeared to be strongly isolated and differentiated from all the other groups, as evidenced by the minimum spanning tree (Fig. 2.1 and 2.2). Both haplotype and nucleotide diversities in Johnston Atoll were low (h=0.972, π=0.011), and there was no signature of a recent population expansion (Tab. 2.2). The pairwise Φ_{ST} comparisons between groups showed it was most differentiated from all other groups (Tab. 2.3a), and finally, the genetic distance between Johnston Atoll and all other populations was higher than expected based on the geographic distance between them (Fig. 2.3a).

The East Pacific group was not strongly differentiated from the rest of the Pacific, but appeared to have gone through a bottleneck or a founder effect. Both haplotype and nucleotide diversities were extremely low in the East Pacific (h=0.736, π=0.003), and there was no signature of a recent population expansion (Tab. 2.2). Moreover, all individuals from the East Pacific group were closely related and belonged to MST-group A (Fig. 2.4a), even sharing a common haplotype with the West Pacific group, despite the large geographic distance between them.

Figure 2.1 (opposite). Genetic structure of the crown-of-thorns starfish in the Pacific: (a) minimum spanning tree (MST; all haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks or number, MST-groups separated by more than 18 steps from the MST-group A are highlighted, the delimitation of MST-subgroups 1 and 2 is not based on mutational differences but indicated for clarity when referencing in the text, circle size is proportional to frequency of occurrence); (b) NeighborNet showing the ambiguities in the data in (a) (MST-groups and MST-subgroups are the same as in (a)); (c) geographic distribution of haplotypes, pie charts indicate the frequency of the four major MST-groups for each sampling site, colours are the same as in (a).


(a)
MST-subgroup 1
MST-group B
MST-group A
12
22
11
MST-
subgroup 2
18
13
15
26
15
10
5
1
MST-group D
MST-group C
(b)
MST-group A
MST-group D
MST-
subgroup 1
MST-group C
MST-group B
MST-
subgroup 2
0.01
(c)

The Central Pacific group displayed a mixed pattern of isolation and gene flow with the West Pacific group. Indeed, haplotypes from the highly differentiated MST-group D were only found in Vanuatu and Kingman (Fig. 2.1), and haplotypes from MST-group C were restricted to Central Pacific populations but for two from the West Pacific. This suggested a past interruption in gene flow between the West and Central Pacific as well as between populations within the latter. However, the haplotypes from many Central Pacific individuals were also scattered throughout the rest of MST-group A, indicating gene flow had resumed with the West Pacific. This mixture of individuals from MST-group A and MST-groups C and D in populations from the Central Pacific group led to its very high nucleotide diversity (π=0.044), but as haplotypes were less differentiated and unique haplotypes fewer overall in the Central than West Pacific, haplotype diversity was lower (Central Pacific: h=0.987, Western Pacific: h=0.997). The Central Pacific group showed no clear signature of a population expansion
(Tab. 2.2), and no correlation between genetic and geographic distances (b=-6.8x10^{-5}, R^2=0.135, p>0.05).

The West Pacific group spanned a large area, and there were high levels of gene flow overall throughout the western Pacific. Haplotype diversity was very high

Table 2.1. AMOVA results for the Pacific and the West Pacific group (significance tested with 50'000 permutations; *** p<0.001).

	Pacific WP vs. CP vs. Joh vs. EP[1]	**West Pacific** WA vs. Mal vs. CWP vs. CeBa vs. EWP[2]
Overall Φ_{CT} (between groups)	0.265***	0.149***
Overall Φ_{SC} (within groups)	0.133***	0.032***
Percent variation:		
Among groups	26.46%	12.09%
Among populations within groups	9.76%	2.81%
Within populations	63.77%	85.10%

[1]WP: West Pacific; CP: Central Pacific; Joh: Johnston Atoll; EP: East Pacific (see Tab. S2.1 for populations within groups).
[2]WA: Western Australia; Mal: Malaysia; CWP: Central-West Pacific; CeBa: Cenderawasih Bay; EWP: Eastern-West Pacific (see Tab. S2.1 for populations within groups).

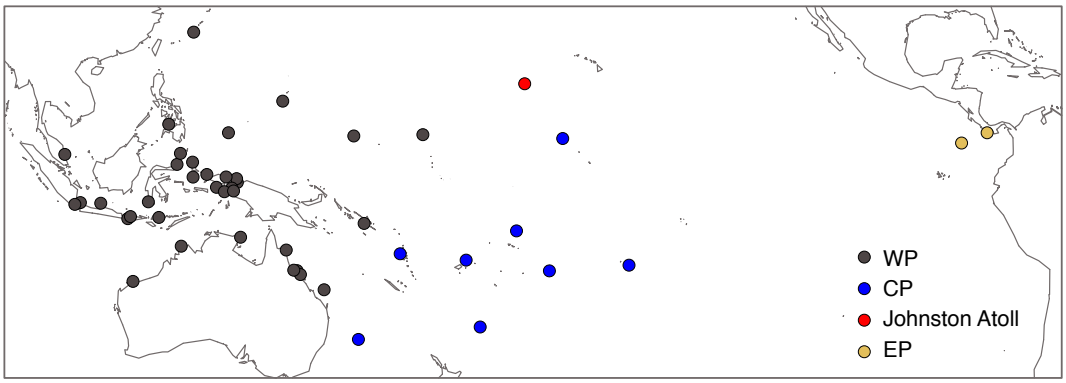


Figure 2.2. Distribution of genetic structure of the Pacific crown-of-thorns starfish: sampling locations coloured according to groups detected by the analyses of molecular variance (WP: West Pacific, CP: Central Pacific, EP: East Pacific).

in this group due to the presence of many recent mutations leading to single haplotypes (Fig. 2.4a), suggesting a recent population expansion, which was further supported by the significance of the statistics F_S, R_2 and D (Tab. 2.2). There was however a correlation between genetic and geographic distances (b=2.6x10^{-5}, R^2=0.087, p<0.01; Fig. 2.3b), indicates some limitation of dispersal between reefs, and significant structure was also detected within this group.

2.3.3. Regional scale: West Pacific

A second series of AMOVAs revealed that the combination of populations that explained most of the genetic variance (Φ_{CT}=0.121, p<0.001; Tab. S2.2b) in the West Pacific was composed of five subgroups: a Western Australian subgroup (3 populations), a subgroup containing all populations in Cenderawasih Bay (4 populations), a subgroup containing only Malaysia, a large central subgroup containing all Indonesian populations (excl. Cenderawasih Bay) as well as the Philippines, Japan, Guam and Palau (Central-West Pacific group, 19 populations), and an eastern subgroup including the Great Barrier Reef, the Solomon Islands, Majuro and Pohnpei (Eastern-West Pacific group, 8 populations) (Fig. 2.5, Tab. 2.1, Tab. S2.1). The overall mixed occurrence of individuals from these subgroups on the minimum spanning tree mostly reflects the high levels of gene flow in the West Pacific, especially within and between the Central-West and the Eastern-West Pacific (Fig. 2.4b), which are also the only two subgroups with a clear signa-

Table 2.2. Summary statistics for the Pacific as a whole, as well as for the regional groups and subgroups identified by the AMOVA analyses: number of populations n_{pop}, number of samples n, number of unique haplotypes n_h, haplotype diversity h, nucleotide diversity π, Fu's F_S, Ramos-Onsins' R_2 and Tajima's D (significant values bold).

Group/Subgroup	n_{pop}	n	n_h	h (± stdev)	π (± stdev)	F_s	R_2	D
All populations	45	673	443	0.997 (±0.0004)	0.026 (±0.0127)	**-23.52**	**0.039**	**-1.68**
West Pacific	35	584	382	0.997 (±0.0004)	0.020 (±0.0110)	**-23.66**	**0.018**	**-1.79**
Western Australia	3	39	29	0.965 (±0.0203)	0.018 (±0.0094)	**-12.70**	0.075	-0.96
Malaysia	1	13	7	0.846 (±0.0758)	0.013 (±0.0075)	1.09	0.142	0.11
Cenderawasih Bay	4	55	31	0.956 (±0.0153)	0.023 (±0.0116)	-6.49	0.073	-0.87
Central-West Pacific	19	340	234	0.996 (±0.0009)	0.022 (±0.0110)	**-23.86**	**0.024**	**-1.68**
Eastern-West Pacific	8	137	102	0.995 (±0.0019)	0.018 (±0.0090)	**-24.46**	**0.033**	**-1.92**
Central Pacific	7	66	51	0.987 (±0.0067)	0.044 (±0.0216)	**-16.30**	0.081	-0.24
Johnston	1	9	8	0.972 (±0.0640)	0.011 (±0.0065)	-2.46	0.110	-0.76
East Pacific	2	14	6	0.736 (±0.1092)	0.003 (±0.0020)	-2.01	0.126	-1.24

ture of population expansion (Tab. 2.2). It is however notable that many haplotypes from Cenderawasih Bay and Western Australia appeared to be differentiated, each belonging to an individual MST-subgroup (respectively MST-subgroups 1 and 2; Fig. 2.4b). These MST-subgroups were also identified in the split graph, but in a different branching order (Fig. 2.1b).

Cenderawasih Bay showed a mixed pattern of being relatively isolated from the rest of the West Pacific, yet of ongoing gene flow as well (haplotypes occurring elsewhere on the minimum spanning tree; Fig. 2.4b). The signal of isolation was not only supported by the haplotypes in MST-subgroup 1 (Fig. 2.4b), but also by the lack of signature of a population expansion (unlike in the surrounding Central-West and Eastern-West Pacific groups), and comparatively low haplotype diversity in this subgroup (h=0.956) (Tab. 2.2). On the other hand, the genetic differentiation between Cenderawasih Bay and the Central-West and Eastern-West Pacific subgroups was low (Tab. 2.5b). Moreover, Cenderawasih Bay and the Central-West Pacific shared haplotypes, both within MST-subgroup 1 (Fig. 2.4b) and throughout the rest of the minimum spanning tree, which led to the high nu-

Table 2.3. Genetic distance between (a) groups in the Pacific and (b) subgroups in the West Pacific: corrected average number of pairwise differences (above diagonal); pairwise Φ_{ST} values (below diagonal); and average number of pairwise differences within populations (diagonal). All values were significant after standard Bonferroni correction (10'000 random replicates). Group codes are WP: West Pacific; CP: Central Pacific; EP: East Pacific; WA: Western Australia; CWP: Central-West Pacific; CeBa: Cenderawasih Bay; EWP: Eastern-West Pacific.

(a) Pacific

	WP	CP	Johnston	EP
WP	11.59	3.03	19.50	3.48
CP	0.20	22.31	16.80	6.04
Johnston	0.62	0.44	5.50	24.84
EP	0.22	0.22	0.89	1.37

(b) West Pacific

	WA	Malaysia	CWP	CeBa	EWP
WA	8.93	2.96	2.11	2.02	4.04
Malaysia	0.26	6.77	1.10	2.57	2.61
CWP	0.16	0.08	11.45	1.21	0.95
CeBa	0.16	0.19	0.10	11.52	2.94
EWP	0.31	0.22	0.08	0.23	8.95

cleotide diversity (π=0.023) and supported the second observation of ongoing gene flow.

A similar pattern was found in relation to the Western Australian group. Isolation from the rest of the Western Pacific was supported by the existence of MST-subgroup 2, composed exclusively of individuals from Western Australia (Fig. 2.4b), the comparatively low haplotype and nucleotide diversities (h=0.965, π=0.018), the lack of recent population expansion (Tab. 2.2) and the comparatively high genetic differentiation between Western Australia and most other subgroups (Tab. 2.3b). However, the lower genetic differentiation between Western Australia and the Central West Pacific (Tab. 2.3b) and the presence of a few haplotypes derived from Central-West Pacific haplotypes (yellow haplotypes in Fig. 2.5b outside of MST-subgroup 2) support occasional gene flow between these areas.

2.4. Discussion

In accordance with previous studies on Pacific COTS (Benzie and Stoddart 1992a;

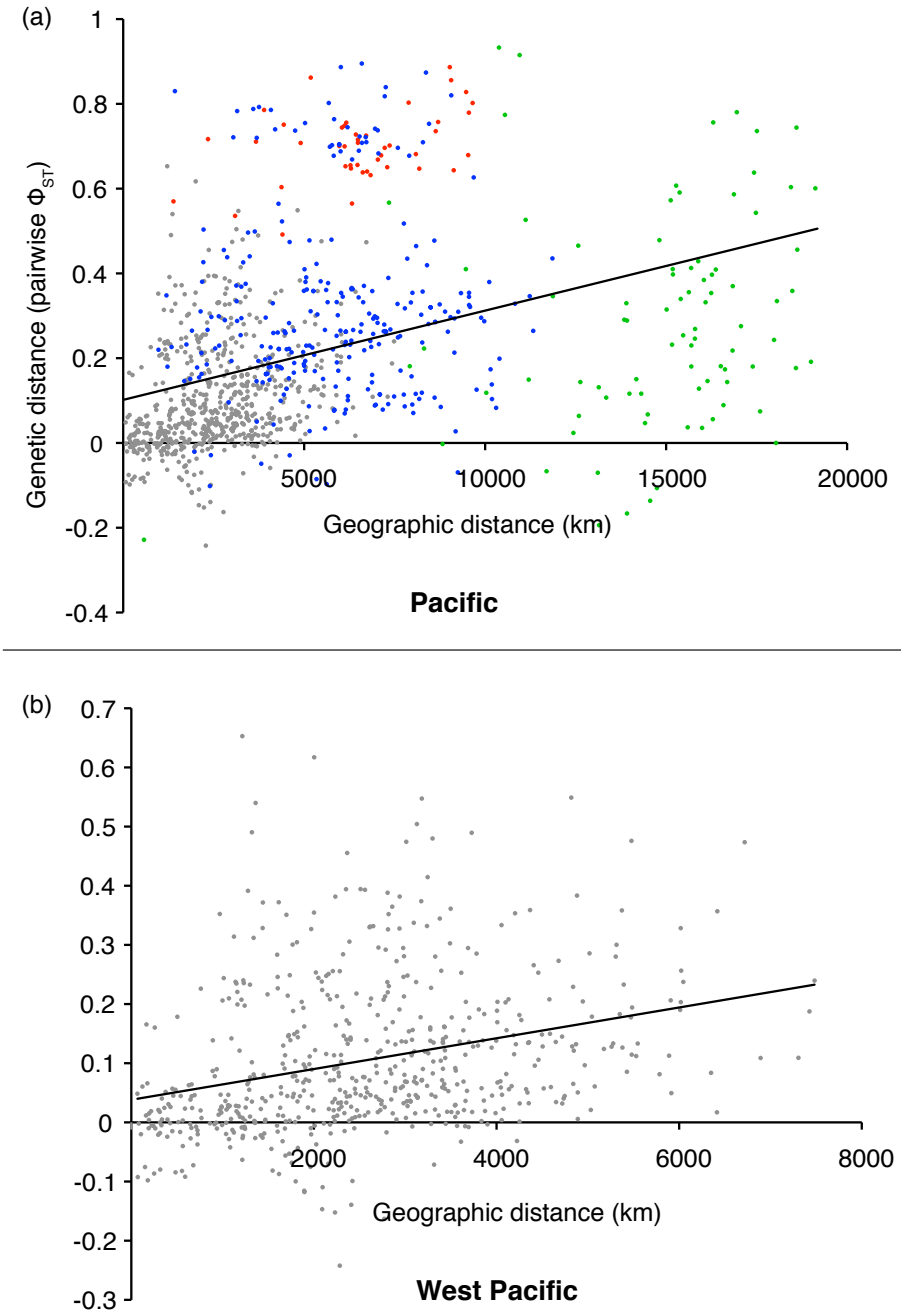


Figure 2.3. Genetic distance (Φ_{ST}) as a function of geographic distance for (a) the Pacific (b=2.1x10^{-5}, R^2=0.145, p<0.01) and (b) the West Pacific group (b=2.6x10^{-5}, R^2=0.087, p<0.01). Colours in (a) represent comparisons involving Johnston Atoll (red), the East Pacific (green), the Central Pacific (blue), and all other comparisons (grey).

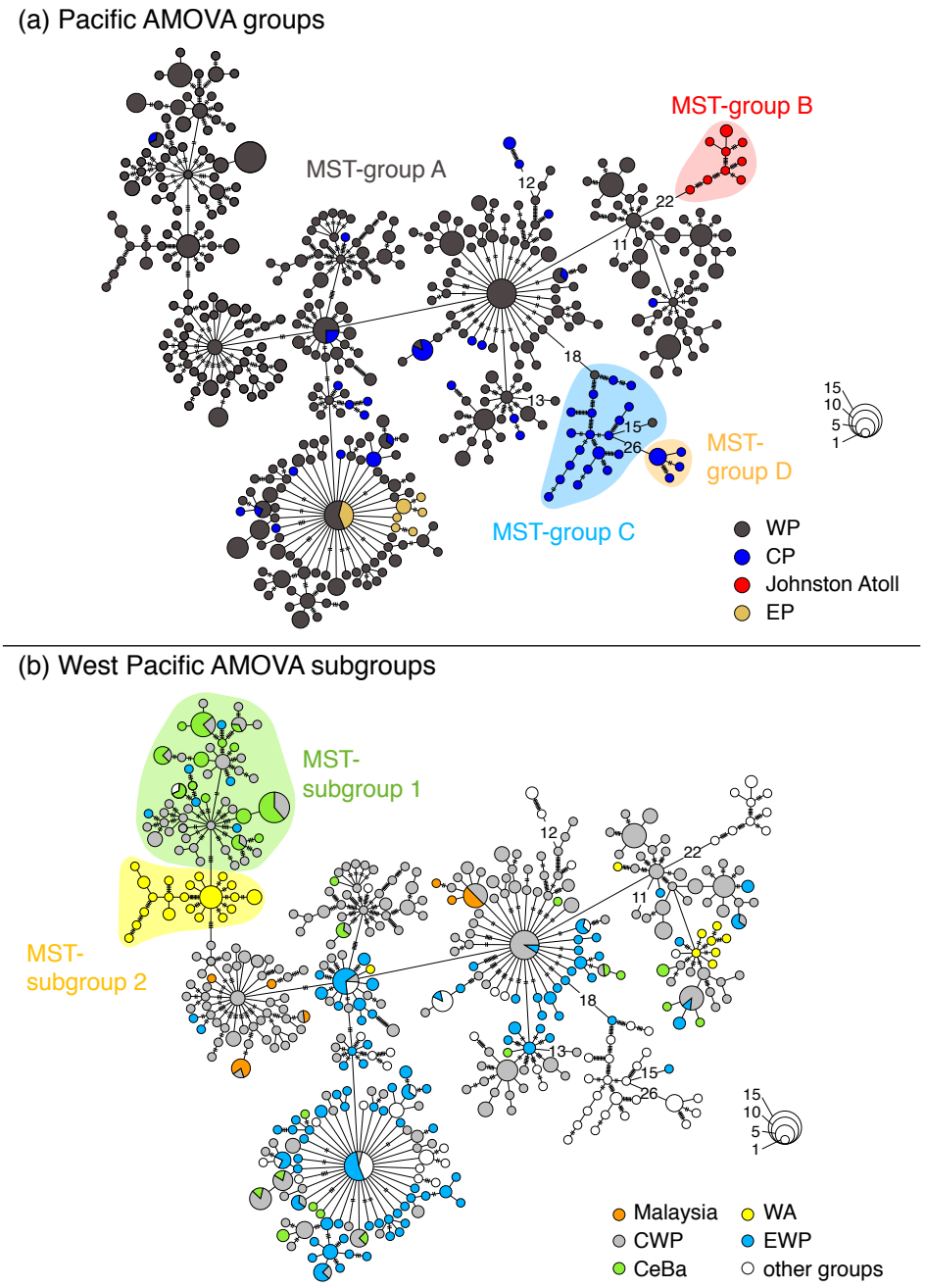


Figure 2.4. Group detected by the analyses of molecular variance plotted onto the minimum spanning tree (Fig. 2.1a) (a) regional groups from the Pacific and (b) subgroups from the West Pacific. Colours and legends are the same as in Figure 2.2 and 2.5 respectively.

Benzie 1999; Nash et al. 1988; Nishida and Lucas 1988; Yasuda et al. 2009), we detected high levels of gene flow in this species across the Pacific. However, we also detected the signature of a complex history of range contraction and extension at both oceanic and regional scales, which enabled assigning the high levels of gene flow to high dispersal rather than a limitation of the genetic markers employed.

2.4.1. Oceanic scale: long-distance dispersal and isolation

At the scale of the Pacific Ocean, we identified both isolated populations and recurrent long-distance dispersal. The population from Johnston Atoll was highly differentiated from the rest of the Pacific, probably as a result of a past founder effect or bottleneck with subsequent genetic drift (Benzie 1992). A similar pattern was identified for COTS from the neighbouring islands of Hawaii with allozymes (Nishida and Lucas 1988). Both areas are known to be quite isolated from the rest of the Pacific for a range of other organisms (Kay and Palumbi 1987; Jokiel 1987), due to both the large distance from other island archipelagos and the lack of favourable currents to transport propagules from possible source populations (Maragos and Jokiel 1986). To a lesser extent, the two areas are also isolated from each other (Maragos and Jokiel 1986), although for organisms with pelagic larval durations of more than 40 days, Johnston Atoll could act as a stepping-stone for colonisation of the Hawaiian Archipelago (Koyabashi 2006). This could apply to the crown-of-thorns starfish, as its larvae can survive from three to four weeks in normal conditions (Yamaguchi 1973) to about seven weeks in marginal food regimes (Lucas 1982), as found throughout most of the Pacific Ocean. The intermediate position of the Johnston Atoll MST-group (MST-group B) between the predominantly West Pacific MST-group (MST-group A) and the Central Pacific MST-groups (C and D) in the split graph (Fig. 2.1b) does unfortunately not allow determining the most likely source of initial propagules that colonised the area.

The East Pacific group stood out, as it also appeared to have gone through a recent bottleneck. Unlike Johnston Atoll however, its haplotypes had not substantially diverged from the rest of the Pacific (Fig. 2.4a). Based on allozymes, Nishida and Lucas (1988) suggested that the genetic homogeneity of COTS either side of the Eastern Pacific Barrier (the 5000+ km expanse of deep water without stepping-stones separating the central from the eastern Pacific) was due to recent

or ongoing gene flow from west to east, through long-distance dispersal of larvae over the North Equatorial Counter Current. Accordingly, our findings support the hypothesis that the eastern Pacific was recently colonised from the west, although it is unclear what islands could have acted as stepping-stones. Indeed, despite the high haplotype diversity of the Pacific as a whole, the East Pacific group shared one of the most common haplotypes with individuals from the western and not the central Pacific (Fig. 2.4a). This could however be a sampling artefact due to the relatively small sample size (14 individuals), but either way supports that Pacific COTS larvae are capable of long-distance dispersal across the "impassable" Eastern Pacific Barrier, in line with a few other marine invertebrates, such as the sea urchin *Tripneustes gratilla* (Lessios et al. 2003), the gastropod *Conus ebraeus* (Duda and Lessios 2009) and the shrimp *Alpheus lottini* (Williams et al. 2002), as well as numerous fish species (e.g. Lessios and Robertson 2006).

Evidence for both long-distance dispersal and isolation was found in the central Pacific group as well. The few central Pacific populations that were included in previous COTS studies appeared to be quite differentiated from the western Pacific (with allozymes: Lord Howe Island (Benzie and Stoddart 1992a; Benzie 1999) and Vanuatu (Benzie 1999), and with microsatellites: Fiji and Moorea (Yasuda et al. 2009)). By increasing the number of populations sampled from this area, we found here that central Pacific populations were probably isolated from the rest of the Pacific in the past, as individuals from two highly differentiated MST-groups (C and D; Fig. 2.1) were identified in these populations. They may have diversified in different areas of the central Pacific during periods of lower connectivity between populations. Today however, the central Pacific appears to receive occasional influx of larvae from the West Pacific, and similarly, rare dispersal events in the opposite direction also seem to occur. A comparable pattern was identified for other organisms, such as the damselfish species complex *Dascyllus trimaculatus* (Leray et al. 2010) and the gastropod *Nerita plicata* (Crandall et al. 2008b), where several differentiated lineages were present in central Pacific populations. Although in both cases the presence of these different lineages could best be explained by the emergence of temporary barriers to dispersal leading to allopatric divergence, the actual barrier(s) could not be identified. Considering the relatively poor connectivity between central and western Pacific

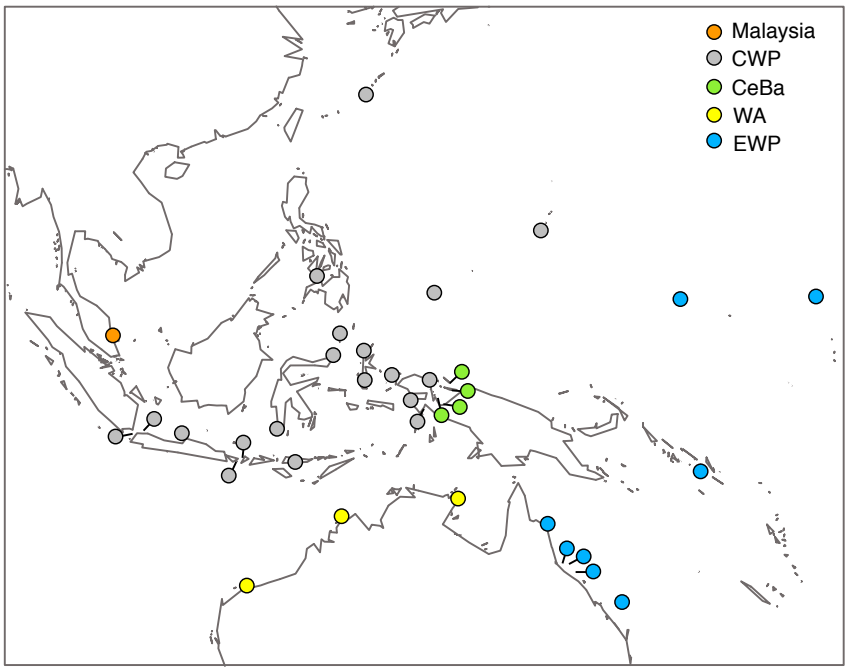


Figure 2.5. Distribution of genetic structure of the Pacific crown-of-thorns starfish in the West Pacific: sampling locations coloured according to subgroups detected by the analyses of molecular variance (CWP: Central-West Pacific, CeBa: Cenderawasih Bay, WA: Western Australia, EWP: Eastern-West Pacific).

reefs based on larval dispersal models however (Treml et al. 2008), larval exchange between these areas probably only occurs sporadically under present oceanic conditions, for example during El Niño years when circulation patterns are altered (Treml et al. 2008). It is therefore conceivable that even slight changes in circulation patterns in this area could have had a strong effect on the connectivity between the western and central Pacific and within the central Pacific, temporarily interrupting gene flow between these areas.

2.4.2. West Pacific: high connectivity and temporary isolation

The high levels of gene flow found in previous studies with allozymes and microsatellites in the western Pacific were also observed using the mitochondrial control region. Both the Central-West Pacific and Eastern-West Pacific groups spanned very large areas and were genetically quite homogeneous. But additionally, by identifying a signature of past isolation and subsequent recolonisation

events between some of the regional subgroups identified in this study, we were able to attribute these high levels of gene flow to a high dispersal potential.
For example, the Cenderawasih Bay group carried both the signature of a past bottleneck and of recent bidirectional exchange with the rest of the Central-West Pacific group. The signature of a bottleneck was also observed in this area for the giant clam *Tridacna crocea* (DeBoer et al. 2008), the starfish *Linckia laevigata* and *Protoreaster nodosus*, and the snail *Thyca crystallina* (Crandall et al. 2008a). DeBoer et al. (2008) suggested that this could either be due to the physical setting of the bay, its deep embayment cutting it off from the South Equatorial Current, or to the increased isolation of the bay during sea-level low stands. As we could identify resumed gene flow with the rest of the Central-West Pacific group, the latter hypothesis appears to apply for Pacific COTS.
A similar pattern could be observed in relation to the Western Australian group. The comparatively lower genetic differentiation between Western Australia and the Central-West Pacific suggests that following isolation from surrounding populations during past sea-level lowstands, subsequent influx of larvae probably occurred from Indonesian rather than from eastern Australian populations. Considering New Guinea and Australia would have been connected by a land bridge even when sea levels were only 10m below present levels (Voris 2000), the Torres Strait would have been closed for much of the Pleistocene, thus interrupting gene flow between eastern and western Australian populations until around 8000 years ago (Galloway and Kemp 1981). A similar genetic break has been found in many other marine organisms, e.g. fish (Gopurenko and Hughes 2002), mud crabs (Chenoweth et al. 1998), prawns (Benzie et al. 2002) and starfish (Williams and Benzie 1998). However, although western Australian populations of the starfish *Linckia laevigata* also appeared to be more closely related to southeast Asian rather than eastern Australian populations (Williams and Benzie 1998), the opposite was true for the giant tiger prawn *Peneaus monodon* (Benzie et al. 2002), indicating that avenues of gene flow have a strong species-specific component in this area.
Exposed shelf areas (Voris 2000) and restricted current patterns during sea level lowstands are thus likely to have led to range contractions and interrupted gene flow with peripheral populations, as well as with central populations isolated due to their complex topographic setting (McManus 1985) (e.g. Cenderawasih

Bay). These small isolated areas probably underwent population bottlenecks, reducing their genetic diversity and altering them through genetic drift from the larger populations that remained in more connected areas. Subsequent increases in sea levels would have offered renewed opportunities for dispersal between the previously isolated populations, especially considering the high density of suitable habitat for COTS in most of the West Pacific and the strong boundary currents that allow maintaining high levels of gene flow between relatively distant populations (e.g. East Australian Current, Kuroshio Current; Yasuda et al. 2009). Although the genetic structure identified in the West Pacific group reflected most of the patterns previously identified with microsatellites (high gene flow, isolation by distance; Yasuda et al. 2009), there were also a few differences. Yasuda et al. (2009) found a differentiation between East Asia (Japan and Philippines), the North Pacific Islands (Palau, Majuro and Pohnpei) and the Great Barrier Reef+Fiji. In the present study, Palau grouped together with Japan and the Philippines as well as most populations from Indo-Australian Archipelago, whereas Majuro and Pohnpei grouped with the Great Barrier Reef, and Fiji with populations from the Central Pacific. These discrepancies could be indicative of the different timescales at which these markers operate, the microsatellites reflecting more recent larval dispersal patterns, whereas the patterns uncovered with mitochondrial DNA were more representative of historical gene flow (Hellberg 2009).

2.5. Conclusion

By investigating the genetic structure of Pacific COTS with a highly variable mitochondrial marker, we were able to confidently establish that the high levels of gene flow previously identified for this organism in the Pacific Ocean were truly due to high dispersal and not to the lack of resolution of the genetic markers employed. Moreover, by extending the sampling range of previous studies to the most complete dataset used until now in a COTS genetic study, we were also able to identify the signature of fluctuating isolation and expansion between population sets. Some features, such as temporarily emerged land due to sea-level changes and unfavourable currents that contributed to isolating populations in the past, were identified as barriers to dispersal in this organism. On the other hand, large distances did not appear to be an impassable barrier to dispersal for Pacific COTS larvae, as large oceanic expanses without stepping-stones are

crossed at least occasionally. This was most likely due to COTS' relatively long pelagic larval duration in the marginal food conditions found in most of the Pacific, and the potential occurrence of a facultative teleplanic larva (Birkeland and Lucas 1990).

Further research will be required to find out whether the genetic structure of the other sister-species of the crown-of-thorns starfish shows similar patterns and are driven by the same mechanisms.

3. Current affairs in cryptic speciation: the crown-of-thorns starfish in the Indian Ocean

This chapter is currently in preparation for standalone publication:

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3. Current affairs in cryptic speciation: the crown-of-thorns starfish in the Indian Ocean

Abstract

Understanding both the processes that lead to and the consequences of cryptic speciation in the marine realm is particularly relevant in organisms that require management. In this first Indian Ocean-wide genetic study of a marine organism, we explored the structure of two sister-species of the crown-of-thorns starfish *Acanthaster planci*, a coral predator infamous for its outbreaks that have devastated reefs through much of its distribution. The first of two main diversification events in the Indian Ocean led to the formation of a southern and northern Indian Ocean sister-species (SIO and NIO) in the late Pliocene-early Pleistocene. The second led to the formation of two internal clades within each species around the onset of the last interglacial. The subsequent demographic history of the two lineages strongly differed, the SIO sister-species showing a signature of recent population expansion and hardly any regional structure, whereas the NIO sister-species maintained a constant size with highly differentiated regional groupings that were asymmetrically connected. Past and present surface circulation patterns in conjunction with ocean primary productivity were identified as key processes in shaping the genetic structure between and within both lineages.

3.1. Introduction

A growing body of research shows that cryptic speciation is ubiquitous in the marine realm (reviewed in Knowlton 2000). Indeed, the discovery through molecular methods of sister-species who are superficially morphologically indistinguishable and are thus classified as a single nominal species (Bickford et al. 2007) is increasingly common. This is even the case in widespread organisms with long-lived pelagic larvae that could be expected to display little genetic structure (Knowlton 2000). Exploring the mechanisms that drive the speciation process can

shed light on the importance of past and present barriers to gene flow in marine systems (Hellberg 2007). Moreover, investigating the extent to which sister-species have diversified can provide information on the likelihood of their biology and ecology having changed too.

Identifying cryptic speciation and understanding the extent of the differences between sister-species is especially important in organisms where the presence of cryptic species could have far-reaching impacts, for example in biological model organisms, in commercially valuable species, in biological indicator species or in organisms that require management, such as threatened species and pests (Knowlton 1993; Bickford et al. 2007).

The corallivorous crown-of-thorns starfish (COTS) *Acanthaster planci* is of particular interest in this regard as it undergoes population outbreaks that have devastated coral reefs through much of its distribution since the 1960s (Birkeland and Lucas 1990). Although outbreaks still amount for a large proportion of the disturbance to Indo-Pacific reefs today (Fabricius et al. 2010), their causes remain debated, as well as appropriate monitoring strategies to predict their occurrence and management plans to reduce their impact (Birkeland and Lucas 1990; Houk et al. 2007; Sweatman 2008; Fabricius et al. 2010). The crown-of-thorns starfish has been shown to be constituted of four highly differentiated lineages with restricted ranges that together form a species complex, located in the Pacific, the Red Sea, the northern and the southern Indian Ocean (Vogler et al. 2008).

Since the overwhelming majority of COTS research has been performed on the Pacific species, Vogler et al. (2008) suggested that failure to recognise the existence of this species complex could have contributed to a lack of understanding of the processes that lead to outbreaks in the different COTS lineages, by extrapolating results obtained from the Pacific studies to COTS' entire distribution for both research and management purposes. Indeed, although outbreaks are also a reason for concern in the Indian Ocean (Celliers and Schleyer 2006; Mendonça et al. 2010) and the Red Sea (Wilkinson 2008), they do not appear to be as massive or widespread as in the Pacific (Zann 2000), which might be indicative of differences between the sister-species.

To understand if and how these sister-species differ, we here focus on COTS' population genetic structure in the Indian Ocean. Although some data is available from other studies (Benzie 1999; Gérard et al. 2008; Yasuda et al. 2009), it has

never been analysed separately with a basin-wide coverage. The aim of this study was thus to address the following questions: (1) What processes led to the diversification of the different species in the Indian Ocean? (2) Is the genetic structure of the two sister-species different? (3) If so, how can these differences be explained?

3.2. Materials and Methods

3.2.1. Sampling and sequencing

COTS samples were collected by SCUBA and snorkel from 18 sites in the Indian Ocean between 1990 and 2010 (Fig. 3.1a, Tab. S3.1). We purposefully did not include any samples from the south-eastern Indian Ocean (Western Australia), as they have been shown to belong the Pacific sister-species (Vogler et al. 2008). The tissue sampled included pyloric caeca (Benzie 1999), gonads (Gérard et al. 2008) and tube feet, and was stored as soon as possible after collection, either at -80°C for the pyloric caeca (Benzie 1999), or in ethanol (>80%), DMSO buffer (Seutin et al. 1991) and on FTA paper (Whatman) for the gonads and tube feet. The DNA was extracted from the pyloric caeca using a MagAttract 96 DNA Plant Core Kit (Qiagen) according to the manufacturer's manual DNA purification protocol, with the following initial steps: the tissue was manually ground in a 1.5ml Eppendorf tube after freezing in liquid nitrogen, then incubated at 35°C for an hour in RLT lysis buffer (Qiagen), vortexed at full speed for 20s, and centrifuged at 8000 x g for 5min. DNA was extracted from the other tissues (gonads, and tube feet) using a DNeasy Tissue Kit (Qiagen) according to the manufacturer's protocol.

A DNA fragment containing the putative mitochondrial control region (CoReg) and the 5' end of the adjacent 16S rRNA gene (Yasuda et al. 2006) was amplified with the following primers: COTS-CoReg-F15635 5'-CAAAAGCTGACGGGTAAGCAA-3' and COTS-CoReg-R114 5'-TAAGGAAGTTTGCGACCTCGAT-3'. DNA sequencing was performed using the PCR reverse primer, and the following internal forward primer: COTS-CoReg-seqIO-F15749 5'-GCTTGTGTTCACGGGAAAGC-3'. Cytochrome Oxidase subunit I (COI) sequences from Vogler et al. (2008) with additional samples from the Chagos Archipelago (Tab. S3.1) were also used. The sequences were assembled using CODONCODE ALIGNER (http://www.codoncode.com/aligner) and

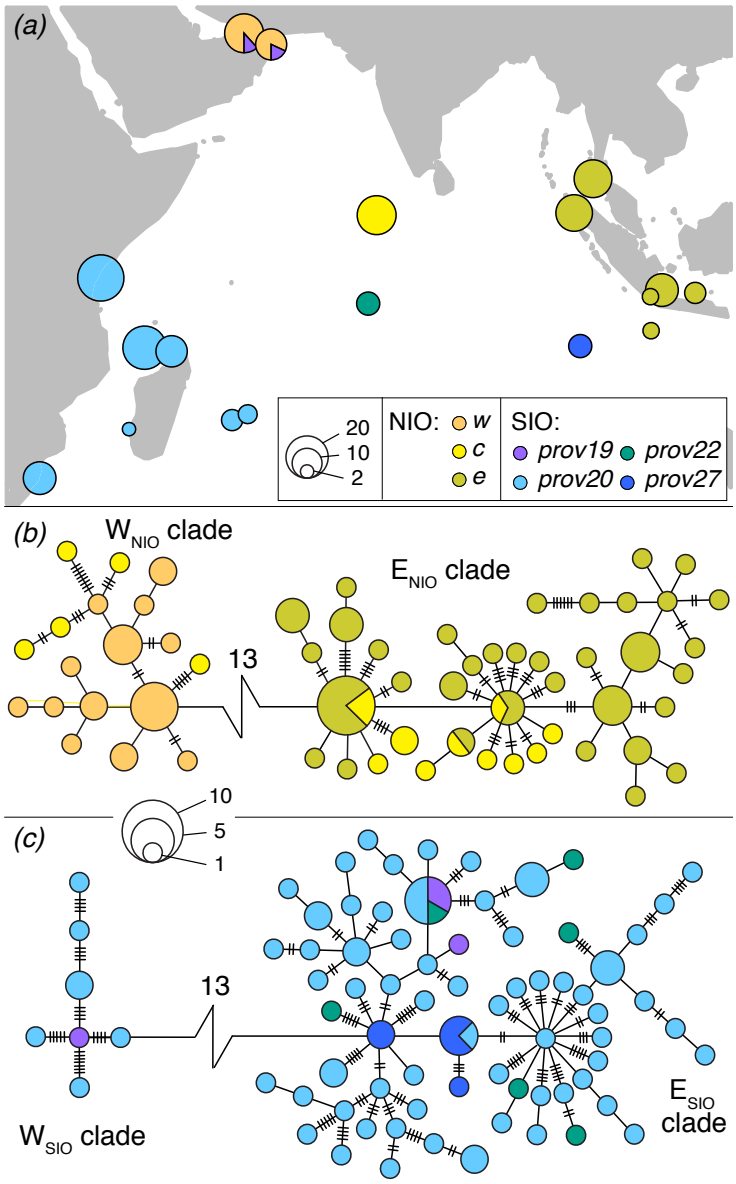

Figure 3.1. Phylogeography of the crown-of-thorns starfish in the Indian Ocean: *(a)* sampling locations from the northern and southern Indian Ocean sister-species (here denoted as NIO and SIO respectively), circles are proportional to sample size, colours indicate the regional grouping of populations that explained the most variance amongst groups following the AMOVA analyses (Tab. 3.2) and that were used for the MIGRATE analyses (see Results, Fig.3.2 and Tab. 3.4). *(b)* and *(c)* Minimum spanning trees of respectively the NIO and SIO sister-species, all haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks, except the clades W_{NIO} and E_{NIO} as well a W_{SIO} and E_{SIO} which are separated by 13 mutational steps. Colours are the same as in *(a)* and circle size is proportional to frequency of occurrence.

aligned in SEAVIEW v4.2 (Galtier et al. 1996) using the built-in MUSCLE software (Edgar 2004).

3.2.2. Time of divergence and demographic patterns

As the CoReg sequences could not be aligned unambiguously between the southern Indian Ocean (SIO) and the northern Indian Ocean (NIO) sister-species (Vogler et al. 2008), the timing of their divergence was estimated using the COI dataset (Tab. 3.1, Tab. S3.1). The net divergence d_A (Nei and Li 1979) between the two species was calculated using Kimura 2-parameter (K2P) distances estimated in PAUP*4.0b10 (Swofford 2003), approximating divergence times by applying the most accurate COI divergence rates available for echinoderms to d_A (3.7±0.8%.Myr^{-1}; Lessios 2008).

Intraspecific patterns of diversification were investigated by estimating minimum spanning trees in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010) for the CoReg sequences of both SIO and NIO, based on pairwise differences and redrawn with ADOBE ILLUSTRATOR. To assess the robustness of the signal in the minimum spanning trees, we also constructed split graphs in SPLITSTREE v4.11.3 (Huson and Bryant 2006) using the NeighborNet method, which allow detecting incongruences in the signal and alternative phylogenetic histories.

The minimum spanning trees revealed a deep internal split, separating two clades in each species (Fig. 3.1). We estimated the net divergence d_A (Nei and Li 1979) between these clades using the CoReg dataset (Tab. S3.1), as the COI sequences did not offer the necessary resolution. After inferring the best-fit nucleotide evolution model using the Akaike Information Criterion as implemented in JMODELTEST v0.1.1 (Posada 2008; TPM1uf+I+G for the NIO sister-species, TrN+I+G for the SIO sister-species), d_A was estimated for the maximum likelihood distances calculated in PAUP*4.0b10 (Swofford 2003).

Since there are no mutation rates available for echinoderm CoReg sequences, we also used a concatenated COI-CoReg dataset to calculate the time to the most recent common ancestor T_{MRCA} of the NIO and SIO sister-species, by estimating Bayesian skyline plots in BEAST v1.5.4 (Drummond et al. 2005; Drummond and Rambaut 2007). We set a strict clock on COI since preliminary tests showed a clocklike behaviour of the data could not be rejected (zero value of uncorrelated relaxed lognormal clock standard deviation within 95% highest posterior density

interval). We used a substitution rate of 1.85±0.4%.Myr^{-1} (normal distribution) in order to incorporate the uncertainty on this rate from the literature (Lessios 2008), and estimated the CoReg uncorrelated relaxed lognormal clock from COI (see Tab. S3.2 for settings).

These Bayesian skyline analyses allowed us to explore the demographic patterns within each of the sister-species, comparing these to statistics that have the ability to detect signatures of recent population expansions: Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989), both calculated using ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010; 50'000 replicates), as well as Ramos-Onzins R_2 (Ramos-Onsins and Rozas 2002), estimated using the R package PEGAS v0.3-1 (Paradis 2010; 10'000 replicates). All these demographic summary statistics were estimated at the species level with the COI dataset, and at the species, clade and population level with CoReg.

3.2.3. Spatial genetic structure and migration patterns

All population level statistics were performed on the CoReg dataset using ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010), unless stated otherwise. We calculated standard measures of genetic diversity (haplotype frequencies, haplotype diversity h and nucleotide diversity π) for each population and sister-species (CoReg and COI), as well as pairwise Φ_{ST}s between population pairs within each sister-species (50'000 random replicates, standard Bonferroni correction for multiple

Table 3.1. Summary statistics per sister-species and dataset: aligned sequence length, number of individuals, haplotype diversity h, nucleotide diversity π, Fu's F_S, Tajima's D and Ramos-Onsins R_2 (significant values are bold).

Dataset	Sequence length (bp)	n	h	π	F_S	D	R_2
Northern Indian Ocean sister-species							
COI	632	48	0.68 (±0.045)	0.004 (±0.0059)	-1.32	-0.13	0.101
CoReg	522	95	0.98 (±0.006)	0.020 (±0.0102)	**-24.44**	-0.28	0.079
Southern Indian Ocean sister-species							
COI	632	57	0.59 (±0.074)	0.002 (±0.0013)	**-12.67**	**-2.08**	**0.036**
CoReg	546	95	0.99 (±0.003)	0.016 (±0.0082)	**-24.65**	**-1.57**	**0.050**

tests). We also used a Mantel test (100'000 permutations) to determine the relationship between genetic and geographic distances within each sister-species following the method recommended by Rousset (1997) for populations in a two-dimensional model, i.e. testing the regression of population pairwise $\Phi_{ST}/(1-\Phi_{ST})$ against the natural logarithm of geographic distances (Rousset 1997). We then used analyses of molecular variance (AMOVA) to identify regional patterns of genetic differentiation (locus-by-locus AMOVA, 50'000 replicates). We tested several different combinations of groups of populations based on geography and published regional provinces (Marine Ecoregions of the World; Spalding et al. 2007), to determine which combination explained the most genetic variation among groups.

In order to understand the connectivity between the regional groups identified by the AMOVA analyses (Tab. 3.2), we estimated migration rates and effective population sizes with MIGRATE v3.1.6 (Beerli and Felsenstein 2001), using a Bayesian search strategy as recommended by Beerli (2006). We established the most likely mutation model within the constraints of MIGRATE by using PAUP*4.0b10 (Swofford 2003) to estimate parameters for site rate variation and the transition/transversion ratio, and performed several exploratory runs to de-

Table 3.2. AMOVA results for the southern and northern Indian Ocean sister-species (significance tested with 50'000 permutations; * p<0.05, ** p<0.01 and *** p<0.001).

	Northern Indian Ocean sister-species	**Southern Indian Ocean sister-species**
	w vs. *c* vs. *e*[1]	*prov19* vs. *prov20* vs. *prov22* vs. *prov27*[2]
Overall Φ_{CT} (between groups)	0.574***	0.056*
Overall Φ_{SC} (within groups)	0.066***	0.025**
Percent variation:		
Among groups	57.37%	5.64%
Among populations within groups	2.82%	2.36%
Within populations	39.81%	92.00%

[1]*w*: UAE, Oman; *c*: Maldives; *e*: Thailand, Aceh, Christmas Island, Pulau Seribu, Krakatau, Karimunjawa

[2]*prov19*: UAE, Oman; *prov20*: Kenya, South Africa, Mayotte, South Madagascar, North Madagascar, Réunion, Mauritius; *prov22*: Chagos; *prov27*: Cocos Keeling Islands

termine appropriate priors (Tab. S3.3). To explicitly evaluate the performance of different migration models, ranging from panmixia to a full migration matrix (Fig. 3.2), we ran the analyses with the following heating scheme: [1 1.5 3 10'000] (1'000'000 generations, 32 replicates), allowing the approximation of marginal likelihoods using thermodynamic integration and hence the estimation of Bayes Factors that allow comparing the performance of different models (Beerli and Palczewski 2010).

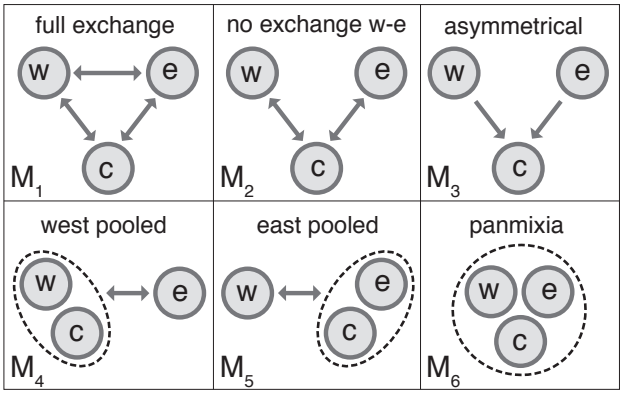

Figure 3.2. Migration models compared in the MIGRATE analysis of the northern Indian Ocean sister-species, ranging from M_1: full exchange to M_6: panmixia. *w*, *e* and *c* represent the regional groupings displayed in Fig. 3.1; arrows indicate direction of migration.

3.3. Results

3.3.1. Sampling and sequencing

Of the 190 samples for which we obtained CoReg sequences, 95 belonged to the northern Indian Ocean (NIO) sister-species (522bp) and 95 to the Southern Indian Ocean (SIO) sister-species (546bp; Tab. 3.1, Tab. S3.1). The corresponding COI dataset (632bp) included 48 individuals in the NIO sister-species, and 57 in the SIO sister-species (Tab. 3.1, Tab. S3.1). Haplotype and nucleotide diversity were high for the CoReg datasets (respectively 0.98 and 0.020 in the NIO sister-species, 0.99 and 0.016 in the SIO sister-species), and lower for the COI dataset (0.68 and 0.004 for the NIO sister-species, 0.59 and 0.002 for the SIO sister-species; Tab. 3.1; see Tab. 3.3 for population level statistics). The COI dataset was thus more appropriate for interspecific analyses, and the CoReg datasets for intraspecific analyses.

3.3.2. Time of divergence and demographic patterns

The time of divergence between the two Indian Ocean sister-species, based on the net divergence d_A of the K2P distances from the COI dataset, was estimated to be 1.86-2.89 Mya, in the late Pliocene-early Pleistocene (divergence rate: 3.7±0.8%.Myr^{-1}; Lessios 2008).

The minimum spanning trees for each of these sister-species showed two clades separated by a large internal split of 13 mutation steps. In the NIO sister-species, one clade consisted of haplotypes found only in the west and central northern Indian Ocean sites (here called W_{NIO}), and the other consisted of haplotypes found only in the eastern and central northern Indian Ocean (E_{NIO}; Fig. 3.1). In the SIO sister-species, one clade consisted of haplotypes found only in western Indian Ocean sites (W_{SIO}), the second consisted of haplotypes spread throughout the southern Indian Ocean but apparently derived from ancestors found in Cocos Keeling Islands, thus of eastern-origin (E_{SIO}; Fig. 3.1). These clades and the central position of the Cocos Keeling haplotypes were also recovered in the Neighbor-Nets (Fig. S3.1), supporting the robustness of this signal. The net divergence d_A between these clades was similar: 3.98% for W_{NIO} vs. E_{NIO}, and 3.50% for W_{SIO} vs. E_{SIO}, as were the T_{MRCA}s for each lineage: 139'600 years ago for the NIO sister-species, 113'700 for the SIO sister-species.

The Bayesian skyline plots showed signs of recent expansions in both sister-species around 15'000 years ago, potentially indicating an expansion after the last glacial maximum (18'000-24'000 years ago), but in both cases with very large variance around the parameter estimates, which limits the interpretability of the data (Fig. S3.2). However, all other demographic statistics showed no signs of a recent population expansion for the NIO sister-species (F_s, D, and R_2 not significant except F_s estimated with the CoReg dataset) whereas the SIO sister-species clearly did (F_s, D, and R_2 significant for both COI and CoReg) (Tab. 3.1).

3.3.3. Spatial genetic structure and migration patterns

The overall Φ_{ST} of the NIO sister-species without *a priori* structure was strong (Φ_{ST}=0.51, p<0.001), whereas in the SIO sister-species it was weak (Φ_{ST}=0.07, p<0.001). Indeed, 14 of the 36 pairwise Φ_{ST} comparisons in the NIO sister-species

were significant after Bonferroni correction, whereas none of the 55 the SIO sister-species comparisons were (Tab. S3.4). There was significant isolation by distance in the NIO sister-species, as revealed by the positive regression between Φ_{ST} /(1-Φ_{ST}) and the logarithm of geographic distances (b=1.28, R^2=0.35, p<0.001; Fig. S3.3a), and no relationship in the SIO sister-species (b=0.11, R^2=0.06, p>0.05; Fig. S3.3b).

Table 3.3. Summary statistics per location based on the CoReg dataset: number of individuals *n*, haplotype diversity *h*, nucleotide diversity π, Fu's F_S, Tajima's *D* and Ramos-Onsins R_2.

Location	*n*	*h*	π	F_S	*D*	R_2
Southern Indian Ocean sister-species						
UAE	2	1.00 (±0.500)	0.004 (±0.0045)	0.69	-	0.500
Oman	2	1.00 (±0.500)	0.032 (±0.0325)	2.83	-	0.500
Reunion	5	1.00 (±0.127)	0.011 (±0.0075)	-1.06	-0.28	0.138
Mauritius	4	1.00 (±0.177)	0.010 (±0.0074)	-0.40	-0.07	0.137
Kenya	24	0.99 (±0.014)	0.017 (±0.0090)	**-11.82**	-1.29	0.077
South Africa	12	1.00 (±0.034)	0.017 (±0.0094)	**-5.33**	**-1.58**	0.099
Mayotte	21	0.99 (±0.018)	0.015 (±0.0082)	**-9.55**	-1.17	0.081
Nth Madagascar	11	1.00 (±0.039)	0.017 (±0.0098)	**-4.40**	-1.38	**0.091**
Sth Madagascar	2	1.00 (±0.500)	0.043 (±0.0436)	3.14	-	0.500
Chagos	6	1.00 (±0.096)	0.016 (±0.0091)	-1.23	-1.14	**0.055**
Cocos Keeling Islands	6	0.73 (±0.155)	0.003 (±0.0024)	0.54	-0.93	0.373
Northern Indian Ocean sister-species						
UAE	15	0.95 (±0.040)	0.0044 (±0.0029)	**-5.67**	-0.60	**0.084**
Oman	9	0.89 (±0.091)	0.0048 (±0.0032)	-1.66	-0.77	0.123
Maldives	17	0.99 (±0.025)	0.0178 (±0.0097)	**-5.51**	0.07	0.119
Christmas Island	3	0.00 (±0.000)	0.0000 (±0.0000)	-	-	-
Aceh	15	0.93 (±0.054)	0.0121 (±0.0068)	-2.48	-1.13	0.093
Thailand	16	0.98 (±0.028)	0.0098 (±0.0057)	**-7.55**	-0.45	0.116
Pulau Seribu	12	0.97 (±0.044)	0.0109 (±0.0063)	-3.21	-0.49	0.122
Karimunjawa	5	0.90 (±0.161)	0.0124 (±0.0083)	0.88	-0.35	0.180
Krakatau	3	1.00 (±0.272)	0.0182 (±0.0144)	1.07	-	0.205

The regional groupings explaining most of the genetic variation according to the AMOVA analyses in the NIO sister-species were composed of a western group (*w*: Oman and UAE), a central group (*c*: Maldives) and an eastern group (*e*: Thailand, Aceh, Christmas Island, Pulau Seribu, Krakatau and Karimunjawa; Fig. 3.1). In the SIO sister-species, they followed the Marine Ecoregions of the World provinces (Spalding et al. 2007): province 19 (*prov19*: Oman and UAE), province 20 (*prov20*: Kenya, Mayotte, North Madagascar, South Madagascar, South Africa, Réunion and Mauritius), province 22 (*prov22*: Chagos) and province 27 (*prov27*: Cocos Keeling Islands; Fig. 3.1). In the NIO sister-species, most of the genetic variation was explained among regional groups (57.37%, Φ_{CT}=0.574, p<0.001) within which variation was low (2.82%, Φ_{SC}=0.066, p<0.001; Tab. 3.2). In the SIO sister-species, although we present the regional combination that maximised genetic variation among groups, this explained little of the total variation (5.64%, Φ_{CT}=0.056, p<0.05), and most occurred between individuals within populations (92%; Tab. 3.2).

The MIGRATE analyses, based on the regional groupings identified by the AMOVA, were first run with a full exchange matrix (i.e. bidirectional exchange of migrants possible between all regional groups) to determine appropriate priors (Tab. S3.3). The process was straightforward for the NIO sister-species, but the results did not converge for the SIO sister-species. Since the groupings in the SIO sister-species were of unequal sizes, we restricted the analysis to the larger populations, i.e. within *prov20* only (excl. South Madagascar). However, the only

Table 3.4. Performance of different gene flow models (Fig. 3.2) between regional groupings in the northern Indian Ocean sister-species, compared against M_3, the best-performing model. l_M: Log marginal likelihood, LBF: Log Bayes factors and ensuing rank.

Model	l_M	LBF	Rank
M_1	-1954.69	-13.4	2
M_2	-1975.56	-34.3	3
M_3	-1941.29	0.0	1
M_4	-1995.19	-53.9	5
M_5	-1991.02	-49.7	4
M_6	-2031.98	-90.7	6

model that converged was the panmixia model, suggesting gene flow was too high within this province to determine individual migration rates between populations and allow a proper comparison of migration models. Therefore, we only present the MIGRATE results for the NIO sister-species. Here, a series of different migration models could be tested (Fig. 3.2).

According to the Log Bayes factors, there was strong support (Beerli and Palczewski 2010) in favour of the asymmetrical migration model M_3, allowing migration from the regional groups *w* (west) and *e* (east) towards *c* (central) but not back to these groups or between them (Tab. 3.4). For this model, the effective number of migrants per generation ($Ne_i m_{j\rightarrow i}=\Theta_i * M_{j\rightarrow i}$) from *w* to *c* was 218, and from *e* to *c* 254 (Tab. 3.5). We also present the results of the second best ranking model, the full exchange model M_1, which essentially revealed the same migration patterns as M_3 but with a stronger contribution to *c*'s gene pool from *e* than from *w* (Tab. 3.5).

Table 3.5. Migration matrix of the two most supported gene flow models in the northern Indian Ocean sister-species (M_3 and M_1; see Fig. 3.2 and Tab. 3.3), showing Θ_i (diagonal) and the number of migrants from regional grouping i to j per generation, followed by the migration rates in brackets. Top numbers in black are the results for model M_3, bottom numbers in grey for model M_1.

from / to	***w***	***c***	***e***
w	0.015	654 (218)	0 (0)
	0.010	178 (68)	23 (0.9)
c	0 (0)	0.333	0 (0)
	60 (0.5)	0.379	77 (3)
e	0 (0)	762 (254)	0.015
	56 (0.5)	693 (262)	0.038

3.4. Discussion

3.4.1. Diversification processes

The diversification event that led to the separation of the northern (NIO) and the southern Indian Ocean (SIO) sister-species of the crown-of-thorns starfish occurred during the late Pliocene-early Pleistocene (1.86-2.89 Mya). Although the exact timing of this event should be interpreted with caution, as no external cali-

bration points were available and the mutation rate we used was inferred from other echinoderms, it coincided with periods of strong climatically-induced sea-level fluctuations. Indeed, global sea levels repeatedly dropped 120m below their present level during glaciations in the early Pleistocene (2.5, 2.2, 2.1 and 1.9 Mya; Miller et al. 2005).
Sea-level changes have frequently been invoked as a driver of speciation on coral reefs (Palumbi 1994; Veron 1995), as the impact of sea-level lowstands during glaciations could have restricted and/or altered the distribution of reef-dwelling organisms (Montaggioni 2005). The present distributions of the NIO and SIO sister-species are largely, but not entirely, restricted to the two main current systems to the north and south of the equator, respectively. The Indian Ocean circulation is characterised by strong, seasonal monsoonal current systems and upwelling patterns in the north, whereas an equatorial gyre dominates the tropical southern half (Fig. 3.3; Schott et al. 2002). As COTS' planktonic larvae display negative geotactic behaviour, i.e. after hatching they swim to the surface and remain there until the late brachiolaria stage (the last stage of their larval cycle before settling; Yamaguchi 1973), ocean surface currents are likely to have an important impact on their dispersal, and changes in these currents can be expected to strongly affect the connectivity between populations. It is therefore tempting to suggest that the divergence of the two species is based on these currents, or changes in these currents during sea level fluctuations.

Consideration of the divergence of the major clades within each of these two sister-species can be constructive with respect to investigating the origin of the two species. The close timing of the intraspecific divergence (113-139'000 years ago) of the two clades suggests these could have been initiated by one single climatic event. Global sea levels also dropped 120m below their current level before the onset of the last interglacial (130'000 years ago, isotopic stage 6; Siddall et al. 2003). There is strong evidence that during glacial periods, the northern Indian Ocean monsoonal system would have been altered, the seasonal southwest (SW) monsoon being weaker whereas the strength of the northeast (NE) monsoon would have increased (Ivanova 2009) in comparison to present-day interglacial patterns (Fig. 3.3). As suggested by Pollock (1993) when investigating interspecific patterns of diversification in spiny lobsters, weaker oceanic circulation could

have increased the retention of larvae in the Arabian Sea, thus promoting the diversification of the west and east clades in the NIO sister-species.

In the SIO sister-species, we also detected a western and eastern-origin clade (Fig. 3.3), suggesting changes in surface circulation resulting from sea-level fluctuations could also have restricted the distribution of COTS in the southern Indian Ocean. However, in this area, past changes in circulation patterns are comparatively poorly documented and still debated. Hutson (1980) suggested that intensified westerly winds would have hindered the penetration of the South Equatorial Current and the Northeast Madagascar Current along the southeast coast of Africa (Fig. 3.3), which could have led to the retention of larvae between the continent and Madagascar, and the subsequent diversification of these populations from other populations of the SIO sister-species. Although more recent findings suggest that temperature and flow in this area were stable for the last 150'000 years, changes in upwelling and eddy formation may still have occurred (Winter and Martin 1990). Where exactly the two clades diverged in the southern Indian Ocean therefore remains unclear, although the central position of the Cocos Keel-

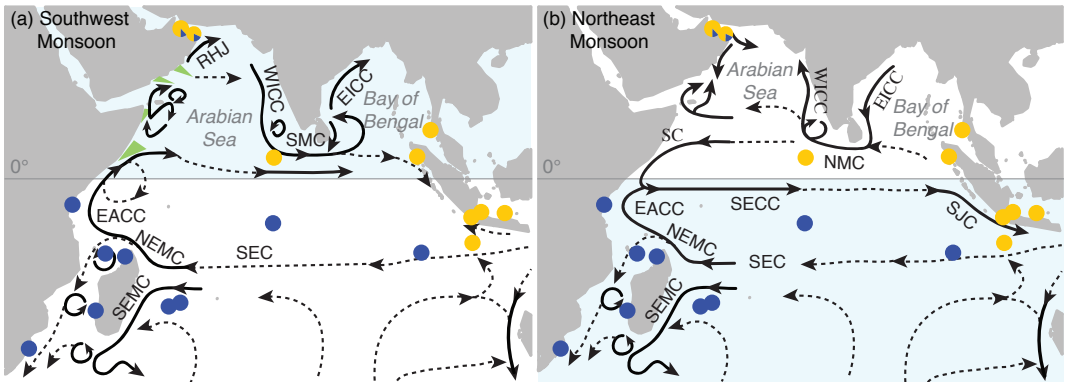

Figure 3.3. Schematic representation of the Indian Ocean surface circulation during the *(a)* southwest (July/August) and *(b)* northeast (December/January) monsoon after Schott and McCreary (2001), in relation to crown-of-thorns starfish sampling locations (yellow circles: NIO sister-species, blue circles: SIO sister-species). Blue shaded areas indicate the area in which COTS larvae would likely be released according to season. Green wedges in *(a)* are upwelling areas. Current branches indicated are the South Equatorial Current (SEC), Southeast and Northeast Madagascar Current (SEMC and NEMC), East African Coast Current (EACC), Somali Current (SC), Ras al Hadd Jet (RHJ), West and East Indian Coast Current (WICC and EICC), Southwest and Northeast Monsoon Current (SMC and NMC), South Java Current (SJC).

ing haplotypes in the minimum spanning tree suggests this area might also have acted as a refugium (*prov27* in Fig. 3.1c).
The substantial evidence in favour of the impact of surface circulation changes on population connectivity and subsequent intraspecific divergence provides some support in favour of similar dynamics having acted in the separation process of the two species. However, at this point there is no evidence to suggest anything more specific than that these currents helped to maintain the isolation of these species following their divergence.

3.4.2. Intraspecific population structure

Following the second diversification event, our results point towards differing genetic structures in the two lineages. Although the Bayesian skyline suggested an expansion after the LGM (18'000-24'000 years ago; Fig. S3.2a), there was a very large variance to those estimates. Clearer signals were obtained from the other demographic statistics, in which the NIO sister-species showed no strong signature of a recent population expansion (Tab. 3.1). There was also a strong signal for genetic structure in the NIO sister-species, dividing it into three regional groupings, *w* (west), *c* (central) and *e* (east; Tab. 3.2). An asymmetric pattern of connectivity was detected between these grouping, from *w* and *e* towards *c* (model M_3 in Fig. 3.2, Tab. 3.5). In other words, the Maldives acted as a sink population whereas the eastern and western regional groups (*w* and *e*) were sources of migrants. In contrast, there was strong evidence that the SIO sister-species went through a population expansion. The populations in the SIO sister-species appeared to be well connected, the entire lineage essentially acting as a panmictic population with extremely high levels of gene flow.
There are several differences between the northern and southern Indian Ocean, some or all of which could impact population genetic structure. The principal of these are (1) landmass distribution: the northern Indian Ocean is bounded by a long coastline on all but its southern margin, and has large numbers of islands in the centre (Maldives) and east (Andamans). In contrast, the southern Indian Ocean has extensive coastlines only on its western and eastern reaches. Numerous islands are found in the west (Madagascar, Comoros, Seychelles, Mascarenes) and very few in the centre (Chagos Archipelago) and the east (Christmas and Cocos Keeling). (2) Habitat availability, i.e. coral reef distribution: coral reefs are dis-

tributed over most of the continental margins and islands of the Indian Ocean, although not continuously. Major breaks in the northern Indian Ocean include upwelling areas off Somalia and Oman, the northern Arabian Sea coast, stretches of the Indian western and eastern coasts, and of the Bay of Bengal. In the southern Indian Ocean, there are some major breaks in southern Mozambique and in Madagascar, close to the southern end of coral distribution (Wilkinson 2008). (3) Currents: as described in detail in the preceding section, the main currents in the northern Indian Ocean reverse according to monsoon, which, along with strong changes in upwelling patterns, leads to a complex current system. Currents in the southern hemisphere are dominated by an equatorial gyre that remains relatively constant throughout the year, except for a slight northwards shift during the southwest monsoon, and a change of direction of the current along the Indonesian coast (Fig. 3.3). (4) Productivity (important for larval development): areas of high productivity (>130 $gC.m^{-2}$) are distributed over a far greater proportion of the northern Indian Ocean (generally associated with the continental margins) than in the southern Indian Ocean, where they are associated with the Seychelles and the Chagos Archipelago (Fig. 3.4).

Figure 3.4. Areas of the Indian Ocean where primary productivity exceeds 130$gC.m^{-2}$ (in grey; modified from Reid et al. (2006), data for 1998–99 (not an El Niño year) after NASA SeaWiFS).

Landmass distribution and habitat availability

A general expectation might suggest that areas with a greater amount and less fragmentation of suitable habitat would lead to higher connectivity between

populations. If that were so, one would expect greater connectivity of populations in the northern Indian Ocean with its greater amount of coastline and islands even given gaps in coral distribution. However, the connectivity between populations in the SIO sister-species was much higher than in the NIO sister-species. This implies that factors other than the distribution of landmasses and suitable habitat determine the population genetic structure.

Currents and productivity

Isolation of populations in restricted areas of circulation has already been postulated to have played a critical role in the evolution of the western and eastern clades in each of the NIO and SIO sister-species in the previous section. At higher latitudes in the Pacific Ocean (>10°N or S), COTS larvae are released during a summer spawning season (Birkeland and Lucas 1990). This would correspond to the SW monsoon (Fig. 3.3a) in the northern Indian Ocean, and corroborates our findings, as currents at this time would facilitate transport of larvae from the regional group *w* to *c* (model M_3 in Fig. 3.2, Tab. 3.5, Fig. 3.3a). Direct data on spawning times for populations near the equator are rare, but data from the Pacific Ocean suggest there is no discrete spawning season (Birkeland and Lucas 1990). This means movement of larvae from *e* to *c* could well occur outside the SW monsoon, when currents flow from east to west (Fig. 3.3b). A simple estimation of current velocity and the duration of COTS larval development in normal conditions (three to four weeks in the Pacific; Yamaguchi 1973) shows that larvae from both the regional groups *w* and *e* could reach the Maldives with no or few stepping-stones. At an average current velocity of 0.5 $m.s^{-1}$ in the Arabian Sea and in the Bay of Bengal during the SW monsoon and NE monsoon respectively (Fig. 3.5; Bonjean and Lagerloef 2002), a pelagic larval duration of four weeks would enable larvae to travel 1200 km on the predominant currents, thus reaching the Maldives from respectively Oman or Aceh either directly or within two generations using a stepping-stone (e.g. western Indian coast or Sri Lanka, respectively). In the NIO sister-species, the patterns of gene flow inferred from the genetic structure are thus consistent with the main directions of oceanographic circulation at the times at which COTS spawn.

In the southern Indian Ocean, the consistent gyre would theoretically enable circulating larvae from east to west and vice-versa throughout the year, independ-

ent of the spawning time, although larvae from the SIO sister-species are thought to be released during the Austral summer (Schleyer 2004). However, panmixia was observed in the SIO sister-species, suggesting high connectivity even among populations that were geographically extremely isolated. Indeed, the Cocos Keeling Islands are separated from their closest downstream neighbour, the Chagos Archipelago, by 2700 km, and the latter from the Seychelles and Rodrigues by another 1600 km. At a speed of 0.5 $m.s^{-1}$ (Fig. 3.5; Bonjean and Lagerloef 2002), larvae would need 65 and 35 days respectively to cover these distances on the predominant currents, which far exceeds their pelagic larval duration in normal conditions (Yamaguchi 1973). To explain the amount of gene flow observed between these populations and the rest of the SIO sister-species, a longer, probably teleplanic, larval stage would be necessary. COTS larvae from the Pacific sister-species have been found to extend their developmental period to seven weeks in marginal food regimes (Lucas 1982), although the occurrence of a facultative teleplanic larva remains to be confirmed (Birkeland and Lucas 1990). Either way, the low primary productivity in the southern Indian Ocean (Reid et al. 2006) could stimulate the extension of the developmental period, thus explaining how connectivity could be maintained across such large distances. And in contrast, larval duration in the northern Indian Ocean is unlikely to exceed that found in normal conditions due to the high levels of primary productivity, thus contributing to the stronger genetic structure observed in the NIO sister-species.

The presence of a few individuals from the SIO sister-species in populations of the NIO sister-species is quite intriguing (Fig. 3.1a). They do not appear to have dispersed into the area during a single founder event, as their haplotypes do not cluster together in the minimum spanning tree (Fig. 3.1c). As no individuals from the SIO sister-species are found in the Maldives, the most likely source of propagules would be the east African coast. Yet the strong upwelling conditions and eddies that accompany the SW monsoon (Fig. 3.3a) seem unsuitable for the transport of larvae from this area to Oman (Glynn 1993), more so because the African coast populations might not spawn at that time of year, although low latitude populations may still contribute propagules, as the timing of spawning in these areas is undetermined. During the NE monsoon, when populations in the higher latitudes of the southern Hemisphere are most likely to spawn, the

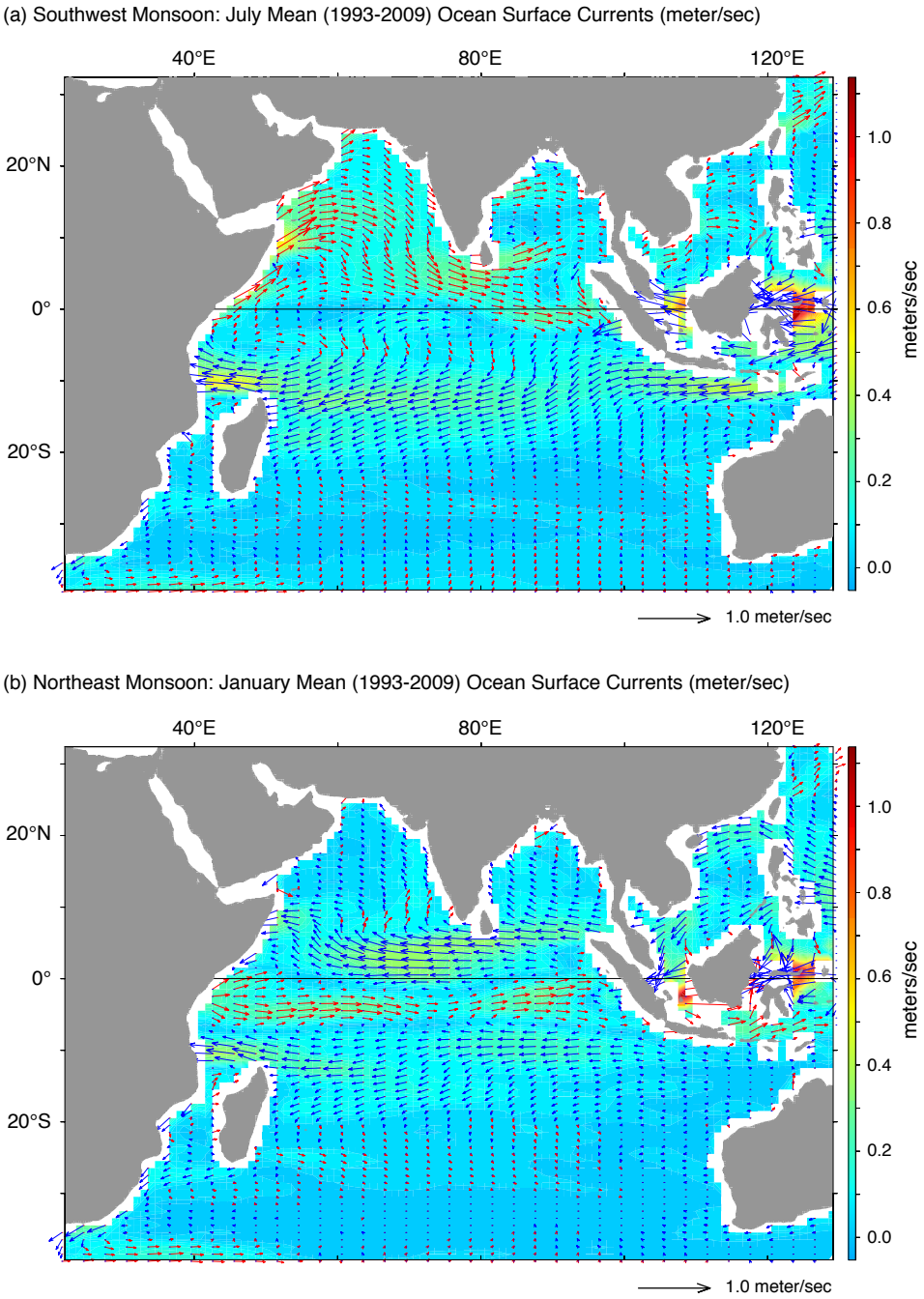

Figure 3.5. Current direction and velocity during the peak of (a) the Southwest Monsoon (January mean from 1993 to 2009) and (b) the Northeast Monsoon (July mean from 1993 to 2009). Arrow colour indicates direction of flow (westward: blue, eastward: red), arrow length and plot background colour indicate current velocity in meters per second. Data obtained from and plots constructed using Ocean Surface Current Analysis – Real time: http://www.oscar.noaa.gov/ (Bonjean and Lagerloef 2002).

southward flowing Somali Current would also hamper the northward dispersal of larvae (Fig. 3.3b). Although such oceanographic barriers to dispersal should prevent larval crossing, it can however not be excluded that a few propagules occasionally survive to the other side, and, as Glynn (1993) suggested for tropical species in this area, they might represent ephemeral populations that experience brief periods of invasion and extinction.

3.5. Conclusion

Present-day circulation patterns, in conjunction with primary productivity, appear to play an important role in generating the differences in genetic structure of the two Indian Ocean species of COTS. These differences indicate they are now on different evolutionary trajectories, but whether this has already led to changes in their biology needs to be further investigated. However, it is conceivable that very different selective pressures are acting on individuals from NIO and SIO, with longer larval phases and better larval dispersal capabilities possibly being selected for in the latter. As the general consensus today seems to be that outbreaks are at least to some extent caused by the effects of primary productivity on larval survival (Birkeland and Lucas 1990; Houk et al. 2007; Fabricius et al. 2010), such differential selection could have far-reaching consequences for differences in outbreak ecology between the southern and northern Indian Ocean sister-species. We would also like to stress the importance of undertaking similar studies for other coral reef-associated organisms in the Indian Ocean. There is only little population genetic information available from this ocean (Ridgway and Sampayo 2005), yet there is a strong need for more research to increase the overall state of knowledge (Sheppard 2000) and devise appropriate conservation strategies (Mora et al. 2006; Graham et al. 2008). By identifying genetic breaks between and within species as well as exploring the connectivity between populations (Palumbi 1996; Hellberg 2009), molecular studies such as this one can not only increase our understanding of the biology of individual organisms, but also contribute to identifying conservation targets, and form the basis for biogeographical classifications and future monitoring (Lourie and Vincent 2004). If patterns similar to those of this study are identified, this could have important implications, for example for the design of an adequate network of marine protected areas.

4. Evolution of the crown-of-thorns starfish species complex

This chapter is currently in preparation for standalone publication:

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4. Evolution of the crown-of-thorns starfish species complex

Abstract

Long thought to be a widespread species, the crown-of-thorns starfish (COTS) *Acanthaster planci* forms a species complex constituted of four highly differentiated lineages with restricted distributions, located in the Pacific, the Red Sea, the Northern and the Southern Indian Ocean. To understand the evolution of this species-complex, we first investigated what could have shaped its distribution, by reviewing what is known about other Indo-Pacific organisms and comparing this to the patterns of genetic structure identified for COTS. We then reviewed the information available on the different COTS sister-species, in order to determine whether there were indications of biological or ecological differences between them that may have been overlooked while COTS was considered a single widespread species.

Sea level changes, land bridges, circulation patterns and cold-water upwelling played an important role in shaping the genetic structure of this species complex, but COTS' unusual life history could have further contributed to the formation of the four species. A few differences in morphology, cryptic behaviour, toxicity, larval and adult temperature tolerance, and in the extent of outbreaks of the four sister-species were uncovered. Their interpretation was limited due to a strong geographic bias in the origin of the data in favour of the Pacific sister-species, but still indicated that the four sister-species have already to diverged in more than their genetic structure. Moreover, we were able to determine future research directions to improve our understanding of this organism that remains a substantial threat to coral reefs.

4.1. Introduction

Marine invertebrates with long-lived planktonic larvae have long been expected to display little genetic structure, due to the apparent lack of barriers in the marine realm. But molecular studies have revealed that many widespread marine invertebrates have highly structured populations, and cryptic speciation is a common phenomenon in the oceans (Knowlton 1993, 2000). This is also the case for the crown-of-thorns starfish (COTS) *Acanthaster planci*, which has been found to be a species complex constituted of four highly differentiated lineages with restricted distributions, located in the Pacific, the Northern (NIO) and the Southern Indian Ocean (SIO), and the Red Sea (Vogler et al. 2008).

Previous investigations have shown that the Pacific sister-species not only had a high dispersal potential, but also achieved it, as evidenced by the signature of ongoing gene flow between areas that were isolated in the past as well as high levels of connectivity, even among distant populations (chapter 2). In the Indian Ocean on the other hand, the genetic structure was much higher, in that COTS were constituted of two separate species (Vogler et al. 2008), which was quite intriguing considering the high dispersal in Pacific COTS and the much smaller size of the Indian Ocean. Moreover, there was also a strong contrast in the genetic structure of the two Indian Ocean sister-species. The NIO sister-species was constituted of highly differentiated regional groupings, whereas the SIO sister-species displayed high levels of gene flow, even over large distances (chapter 3).

To further understand what could have driven the evolution and distribution of this species complex, we first investigated whether the patterns of genetic structure found in COTS were similar to those of other Indo-Pacific organisms, and whether the likeness or differences with other species could shed more light on this organism that remains a substantial threat to coral reefs (Fabricius et al. 2010).

Indeed, the crown-of-thorns starfish is known mostly for its population outbreaks that have devastated coral reefs through much of its distribution since the 1960s (Birkeland and Lucas 1990). Although outbreaks still amount for a large proportion of the disturbance to Indo-Pacific reefs today (Fabricius et al. 2010), their causes remain debated, as well as appropriate monitoring strategies to predict their occurrence and management plans to reduce their impact (Birkeland and Lucas 1990; Houk et al. 2007; Sweatman 2008; Fabricius et al. 2010). It was

suggested that failure to recognise the existence of a species complex in this organism may have contributed to a lack of understanding of the processes that lead to outbreaks in the different COTS lineages (Vogler et al. 2008).
The second aim of this chapter was therefore to compare the information available on the different sister-species, in order to determine whether there were indications of biological or ecological differences between them that may have been overlooked while COTS was considered a single widespread species.

4.2. Comparison with other Indo-Pacific organisms

The main patterns of genetic structure found for COTS were (1) the break between the Pacific and the Indian Ocean sister-species, (2) the absence of strong genetic breaks in the Pacific, (3) the break within the Indian Ocean, (4) the break between the Indian Ocean and the Red Sea sister-species and (5) the notable absence of COTS from the Arabian Gulf.

4.2.1. Pacific and Indian Ocean break

The major genetic differentiation of COTS from the Pacific and Indian Oceans was first discovered using allozymes (Benzie 1999), and subsequently identified as the break between the NIO sister-species and the Pacific sister-species using mitochondrial DNA (Vogler et al. 2008). This Indo-Pacific barrier (IPB) has also been identified in a range of other organisms, based both on morphological (Randall 1998; Woodland 1983; Briggs 1999 and references therein) and molecular data (e.g. McMillan and Palumbi 1995; Williams and Benzie 1998; Duda and Palumbi 1999; Nelson et al. 2000; Lessios et al. 2001; Bay et al. 2004; Crandall et al. 2008b; Meyer 2003). During Pliocene and Pleistocene glacial cycles, repeated sea levels lowstands (up to 120 m below present levels) imposed a nearly complete barrier between the two oceans (Fig. 4.1). Cold-water upwelling probably enhanced the effectiveness of the IPB by further limiting the dispersal of tropical marine organisms by reducing the availability of suitable habitat (Voris 2000; Fleminger 1985).
The exact location of the IPB does however vary between species, as well as the extent to which Indian and Pacific lineages overlap in the contact zone. For COTS, the northern part of the boundary between the Pacific and NIO sister-species runs along Peninsular Malaysia and Sumatra, and the southern part be

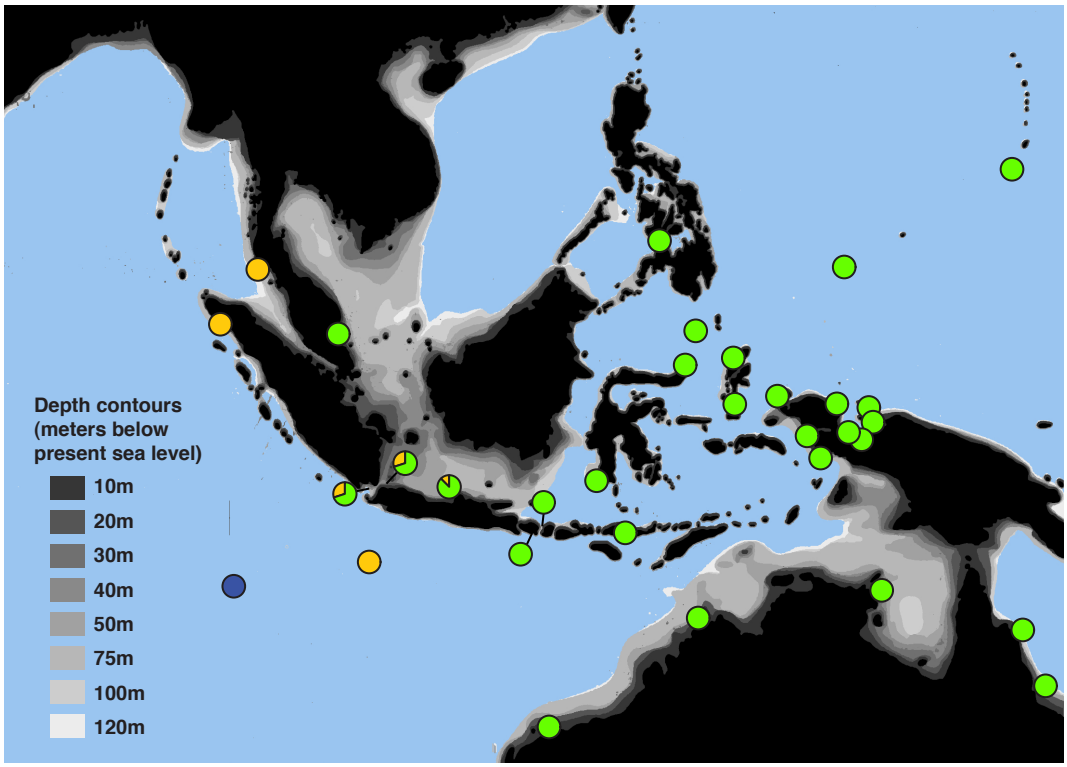

Figure 4.1. Distribution of the Pacific (green), Northern (yellow) and Southern Indian Ocean (dark blue) sister-species in the Indo-Australian Archipelago. Pie charts indicate relative frequency of each species per sampling location. Depth contours show emerged land during lower sea levels.

tween western Australia and Christmas Island (Fig. 4.1). Both species co-occur at a few sites between the islands of Sumatra and Java (Pulau Seribu and Krakatau), and individuals from the NIO sister-species are only found as far into the Java Sea as Karimunjawa (Fig. 4.1).

Northern section of the IPB

Only a few datasets had a high enough coverage in the northern part of the break for comparative purposes: the starfish *Linckia laevigata* (Williams 2000; Crandall et al. 2008a), the giant tiger shrimp *Penaeus monodon* (Benzie et al. 2002; You et al. 2008) and the gastropod *Nerita albicilla* (Crandall et al. 2008b).

Individuals of the Indian Ocean clade of *L. laevigata* and *P. monodon* occurred much further into the Pacific Ocean than COTS (Guam and Papua New Guinea for *L. laevigata*, and eastern Thailand and Vietnam for *P. monodon*). This suggests

there are no present oceanographic barriers that prevent larvae from dispersing further into the Coral Triangle. Considering that what is known on the biology and ecology of larvae of COTS and the starfish *L. laevigata* suggest they are quite similar (Yamaguchi 1973), something else may prevent the dispersal of COTS larvae from the Indian Ocean into the Pacific Ocean.
An overlap of the same magnitude as in COTS was found in *N. albicilla* (Crandall et al. 2008b), for which the Indian and Pacific Ocean clades only co-occurred in Pulau Seribu and Krakatau. Crandall et al. (2008b) suggested that the lack of introgression between the two clades was most likely due to pre- or post-zygotic barriers to reproduction between individuals of these clades. This may also be the case for COTS, as the high dispersal achieved in other areas of its distribution suggests that post-dispersal processes are more likely to provide an explanation for the maintenance of this phylogeographic break than are barriers to dispersal. This also raises the question of reproductive barriers between the different COTS species, which will be addressed in the last part of this chapter (see section 4.3.5).

Southern section of the IPB

For COTS, the southern part of the break between the Pacific and Indian Ocean sister-species runs between western Australia and Christmas Island (Fig. 4.1). For many other species that show an Indian/Pacific genetic break, western Australian populations also belong to the Pacific genetic pool rather than the Indian Ocean one (e.g. Williams and Benzie 1998; Benzie et al. 2002; Bay et al. 2004; Portnoy et al. 2010). Based on extensive studies of the molecular systematics of cowries (Meyer 2003, 2004), it appeared that more than 90% of the cowries found in western Australia belong to Pacific clades (Christopher Meyer *pers. comm.*). Williams and Benzie (1998) suggested that the closer affinity of western Australian populations with the Pacific rather than Indian Ocean populations was due to cold-water upwelling regions off the northwest shelf of Australia (Schott and McCreary 2001), that would have restricted larval dispersal between western Australia and the rest of the Indian Ocean.
There are however a number of species for which the boundary runs further west, as was found in a recent study on 11 sister-species pairs of coral reef fishes with an Indian/Pacific split. For these species, the Cocos Keeling and Christmas Islands constituted the contact and hybridisation zone (Hobbs et al. 2009). This

was also reflected for a number of cowries, where populations from Cocos Keeling and Christmas Islands often showed admixture of both Pacific and Indian Ocean clades. From the perspective of faunal composition, the overall biota of these two islands was found to be principally derived from the Indo-West Pacific and only to a lesser extent from the Indian Ocean (Woodroffe and Berry 1994). For COTS, these two islands were also an interesting area, as Christmas Island samples belonged to the NIO sister-species, and the Cocos Keeling Islands samples to the SIO sister-species (Vogler et al. 2008). However, the sample sizes from both areas were small (3 and 6 individuals respectively), which does not exclude the possibility of overlap of the different species on these islands, as was suggested by Hobbs and Salmond (2008) based on colour morphs (see section 4.3.1).

The Pacific/Indian break found in COTS thus reflected a pattern present for many other Indo-Pacific species, highlighting the importance of not only land barriers but also cold-water upwelling in limiting COTS dispersal. The importance of cold-water upwelling as a barrier to dispersal in COTS was further supported by the location of the southern section of the IPB, delimited by upwelling off the coast of western Australia. Moreover, the discrepancy with the starfish *Linckia laevigata* in the northern section of the break supported the existence of barriers to reproduction between the Pacific and NIO sister-species.

4.2.2. Structure within the Pacific Ocean

For COTS, Johnston Atoll (and probably Hawaii; Nishida and Lucas 1988) was identified as the only really isolated area in the Pacific Ocean. Both areas are known to be isolated from the rest of the Pacific for a range of other organisms (Briggs 1974; Kay and Palumbi 1987; Jokiel 1987), due to both the large distance from other island archipelagos and the lack of favourable currents to transport propagules from possible source populations (Maragos and Jokiel 1986). Other otherwise highly connected organisms are isolated in this area too (e.g. Leray et al. 2010).

As originally suggested by Nishida and Lucas (1988) and shown in chapter 2, COTS larvae are however capable of dispersing to the eastern Pacific despite the presence of the "impassable" Eastern Pacific Barrier (EPB), the 5000+ km expanse of deep water without stepping-stones separating the central from the eastern

Pacific. This barrier is considered to be responsible for one of the most pronounced breaks in circumtropical shore fauna, second only to continents (Briggs 1961 and references therein; Grigg and Hey 1992). Only a few other marine invertebrates have been identified to be able to disperse over this barrier: the sea urchins *Echinotrix diadema* (Lessios et al. 1998) and *Tripneustes gratilla* (Lessios et al. 2003), the gastropod *Conus ebraeus* (Duda and Lessios 2009) and the shrimp *Alpheus lottini* (Williams et al. 2002). Particularly interesting is however that, contrarily to COTS, these species did not display an Indian/Pacific break or strong genetic structure within the Indian Ocean (when data was available from these areas: Lessios et al. 2003; Williams et al. 2002).

Very long distances did not represent an impassable barrier to dispersal for COTS in the Pacific, but the aforementioned contrast in genetic structure with other species capable of dispersing over such long distances suggests that other factors may play a strong role in limiting the dispersal of COTS larvae.

4.2.3. Structure within the Indian Ocean

COTS in the Indian Ocean are constituted of two sister-species, the NIO and SIO sister-species (Vogler et al. 2008), between which large genetic distances have also been detected using allozymes (Benzie 1999). The genetic structure of the two sister-species was strongly contrasted (Tab. 4.1), the NIO sister-species being constituted of highly differentiated regional groupings, whereas the SIO sister-species displayed high levels of gene flow, even over large distances (chapter 3).
Faunal distributions led to suggesting that the Western Indian Ocean (WIO) formed a coherent subdivision of the tropical Indo-Pacific, rather than the Indian Ocean as a whole (Briggs 1974; Sheppard 1998, 2000). The main reason that was brought forward for this differentiation was the great distance and lack of suitable habitat between the Indo-Australian Archipelago and the WIO, leading to its differentiation. A recent review of population genetic studies in the Indian Ocean suggested that these also supported the WIO as a distinct subdivision of the tropical Indo-Pacific, and that there was a major disjunction between WIO and Eastern Indian Ocean (EIO) groups (Ridgway and Sampayo 2005). However, in the studies reviewed, this disjunction reflected the isolation of western Australia from the rest of the Indian Ocean, which as mentioned before has more affinities

Table 4.1. Summary statistics per species and dataset (COI and CoReg; see "supplementary methods and results for chapter 4" in the Appendix): aligned sequence length, number of populations n_{pops}, number of individuals n, haplotype diversity h, nucleotide diversity π, Fu's F_S, Ramos-Onsins R_2 and Tajima's D (significant values in bold), Φ_{ST} with no *a priori* structure.

	Species	Sequence length	n_{pops}	n	h	π	F_S	R_2	D	Φ_{ST}
COI	Pacific	632	15	108	0.874 (±0.0221)	0.005 (±0.0029)	**-18.76**	**0.039**	**-1.72**	-
	Red Sea	632	2	29	0.791 (±0.0839)	0.002 (±0.0013)	**-6.20**	**0.054**	**-1.75**	-
	NIO	632	6	48	0.679 (±0.0448)	0.004 (±0.0059)	-1.32	0.101	-0.13	-
	SIO	632	8	57	0.592 (±0.0745)	0.002 (±0.0013)	**-12.67**	**0.036**	**-2.08**	-
CoReg	Pacific	530	45	673	0.997 (±0.0004)	0.026 (±0.0127)	**-23.52**	**0.039**	**-1.68**	**0.198**
	Red Sea	506	2	33	0.992 (±0.0104)	0.014 (±0.0073)	**-24.31**	**0.058**	**-1.50**	0.002
	NIO	522	9	95	0.981 (±0.0059)	0.020 (±0.0102)	**-24.44**	0.079	-0.28	**0.511**
	SIO	546	11	95	0.993 (±0.0035)	0.016 (±0.0082)	**-24.65**	**0.050**	**-1.57**	**0.068**

with the Pacific. It is therefore not clear whether the pattern detected by Ridgway and Sampayo (2005) represents an isolation of the WIO, or whether this reflects a Pacific/Indian Ocean split closer to the eastern margin of the Indian Ocean near Australia.

To further understand what patterns were present from a genetic perspective in the Indian Ocean, we examined the studies published since Ridgway and Sampayo's review (2005) that included more than one population from the Indian Ocean, and re-examined some of the publications that had already been reviewed (Tab. S4.1). These were only few, and the sample coverage was mostly very low (average of three localities from the Indian Ocean per study; Tab. S4.1). There was a strong bias in favour of the southwestern Indian Ocean and northwestern Australia (Fig. 4.2). This was on the one hand due to the fact that the Indian Ocean itself was not the focus of most of these studies, and sampling was oriented towards comparisons between the Pacific and Indian Ocean rather than within the latter, and on the other hand to the fact that most of the studies that did focus on

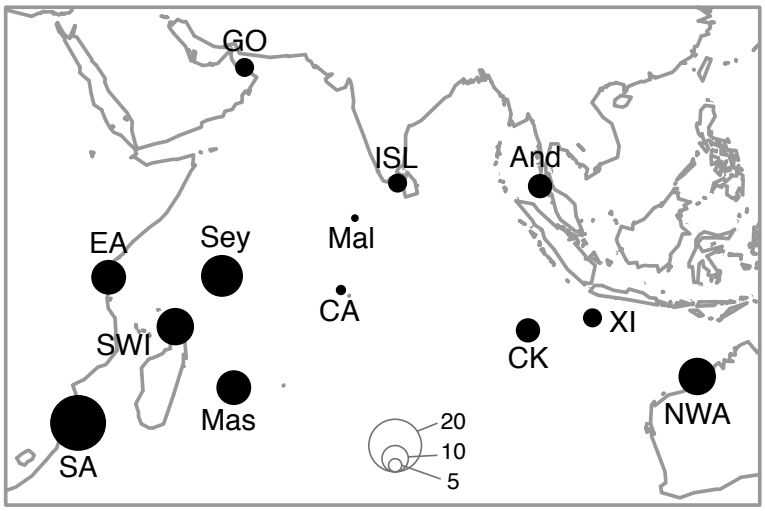

Figure 4.2. Distribution of phylogeographic and population genetic studies in the Indian Ocean, circle size is proportional to the number of studies including samples from each location. Locations are defined as: SA: South Africa+Mozambique; EA: Tanzania (incl. Zanzibar)+Kenya; SWI: south-western islands; Mas: Mascarene Islands; Sey: Seychelles; GO: Gulf of Oman; CA: Chagos Archipelago; Mal: Maldives; ISL: India+Sri Lanka; And: Andaman Sea; CK: Cocos Keeling Islands; XI: Christmas Island; NWA: north-western and western Australia.

the Indian Ocean were restricted to a relatively small area, thus limiting their use for detecting patterns at full ocean scale (Tab. S4.1).

Of the few studies that did have a larger coverage in the Indian Ocean, many of them revealed no structure at all (Tab. S4.1), which was to be expected for the highly vagile species (e.g. Gaither et al. 2010; Theisen et al. 2008) and the species with long larval stages (e.g. Horne et al. 2008; Crandall et al. 2008b; Leray et al. 2010), but is harder to explain for species that do not have such a high dispersal potential (e.g. Williams and Reid 2004; Lessios et al. 2003).

A few datasets that did uncover structure at the scale of the Indian Ocean and had an extensive enough coverage for comparative purposes were however identified. We examined them to find out whether they also revealed that (i) populations in the Indian Ocean were more structured than in the Pacific Ocean; (ii) there was evidence for a north-south break as identified for COTS and/or (iii) for a west-east break, and if yes, whether the delimitation of these breaks could be identified.

Differences in structure between the Indian and Pacific Oceans

Two studies found greater genetic structure in the Indian compared to the Pacific Ocean, one on the damselfish *Dascyllus trimaculatus* (Leray et al. 2010) and the other on the starfish *Linckia laevigata* (Williams and Benzie 1998). In the first, the genetic structure within the Indian Ocean was not further detailed or investigated by the authors. The second however revealed that populations from South Africa and Thailand were strongly differentiated from each other (Williams and Benzie 1998), and to a larger extent from the Pacific Ocean. This could reflect either or both a north-south and east-west break, and a more extensive coverage will be required to understand the processes that could have led to this pattern.

Evidence for a north-south break

Further potential support for the presence of a north-south break in the Indian Ocean was found for the giant tiger prawn *Penaeus mondon*, limpets of the *Patelloida profunda* group (Kirkendale and Meyer 2004) and the turbinid gastropod *Astralium rhodostomum* (Meyer et al. 2005). *Penaeus monodon* populations from the northeastern and southwestern Indian Ocean were strongly differentiated (You et al. 2008), although not to the same degree as COTS. There was also more dispersal between *Penaeus monodon* populations across the Indian/Pacific break than for COTS (You et al. 2008). Nonetheless, the genetic structure of *Penaeus monodon* in the Indian Ocean might have been shaped by similar mechanisms as COTS.

Patelloida profunda populations were highly structured throughout their Indo-Pacific range, most likely due to the limpets' poor dispersal potential (Kirkendale and Meyer 2004). In the Indian Ocean, independent lineages were detected in each of the sampling locations (Zanzibar, Mauritius, South Africa and Oman). Biogeographical inference from this data was limited due to ambiguities in the branching order of the different lineages, but the authors suggested that upwelling regions could have resulted in genetic breaks along the southern coast of Africa and between the east coast of Africa and the Arabian region. In a study on another organism with limited dispersal potential, *Astralium rhodostomum*, populations from Thailand were strongly differentiated from populations from the Cocos Keeling and Christmas Islands, and the latter were more closely related to each other than to the other Pacific populations (Meyer et al. 2005). Both these

studies thus also hinted towards the presence of a north-south break in other organisms, although both of these had poor dispersal abilities.

Evidence for a west-east break

Meyer (2003) compared the distributions of nearly all known cowrie species, and determined that the break between 15 pairs of evolutionary significant units was located in the central Indian Ocean (Christopher Meyer *pers. comm*.). Populations from the Lakshadweep Islands, the Maldives, India and Sri Lanka all belonged to EIO evolutionary significant units, which in some cases were panmictic with the Pacific Ocean (Christopher Meyer *pers. comm*.). We could not obtain any information on the affiliation of populations from the Gulf of Oman or the Chagos Archipelago, but depending on whether they belong to EIO or WIO evolutionary significant units, the west-east break may actually be more similar to the north-south break identified for COTS.

Although very little population genetic studies are available from the Indian Ocean, there are some indications that the higher structure found in the crown-of-thorns starfish in comparison to the Pacific Ocean was also present in other organisms. However, because the location of genetic breaks is unclear for most species, due essentially to the low geographic coverage of these studies, determining whether the factors suggested to have shaped the genetic structure of COTS in the Indian Ocean (mainly circulation patterns, upwelling areas and the distribution of primary productivity; see chapter 3) have also played a role for other species, or if other factors can be identified that may have been overlooked for COTS, is impossible at this point. Moreover, many of the species that showed similar patterns were poor dispersers, and only *Linckia laevigata*, *Dascyllus trimaculatus* and *Penaeus monodon* were organisms with a high dispersal potential that also displayed greater structure in the Indian Ocean, yet not of the same intensity as COTS. This again suggests that COTS are either particularly sensitive to some barriers to dispersal (e.g. upwelling areas), or possibly that the genetic differentiation between the NIO and SIO sister-species (chapter 3) very soon led to reproductive barriers, thus limiting remixing between the two species, and possibly even leading to competition between them. However, these interpretations are based on a very small number of studies and should therefore be taken

with caution. Future comparative studies with a targeted sampling effort in the Indian Ocean and a broad geographic coverage should enable understanding more about the dynamics that drive the distribution of biodiversity and connectivity in this ocean.

4.2.4. Indian Ocean and Red Sea break

COTS from the Red Sea belong to the fourth sister-species, which was found to have diverged in the late Pliocene-early Pleistocene (Vogler et al. 2008). Although the Red Sea was originally considered an extension of the Indian Ocean zoogeographic region by ichthyologists (reviewed in Klausewitz 1972), the high levels of endemism across multiple taxa that characterise its fauna led to defining it as a separate entity (Klausewitz 1972; Briggs 1974), and the Red Sea recently earned the status of marine biodiversity hotspot (Roberts et al. 2002). These high levels of endemism have been attributed to the Red Sea's unique and complex paleogeographic history. Indeed, the biogeographic origin of the present Red Sea fauna consists of (1) Tethys relicts and Miocene immigrants from the Mediterranean, (2) Pliocene-Pleistocene pre-glacial Indo-Pacific fauna and (3) newly settled post-glacial Indo-Pacific immigrants (Klausewitz 1972). The distinction between pre- and post-glacial in the fauna of Indo-Pacific origin is due to the repeated sea-level changes that were brought on by the glaciations of the Pliocene and Pleistocene.
The Red Sea is connected to the Indian Ocean by a shallow sill at its southern end (137 m deep), to the north of the Bab el Mandab Strait (Fig. 4.3). As sea levels repeatedly dropped 120 m below present levels during these glaciations (Miller et al. 2005; Siddall et al. 2003), this would have strongly restricted water mass exchange through the strait, although not interrupting it (Rohling et al. 1998; Fernandes et al. 2006). Paleoceanographic reconstructions indicate that during glaciations, environmental conditions were strongly altered as a consequence of the restricted water exchange. Salinity increased to 50±2‰ (Rohling et al. 1998), and winter sea surface temperatures dropped, although probably not below 17°C (Fenton et al. 2000). Species that were able to adapt to these environmental changes would thus have survived Pliocene-Pleistocene glaciations in the Red Sea, increasingly differentiating themselves from the populations of the Indian Ocean if interglacial exchange was not sufficient to re-establish gene flow, and even more so if the remaining populations were restricted to refugia, leading to

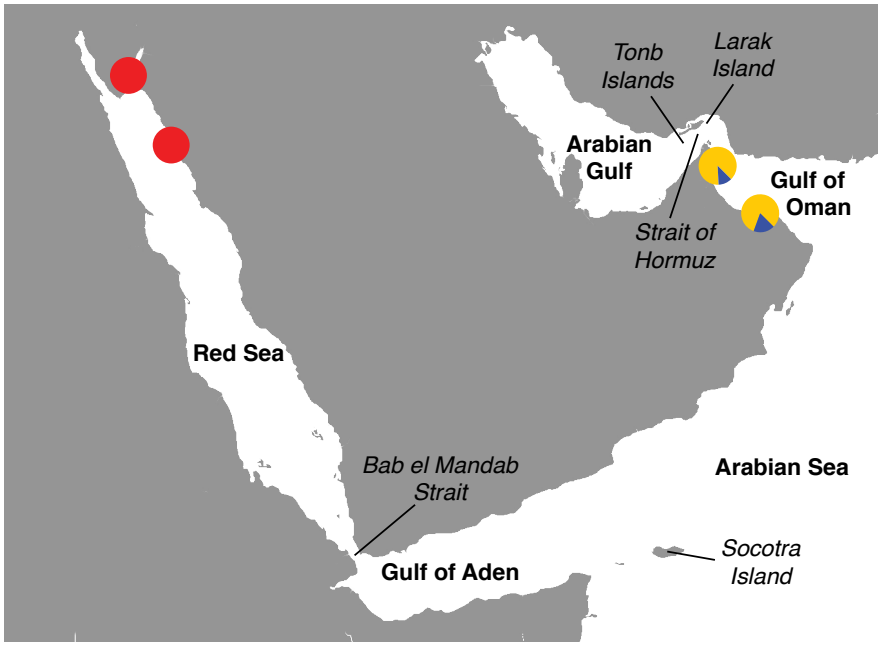

Figure 4.3. Distribution of the crown-of-thorns starfish sister-species around the Arabian Peninsula (Red Sea sister-species: red, NIO sister-species: yellow, SIO sister-species: blue). Pie charts indicate relative frequency of each species per sampling location.

genetic drift. Species that were not able to adapt but are found in the Red Sea today as part of widespread ranges most likely represent post-glacial immigrants from the Indian Ocean.

This differential pattern of survival in the Red Sea is also reflected in the very few population genetic, phylogeographic and phylogenetic studies that have included samples from this area. Some of these studies found isolated populations or sister-species in the Red Sea versus the Indian Ocean (McMillan and Palumbi 1995; Lessios et al. 2001; Meyer 2003; Wörheide et al. 2008). Of these, the butterfly fish of the *Chaetodon 'rhombochaetodon'* species complex (McMillan and Palumbi 1995), the cowries from the *Cypraea* genus (Meyer 2003) and the sponge *Leucetta chagosensis* (Wörheide et al. 2008) also displayed a Pacific/Indian Ocean break whereas the sea urchin *Diadema setosum* on the other hand displayed no such break, which might indicate that the Red Sea/Indian Ocean break was more substantial than the Indian/Pacific Ocean break, although this would need to be further investigated.

On the other hand, some species had high levels of connectivity between the In-

dian Ocean and the Red Sea, or the signature of a very recent colonisation of the Red Sea (Meyer 2003; Kochzius and Blohm 2005; McCafferty et al. 2002; Teske et al. 2005). This suggests these species did not have the potential to survive the environmental changes during restricted water exchange in the Red Sea, and only subsequently (re)colonised it, although it cannot be excluded that gene flow was maintained between the Red Sea and the Indian Ocean during glaciations for these species.

The timing of divergence of the COTS Red Sea sister-species thus supports the survival of COTS populations in the Red Sea during the environmental changes that occurred in this area, despite the extreme temperature and salinity conditions (minimum 17°C and 50‰). COTS larvae from the Pacific have been found to tolerate salinity values up to 45-50‰, although this considerably lowered their development rate and probability of survival (reviewed in Birkeland and Lucas 1990). Moreover, experimental studies found that larval development, at least from the Great Barrier Reef and Guam, only occurred over a very narrow temperature range, between 26 and 31°C (Lucas 1973; Yamaguchi 1973). The only data on larval development available from the Red Sea indicates that larvae were successfully reared at 28-29°C (Ormond and Campbell 1974), their tolerance to colder temperatures not being tested. The extremes of 17°C in the Red Sea during glaciations thus seem highly unfavourable for larval development, but restricting breeding to the summer months and possibly surviving in less extreme local refugia, which may have been present in the southernmost Red Sea (Behairy and Yusuf 1984), could have contributed to the survival of populations. Indeed, although our dataset only comprised two populations from the Red Sea, the low levels of diversity and the signature of a recent population expansion (Tab. 4.1) suggest that populations were more restricted in the past and recently expanded, possibly after the last glacial maximum when conditions became more favourable, as was observed for the endemic wrasse *Larabicus quadrilineatus* (Froukh and Kochzius 2007). Nonetheless, some degree of local adaptation to the more extreme salinity and temperature conditions must have occurred, considering many species that were not tolerant enough did not survive the glaciations (Fenton et al. 2000).

Extending the present sampling to the southern Red Sea, the Gulf of Aden and Socotra Island would probably allow shedding further light on these questions,

as well as identifying whether there is any current dispersal of larvae between the Indian Ocean and the Red Sea, or whether the Red Sea species is solely restricted to this basin.

4.2.5. The case of the Arabian Gulf

It is interesting to note that COTS appear to be virtually absent from the Arabian Gulf (Bernhard Riegl, Kaveh Samimi and Andrew Willson *pers. comm*.). Although Price and Rezai (1996) claimed to have found two COTS individuals at Tonb and Larak Islands (Fig. 4.3), this remains questioned to date or may only have been an exceptional sighting (Bernhard Riegl and Kaveh Samimi *pers. comm*.), which additionally was made at the entrance of the Gulf.

Because water depth in the Arabian Gulf does not exceed 100m, the Arabian Gulf was free of any marine influence during the last glacial maximum, and recolonisation by marine organisms could only have started 14'000 years ago as the Strait of Hormuz reopened with rising sea levels (Fig. 4.3; Lambeck 1996). The Arabian Gulf now supports coral reef communities, although lower in diversity than those in the adjacent Gulf of Oman, due to stressful temperatures (lowest ranging from 11.4 to 16°C in different parts of the Arabian Gulf, highest from 31.5 to 36.2°C) and salinity conditions (40-50‰; Sheppard et al. 2000). COTS' preferred preys in the Gulf of Oman (*Acropora* and *Montipora*; Glynn 1993) are in each case both present in the Arabian Gulf (Sheppard et al. 2000). Therefore, the absence of COTS in this area can either be explained by limitations in COTS' ability to disperse into this area, or by its inability to settle, survive or reproduce here after dispersal occurs.

Not only have corals recolonised the Arabian Gulf, but other organisms with similar distributions to COTS are also found in this area, such as the sea urchin *Diadema setosum*, which is thought to have entered the Arabian Gulf from the Red Sea following the opening of the Strait of Hormuz (Lessios et al. 2001).

This suggests that there is no obvious present-day barrier that would prevent larvae from dispersing into the Gulf. Indeed, there is inflow of surface waters from the Indian Ocean both during the Southwest and Northeast monsoons (Schott and McCreary 2001), which could transport COTS' negatively geotactic larvae (Yamaguchi 1973) into the Arabian Gulf. It is therefore tempting to suggest that the absence of COTS is due to its intolerance of the prevailing conditions in

the area. But COTS appear to have survived in similar conditions in the Red Sea during past glaciations (temperatures down to 17°C and salinity up to 50‰). However, the closest COTS populations to the Arabian Gulf are composed of individuals from the two Indian Ocean sister-species (Fig. 4.3). Unlike the Red Sea sister-species, they would not have gone through a history of exposure to extreme conditions, and may therefore not produce tolerant enough larvae that could cope with the high salinity and fluctuating temperatures of the Arabian Gulf. Differences in tolerance between the Red Sea and Indian Ocean sister-species might therefore explain or at least factor into the absence of COTS in this area.

4.2.6. Conclusions on the comparisons with other Indo-Pacific organisms

Comparing the four COTS sister-species' present genetic structure to that of other Indo-Pacific species allowed identifying what barriers contributed to shaping their present distribution. Distance alone did not seem to play a major role due to COTS' high dispersal potential and possibly the existence of a facultative teleplanic larva. On the other hand, not only land barriers and circulation patterns, but also cold-water upwelling appeared to play an important role in isolating populations, more so than in other species with at least superficially similar larval biology. The narrow temperature range within which COTS larvae from the Pacific were able to develop (26 to 31°C; Lucas 1973; Yamaguchi 1973) provided further support for a potentially profound impact of cold-water upwelling as a barrier to dispersal.

If the outbreaks that COTS are infamous for are not just a recent phenomenon (Birkeland and Lucas 1990), it is also conceivable that the stronger genetic structure observed within the Indian Ocean and in relation to other oceans in COTS, in comparison to other marine invertebrates with a high dispersal potential, was a consequence of this distinctiveness. Indeed, boom-and-bust taxa such as COTS that go through high population density fluctuations are expected to be particularly vulnerable to Allee effects, as their fertilisation rates exponentially decline with decreasing density (Uthicke et al. 2009). This would make them particularly vulnerable to adverse environmental conditions (Knowlton 2001), as for example encountered during glaciations. Small remaining populations may then be subjected to stronger genetic drift, accentuating the diversification process in con-

trast to species with different life histories.
The interpretation of these findings was however limited by the lack of comparative data, especially from the Indian Ocean. This highlighted the importance of performing equivalent studies with an extensive geographic coverage, as incomplete sample coverage can lead to a bias in the interpretation from such studies, as was apparent from the ambiguity surrounding the location and denomination of breaks in the Indian Ocean.
These comparisons also suggested that the Red Sea sister-species might have evolved a higher tolerance to extreme conditions than both Indian Ocean sister-species. To further investigate whether there were other indications of differences between the COTS species, the following section focussed on comparing the available information from the four sister-species.

4.3. Comparisons between the four sister-species

In this section, we aimed to determine whether information on differences between the COTS sister-species could be extracted from the considerable amount of research that has been performed on this species-complex. There were nearly a thousand articles published on COTS until 1990 (Birkeland and Lucas 1990), but there was a strong bias in this research in favour of the western Pacific. Birkeland and Lucas' comprehensive review (1990) was used here as the reference for Pacific studies until 1990.
Most aspects of COTS biology and ecology were covered either by extensive field and/or experimental studies in the Pacific, whereas only very little information was available from the other three sister-species, and a large proportion of it was based on occasional observations rather than thorough studies. This strong bias in favour of the Pacific Ocean could mostly be attributed to the fact that not only the first but also the most extensive outbreaks occurred in the Pacific Ocean, and that as a consequence large amounts of funding were made available and dedicated research groups set up to study COTS (Birkeland and Lucas 1990). Moreover, as COTS was until recently considered a single widespread species, similar biology and ecology was assumed throughout its range, and there was no apparent reason to reproduce the same type of studies elsewhere. And finally, the infrastructure and funding for research are much lower in both the Indian Ocean and the Red Sea, as already evidenced by the considerably lower number of stud-

ies on the phylogeography and population genetic structure of marine organisms from these areas.
There was a bit more information available from the Red Sea sister-species than the Indian Ocean ones, as in the early 1970s Cambridge University had a dedicated Coral Starfish Research Group working on COTS from the Red Sea. However, in contrast to the research performed over the next two decades mainly in Japan, Australia and Guam on the Pacific species, the data from these studies was mostly introductory, and again limited the potential for comparison.
We did however find a few differences or indications of potential differences between some or all of the four species. These were (1) morphological, (2) in some aspects of larval biology, (3) in some aspects of adult biology and (4) in the extent and intensity of outbreaks.

4.3.1. Morphology

There were some indications of differences in the number of arms between the different sister-species (Pacific: 23 max; Red Sea: 13 max). However, as pointed out by Birkeland and Lucas (1990), the aspect of morphological variation of particular interest was the difference in colour detected between the Pacific and the Indian Ocean. COTS in the Pacific Ocean are variable in colour but cryptic: they are usually gray-green to gray-purple, often with reddish papulae, that typically take on a 'bulls-eye' appearance due to two rings of darker papulae (Fig. 4.4a; Birkeland and Lucas 1990). Red Sea individuals are of a similar type of colour (Fig. 4.4b; JB *pers. obs.*; Birkeland and Lucas 1990). Individuals found in the northern Indian Ocean on the other hand tend to have a very different colour, sometimes referred to as 'electric blue' (Fig. 4.4c, 4.4d; CV, JB, GW *pers. obs.*; Birkeland and Lucas 1990). This 'electric blue' is however not found in the southern Indian Ocean, which, based solely on personal observations (CV, JB), appear to range from a light blue to a rusty colour (Fig. 4.4e, 4.4f). Whether these colours exactly match the genetic affiliation of each individual, especially in the contact zones of two species, is not clear.
There was no other information available on morphological characters that characterise the differences between *A. brevispinus* and *A. planci*, such as the shape of pedicellariae or spines (Madsen 1955), and thorough morphological comparisons may uncover new differences between the sister-species that were overlooked

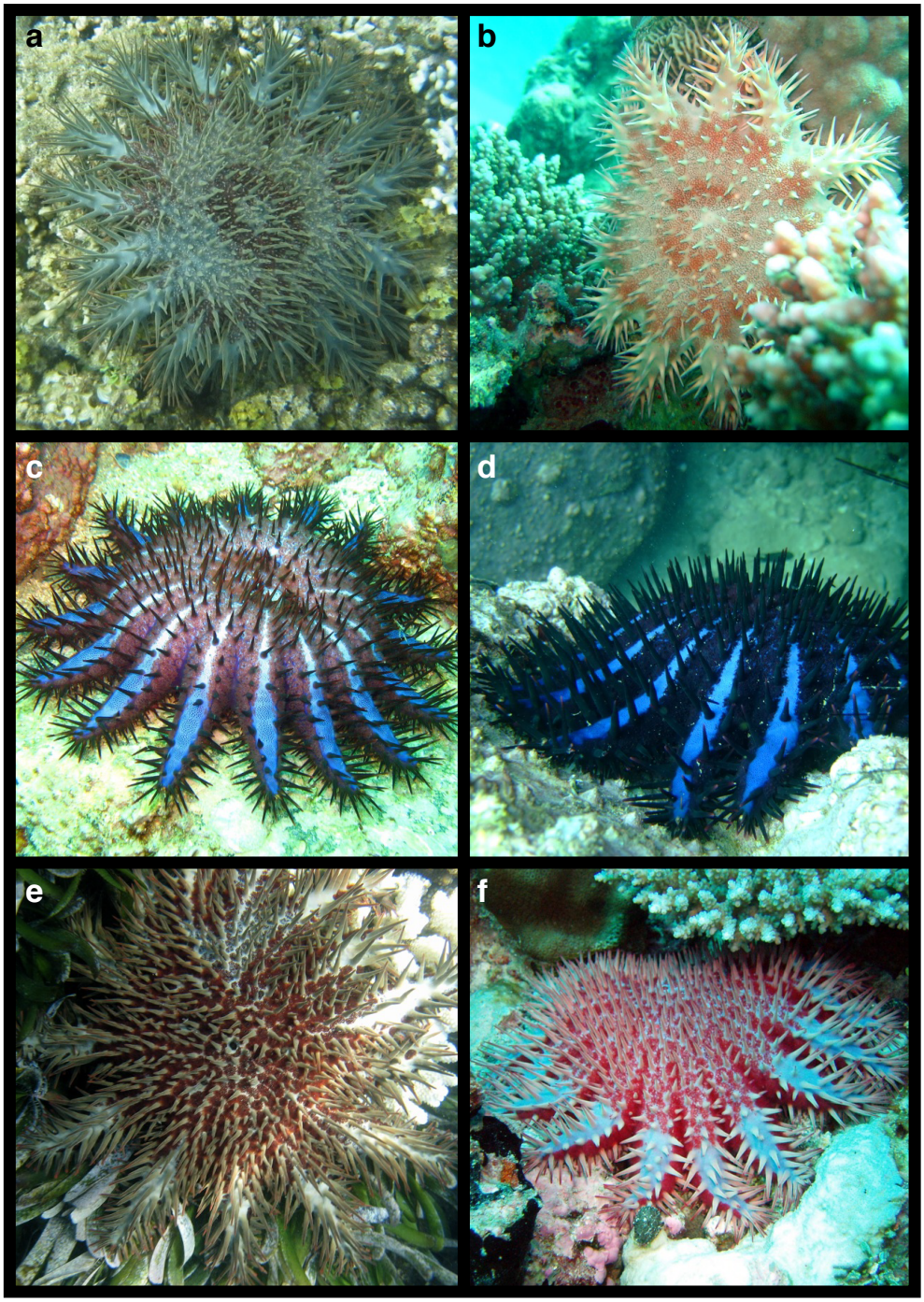

Figure 4.4. The four 'typical' colour morphs found in the sister-species: (a) Pacific (Fiji, credit: Nina Yasuda), (b) Red Sea (Egypt, credit: Jessica Bouwmeester), (c) and (d) Northern Indian Ocean (c: UAE, credit: Maral Shuriqi; d: Oman, credit: David Mothershaw), (e) and (f) Southern Indian Ocean (e: Kenya, credit: Kevin Ransom; f: Chagos Archipelago, credit: Anne Sheppard).

until now.

4.3.2. Larval biology

There was very little information available on larval morphology, dispersal, behaviour, physiology and feeding for any of the sister-species except the Pacific one. Larvae of the Red Sea sister-species were reared in the laboratory, however their physiological limits were not tested (Ormond and Campbell 1974). At 28-29°C, they settled after 17 days, which falls in the range found for Pacific COTS larvae (Birkeland and Lucas 1990). Pacific COTS have a very narrow temperature range in which they can be reared (between 26 and 31°C Lucas 1973; Yamaguchi 1973). The survival of COTS in the extreme conditions of the Red Sea during the Pleistocene sea-level changes suggests Red Sea COTS may have a larger tolerance than Pacific COTS, whereas the Indian Ocean sister-species are probably also quite sensitive to extreme temperatures, as neither of them occurs in the Arabian Gulf.

Pacific COTS larvae have been found to extend their developmental period to seven weeks in marginal food regimes (Lucas 1982), although the occurrence of a facultative teleplanic larva remains to be confirmed (Birkeland and Lucas 1990). Based on genetic data (chapter 3), COTS from the SIO sister-species were shown to also have the ability to extend their larval stage. It remains to be investigated whether this is the case for the other two species as well, as this might not be required due to smaller distances between reefs and thus not necessarily selected for in the long term (chapter 3).

4.3.3. Adult biology

There was a bit more information available on adult biology from areas outside the Pacific Ocean, however most of this information, especially that from the Indian Ocean, was based on field observations rather than controlled experiments. This limited the extent to which potential differences could be interpreted as real differences between the species rather than differences in the environment. Nevertheless, there was some support for differences in physiology, feeding preferences, behaviour and toxicity.

Physiology

Adults from the Red Sea may have an increased temperature tolerance to those from the Pacific Ocean, for the same reasons as mentioned before for the larvae. Indeed, Pacific COTS were found to stop feeding at 16°C (Birkeland and Lucas 1990), so the long-term survival of COTS in the Red Sea during past glaciations would probably have required a greater tolerance to cold temperatures.

Feeding preferences

Adult COTS feed on hermatypic scleractinian corals in all four sister-species (Birkeland and Lucas 1990; Ormond and Campbell 1971; Glynn 1993; Mendonça et al. 2010; Schleyer 2004). All four showed a marked preference for acroporids (*Acropora* and *Montipora*). However, Ormond and Campbell (1974) listed *Turbinaria* as a favoured species of coral. Pacific COTS almost never eat this species (Birkeland and Lucas 1990), which might point towards some differences in diet between the species, although it is unfortunately not completely clear whether *Turbinaria* were favoured in the Red Sea for shelter or as food.

Behaviour

There were however substantial behavioural differences between Red Sea and Pacific COTS. Red Sea COTS were clearly nocturnal, feeding at night and seeking shelter under coral during the day (Goreau 1964; Campbell and Ormond 1970; Ormond and Campbell 1974). The feeding and cryptic behaviour of Pacific COTS on the other hand was size-dependent, smaller individuals feeding nocturnally and remaining cryptic during the day, whereas larger individuals fed primarily during the day and were rarely cryptic (Keesing 1995). Keesing (1995) suggested that these patterns evolved as a predator avoidance strategy, with larger individuals being protected by their size and not requiring shelter. The little information available on predators revealed that the proportion of injured COTS in the Red Sea (25%; Ormond and Campbell 1974) was not higher than in the Pacific Ocean (17-60%; Birkeland and Lucas 1990), and the predators identified in the Red Sea were all also COTS predators in the Pacific (Ormond and Campbell 1974; Birkeland and Lucas 1990). This does however not exclude that predator pressure on large individuals is higher in the Red Sea, thus requiring this cryptic, nocturnal behaviour. Possible nocturnal behaviour was also suggested for COTS from

the NIO sister-species based on the disproportionate amount of feeding scars observed relative to the very few individuals spotted during the day (Glynn 1993).

Toxicity

The toxicity of Red Sea and Pacific COTS might also be different. There was no evidence for strong venom in Red Sea COTS, based on the reaction of divers to the penetration of spines: "although most members of the expedition were often pricked by their sharp spines, only one animal gave any real discomfort to both of the people who handled it" (Campbell and Ormond 1970). Pacific COTS on the other hand were found to inflict a range of different symptoms, from severe pain for several hours to persistent nausea and fever for several weeks or even permanent abscesses and bone-destroying processes (Birkeland and Lucas 1990). This was dependent on the number of spines penetrating, whether they broke off in the wounds, possible variability between individual starfish and different sensitivities of the victims (Birkeland and Lucas 1990). This suggests that either the Cambridge Coral Starfish Research Group members were all particularly insensitive to COTS-inflicted wounds, or that there was a difference in toxicity between the Red Sea and the Pacific COTS.

As toxicity is likely to evolve as a defence against predation, this could even be part of the reason why Red Sea COTS are nocturnal, as their defences may not be as efficient as Pacific COTS'. As overexploitation of predators was long one of the favoured possible causes of outbreaks (Birkeland and Lucas 1990) and has recently regained some support (Sweatman 2008), these differences in cryptic behaviour and toxicity may not be completely trivial. Indeed, outbreaks are much more intensive in the Pacific Ocean than in the Red Sea, and investigating whether predator pressure is higher in the latter may shed new light on this question.

4.3.4. Outbreak patterns

Zann (2000) observed that there had not been massive and widespread outbreak episodes of COTS in the Indian Ocean and the Red Sea, unlike what occurred in parts of the western Pacific. However, the limited and patchy information available restricted its interpretability. Since then, an increasing number of countries have reported 'outbreaks', 'aggregations', 'infestations', 'spot outbreaks', 'high

numbers' and 'increased densities' of COTS in different areas of the Indo-Pacific, not only from many Pacific Ocean locations but also from the Red Sea and the east African coast (Wilkinson 1998, 2000, 2002, 2004, 2008). The extent to which outbreak patterns can be compared between the four sister-species remains limited, due to the very patchy information, the absence of systematic surveys as well as the ambiguous definitions of what constitutes an outbreak. Nevertheless, the main trend identified by Zann (2000), that there were no reports of widespread, chronic infestations of COTS in the Indian Ocean and Red Sea, still appears to hold. It is conceivable that this is due to differences between the sister-species, but differences between the oceans and the presence, absence or differing intensity of outbreak triggers may also play a role.

Alhtough the causes of outbreaks are still unclear, the two hypotheses that have gained most support to date are (1) the 'larval starvation hypothesis', which argues that increased nutrient levels favour the survival of COTS larvae (Birkeland and Lucas 1990; Fabricius et al. 2010; Houk et al. 2007) and (2) the 'predator removal hypothesis', which suggests that the overexploitation of predators allows more larvae and juveniles to survive to maturity (Birkeland and Lucas 1990; Sweatman 2008). It is conceivable that some of the factors that are thought to play an important role in triggering outbreaks in the Pacific are of differing intensity in the other basins (e.g. terrestrial run-off, ocean productivity, predator removal). Comparative studies between the Red Sea, Indian and Pacific Oceans would allow gaining more insight into whether these hypotheses hold throughout the Indo-Pacific.

4.3.5. Conclusions on the differences between the four sister-species

Despite the limited amount of information available on COTS from the Red Sea and the Indian Ocean, several differences were identified that provide at least a little support for divergent biology and ecology of the four sister-species, and definitely warrant further investigation to assess whether these rather anecdotal observations truly hold.

Considering the suggested differences in colour patterns between the four sister-species, thorough morphometric analyses may uncover further morphological differences that characterise each species. The differences in adult behaviour and toxicity, as well as the potential differences in larval physiology, should also be

looked into more thoroughly, as these could both affect outbreak patterns according to the currently prevailing hypotheses on the causes of outbreaks ('predator removal hypothesis' and 'larval starvation hypothesis').

The larval and/or juvenile stages are thought to play a critical role in the causes of outbreaks (Birkeland and Lucas 1990; Houk et al. 2007; Sweatman 2008; Fabricius et al. 2010). These stages of COTS' life history were however the ones for which least comparative data were available outside the Pacific Ocean, and should ideally be a focus of future research.

Finally, to understand differences between the sister-species and the degree to which they have diverged, finding out to what extent they are still capable of interbreeding is also important. There was admixture between COTS species in two different areas: the Gulf of Oman, where the NIO and SIO sister-species co-occur (Fig. 4.3) and at the Indian/Pacific break, where individuals from the NIO and the Pacific sister-species were both present in only a few populations (Fig. 4.1). As mentioned before, this was remarkable, given the high dispersal achieved by COTS in other areas of its distribution (see section 4.2.1). This suggested that post-dispersal processes were more likely to provide an explanation for the maintenance of only a restricted overlap of these species than were barriers to dispersal. Unfortunately, due to its maternal inheritance mode, data from mitochondrial DNA did not allow unambiguously determining whether reproductive barriers were present.

Data from biparentally inherited allozymes suggested there was little gene exchange between the Indian and Pacific Ocean populations, but that there may have been introgression in the mixed population of Pulau Seribu (Benzie 1999). However, the data was deemed inconclusive due to small sample sizes, and the potential incomplete segregation of ancestral genotypes (Benzie 1999). Further research will thus be necessary to determine whether there are hybridisation zones in the contact areas.

Different methods could be used to test for hybridisation between the sister-species. Mating trials would allow determining whether COTS from different species can produce viable offspring. Lucas and Nash (1985) crossed *A. planci* (from the Pacific sister-species) with *A. brevispinus*, the short-spined sister-species of the COTS species complex. They found that first-generation hybrids were viable, and reared them to maturity to produce second-generation hybrids and

back-crosses. Both the second-generation hybrids and the back-crosses were of poor viability and some of them showed morphological abnormalities, which suggested there were barriers to reproduction between *A. brevispinus* and COTS. These experiments were however very time- and resource-intensive (they ran for over eight years). Using indirect genetic methods to assess the extent to which hybridisation may occur between the COTS sister-species may therefore be preferable, for example using rapidly evolving nuclear markers such as microsatellites to determine if there is genetic introgression in the areas where two species overlap.

4.4. Final conclusions

By reviewing the information available from other Indo-Pacific organisms and comparing it to our findings from the three previous chapters, we determined that past sea level changes, land bridges, circulation patterns and cold-water upwelling played an important role in shaping the crown-of-thorns starfish species complex. But the strong population fluctuations that it is infamous for could have further contributed to the formation of the four species, as the genetic structure of the crown-of-thorns starfish was more pronounced than that of other invertebrates with similar life histories. The interpretation of these observations was however strongly limited by the lack of comparative data, and highlighted the importance of performing equivalent studies that include species' entire ranges to further increase our understanding of evolution in the marine realm.

We then reviewed and compared the information available on the biology and ecology of the four different sister-species, to determine whether differences between them may have been overlooked while the crown-of-thorns starfish was considered a single widespread species. As the overwhelming majority of studies stemmed from the Pacific Ocean, very little comparative material was available, but we were nonetheless able to uncover a few differences in morphology, cryptic behaviour, toxicity, larval and adult temperature tolerance, and in the extent of outbreaks. It cannot be excluded that differing local conditions were driving some of these differences. Nonetheless, the differences in for example morphology (colour) and defence mechanisms (toxicity and cryptic behaviour) are unlikely to be due to differing local conditions, and suggest the crown-of-thorns starfish sister-species have diverged in more than their genetic structure.

Summary of results

By exploring the phylogeography of the crown-of-thorns starfish *Acanthaster planci* throughout its entire Indo-Pacific range with a mitochondrial marker that is commonly used to discriminate species (e.g. in DNA barcoding studies), we first discovered that, unlike what was previously thought, *A. planci* was not a single widespread species but instead formed a species complex (**chapter 1**). Although cryptic speciation is a widespread phenomenon in the marine realm, this finding was quite surprising for an organism as extensively studied as *A. planci* over the last decades.

We then focused on the phylogeography of the Pacific sister-species (**chapter 2**), which had already been the subject of numerous studies investigating its genetic structure. However, by increasing the sample coverage and using a mitochondrial marker (the putative control region) with a higher resolution than the allozymes used in previous studies, we were able to establish that the Pacific sister-species not only had a high dispersal potential, but also achieved it, as evidenced by the signature of ongoing gene flow between areas that were isolated in the past, and by the high levels of connectivity, even among distant populations.

In **chapter 3**, we concentrated on the two sister-species found in the Indian Ocean. This was the first comprehensive phylogeographic study of a coral reef organism in this comparatively under-researched ocean, and thus a unique opportunity to explore the potential factors that shape the genetic structure of organisms in this area. Past and present surface circulation patterns in conjunction with ocean primary productivity were identified as key processes in shaping the genetic structure between and within both sister-species, and the strong contrasts between them indicated they are now on different evolutionary trajectories.

In **chapter 4**, we reviewed the information available on other Indo-Pacific organisms and on the biology of the different crown-of-thorns starfish sister-species to

understand more about the evolution of this species complex. We determined that past sea level changes, land bridges, circulation patterns and cold-water upwelling played an important role in shaping its genetic structure. But the strong population fluctuations that the crown-of-thorns starfish is infamous for could have further contributed to the formation of the four species, as its genetic structure was more pronounced than that of other invertebrates with similar life histories. We also uncovered differences in the biology and ecology of the four sister-species, although a lack of comparative data strongly limited the interpretability of these findings, but nonetheless indicated that the sister-species are not only on different evolutionary trajectories but have also diverged in some aspects of their biology (e.g. morphology and defence mechanisms).

Although the crown-of-thorns starfish is arguably one of the most researched marine organisms to date, by performing a thorough phylogeographic study including this organism's entire distribution and markers with different resolutions, we were able to uncover new and unexpected aspects of crown-of-thorns starfish biology, as well as determine future research directions that will hopefully allow increasing our understanding of this threat to coral reefs. Moreover, our extensive dataset also revealed some new phylogeographic patterns in comparatively under-researched areas of the Indo-Pacific. Performing similar analyses with extensive datasets for other organisms should allow increasing our understanding of what drives evolution in the marine realm.

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Appendixes

Table S1.1. Sampling locations of crown-of-thorns starfish individuals, with coordinates (decimal degrees), number of COI sequences (*n*) per clade and location, and EMBL accession numbers. Locations preceded by an asterisk are represented in two clades. Numbers in square brackets indicate references for previously sampled material: [1] (Benzie 1999), [2] (Benzie and Wakeford 1997), [3] (Johnson and Stoddart 1988), [4] (Yasuda et al. 2006), [5] (Himeno et al. 1987); and the collectors of additional samples are identified as follows: (a) Harilaos Lessios, (b) Molly Timmers, (c) Serge Planes, (d) Gustav Paulay, (e) Peter Schupp, (f) Elisabeth Illidge-Evans, (g) Gordon Kirkwood, (h) Catherine Vogler, (i) Sven Uthicke, (j) Lyndon de Vantier, (k) Alexander Keck.

Location per clade	Latitude	Longitude	Sampled by	*n*	Accession number
Pacific				**108**	
Isla del Coco	5.534	-87.060	(a)	13	FM202076-88
Gulf of Chiriqui	7.473	-82.235	(a)	2	FM202089-90
Hawaii Island	19.696	-155.387	(a)	5	FM202070-74
Johnston Reef	16.727	-169.533	(b)	7	FM174472-78
Kingman Reef	6.386	-162.354	(b)	8	FM174479-86
Swains Is.	-11.056	-171.076	(b)	8	FM174487-94
Moorea	-17.533	-149.839	(c, d)	6	FM174495-99, 202075
Guam	13.444	144.794	(e)	8	FM174500-07
Japan	26.472	127.983	[1]	6	FM174508-13
Lizard Is.	-14.673	145.453	[2]	8	FM174514-21
Enderby Is.	-20.595	116.525	[3]	8	FM174522-29
Gove	-12.254	136.837	[1]	7	FM174530-36
Vanuatu	-15.377	166.959	[1]	7	FM177190-96
Philippines	9.082	123.273	[1]	7	FM177197-203
*Pulau Seribu	-5.712	106.597	[1]	8	FM174537-44
Northern Indian Ocean				**48**	
*Pulau Seribu	-5.711	106.597	[1]	2	FM174545-46
Christmas Is.	-10.447	105.690	(f)	3	FM174547-49
Thailand	7.580	98.522	[1]	11	FM174550-60
Maldives	3.203	73.221	[1]	13	FM174561-73
*UAE	25.483	56.362	(g, h)	10	FM174574-83
*Oman	23.828	58.143	(h)	9	FM174584-92
Southern Indian Ocean				**52**	
*UAE	25.323	56.382	(g, h)	2	FM174593-94
*Oman	23.828	58.143	(h)	2	FM174595-96
Kenya	-4.053	39.673	(h)	23	FM174597-619
South Africa	-27.915	32.564	[1]	9	FM174620-28
Reunion	-21.115	55.536	(i)	5	FM174629-33
Mauritius	-20.348	57.552	[1]	5	FM174634-38
Cocos-Keeling Is.	-12.164	96.871	[1], (f)	6	FM174639-44
Red Sea				**29**	
Wajh Bank	26.227	36.380	(j)	20	FM174645-64
Sinai	28.508	34.516	(k)	9	FM174665-73
Outgroups					
Acanthaster brevispinus				**3**	
Great Barrier Reef			[1]	2	FM174674-75
Japan			[4]	1	AB231476
Patiria pectinifera			[5]	1	D16378

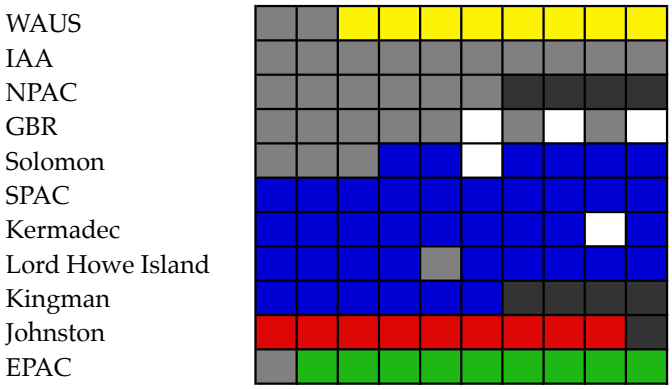
Table S2.1 (next page)**.** Sampling locations of crown-of-thorns starfish individuals from the Pacific sister-species, with population number for AMOVA test (#; Tab. S2.2), coordinates (decimal degrees), collector or reference and number of samples *n*. Locations followed by an asterisk are shared with the Northern Indian Ocean sister-species (chapter 3).

Group Subgroup	Location	#	Lat	Long	Sampled by/ref	*n*
West Pacific						584
Western Australia						39
	Enderby Island	1	-20.595	116.525	Johnson and Stoddart 1988	20
	Cassini	2	-13.946	125.634	WA Museum	3
	Gove	3	-12.254	136.837	Benzie 1999	16
Malaysia		4	3.388	103.656	Benzie 1999	13
Central-West Pacific						340
	Krakatau*	5	-6.047	105.549	P. Barber	4
	Pulau Seribu*	6	-5.711	106.597	P. Barber, Benzie 1999	28
	Karimunjawa*	7	-5.835	110.439	P. Barber	35
	Bali	8	-8.746	115.545	P. Barber	2
	Lombok	9	-8.351	116.050	P. Barber	3
	South Sulawesi	10	-5.566	119.427	P. Barber	23
	Flores	11	-8.515	121.411	P. Barber	19
	Philippines	12	9.082	123.273	Benzie 1999	14
	North Sulawesi	13	1.496	124.838	P. Barber	17
	Talaud Islands	14	3.581	125.480	P. Barber	16
	North Halmahera	15	1.926	127.776	P. Barber	17
	South Halmahera	16	-0.899	127.865	P. Barber	13
	Raja Ampat	17	-0.405	130.471	P. Barber	32
	Fakfak	18	-2.826	132.280	P. Barber	17
	Kaimana	19	-3.661	133.775	P. Barber	20
	Manokwari	20	-0.875	134.100	P. Barber	17
	Japan	21	26.472	127.983	Benzie 1999	19
	Palau	22	7.478	134.549	N. Yasuda	28
	Guam	23	13.444	144.794	P. Schupp	16
Cenderawasih Bay						55
	Teluk Cenderawasih	24	-2.391	134.553	P. Barber	14
	Nabire	25	-3.361	135.496	P. Barber	4
	Biak	26	-1.189	136.084	P. Barber	24
	Yapen	27	-1.910	136.224	P. Barber	13
Eastern-West Pacific						137
	Lizard Island	28	-14.673	145.453	Benzie and Wakeford 1997	13
	Rib Reef	29	-18.478	146.873	Lyndon de Vantier	14
	Helix Reef	30	-18.563	147.501	Benzie and Stoddart 1992a	16
	Stanley Reef	31	-19.298	148.135	Benzie and Stoddart 1992a	12
	Swains Reef	32	-22.174	152.604	Benzie and Stoddart 1992a	14
	Solomon Islands	33	-9.646	160.156	P. Barber	14
	Pohnpei	34	6.867	158.209	N. Yasuda 2007	28
	Majuro	35	7.115	171.247	N. Yasuda 2007	26
Central Pacific						66
	Lord Howe Island	36	-31.552	159.081	De Vantier and Deacon 1990	7
	Vanuatu	37	-15.377	166.959	Benzie 1999	9
	Fiji	38	-16.578	179.414	P. Barber	4
	Kermadec Islands	39	-29.206	-177.934	R. Babcock	7
	Swains Island	40	-11.056	-171.076	M. Timmers	10
	Moorea	41	-17.533	-149.839	S. Planes	5
	Kingman Reef	42	6.386	-162.354	M. Timmers	24
Johnston Atoll		43	16.727	-169.533	M. Timmers	9
East Pacific						14
	Isla del Coco	44	5.534	-87.060	H. Lessios	12
	Gulf of Chiriqui	45	7.473	-82.235	H. Lessios	2

Table S2.2. Design of the ten best performing AMOVA tests (a) within the Pacific, (b) within the West Pacific, ranked according to the percent variation among regional groups they explained. Identical colours indicate the composition of the different groups in each test. The population within each group are detailed under the tables, the numbers refer to population numbers in Tab. S2.1.

(a)

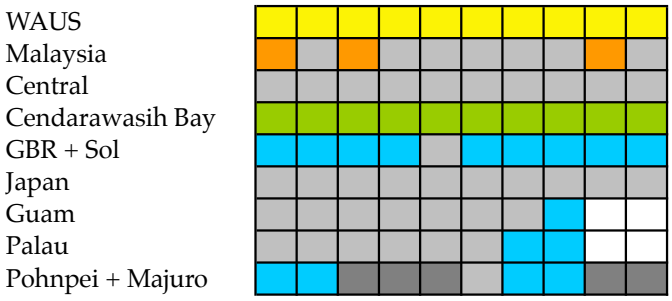
Rank	1	2	3	4	5	6	7	8	9	10
Percent variation between groups	27.99	26.46	24.12	21.27	20.94	19.34	15.42	14.92	13.01	9.62



WAUS: 1-3; IAA: 4-20 & 24-27; NPAC: 21-23 & 34-35;
GBR: 28-32; SPAC: 37-38 & 40-41; EPAC: 44-45

(b)

Rank	1	2	3	4	5	6	7	8	9	10
Percent variation between groups	12.09	12.06	11.66	11.59	11.37	11.28	11.16	10.90	10.81	10.66



WAUS: 1-3; Central: 5-20; Cenderawasih Bay: 24-27;
GBR+Sol: 28-33

Table S2.3 (next page)**.** Summary statistics for crown-of-thorns starfish sampling locations of the Pacific sister-species: number of samples n, number of unique haplotypes n_h, haplotype diversity h, nucleotide diversity π, Fu's F_S and Ramos-Onsins R_2 (significant values in bold). Locations followed by an asterisk are shared with the Northern Indian Ocean sister-species (chapter 3).

Group	Subgroup	Location	n	n_h	h (± stdev)	π (± stdev)	F_s	R_2
West Pacific								
	Western Australia							
		Enderby Island	20	12	0.874 (± 0.0653)	0.007 (± 0.0040)	**-4.55**	0.087
		Cassini	3	3	1.000 (± 0.2722)	0.010 (± 0.0086)	0.46	0.386
		Gove	16	14	0.983 (± 0.0278)	0.021 (± 0.0114)	-3.64	0.136
	Malaysia		13	7	0.846 (± 0.0758)	0.013 (± 0.0075)	1.09	0.142
	Central-West Pacific							
		Krakatau*	4	3	0.833 (± 0.2224)	0.009 (± 0.0065)	1.34	0.280
		Pulau Seribu*	28	25	0.989 (± 0.0138)	0.021 (± 0.0108)	**-12.32**	**0.056**
		Karimunjawa*	35	32	0.993 (± 0.0094)	0.023 (± 0.0118)	**-18.89**	**0.061**
		Bali	2	2	1.000 (± 0.5000)	0.031 (± 0.0323)	2.77	0.500
		Lombok	3	3	1.000 (± 0.2722)	0.024 (± 0.0205)	1.44	0.164
		South Sulawesi	23	21	0.992 (± 0.0154)	0.024 (± 0.0123)	**-8.43**	0.080
		Flores	19	13	0.953 (± 0.0305)	0.020 (± 0.0108)	-1.11	0.084
		Philippines	14	14	1.000 (± 0.0270)	0.026 (± 0.0138)	**-5.32**	**0.072**
		North Sulawesi	17	16	0.993 (± 0.0230)	0.019 (± 0.0104)	**-6.69**	**0.080**
		Talaud Islands	16	16	1.000 (± 0.0221)	0.020 (± 0.0109)	**-8.22**	**0.071**
		North Halmahera	17	17	1.000 (± 0.0202)	0.023 (± 0.0124)	**-8.42**	**0.076**
		South Halmahera	13	13	1.000 (± 0.0302)	0.023 (± 0.0123)	**-5.12**	**0.086**
		Raja Ampat	32	27	0.984 (± 0.0144)	0.025 (± 0.0130)	**-10.61**	0.077
		Fakfak	17	15	0.985 (± 0.0252)	0.025 (± 0.0131)	-3.64	**0.075**
		Kaimana	20	16	0.974 (± 0.0250)	0.025 (± 0.0130)	-2.86	0.079
		Manokwari	17	15	0.978 (± 0.0313)	0.025 (± 0.0132)	-3.81	0.083
		Japan	19	18	0.994 (± 0.0193)	0.018 (± 0.0096)	**-9.03**	**0.059**
		Palau	28	25	0.992 (± 0.0118)	0.023 (± 0.0117)	**-11.42**	0.076
		Guam	16	16	1.000 (± 0.0221)	0.019 (± 0.0102)	**-8.56**	0.087
	Cenderawasih Bay							
		Teluk Cenderaw.	14	9	0.934 (± 0.0448)	0.019 (± 0.0106)	0.48	0.106
		Nabire	4	4	1.000 (± 0.1768)	0.022 (± 0.0156)	0.48	0.108
		Biak	24	18	0.953 (± 0.0320)	0.023 (± 0.0121)	-3.31	0.100
		Yapen	13	11	0.974 (± 0.0389)	0.023 (± 0.0139)	-1.27	0.118
	Eastern-West Pacific							
		Lizard Island	13	13	1.000 (± 0.0302)	0.017 (± 0.0095)	**-6.66**	0.094
		Rib Reef	14	13	0.989 (± 0.0314)	0.015 (± 0.0085)	**-5.35**	**0.087**
		Helix Reef	16	16	1.000 (± 0.0221)	0.020 (± 0.0109)	**-8.40**	**0.083**
		Stanley Reef	12	12	1.000 (± 0.0340)	0.016 (± 0.0091)	**-5.70**	**0.080**
		Swains Reef	14	14	1.000 (± 0.0270)	0.021 (± 0.0115)	**-6.41**	0.130
		Solomon Islands	14	14	1.000 (± 0.0270)	0.017 (± 0.0092)	**-7.45**	**0.081**
		Pohnpei	28	23	0.984 (± 0.0144)	0.018 (± 0.0095)	**-6.67**	0.075
		Majuro	26	22	0.985 (± 0.0160)	0.018 (± 0.0094)	**-9.80**	0.083
Central Pacific								
		Lord Howe Island	7	7	1.000 (± 0.0764)	0.043 (± 0.0248)	-0.40	0.160
		Vanuatu	9	9	1.000 (± 0.0524)	0.052 (± 0.0286)	-0.91	0.152
		Fiji	4	3	0.833 (± 0.2224)	0.023 (± 0.0160)	2.91	0.283
		Kermadec Islands	7	6	0.952 (± 0.0955)	0.004 (± 0.0030)	**-3.27**	**0.109**
		Swains Island	10	9	0.978 (± 0.0540)	0.042 (± 0.0227)	-0.06	0.162
		Moorea	5	5	1.000 (± 0.1265)	0.045 (± 0.0279)	0.67	0.154
		Kingman Reef	24	12	0.906 (± 0.0380)	0.034 (± 0.0175)	3.17	0.138
Johnston			9	8	0.972 (±0.0640)	0.011 (±0.0065)	-2.46	0.110
East Pacific								
		Isla del Coco	12	6	0.758 (± 0.1221)	0.003 (± 0.0022)	-2.15	0.131
		Gulf of Chiriqui	2	2	1.000 (± 0.5000)	0.002 (± 0.0028)	0.00	0.500

Table S3.1. Sampling locations of crown-of-thorns starfish individuals, with coordinates (decimal degrees), collector or reference, number of CoReg sequences (n_{Coreg}) and number of COI sequences (n_{COI}) per clade and location. Locations preceded by an asterisk are represented in both Indian Ocean sister-species, locations preceded by a dash are shared with the Pacific sister-species (chapter 2).

Location	Latitude	Longitude	Sampled by/ref	n_{CoReg}	n_{COI}
Southern Indian Ocean				**95**	**57**
*UAE	25.323	56.382	G. Kirkwood, C. Vogler	2	2
*Oman	23.828	58.143	C. Vogler	2	2
Réunion	-21.115	55.536	S. Uthicke	5	5
Mauritius	-20.348	57.552	Benzie 1999	4	4
Kenya	-4.053	39.673	C. Vogler	24	23
South Africa	-27.915	32.564	Benzie 1999	12	9
Mayotte	-12.724	45.149	K. Gérard	21	-
Nth Madagascar	-12.891	48.604	K. Gérard	11	-
Sth Madagascar	-22.075	43.242	C. Sheppard	2	-
Chagos	-6.638	71.314	C. Sheppard	6	6
Cocos Keeling Islands	-12.164	96.871	L. Illidge-Evans, Benzie 1999	6	6
Northern Indian Ocean				**95**	**48**
*UAE	25.323	56.382	G. Kirkwood, C. Vogler	15	10
*Oman	23.828	58.143	C. Vogler	9	9
Maldives	3.203	73.221	Benzie 1999	17	13
Christmas Island	-10.447	105.690	L. Illidge-Evans	3	3
Aceh	3.954	96.649	P. Barber	15	-
Thailand	7.580	98.522	Benzie 1999	16	11
#Pulau Seribu	-5.711	106.597	Benzie 1999, P. Barber	12	2
#Karimunjawa	-5.835	110.439	P. Barber	5	-
#Krakatau	-6.047	105.549	P. Barber	3	-

Table S3.2. Run conditions for the Indian Ocean sister-species MIGRATE analyses.

	SIO	**NIO**
Mutation model	F84	F84
Transition/transversion ratio	17.243159	11.329992
Site rate modifier (4 groups)	0.322599 1.744942 4.533686 9.388189	0.323263 1.734373 4.495796 9.299330
Probabilities of site rates	0.602957 0.357582 0.038922 0.000540	0.600390 0.359699 0.039363 0.000548
Prior distribution for mutation-scaled population size	Uniform with range 0.0 to 1.5	Uniform with range 0.0 to 1.0
Prior distribution for mutation-scaled migration rates	Uniform with range 0.0 to 5000	Uniform with range 0.0 to 2000
Proposal distribution for parameters and geneaologies	Slice sampling	Slice sampling
Increment between samples	200	200
Samples per replicate	5'000	5'000
Burn-ins per replicate	100'000	100'000
Replicates	32	32

Table S3.3. Pairwise Φ_{ST} values for the (a) Northern and (b) Southern Indian Ocean sister-species.

(a) Northern Indian Ocean

	UAE	Oman	Maldives	Thailand	Aceh	XmasIs	Seribu	Krakatau
Oman	0.010							
Maldives	**0.551**	**0.505**						
Thailand	**0.776**	**0.757**	**0.157**					
Aceh	**0.741**	**0.716**	0.077	0.039				
Christmas Island	**0.893**	0.905	0.433	0.372	**0.454**			
Seribu	**0.777**	**0.756**	0.129	-0.037	0.025	0.401		
Krakatau	**0.799**	0.776	0.116	-0.050	0.002	0.333	-0.005	
Karimunjawa	**0.796**	**0.777**	0.068	0.075	-0.010	0.524	-0.002	0.021

Values in bold were significant after Bonferroni correction: p<0.00139.

(b) Southern Indian Ocean

	UAE	Oman	Chagos	CocosIs	SthAfrica	Kenya	Mauritius	Reunion	Mayotte	NthMadagascar
Oman	0.000									
Chagos	0.070	0.149								
CocosIs	0.623	0.508	0.249							
SthAfrica	0.032	0.086	-0.013	0.174						
Kenya	-0.059	0.091	-0.017	0.144	0.013					
Mauritius	0.399	0.263	0.069	0.545	-0.012	0.079				
Reunion	0.116	0.178	-0.059	0.306	-0.035	-0.015	0.177			
Mayotte	-0.108	0.054	0.005	0.167	0.021	-0.002	0.137	-0.015		
NthMadagascar	-0.143	0.016	-0.020	0.177	0.029	-0.023	0.117	0.006	-0.037	
SthMadagascar	-0.042	-0.667	0.165	0.444	0.148	0.128	0.258	0.194	0.122	0.049

Values in bold were significant after Bonferroni correction: p<0.0009.

Figure S3.1. NeighborNet analyses of the (a) Northern and (b) Southern Indian Ocean sister-species. The two main clades within each species are highlighted, and the central Cocos-Keeling Island haplotypes in the E_{SIO} clade are surrounded by a grey box.

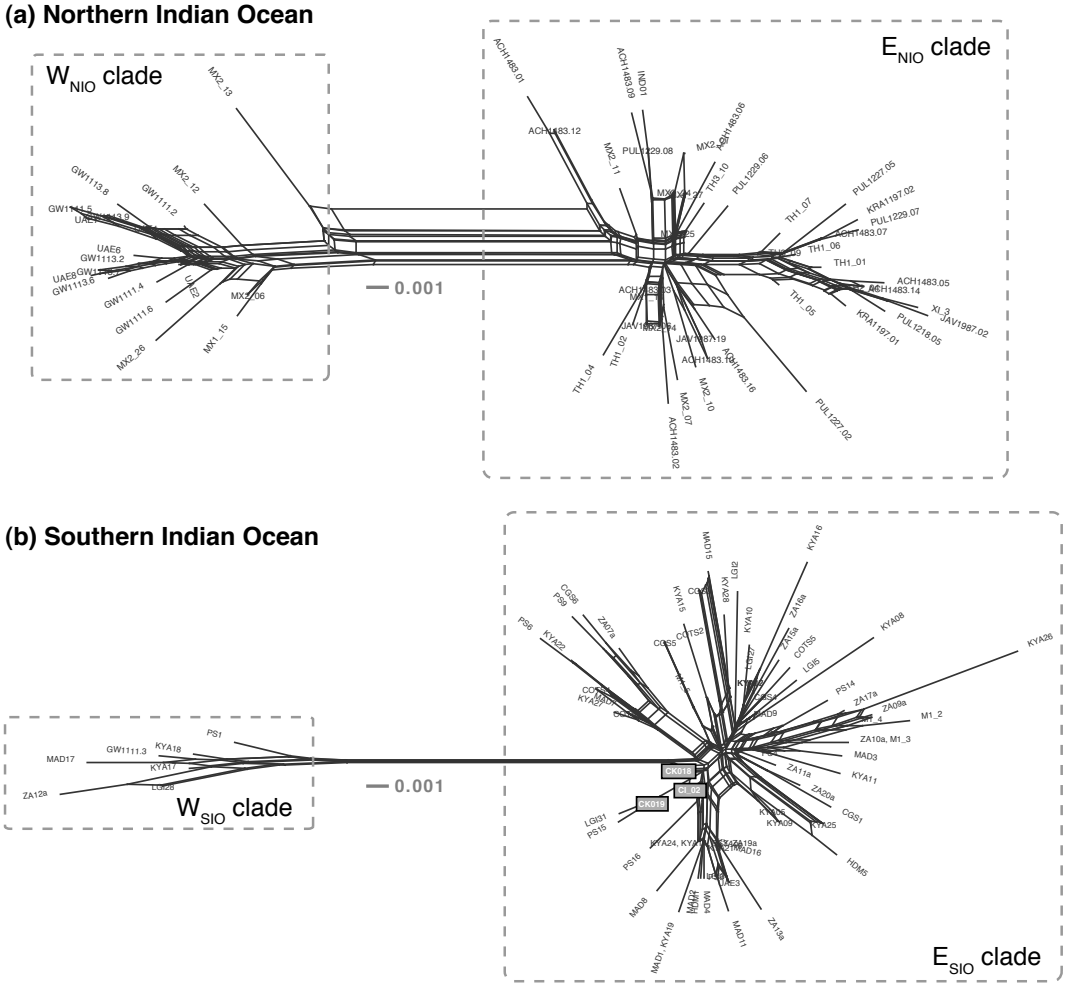
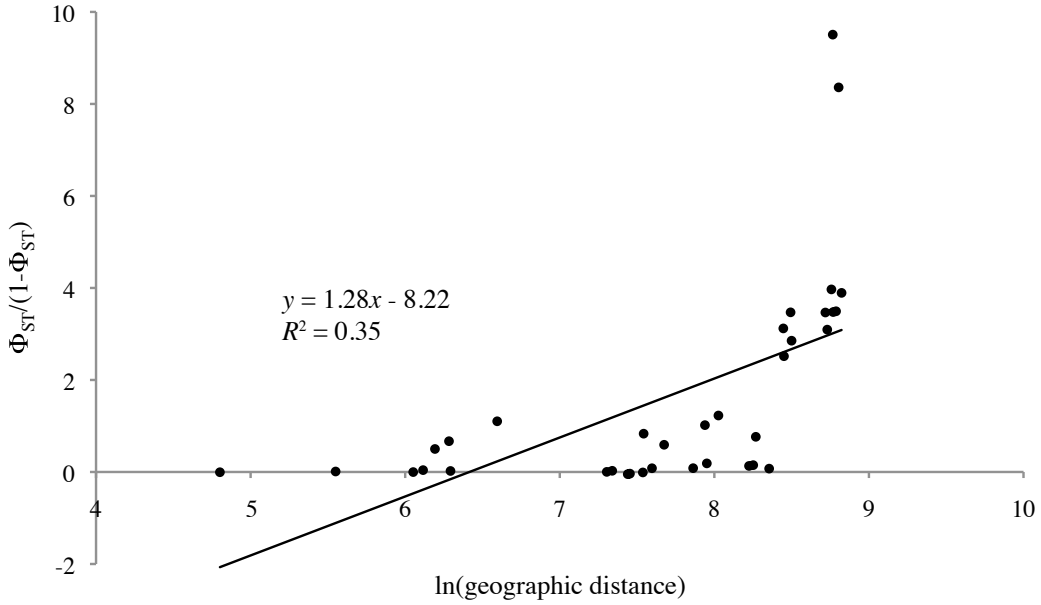


Figure S3.2. Genetic distance $\Phi_{ST}/(1 - \Phi_{ST})$ as a function of the natural logarithm of geographic distance (in km) for the (a) Northern and (b) Southern Indian Ocean sister-species.

(*a*) **Northern Indian Ocean**



(*b*) **Southern Indian Ocean**

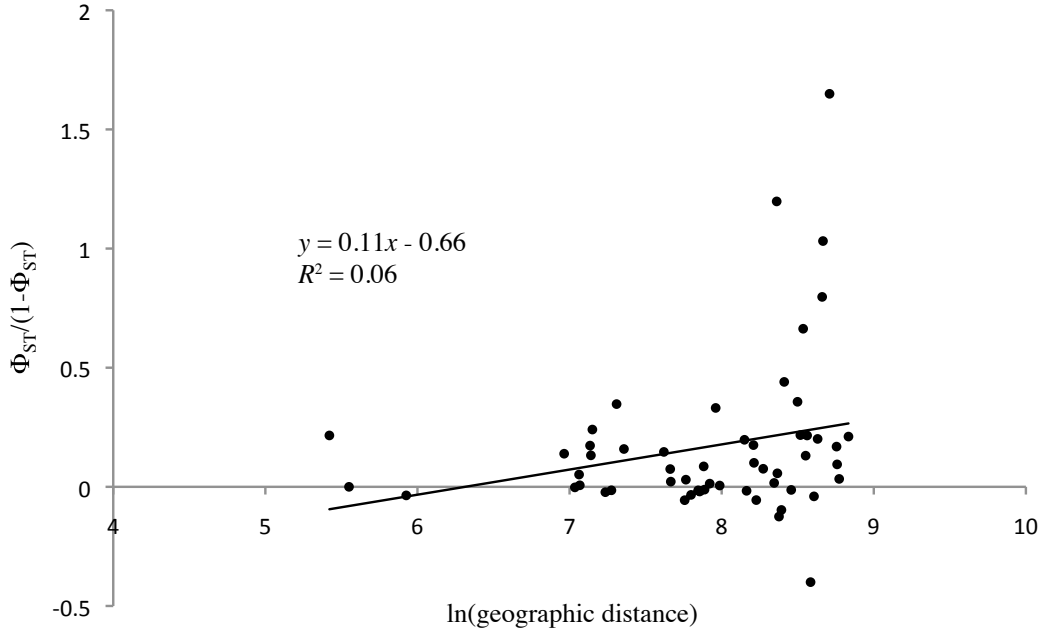
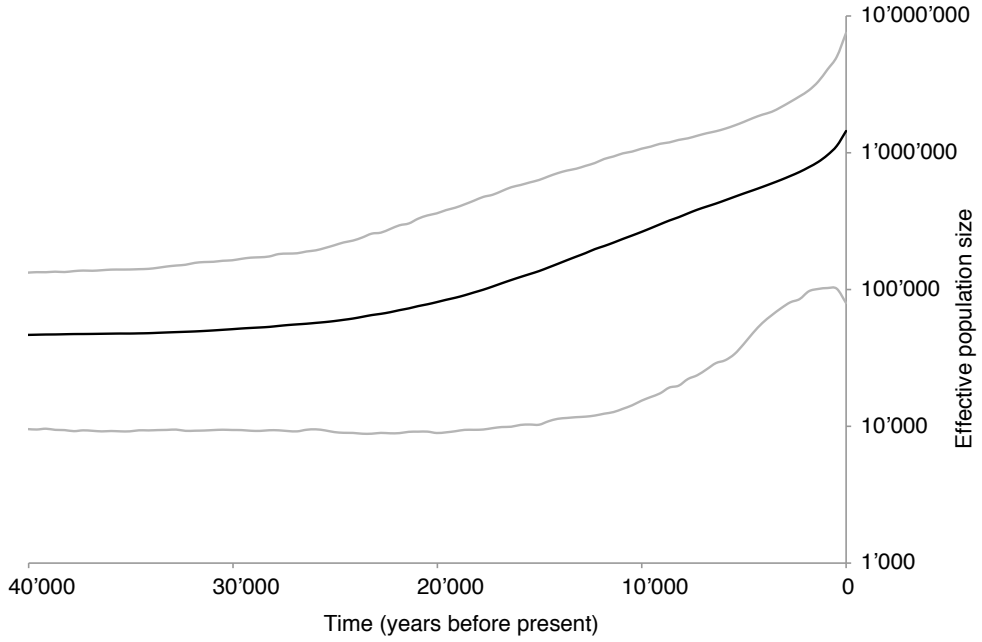


Fig. S3.3. Bayesian skyline plots for the (a) Northern and (b) Southern Indian Ocean sister-species. Black lines are an estimate of effective population size as a function of time, grey lines indicate the 95% upper and lower highest posterior probability interval.

(a) **Northern Indian Ocean**



(b) **Southern Indian Ocean**

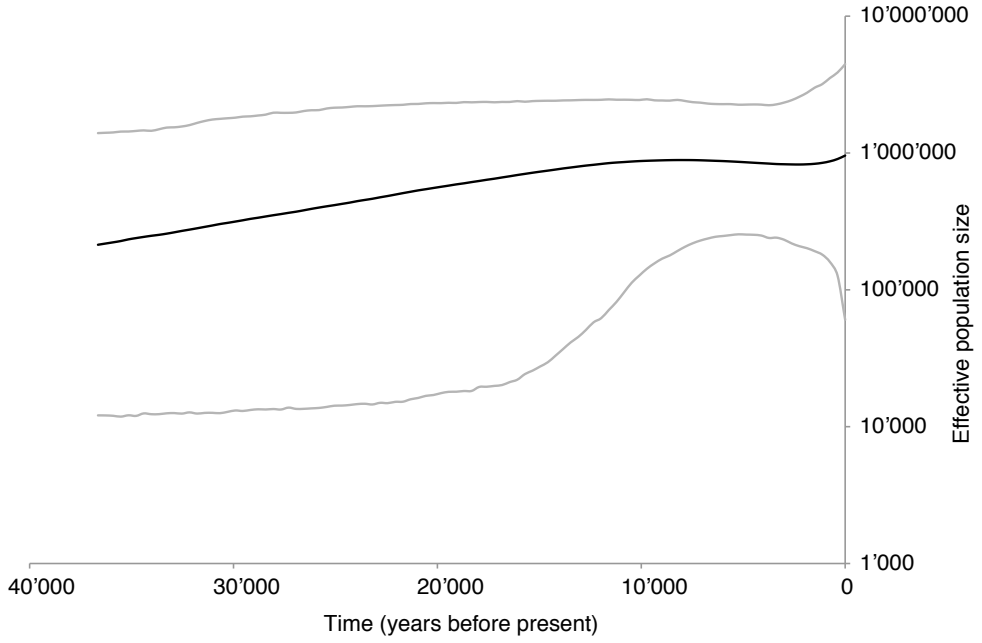


Table S4.1 (next pages)**.** Population genetic and phylogeographic studies containing more than two populations from the Indian Ocean. Regions are defined as: IO: Indian Ocean, Pac: Pacific Ocean, Atl: Atlantic Ocean, RS: Red Sea, Med: Mediterranean Sea. Locations are defined as: SA: South Africa+Mozambique; EA: Tanzania (incl. Zanzibar)+Kenya; SWI: south-western islands; Mas: Mascarene Islands; Sey: Seychelles; GO: Gulf of Oman; CA: Chagos Archipelago; Mal: Maldives; ISL: India+Sri Lanka; And: Andaman Sea; CK: Cocos Keeling Islands; XI: Christmas Island; NWA: north-western and western Australia.

Table 4.2. continued

	Species	Common name	Group	Study coverage	Localities IO	Marker	Reference
Pelagic organisms	*Acanthocybium solandri*	pelagic wahoo	Fish	IO, Pac, Atl	SA, Sey, And	mtDNA (cytB) and nuclear intron	(Theisen et al. 2008)
	Megaptera novaeangliae	humpback whale	Whale	IO, Atl	SA, SWI, GO	mtDNA (CoReg)	(Rosenbaum et al. 2009)
	Rhincodon typus	whaleshark	Fish	IO, Pac, Atl	SA, EA, NWA	mtDNA (CoReg)	(Castro et al. 2007)
	Xiphias gladius	swordfish	Fish	IO, Pac, Med	SWI, Mas, CA, And, NWA	mtDNA (CoReg)	(Lu et al. 2006)
	Xiphias gladius	swordfish	Fish	IO	SWI, Sey, Mas	mtDNA (CoReg) and microsats	(Muths et al. 2009)
Homogeneous across Indo-Pacific	*Echinolittorina reticulata*	snails	Mollusc	IO, Pac, RS	SWI, Mas, ISL	mtDNA (COI and 12S) and nuclear (28S)	(Reid et al. 2006)
	Gymnothorax spp.	moray eels	Fish	IO, Pac	SA, Sey	mtDNA (COI and cytB) and nuclear loci	(Reece et al. 2010)
	Lutjanus kasmira	snapper	Fish	IO, Pac	SA, Sey, CA, CK, XI	mtDNA (cytB) and nuclear intron	(Gaither et al. 2010)
	Naso unicornis	surgeonfish	Fish	IO, Pac	Sey, CK, XI, NWA	mtDNA (CoReg)	(Horne et al. 2008)
	Naso brevirostris	surgeonfish	Fish	IO, Pac	Sey, CK, XI, NWA	mtDNA (CoReg)	(Horne et al. 2008)
	Tripneustes spp.	sea urchins	Echinoderm	IO, Pac, Atl	SA, SWI, Mas, Sey	mtDNA (COI)	(Lessios et al. 2003)

Table S4.2. continued

		Species	Common name	Group	Study coverage	Localities IO	Marker	Reference
Homogeneous across Indian Ocean		*Dascyllus trimaculatus*	damselfish	Fish	IO, Pac, RS	EA, SWI, Sey, GO, CK	mtDNA (CoReg) and microsats	(Leray et al. 2010)
		Linckia laevigata	starfish	Echinoderm	IO, Pac	SA, Sey, And	mtDNA (COI)	(Williams et al. 2002)
		Naso vlamingii	unicornfish	Fish	IO, Pac	Sey, CK, XI	mtDNA (CoReg)	(Klanten et al. 2007)
		Nerita albicilla		Mollusc	IO, Pac	SA, EA, SWI, Mas, GO, And	mtDNA (COI)	(Crandall et al. 2008b)
Restricted distribution	OIN	*Hippocampus* spp.	seahorse	Fish	IO	ISL	mtDNA (cytB)	(Goswami et al. 2009)
		Penaeus monodon	tiger prawn	Crustacean	IO	ISL	mtDNA (CoReg)	(Kumar et al. 2007)
		Uca annulipes	fiddler crab	Crustacean	IO, Pac, RS	And	mtDNA (COI) and 16S rDNA	(Shih et al. 2009)
	SWIO	*Brachidontes variabilis*	mussel	Mollusc	IO, Pac, RS, Med	SA, SWI	mtDNA (COI) and 16S rDNA	(Sirna Terranova et al. 2007)
		Chelonia mydas	sea turtle		IO	SWI	mtDNA (CoReg)	(Bourjea et al. 2007)
		Neosarmatium meinerti	mangrove crab	Crustacean	IO	SA, EA, Sey	mtDNA (COI)	(Ragionieri et al. 2010)
		Nerita plicata		Mollusc	IO, Pac	SA, EA, SWI, Mas	mtDNA (COI)	(Crandall et al. 2008b)
		Pocillopora damicornis		Coral	IO	EA	microsats	(Souter et al. 2009)
		Penaeus monodon	tiger prawn	Crustacean	IO, Pac	EA, SWI, Mas, NWA	nuclear intron	(Duda & Palumbi 1999)
		Penaeus monodon	tiger prawn	Crustacean	IO, Pac	SA, SWI, NWA	mtDNA (RE)	(Benzie et al. 2002)

Table S4.2. continued

	Species	Common name	Group	Study coverage	Localities IO	Marker	Reference
Restricted distribution SWIO and NWA	*Carcharhinus plumbeus*	sandbar shark	Fish	IO, Pac, Atl	SA, NWA	mtDNA (CoReg) and microsats	(Portnoy et al. 2010)
	Chlorurus sordidus	parrotfish	Fish	IO, Pac	SWI, Sey, NWA	mtDNA (CoReg)	(Bay et al. 2004)
	Holothuria nobilis	sea cucumber	Echinoderm	IO, Pac	Mas, NWA	mtDNA (COI)	(Uthicke & Benzie 2003)
	Scylla serrata	mud crab	Crustacean	IO	SA, EA, Sey	mtDNA (COI)	(Fratini et al. 2010)
Very few populations	*Alpheus lottini*	snapping shrimp	Crustacean	IO, Pac, RS	Sey, CA, NWA	mtDNA (COI) and allozymes	(Williams et al. 2002)
	Astralium rhodostomum	turbinid	Mollusc	IO, Pac	CK, XI, And	mtDNA (COI)	(Meyer et al. 2005)
	Echinolittorina trochoides A	snails	Mollusc	IO, Pac	ISL, And	mtDNA (COI)	(Reid et al. 2006)
	Hippocampus kuda	seahorse	Fish	IO, Pac, RS	SA, EA, ISL	mtDNA (CoReg)	(Teske et al. 2005)
	Linckia laevigata	starfish	Echinoderm	IO, Pac	SA, And	mtDNA and allozymes	(Williams & Benzie 1998)
Structure detected and high coverage	*Acanthaster planci*	crown-of-thorns starfish	Echinoderm	IO, Pac	SA, Mas, Mal, CK, And	allozymes	(Benzie 1999)
	Acanthaster planci	crown-of-thorns starfish	Echinoderm	IO, Pac, RS	SA, EA, Mas, GO, Mal, And, CK, XI, NWA	mtDNA (COI)	(Vogler et al. 2008)
	Penaeus monodon	tiger prawn	Crustacean	IO, Pac	EA, SWI, ISL, And, NWA	mtDNA (CoReg) and microsats	(You et al. 2008)
	Patelloida profunda	limpet	Mollusc	IO, Pac, Atl	SA, EA, Mas, GO	mtDNA (COI and 16S)	(Kirkendale & Meyer 2004)

Supplementary materials and methods for chapter 4

A DNA fragment containing the putative mitochondrial control region (CoReg) and the 5′ end of the adjacent 16S rRNA gene was amplified from the 33 COTS samples from the Red Sea (Vogler et al. 2008) with the following primers: COTS-CoReg-F15635 5'-CAAAAGCTGACGGGTAAGCAA-3' and COTS-CoReg-R114 5'-TAAGGAAGTTTGCGACCTCGAT-3'. DNA sequencing was performed using the PCR reverse primer, and the following internal forward primer: COTS-CoReg-seqPac-F15735 5′-TTAGCTGGG-TATAAG-3′. Sequences were assembled using CODONCODE ALIGNER and aligned in SEAVIEW v4.2 (Galtier et al. 1996) using the built-in MUSCLE software (Edgar 2004).

For this CoReg dataset as well as the Red Sea and Pacific Ocean COI dataset from Vogler et al. (2008), ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010) was used to estimate haplotype and nucleotide diversities, Fu's F_S (Fu 1997; 50′000 replicates), Tajima's D (Tajima 1989; 50′000 replicates). Ramos-Onzins' R_2 (Ramos-Onsins and Rozas 2002) was estimated using the R package PEGAS v0.3-2 (Paradis 2010; 10′000 replicates). The overall Φ_{ST} value without a priori structure (10′000 replicates) was estimated for the CoReg dataset only using ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010; 10′000 replicates).

The results can be found in table 4.1, alongside the results already obtained from chapters 2 and 3.

Erklärung über eigene Leistungen

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe. Die Stellen, die anderen Werken wörtlich oder sinngemäß entnommen sind, sind als solche kenntlich gemacht. Eigene Beiträge im Verhältnis zu denen von Koautoren in bereits publizierten oder zur Publikation einzureichenden Teilen dieser Arbeit sind wie folgt:

Kapitel 1: Catherine Vogler konzipierte die Studie in wesentlichen Teilen, führte einen Teil der Feldarbeiten durch, generierte, analysierte und interpretierte die Daten und verfasste das Manuskript. Gert Wörheide trug zur Konzeption der Studie und zur Verbesserung des Manuskripts durch kritische Kommentare bei. John Benzie, Harilaos Lessios und Paul Barber führten einen Teil der Feldarbeiten durch und trugen zur Verbesserung des Manuskripts durch kritische Kommentare bei.

Kapitel 2: Catherine Vogler konzipierte die Studie in wesentlichen Teilen, generierte den größten Teil der Sequenzdaten, analysierte und interpretierte die Daten und verfasste das Manuskript. Gert Wörheide und John Benzie trugen zur Konzeption der Studie und zur Verbesserung des Manuskripts durch kritische Kommentare bei. John Benzie, Paul Barber, Molly Timmers, Harilaos Lessios, Peter Schupp und Kazuo Nadaoka führten einen Teil der Feldarbeiten durch. Kimberley Tenggardjaja, Janna Groeneveld und Nina Yasuda generierten einen Teil der Sequenzen. Lisa Peplow und Elizabeth Ballment gaben technische Hilfestellung.

Kapitel 3: Catherine Vogler konzipierte die Studie in wesentlichen Teilen, führte einen Teil der Feldarbeiten durch, generierte den größten Teil der Sequenzdaten, analysierte und interpretierte die Daten und verfasste das Manuskript. Gert Wörheide und John Benzie trugen zur Konzeption der Studie und zur Verbesserung des Manuskripts durch kritische Kommentare bei. John Benzie, Paul Barber und Charles Sheppard führten einen großen Teil der Feldarbeiten durch. Kimberley Tenggardjaja und Karin Gérard generierten einen Teil der Sequenzdaten.

Kapitel 4: Catherine Vogler konzipierte die Studie in wesentlichen Teilen, generierte, analysierte und interpretierte die Daten und verfasste das Manuskript. Gert Wörheide und John Benzie trugen zur Konzeption der Studie und zur Verbesserung des Manuskripts durch kritische Kommentare bei.

Curriculum Vitae

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2000-2005 **University of Lausanne, Switzerland**
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2004 **University of Technology, Sydney, Australia**
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First year of veterinary medicine

1996-1999 **International Lycée of Ferney-Voltaire, France**
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Vogler C, Benzie J, Lessios H, Barber P and Wörheide G **2008**. A threat to coral reefs multiplied? Four species of crown-of-thorns starfish. *Biology Letters* 4(6) 696-699.

Publications: others

Vogler C 2009 The crown-of-thorns starfish. In *The Biology of Coral Reefs* (eds. Sheppard CRC, Davy SK, Pilling GM) Oxford University Press, USA. P.179-180.

Vogler C, Voigt O and Wörheide G **2008**. Der Dornenkronenseestern: Massenmörder oder missverstandenes Opfer von Umständen? In *Abgetaucht* (eds. Leinfelder R, Heiss G, Mildrzyk U, for the International Year of the Reef 2008) Museum of Natural History of the Humboldt University Berlin. Konradin Verlag, pp. 139-147.

Teaching experience

Introduction to ecology (SS2009 and WS2009-2010)
lecture for BSc students, with Dr. Voigt

Earth-life interactions I: evolutionary and ecological biogeography (WS2009-2010)
lecture for MSc students

Advanced methods in palaeobiology (WS2009-2010 and SS2010)
molecular lab course for BSc and MSc students, with Prof. Wörheide, Dr. Erpenbeck and Dr. Voigt

Geobiology and biodiversity of fossil and recent coral reefs and geology of the Sinai, Egypt (SS2009)
field course for BSc and MSc students, with Prof. Wörheide and Dr. Voigt

Geobiology of coastal ecosystems, Banyuls, France (SS2010)
field course for BSc and MSc students, with Prof. Wörheide, Prof. Haszprunar and Dr. Voigt

Scientific communications

Vogler, C and Wörheide, G **2009** Marine invertebrate biodiversity in the Southeast Asian Gateway: examples from sponge and starfish phylogeography. Southeast Asian Gateway Evolution Meeting, London, UK, 15-17 September 2009 (oral presentation)

Vogler, C and Wörheide, G **2009** Scientific diving for biodiversity research. International Workshop on Research in Shallow Marine and Freshwater Systems, Freiberg, Germany, 14-16 May 2009 (oral presentation)

Vogler C, Tenggardjaja K, Barber P, Benzie J and Wörheide G **2008** On the trail of a voracious predator – the phylogeography of *Acanthaster planci*. 11th International Coral Reef Symposium, Fort Lauderdale FL, USA, 7-11 July 2008 (poster)

Vogler C, Benzie J, Barber P and Wörheide G **2008** Uncovering the trail of a voracious predator – the phylogeography of *Acanthaster planci*. Systematics2008, Göttingen, Germany, 7-11 April 2008 (poster)

Vogler C, Erpenbeck D and Wörheide G **2007** Detection of marine bioinvaders in a Mediterranean industrial port by DNA barcoding. Second International Barcode of Life Conference, Taipei, Taiwan, 18-20 September 2007 (poster)

Vogler C and Wörheide G **2007** Detection of non-indigenous marine invertebrates in a Mediterranean industrial port using DNA assisted identification techniques. Fifth International Conference on Marine Bioinvasions, MIT, Boston MA, USA, 21-24 May 2007 (oral presentation)

Vogler C, Berset-Brändli L, Jaquiery J, Broquet T and Perrin N **2006**. Connectivity threshold in a treefrog (*Hyla arborea*) metapopulation. Biology06, Geneva, Switzerland, 16-17 February 2006 (poster)

Training

09.2009	**Transferrable skills – HOTSPOTS university training module** Germany: Munich, organised by Prof. Gert Wörheide and Catherine Vogler
03.2009	**Genetic project leader and biosafety officer training** Germany: Regensburg, organised by Prof. Susanne Modrow, Universität Regensburg
09.2008	**Workshop on biodiversity hotspots – Tropical Biology Association (TBA)** Tanzania: Amani, organised by Dr. Rosie Trevelyan, TBA
01.2008	**Bioinformatics – HOTSPOTS winter school** Switzerland: Lausanne, organised by Dr. Nicolas Salamin, Université de Lausanne
08.2007	**Tropical biology field course – Tropical Biology Association** Kenya: Mpala & Naivasha, organised by TBA
04.2007	**Phylogeny and comparative methods course** Switzerland: Lausanne, organised by Dr. Nicolas Salamin, Université de Lausanne
09.2006	**Community ecology, modelling and statistics – HOTSPOTS university training module** France: Moulis, organised by Dr. Christophe Thébaud, Université de Toulouse
07.2006	**Conservation organisations and policies – HOTSPOTS summer school** UK: London & Cornwall, organised by Dr. Vincent Savolainen, Imperial College
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Languages

French	mother tongue
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