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Corals through the light : phylogenetics, functional diversity and adaptive strategies of coral-symbiont associations over a large depth range

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CHAPTER 1

Introduction



THE WEIGHT OF HISTORY...

Exactly 150 years have passed since Charles Darwin (1859) published a theory that explains the origin of diversity in living organisms. Evolution, by means of natural selection, is the process through which species change and adapt to their environment. Less than a decade later, Gregor Mendel (1865) presented evidence for an inheritance mechanism, a key factor as it provided the raw material on which natural selection was supposed to act. Surprisingly, it took almost a century for the scientific community to combine Darwinism and the discipline of genetics into a “modern evolutionary synthesis” (e.g. Huxley 1942). From the discovery of the double helix structure of the DNA molecule (Watson and Crick 1953), evidence granted by disciplines such as genetics, palaeontology or ethology has piled up to confirm and enhance the evolutionary ideas of Darwin, attesting his consistent theory as the one that allows scientists to explore the amazing complexity of life.

CORAL-SYMBIONT ASSOCIATIONS

Years before the publication of “On the origin of species by means of natural selection”, Darwin (1842) had the opportunity of expressing the astonishment that struck him when he first came across the coral reef ecosystem, “vast rings of coral-rock, often many leagues in diameter” that were built by “apparently insignificantly creatures”. What Darwin did not know is that, in fact, tropical reef-building corals represent a remarkable case of evolution. They are the result of mutualistic symbiotic associations between colonial cnidarian invertebrates (order Scleractinia) and endocytic dinoflagellate algae (phylum Dinoflagellata) of the genus *Symbiodinium* Freudenthal (Figure 1.1). This symbiosis underpins the success of coral reef ecosystems, among the most biodiverse in the world (Hallock 2001). The obligatory animal-algal symbiosis forms a unit, the coral holobiont, whose integrated adaptive properties are more than just the sum of its parts. A complex set of morphological, physiological and molecular properties of the two organisms has been shaped by selection and confers mutual benefit (Trench 1993; Stanley Jr. and van de Schootbrugge 2009), which ultimately relates to the differential reproduction of implicated genes (Dawkins 1990). Accordingly, coral holobionts are likely to constitute important vehicles for selection (Reeve and Keller 1999), for which the possibility of coevolution of the components involved (corals and their symbionts) is not to be excluded (Coffroth and Santos 2005). The dynamics of trait evolution within mutualistic symbioses are also poorly understood (Wade 2007).

Due to their adaptive characteristics, scleractinian corals have come to dominate well lit, shallow-water, tropical coastal areas, producing a diverse assortment of colonial growth forms and morphologies. It is the ability of secreting aragonite skeletons after extracting calcium (Ca^{2+}) and carbonate (CO_3^{2-}) from seawater that allows symbiotic corals to construct the reef framework upon which all the coral reef ecosystems thrive (Stanley Jr. and van de Schootbrugge 2009). Although the scleractinian fossil record begins some 245 million years ago, little is known about the evolution of their photosymbionts (Stanley Jr. 2003), but they have likely evolved from a single adaptive radiation (Wood 1998).

Physiologically, the endosymbiont dinoflagellates play a crucial role in host sustenance and survival in nutrient-poor tropical waters (Muscatine and Porter 1977). The photosynthetically fixed carbon (typically glycerol or other low molecular weight compounds; Figure 1.2)

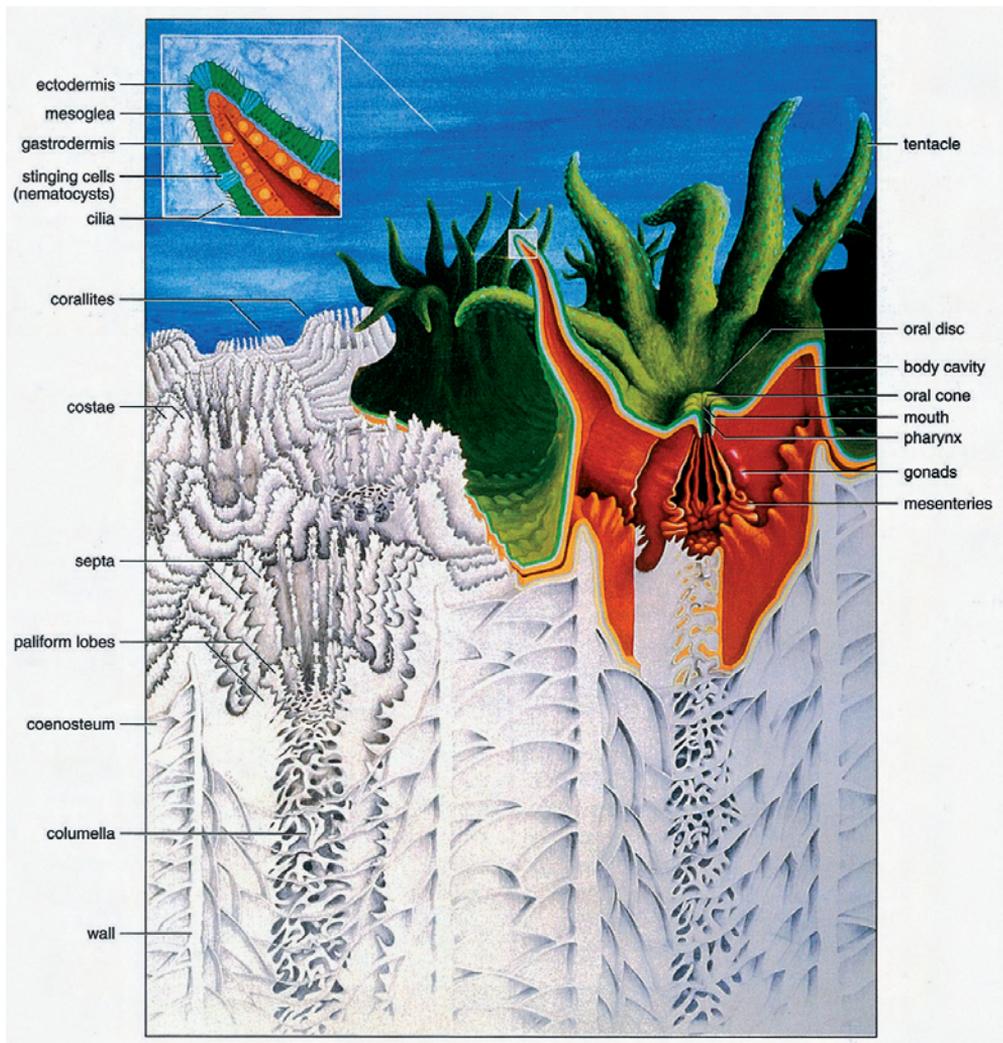


Figure 1.1 General structure of the coral polyp and underlying skeleton (after Veron 2000). Note the endocytic symbionts (gold) in the gastrodermis layer. For full colour version see Appendix (page 129).

translocated from the algae can cover most or the entire respiratory demands of the host (Muscatine *et al.* 1984) and is considered to promote the high coral skeleton calcification rates necessary to build the reef edifice (Pearse and Muscatine 1971). It is also known that the presence of the algal symbionts assists the assimilation or recycling of nitrogen (Burriss 1983; Goodson *et al.* 2001), a limiting resource in the ecosystem (Muscatine and Porter 1977; Muller-Parker and D'Elia 1997). In exchange, the symbionts are kept within the photic zone, sheltered from threats such as ultraviolet (UV) radiation damage and maintain high population densities. Carbon dioxide (CO_2) and other animal metabolites which are source of nutrients (NH_3 , PO_4^{3-}) are also used by the algal partner (Muller-Parker and D'Elia 1997; Yellowlees *et al.* 2008). The close proximity of the autotrophic and heterotrophic components facilitates molecular exchange and prevents nutrient losses (Tanaka *et al.* 2006), another adaptation to the oligotrophic conditions of the surrounding environment.

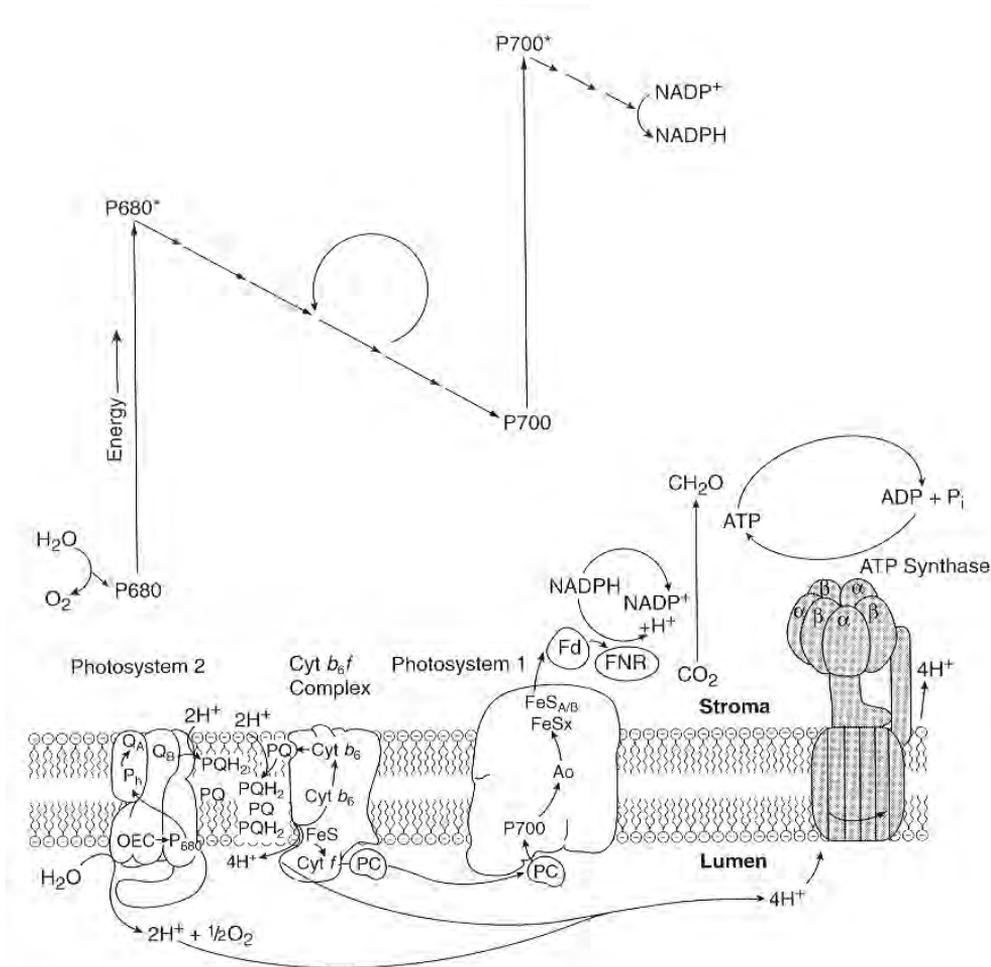


Figure 1.2 Schematic view of the electron transfer pathway found in oxygenic photosynthetic organisms such as *Symbiodinium* sp. (after Blankenship 2002). The upper diagram shows an energetic perspective of the electron transfer pathway incorporating the two photochemical reaction centre complexes: photosystems 1 (P700) and 2 (P680). The lower diagram gives a spatial view of the major protein complexes in the thylakoid membrane of the chloroplasts.

THE DEPTH-LIGHT GRADIENT AND PHOTOADAPTATION

Coral reefs are spread over large geographical regions in the tropics and sub-tropics (Veron 2000). Nevertheless, the most ecologically limiting of a broad set of abiotic parameters to which symbiotic coral species have to adapt - light - acts acutely at the local scale (Veron 1995). Light intensity is dramatically reduced with depth down the reef slopes, depending on local characteristics such as concentration and composition of suspended and dissolved matter (Jerlov 1966; Kirk 1994). Linked to this vertical attenuation of light, there are important spectral distribution changes (Falkowski and Laroche 1991; Stomp *et al.* 2007).

Symbiotic corals thrive across these large depth ranges, being present throughout all the euphotic zone (Veron 2000). Just as plants living under the canopy of a tropical forest, phototrophic corals have adapted to a wide range of light habitats. Most individual coral species show limited vertical distributions and this relates to their physiological tolerances. Holobionts with

broader depth ranges generally have a greater potential for acclimatisation, or, in other words, are more environmentally plastic (Anthony and Hoegh-Guldberg 2003; Todd 2008). This broader plasticity corresponds, in a wider temporal scale, or evolutionarily speaking, to being more adapted to environmental gradients, or having a broader physiological niche. The varied suit of adaptation/acclimatisation features that allows corals and their symbionts to cope with the large light gradients that exist over reef slopes sets the stage for this thesis.

THE ANIMAL HOST

In fact, both symbiotic partners play roles in photoadaptation or acclimatisation. It is interesting to note that coral holobionts are to a great extent phototrophic, but this energy income may be supplemented by or even shifted towards heterotrophic feeding, depending on prey and suspended particulate matter availability (Bak *et al.* 1998; Anthony and Fabricius 2000; Ferrier-Pages *et al.* 2003). Usually during the night, these photosymbiotic benthic suspension feeders extend their polyps and stinging tentacles are used to prey on zooplankton (Sebens *et al.* 1996). Recent evidence shows that heterotrophically acquired carbon may play a much larger role in the daily carbon budget of corals (up to 46 % of the total for some coral species) than previously estimated (Palardy *et al.* 2008). Given the degree of evolutionary interaction between the animal and algal components, it is not surprising that heterotrophy levels can have an effect on the photosynthetic activity of the symbionts (Fitt and Cook 2001; Houlibreque *et al.* 2003; Borell *et al.* 2008). Apart from carbon input, feeding provides nutrients such as nitrogen and phosphorus that cannot be supplied by photosynthesis itself (Houlibreque and Ferrier-Pages 2009).

There are adaptations to available light that are typically mediated by the animal component. These are usually seen at the intra-specific level and may include morphological, micro-skeletal, cellular or even behavioural characteristics. Respiration rates are known to be reduced with increasing depth allowing a positive rate of daily net photosynthesis in darker habitats (McCloskey and Muscatine 1984; Anthony and Hoegh-Guldberg 2003). Changes in colony geometry have been reported for several reef-building species, with flatter and more horizontal colonies being assigned to low light environments (Dustan 1975; Anthony *et al.* 2005) and to a reduced tissue versus light-flux ratio (Stambler and Dubinsky 2005). This contrasts with more hemispheric, branching or vertical plated colonies in the well lit habitats. Varying skeletal micro-morphology is also thought to be involved in light modulation (Anthony and Hoegh-Guldberg 2003; Enriquez *et al.* 2005). Furthermore, tissue and polyp behaviour have been referred to as possible mediators of processes causal to photosynthesis, such as light and nutrient levels available for the symbionts (Brown *et al.* 2002a; Levy *et al.* 2006a). Some animal adaptations are as remarkable as the production of fluorescent proteins that can regulate the light environment within the host tissue (Dove *et al.* 2006; Field *et al.* 2006; Oswald *et al.* 2007). These pigments are supposedly capable of shading the symbionts (Salih *et al.* 2000) or enhancing the light available inside the animal tissue (Schlichter *et al.* 1994). In this latter case, pigments are thought to be involved in capturing short-wavelength photons and re-emitting at suitable spectral regions for algal photosynthesis (Schlichter and Fricke 1991), a case of photoadaptation to dark and deeper habitats. This constitutes a defining example of how mutualism can contribute to the creation of new ecological niches (Stachowicz 2001).

SYMBIONT FUNCTIONAL DIVERSITY

Due to its crucial nutritional function, the phototrophic component of the association plays a major role in coral niche occupation. Specifically, algal symbiont functional diversity has been hypothesised to regulate the vertical distribution of corals (Iglesias-Prieto and Trench 1994; Iglesias-Prieto *et al.* 2004).

Originally, the term “zooxanthellae” was used to classify the yellow-brown algae associated with animal cells. Although still in use, this term is bare in taxonomic quality. The dinoflagellate identity of coral symbionts was established after the observation of motile “swarmers” (with a typical gymnodinoid dinoflagellate morphology) in cultures originated from cnidarian-isolated algae (Kawaguti 1944). Freudenthal (1962) put up the genus *Symbiodinium*, to taxonomically describe the symbiotic dinoflagellates associated with a wide range of invertebrate hosts, and further described their life cycle and morphology. From here, the long-standing paradigm that a single pandemic symbiont species, *Symbiodinium microadriaticum* Freudenthal, was present in all host species (Taylor 1974), was first challenged by biochemical, morphological and physiological studies (Trench 1971b; Chang *et al.* 1983; Trench and Blank 1987). Describing the diversity of coral dinoflagellate symbionts was challenging due to difficulties in culturing the isolated algae and the lack of clear morphological differences. The diverse nature of the genus was only later confirmed by molecular genetics (Rowan and Powers 1991b).

Since then, *Symbiodinium* has been unravelled as a highly diverse taxon and several reviews have comprehensively addressed the questions of its diversity, ecology, evolution and biogeography (Trench 1993; Baker 2003; Coffroth and Santos 2005). The genus is currently divided into at least eight genetically divergent lineages, or clades, named A to H (Rowan and Powers 1991a; Baker and Rowan 1997; Pochon *et al.* 2004), a distinction first inferred based on the small (SSU or 18S) and large subunit (LSU or 28S) nuclear ribosomal DNA (rDNA) and then supported by studies of cytoplasmic DNA markers, such as chloroplastic and mitochondrial genes (Santos *et al.* 2002; Takabayashi *et al.* 2004; Pochon *et al.* 2006).

These subgeneric clades are further divided into an unknown number of types, first defined by sequence variation in the more rapidly evolving internal transcribed spacer (ITS) regions of the rDNA (Hunter *et al.* 1997; LaJeunesse and Trench 2000; LaJeunesse 2001). Follow-up studies on the distribution of symbiont ITS types provided evidence that these divergent phylogenetic lineages also related to distinct ecological and biogeographical units (Santos *et al.* 2001; van Oppen *et al.* 2001a; LaJeunesse 2002), which likely corresponded to the species level. Further sequence-based studies using DNA markers such as portions of the D1/D2 domain of the LSU rDNA (Loh *et al.* 2001; Rodriguez-Lanetty *et al.* 2001; Chen *et al.* 2005), regions of domain V of the chloroplast 23S rDNA (Santos *et al.* 2003a) and microsatellite flanking regions (Santos *et al.* 2004) also confirmed the coarse taxonomic power of clade classification, stressing the need to address algal symbiont diversity below the cladal level. Over the last years, studies applying subcladal ITS rDNA variation to understand the diversity in coral-symbiont associations have proliferated. Specifically, the ITS2 region became a popular molecular marker that has revealed hundreds of distinct *Symbiodinium* variants. These have partially been assigned to distinctive biogeographical distributions and ecological patterns, such as environmental zonation or host specificity (Rodriguez-Lanetty *et al.* 2001; Diekmann *et al.* 2003; LaJeunesse *et al.* 2003; Rodriguez-Lanetty and Hoegh-Guldberg 2003;

Ulstrup and van Oppen 2003; LaJeunesse *et al.* 2004; Thornhill *et al.* 2006; Warner *et al.* 2006; LaJeunesse *et al.* 2007; Sampayo *et al.* 2007; Macdonald *et al.* 2008).

Indeed, coral-symbiont associations are nonrandom in terms of partners involved (Trench 1997) and there is a varying level of host and symbiont specificity among, respectively, symbionts and hosts (Baker 2003; Coffroth and Santos 2005). Several authors have established a relation between symbiont diversity within single host species and the way their symbionts are transmitted from generation to generation (e.g. LaJeunesse *et al.* 2004). Given that coral larvae (planulae) acquire their symbiont populations by both vertical (inherited from the parental colony) and horizontal (from the external environment) transmission modes (Figure 1.3), it is reasonable to hypothesize that the latter strategy maximizes the chance for more diverse or, at least, less host-specific symbiont assemblages. Although this hypothesis has not yet been fully tested and previous studies yielded mixed results (van Oppen 2004; Stat *et al.* 2008b), it is likely that the greatest chance for recombination between symbiont types and their hosts takes place in the early life-stages of horizontally-transmitting broadcast spawners (Little *et al.* 2004). Interestingly, several authors have explored the histological process of acquisition of symbionts during stages of larval development (Hirose *et al.* 2001; Hirose *et al.* 2008; Huang *et al.* 2008b).

Symbiont ecological niche partitioning, usually mediated by either host or environment, has its most meticulous evidence in examples of *Symbiodinium* zonation over the surface of individual colonies. Multiple symbiont lineages are distributed over the colony landscape in patterns that resemble depth zonation over reef slopes (Rowan and Knowlton 1995; Rowan *et al.* 1997; Ulstrup and van Oppen 2003). This strongly indicates that light is an important axis for symbiont niche diversification (Iglesias-Prieto and Trench 1997a). Besides, it suggests an intricate symbiotic relationship, in which host properties, such as colony topography, have a

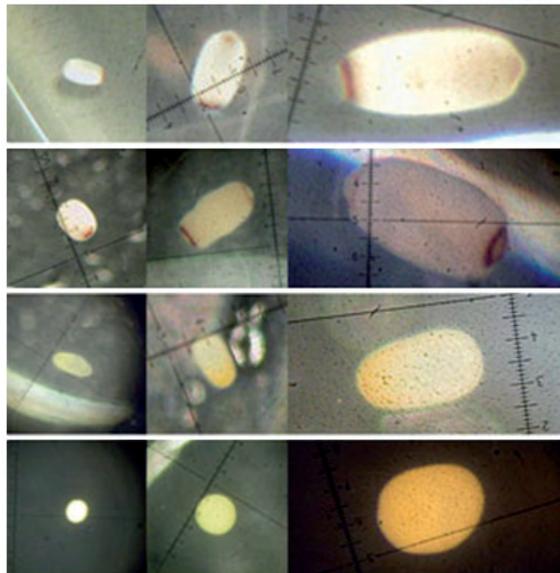


Figure 1.3 *Madracis* spp. planulae under increasing magnification (from left to right). Note a brown ring consisting of symbiont cells on the oral end of the two uppermost planulae, evidence for vertical symbiont transmission mode between coral generations. Maximum diameter is c. 400 μm (pictures by P. Bongaerts). For full colour version see Appendix (page 130).

decisive effect on symbiont population distribution (Kaniewska *et al.* 2008) and hypothetically on their photosynthetic activity and output. The central role of irradiance in the physiology of coral symbionts has also been confirmed by photoacclimation studies performed on algal isolates originating from different cnidarian hosts (Chang *et al.* 1983; Iglesias-Prieto and Trench 1994, 1997b). *Symbiodinium* encompasses a broad physiological diversity and specific symbiont lineages, such as the ones defined by ITS2 types, appear to be adapted to particular light regimes and differ on light use properties. This has recently been confirmed using both intact symbioses (Iglesias-Prieto *et al.* 2004; Abrego *et al.* 2008) and isolated symbionts (Goulet *et al.* 2005; Robison and Warner 2006; Hennige *et al.* 2009), by diverse functional mechanisms such as photosynthetic pigment contents or photosynthetic activity (as measured by oxygen fluxes or state-of-the-art noninvasive chlorophyll *a* fluorescence bio-physical techniques). Physiological differences between symbiont lineages have also been supported by other sorts of evidence such as the production of mycosporine-like amino acids (MAAs), relevant photoprotective pigments against the effect of UV radiation (Banaszak *et al.* 2000; Banaszak *et al.* 2006). Loram *et al.* (2007a) have shown that symbiont genotypes differ in the transfer of photosynthetically fixed carbon to their cnidarian host. As corals and coral reefs face critical changes due to a rapid changing climate (Hoegh-Guldberg *et al.* 2007), the need to understanding symbiont functional diversity and its contribution to holobiont acclimatisation is now extremely important.

CHANGING CLIMATE AND BLEACHING

Coral reefs face widespread degradation, seen for instance in major losses of coral cover worldwide (Wilkinson 1999; Bak *et al.* 2005). In addition to local anthropogenic causes, a rapidly changing global climate is predicted to be shifting the face of coral reefs as we know them, carrying along important ecological and socioeconomic alterations (Walther *et al.* 2002; Hoegh-Guldberg *et al.* 2007). One of the most important causes for coral reef decline is coral bleaching, a loss or reduction of the symbiont populations or their photosynthetic pigments which often results in host mortality (Donner *et al.* 2007; Lesser 2007; Carpenter *et al.* 2008). This phenomenon has occurred with increasing frequency in the last twenty years and mostly during periods of El Niño Southern Oscillation (Hoegh-Guldberg 2004). Bleaching most often results from physiological stresses caused by the synergistic effect of elevated seawater temperature and high light intensity (Brown 1997; Douglas 2003; Lesser and Farrell 2004), which are thought to disrupt the proper functioning of photosystem II or of electron transfer pathways located downstream from photosystem II (Warner *et al.* 1999; Fitt *et al.* 2001; Jones and Hoegh-Guldberg 2001). Although there is still uncertainty over what the initial site of damage to algal photosystems is (Venn *et al.* 2008a), the loss of balance between rates of light collection and light use results in the production of reactive oxygen species (ROS, Figure 1.4), which are damaging to protein function and membrane integrity (Blankenship 2002). Oxidative stress threatens both the photosymbiont and the animal host by provoking a wide range of cellular responses that are known to be involved in bleaching, such as exocytosis of algae from host cells or the activation of programmed cell death pathways (Brown *et al.* 1995; Martindale and Holbrook 2002; Franklin *et al.* 2004). Recently, it was suggested that hydrogen peroxide produced by the photosymbionts may have a role in coral bleaching (Smith *et al.* 2005; Suggett *et al.* 2008), a mechanism proposed by Sandeman (2006) to be mediated by the breakdown of the calcium ions exclusion systems in the cytoplasmic membranes of

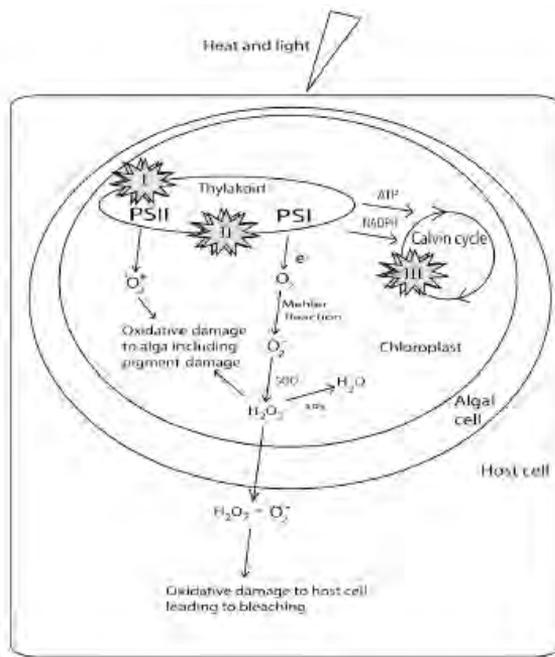


Figure 1.4 Three hypothesized impacts of elevated temperature on the photosystems of symbiotic algae *in hospite* (after Venn *et al.* 2008a). I - Dysfunction of PSII and degradation of the D1 protein; II - Energetic uncoupling in the thylakoid membranes; III - Damage of the Calvin cycle. During bleaching, reactive oxygen species (ROS) such as superoxide (O_2^-) are produced via the Mehler reaction from oxygen (O_2). ROS can be detoxified by superoxide dismutase (SOD) and ascorbate peroxidase (APX) but if the rate of production is too elevated then oxidative damage can occur. Singlet oxygen ($^1O_2^*$) can be produced at impaired PSII reaction centres or in the photosynthetic antennae causing pigment photobleaching.

the gastrodermal layer. This process was partially confirmed by a study on gene expression changes associated with bleaching (Desalvo *et al.* 2008).

Intact symbioses vary in their bleaching susceptibility and much of this variation has been attributed to differences in the tolerance of symbiont types towards temperature (Kinzie III *et al.* 2001; Bhagooli and Hidaka 2003; Berkelmans and van Oppen 2006; Ulstrup *et al.* 2006b; Reynolds *et al.* 2008; Sampayo *et al.* 2008; Thornhill *et al.* 2008). Resistance of algal cells to thermal stress has often been related to a more stable lipid composition of their thylakoid membranes (Tchernov *et al.* 2004) or to nonphotochemical quenching (NPQ), a photoprotective mechanism which can divert and dissipate excessive excitation energy (Warner *et al.* 1996; Brown *et al.* 1999; Muller *et al.* 2001). Ulstrup *et al.* (2008) showed that variation in algal photoprotective mechanisms measured for corals hosting mixed symbiont assemblages is small relative to corals with high fidelity to one symbiont type. Given the fact that symbiont populations may have a seasonal dynamic nature (Fitt *et al.* 2000; Thornhill *et al.* 2006; LaJeunesse *et al.* 2007), a multiple symbiont assemblage likely increases the chance of having the right symbiont at the right time (Ulstrup *et al.* 2008; Venn *et al.* 2008b). The hypothetical evolutionary potential of such a dynamic system constitutes the core of one of the most interesting and controversial debates in the contemporary coral reef science and conservation community (Baker 2001,2002; Hoegh-Guldberg *et al.* 2002; Goulet 2006; Baker and Romanski 2007). Following the publication of the adaptive bleaching hypothesis (Buddemeier and Fautin 1993), several authors have offered pieces of evidence

to argue that bleaching may have an adaptive role and eventually allow corals to cope with a changing climate. The underlying evidence is that bleaching allows the symbiotic populations of certain coral species or communities to become repopulated or dominated by symbiotic lineages that are more competent under the new environmental circumstances (Baker 2001; Fautin and Buddemeier 2004; Rowan 2004; Berkelmans and van Oppen 2006). Repopulation may be originated from the residual symbionts left inside the host tissues or from exogenous populations (Jones and Yellowlees 1997; Lewis and Coffroth 2004). A recent study suggests that heterologous symbionts may infect coral primary polyps through their epidermis, a process for the first time revealed for vertical transmitting hosts (Zurel *et al.* 2008).

Whether bleaching is really adaptive and whether it can offer widespread hope for coral reef resilience to climate change are questions that still need to be completely addressed. The first question relates to the adaptive potential of a phenotypic change (acclimatization through dominance of new symbionts) and whether it can provide new genetic combinations for evolution (Hoegh-Guldberg *et al.* 2002). The second question relates to whether the physiological and time scales of adaptation are suitable to the rate of environmental change (Baker 2002). These questions will be addressed in more detail in the general discussion at the end of this thesis.

SPECIES CONCEPTS

A crucial aspect underpinning the understanding of diversity, selection and evolution of coral-symbiont associations is the concept of species itself (Coffroth and Santos 2005). Originally applied based on morphological characters, taxonomy has in the last decades seen developments through the use of DNA barcoding and biodiversity assessment by the use of orthologous DNA sequences. Recent molecular techniques have become a powerful tool to test morphological taxonomy. Specifically in scleractinians, there are often disagreements between morphological and molecular phylogenies (van Oppen *et al.* 2001b). One initial problem refers to the high degree of environmental plasticity present in coral species (Todd 2008). Often, the same genetic entity may assume different morphologies depending on the habitat (Gates and Edmunds 1999). However, the main source of conflict lies, according to Veron (1995), in the arbitrary nature of species boundaries. Hence, it is worth to briefly introduce the debate on what are species.

The morphological species concept, following the Linnaean classification (Linnaeus 1758) dating back to the 18th century, is almost exclusively based on morphological discontinuity. New specimens are compared against a described standard, the holotype. The main problems with the morphological concept are that there is no objective distinction between intra and interspecific variation and that it is unable to separate homology (similarity due to shared ancestry) from homoplasy (due to convergence). The biological species concept (Mayr 1942) defines species as groups of individuals which have the capacity to interbreed, with boundaries between species being defined by barriers to gene flow that have a genetic basis. A problem with the application of this concept to corals is that they are modular organisms that grow by clonal propagation of their polyps. The phylogenetic species concept (Donoghue 1985) focuses on shared identity by descent and recognizes species on the basis of shared homologous characters that form monophyletic groups, based on either morphological or molecular datasets. Usually, the morphological datasets are scarce compared to the vast

possibilities given by molecular data. Phylogenetic resolution is usually subdued in datasets characterised by too much or too little variation and as such, the level of sequence variation has to be appropriate for the species level.

A major issue in coral species determination, which is problematic to the phylogenetic but manifest to the biological species concept, is that reproductive isolating barriers are apparently weak in corals, and hybridization events have played an important role in coral evolution (Veron 1995; Willis *et al.* 2006). Mass spawning, the synchronous release of gametes between sympatric broadcast spawners (Harrison *et al.* 1984), creates numerous opportunities for interspecific hybridization. Hybridization has also been suggested for brooders (Diekmann *et al.* 2001). Veron (1995) proposed that corals experience reticulate evolution, with populations being intermittently fused together and split apart depending on processes such as changing sea level and surface currents (Figure 1.5). This surface circulation vicariance model suggests that the scale and timing of fusion and fission processes determine whether corals speciate to form monophyletic groups, which can even be reproductively isolated (real species, according to the biological concept), or whether they constitute paraphyletic groups or species complexes. Obviously, understanding genetic diversity and species relationships in corals has important consequences in defining appropriate conservation measures for coral reefs (Miller and Ayre 2008a).

THE GENUS *MADRACIS*

The coral genus *Madracis* Milne Edwards and Haime 1849 (Scleractinia, Pocilloporidae) and its dinoflagellate photosymbiont assemblages constitute the main biological model of the research here reported. Although not as visually conspicuous as the main Caribbean reef-building coral genus *Montastraea*, *Madracis* is one of the most abundant scleractinian genera in the region (Vermeij and Bak 2003). The genus has a wide depth distribution, ranging throughout the euphotic zone from less than 2 m to more than 100 m on Caribbean reefs (Wells 1973a; Vermeij and Bak 2002), which makes it particularly suitable for depth-related studies. Recently, extensive knowledge on the biology and ecology of *Madracis* has been acquired by research of Bak and coauthors (e.g. Nagelkerken *et al.* 2000; Diekmann *et al.* 2001; Meesters *et al.* 2001; Vermeij and Bak 2002; Bak *et al.* 2005; Kaandorp *et al.* 2005), providing the necessary background for a robust photoecological study.

Madracis (Figure 1.6) currently comprises six photosymbiotic species on Caribbean reefs (Wells 1973a,b; Vermeij *et al.* 2003a). The genus is characterised by morphological variation between species, such as in colony shape and in skeletal septal numbers, and shows high levels of morphological plasticity within some of the species (Wells 1973a; Fenner 1993; Bruno and Edmunds 1997). Although occurring in biogeographic sympatry, the species also show important differences with respect to their depth distributions (Vermeij and Bak 2003). *Madracis pharensis* (Heller 1968) and *Madracis senaria* (Wells 1973a) are encrusting or submassive depth generalist species (5 to > 60 m depth) differing in the number of primary septa (10 and 6, respectively). *Madracis decactis* (Lyman 1859) is nodular, has 10 primary septa, and occurs from 5 to 40 m. *Madracis mirabilis* Duchassaing and Michelotti 1861, *Madracis carmabi* (Vermeij *et al.* 2003) and *Madracis formosa* (Wells 1973a) are branching species with 10, 10 and 8 primary septa, and are restricted to shallow (2 to 25 m), mid (20 to 40 m) and deep water (> 30 m), respectively. Furthermore, there is a wide range of nondiagnostic

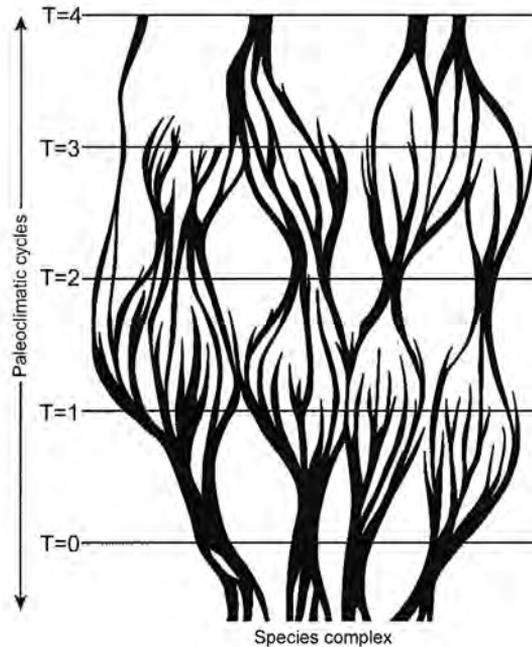


Figure 1.5 General structure of the coral polyp and underlying skeleton (after Veron 2000). Note the endocytic symbionts (gold) in the gastrodermis layer. Veron's surface circulation vicariance model for reticulate speciation in corals (modified after Veron 1995). Scheme represents the evolution of a species complex over time and paleoclimatic cycles. High levels of genetic connectivity resulting in small numbers of well defined species are generated during periods of strong surface circulation (at times $T = 0, 2, 4$). Periods of weak circulation ($T = 1, 3$) result in large numbers of indefinable species complexes due to fragmentation of populations.

colour variation among the species. *Madracis pharensis* shows the most expressive variation, with diverse morphs described (Vermeij *et al.* 2002; Sheppard *et al.* 2007). Using sequence variation of the rDNA ITS, Diekmann *et al.* (2001) showed that the morphological species distinction only corresponds to monophyletic groups for *M. mirabilis* and *M. senaria*, while all the other morphospecies form a paraphyletic "species" complex. *Madracis decaetis* overlaps morphologically with *M. pharensis* and their species status is under debate (Fenner 1993; Diekmann *et al.* 2001; Vermeij *et al.* 2007a). All studied *Madracis* species are brooders and release planulae from April to December. Both vertical and horizontal symbiont transmission have been reported (Vermeij *et al.* 2003b). All *Madracis* specimens sampled to date harbour clade B *Symbiodinium* (Diekmann *et al.* 2002) and three subcladal ITS types have been provisionally described (Diekmann *et al.* 2003).

OBJECTIVE AND THESIS OUTLINE

The main objective of this thesis is to understand how coral holobionts thrive across light gradients spanning large depth ranges on reef slopes. Because it is not always clear what the ecological and evolutionary units of corals and their symbionts are, accomplishing the objective firstly implicates comprehending their nature and studying the diversity and phylogenetic relationships involved in each symbiotic partner. Secondly, this thesis aims to unravel processes regulating potential host-symbiont combinations, their specificity and

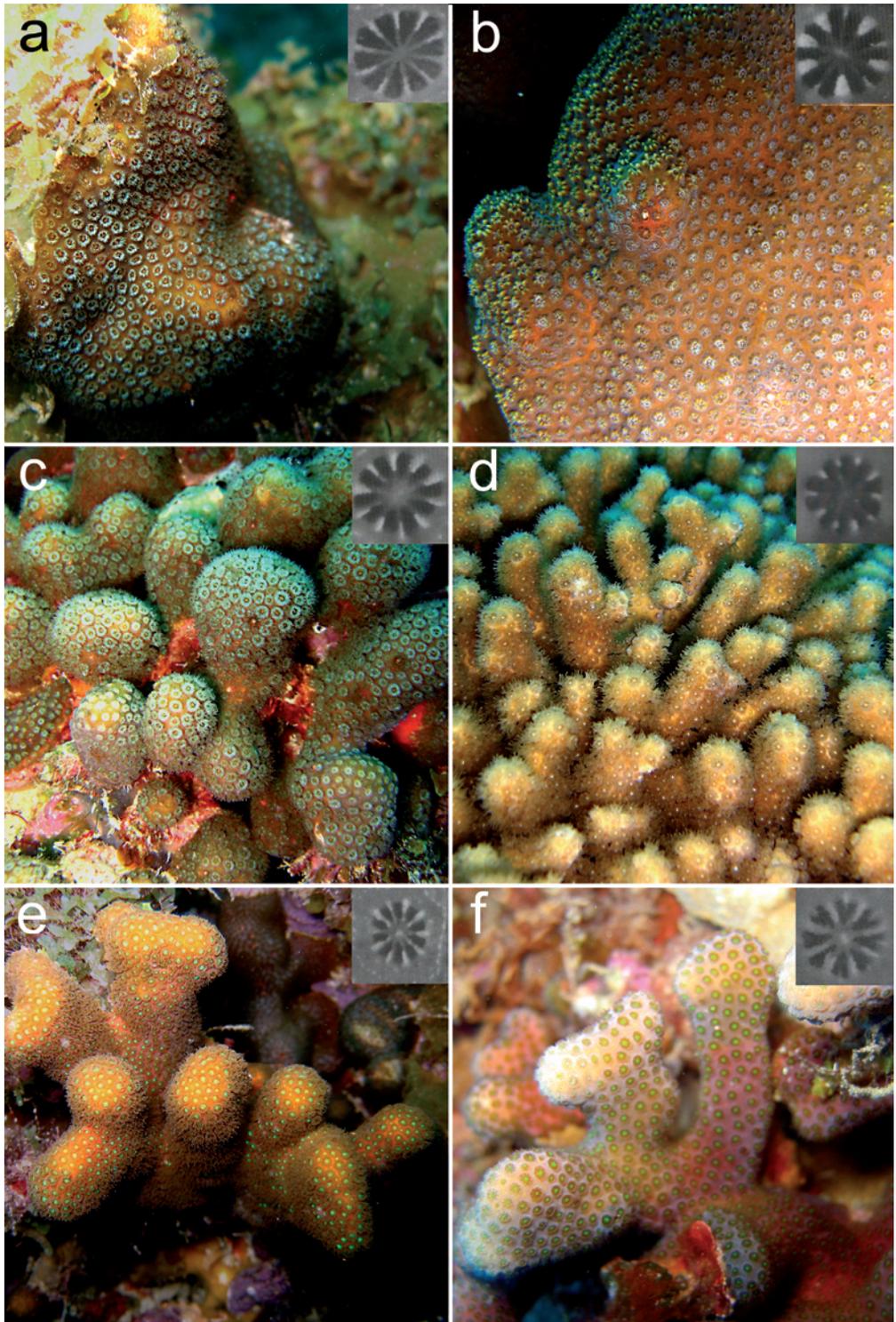


Figure 1.6 *Madracis* spp. The six currently recognized photosymbiotic morphospecies in the Caribbean region. (a) *M. pharensis*, (b) *M. senaria*, (c) *M. decactis*, (d) *M. mirabilis*, (e) *M. carmabi*, (f) *M. formosa*. Species distinction is based on colony shape and skeletal septal numbers (see inserts representing corallites). For full colour version see Appendix (page 131).

ecological zonation. Finally, the main objective could not be achieved without looking at the physiological and functional mechanisms that allow these symbioses to cope with the specific ecological niches they occupy. A deeper comprehension of the acclimatisation and adaptation extent of coral-symbiont associations under extreme environmental gradients will contribute to a better understanding of holobiont response and resilience under climatic changes. The coral genus *Madracis* was used as a biological model. All fieldwork was performed at the island of Curaçao (Netherlands Antilles) in the Caribbean Sea.

What is the genetic variation among *Symbiodinium* in the coral genus *Madracis* over large depth ranges, and is there a relationship between the composition of *Symbiodinium* genotypes and the depth distribution of the host?

In the context of coral adaptation to light gradients, the functional diversity of coral-algal associations distributed over large vertical ranges constitutes an overlooked issue. With a few exceptions (Toller *et al.* 2001; Warner *et al.* 2006; Sampayo *et al.* 2007) there are no extensive data on *Symbiodinium* variation below depths of 10 - 15 m. The starting topic of this thesis is the genetic variation of *Symbiodinium* in corals with large vertical distributions. Thus, in **Chapter 2** I address the genetic composition of symbiont assemblages associated with six *Madracis* morphospecies for a depth range of 5 – 60 m on a reef site in Curaçao (Frade *et al.* 2008c). Two different colony surface positions were studied to address intra-colonial variation. Using polymerase chain reaction and denaturing gradient gel electrophoresis of the rDNA ITS2, three symbiont genotypes were identified, with distributions that suggest ecological niche partitioning, involving distinct levels of host specificity and depth-based zonation.

What is the physiological plasticity hold by the symbiotic associations in *Madracis* across the reef slope, and what are the respective roles of host and symbiont in the holobiont response?

Although it is known that both animal host and algal endosymbiont have varied photoacclimatisation mechanisms (McCloskey and Muscatine 1984; Iglesias-Prieto and Trench 1994; Gorbunov *et al.* 2001), holistic approaches to address the variation in the interactive physiology of different coral-symbiont assemblages have only been vaguely explored (Anthony and Fabricius 2000). Besides, the level of resolution often applied to identify the photosymbiotic component (clades instead of types) may lead to inconclusive results. The integrated photophysiology of coral-algal associations is well within the scope of this thesis and in **Chapter 3** I present a multivariate approach to address the roles of host and symbiont on the *in situ* physiological response of *Madracis* holobionts towards light (Frade *et al.* 2008b). Several functional variables were measured for four *Madracis* morphospecies and three *Symbiodinium* genotypes across a large depth gradient (5 – 40 m) on a reef location in Curaçao. The study reveals both genetically and environmentally regulated mechanisms, and highlights the role of host properties in adjusting the internal environment for their endosymbionts. Distinct holobiont strategies varying in their optimization of light use are discussed in the scope of host-species distribution and dominance over the reef slope.

What is the functional variation between symbiont genotypes in *Madracis pharensis*, and

is there a role for colourful niches in regulating symbiont distribution?

Because there was inconclusive evidence for functional differences among symbionts, in **Chapter 4** I re-address the role of symbiont variation in the photobiology of reef corals (Frade *et al.* 2008a). To do so, a single host species, *M. pharensis*, was re-sampled at two depths (10 and 25 m) and two different light habitats, for several functional parameters such as pigment contents and photosynthetic activity. *Madracis pharensis* has three main colour morphs (Vermeij *et al.* 2002; Sheppard *et al.* 2007) which may hypothetically have a role in modulating the light intensity and distribution reaching the symbiont assemblage (Salih *et al.* 2000; Oswald *et al.* 2007). Two *Symbiodinium* ITS2 types were present showing depth- and colour morph-related distributions. This study suggests a role for spectral light niches in determining symbiont occurrence. A reciprocal depth transplantation experiment indicated steady symbiont populations after environmental change. Besides, there were unambiguous differences between several symbiont functional parameters revealing distinct ecological lineages based on light adaptation.

Are betaines abundant in reef-building corals, and if so, do they correlate to light gradients over the reef slope suggesting a role in oxidative stress defence?

Synergistic effects of temperature and light stresses threaten reef-building corals with bleaching (Douglas 2003), possibly due to cellular damage caused by oxidative stress at the level of photosystem II (Warner *et al.* 1999). Betaines are well known metabolites in vascular plants and free-living algae (McNeil *et al.* 1999), where they exert protein and membrane stabilizing effects, acting as photosystem II protective agents (Papageorgiou and Murata 1995). Besides, betaines have even been involved in genetic engineering procedures aiming to protect crops from cellular stresses caused by high temperature and high irradiance (Chen and Murata 2002). These metabolites may have a bleaching-protective role in reef-building corals, which face worldwide degradation due to a rapidly changing climate (Hoegh-Guldberg 2004). The study reported in **Chapter 5** results from collaborative research with Dr. Richard Hill (Michigan State University) and addresses the presence of betaines along a light gradient in ten reef-building corals, the core of which are *Madracis* species. The study demonstrates that these metabolites are present in physiologically relevant concentrations, are phenotypically plastic and likely participate in coral acclimatisation and stress-defensive processes, opening a line of investigation for future research.

Are morphology and molecular phylogenetics congruent in the coral genus *Madracis*, and is there evidence for a role of hybridization in the evolution of brooding corals?

Fully understanding the diversity and photobiology of coral-algal associations, or processes such as host-symbiont specificity, involves addressing the nature of the evolutionary units that constitute them. Specifically in the scleractinian host, often the morphologically described species do not match with molecular phylogenies (van Oppen *et al.* 2001b). One important reason is that reproductive barriers between species are weak in corals and this opens up a chance for hybridization events (van Oppen *et al.* 2002) which ultimately decouple morphology from phylogenetic DNA patterns. Although mass spawning events are known to maximize hybridization opportunities (Willis *et al.* 2006), introgressive hybridization has also been suggested for brooding corals (Diekmann *et al.* 2001) but its prevalence is still inconclusive. In

Chapter 6 I address the species boundaries in the genus *Madracis* and the role of hybridization in this brooding system. Phylogenetic inference and statistics of population structure based on three molecular genetic markers, one mitochondrial (*nad5*) and two nuclear (*ATPSa* and *SRP54*) intron regions, were applied to six putative *Madracis* species. Suggested host lineages are compared with symbiont signatures and interpreted based on known reproductive traits of the coral animal. *Madracis* host-symbiont assemblages are re-discussed. The study suggests an important role for introgressive hybridization in the evolution of the genus, but also provides evidence for symbiont-mediated disruptive selection.

Chapter 7 constitutes the general discussion. It addresses questions that are still unanswered in the field of coral photoecology, such as perspectives on adaptation of corals and their symbionts to bleaching. Furthermore, because the field of coral-symbiont associations sees new developments almost on a daily basis, I introduce a discussion on *Symbiodinium* species taxonomy based on recent interpretations given by other authors. Then I include a section about ongoing research, comprising a short summary of preliminary results obtained during this PhD study that have not yet been completely analysed or fitted into a manuscript for publication. Finally I present some guidelines for future research.

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