The coral genus Madracis. Speciation in corals and their symbionts

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Chapter 1

General Introduction

Coral reefs

Coral reefs are dominant ecosystems in shallow tropical oligotrophic seas and can be compared with tropical rainforests for their biodiversity and complexity (Ray 1988, Porter & Tougas 2001). The carbonate framework, which provides the matrix for reef life, is formed by the key organisms that produce it: the stony corals (Scleractinia) (Bak & Luckhurst 1980, Meesters & Bak 1993, Bak & Nieuwland 1995). These ecosystem engineers (Jones 1994, Coleman & Williams 2002) are peculiar organisms for two reasons. First they live in symbiosis with unicellular algae and are, at least partially, phototrophic. Second, corals are modular organisms where individual polyps form colonies by self-replication or clonal growth. The living coral animal is only a millimeters-thin veneer of living tissue overlaying the calcareous skeleton that is excreted by the coral animal as it grows. As a consequence, the colony rather than the individual polyp is the functional ecological unit. Features of the colonial life style include partial mortality, fission and fusion of colonies, and decoupling of colony size and age. As a result, corals display considerable variation in their morphology, ecology and, presumably, in their genetics.

Corals

Corals are highly variable taxonomic units. Within and between species of a single genus one can find remarkable variation in colony form, skeletal micromorphology (Budd 1990, Veron 1995, Knowlton & Budd 2001), reproductive biology (Harrison & Wallace 1990, Van Veghel 1993), types of symbiotic dinoflagellates (Rowan 1998) and competitive behaviour (Lang 1971, Van Veghel & Bak 1993). As a consequence of this remarkable variability in form as well as function, species boundaries remain equivocal in practically all coral genera. The balance between historical genetic constraints on morphological form and new adaptations (Brooks & McLennan 2002), as well as the balance between natural morphological plasticity and ecotypic differentiation (Pigliucci 2001) have been discussed for corals by many investigators (Yonge 1968, Foster 1979, Willis & Ayre 1985, Van Veghel & Bak 1993, Veron 1995) with little consensus. In fact, we do not know to what extent morphology may be safely coupled to species identification. As the number of molecular phylogenetic studies in corals has increased in recent years, it has become clear that there are: 1) many sibling species, i.e., taxa characterized by morphologically fuzzy boundaries and genetic paraphyly (Knowlton 1993); 2) cryptic species, i.e., taxa characterized by being morphologically indistinguishable yet genetically mono- or paraphyletic (Van Oppen et al. 2001) and; 3) pseudo-species, i.e., taxa characterized by being morphologically different in appearance but genetically indistinguishable (Miller & Benzie 1997). These kinds of observations reinforce the need for careful reassessments of species concepts in corals and better operational definitions of coral species because many ecological studies are based

Table 1. Described species of *Madracis* and their geographic distribution. Authors: 1) Cairns (1999); 2) Veron (2000), 3) Vermeij et al. (2002). Grey highlight indicates taxa considered in the present study. The four Caribbean-Eastern Atlantic taxa not considered in the present study are deepwater species or of uncertain status. Indian Ocean and Pacific samples were not available.

Species	Author	Western Atlantic (Caribbean)	Eastern Atlantic (Mediterranean)	Red Sea and Indian Ocean	Western and Central Pacific	Eastern Pacific
Madracis formos Wells 1973a	1,2	x				
Madracis mirabilis Duchassaing & Michelotti 1861	1,2	x				
Madracis senaria Wells 1973b	1,2	x				
Madracis decactis (Lyman 1859)	1,2	x	x			
Madracis pharensis (Heller 1868)	1,2	x	x		?	?
Madracis carmabi Vermeij et al. 2002	3	x				
Madracis asperula M. Edward & Haime 1849	1,2	x	x			
Madracis brueggemani (Ridley 1881)	1	x				
Madracis myriaster (M. Edward & Haime 1849)	1	x				
Madracis profunda Zibrowius 1980	1		x			
Madracis hellana M. Edward & Haime 1849	1			x		
Madracis interjecta Marenzeller 1907	1			x		
Madracis kauaiensis Vaughan 1907	1			?	x	
Madracis kirbyi Veron & Pichon 1976	1,2			x	x	
Madracis asonoi Yabe & Sugiyama 1936	1,2				x	
Madracis singularis Rehberg 1892	1				x	

on single-species assumptions that may not be met (Knowlton & Jackson 1994). On a more theoretical level, the speciation process itself is poorly understood in corals (Veron 1995) and at the practical level, issues related to potential interbreeding among "species" are relevant to reef management (Van Oppen et al. 2001).

The coral genus *Madracis*

Approximately 20 genera of corals representing 12 families and »50 species are present in the Caribbean. Among these, the genus *Madracis* (Scleractinia; Astrocoeniina; Pocilloporidae) Milne-Edwards & Hyme 1849, is one of the most important. Although visually less conspicuous than the putatively most abundant genus, *Montastraea*, semi-quantitative line transects (Vermeij unpublished data) suggest that it may rank second in abundance in some areas.

Madracis comprises a group of between 8 (Veron 2000) and 15 (Cairnes 1999) described scleractinian morphospecies (Table 1). There is a wide range of color forms and colony morphologies within each species (see inside cover). The genus is distributed throughout the tropics (Zibrowius 1980, Veron 1993, Swedburg 1994)

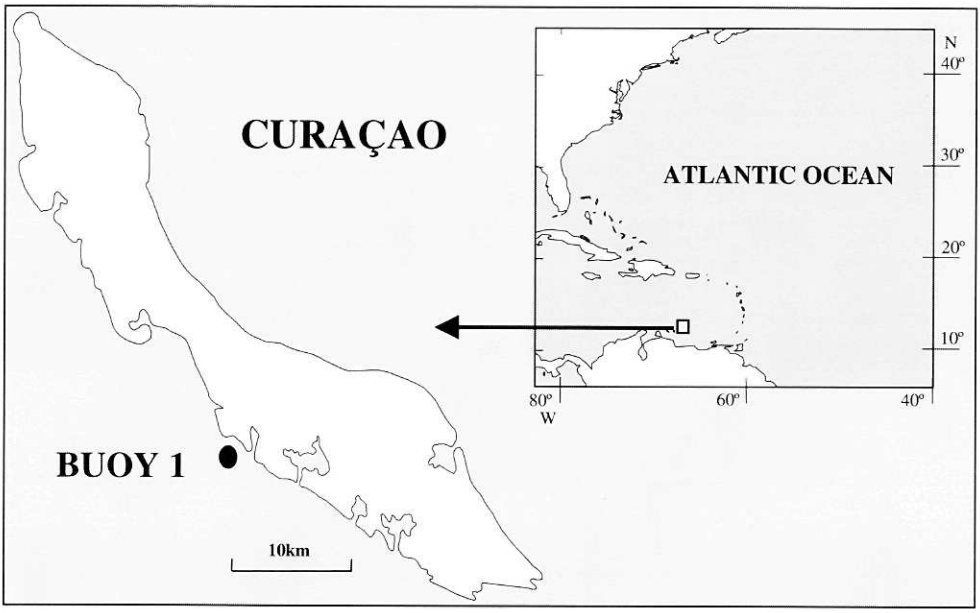

Figure 1. Curaçao, Netherlands Antilles, showing the Buoy-1 study site

being most diverse in the Caribbean-Western Atlantic followed by the Eastern Atlantic, Mediterranean, Red Sea, and Indo-West Pacific. Five morphospecies are typically recognized in the Caribbean with a range extending from Southern Florida (USA) to Brazil. These include *M. mirabilis* Duchassaing & Michelotti 1861, *M. decactis* (Lyman 1859), *M. formosa* (Wells 1973a), *M. senaria* (Wells 1973b) and *M. pharensis* (Heller 1868). A sixth morph *M. carmabi,* which has recently been identified (Vermeij et al. 2002), is morphologically different then other Caribbean species. Three additional Caribbean species—which will not be considered in this thesis—have been described but are either rare or found only in very deep water, e.g., *Madracis asperula* M. Edward & Haime 1849 or are known only from very old literature, e.g., *Madracis asonoi* (Yabe & Sugiyama 1936) *Madracis myriaster* M. Edward & Haime 1849. Species may be found at depths of <2 m to >200 m.

The five common morphospecies of *Madracis* found in the Caribbean generally occur in biogeographic sympatry. On a given reef, the species may occur in sympatry or parapatry with widely overlapping—but not identical—habitats (Vermeij and Bak 2002). This is the case at the Buoy 1 study site on Curaçao, Netherlands Antilles (Figs. 1 and 2). This reef, close to the Ecological Institute Carmabi, has a long-established history of coral reef research (e.g. Bak 1975, Bak and Engel 1979, Bak and Criens 1982, Bak et al 1982, Van Duyl 1985, Meesters 1995, Vermeij et al 2002). Studies focusing specifically on *Madracis* are relatively few and those

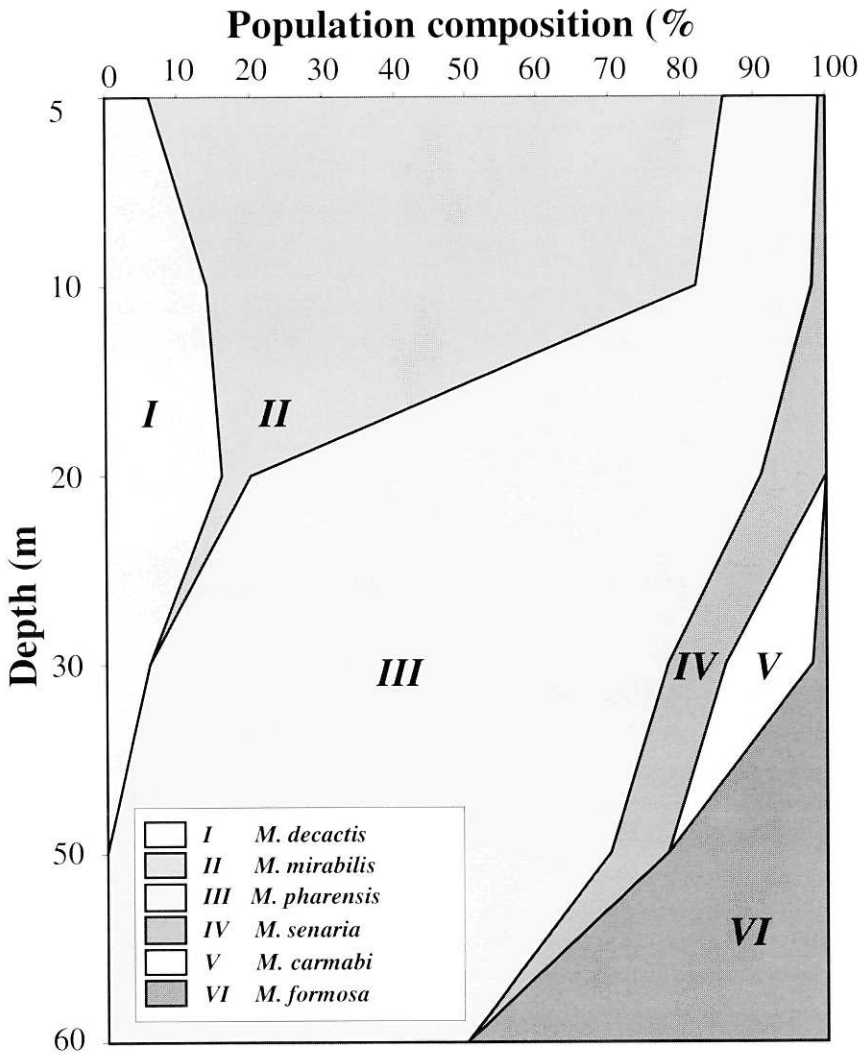

Figure 2. Distribution of *Madracis* morphospecies at Buoy 1 (Curaçao, Netherlands Antilles). Modified after Vermeij and Bak (2002).

available have addressed questions related to the role of fragmentation and feeding behavior of *M. mirabilis* (Bak and Criens 1981, Sebens et al 1996, 1997, Bruno 1998, Nagelkerken et al. 2000) rather than questions related to common ancestry and distribution of zooxanthallae symbionts. Recent work by Mark Vermeij (2002) and the present thesis will change the status of *Madracis* as an understudied genus.

How old is *Madracis*?

The earliest described fossils of Caribbean reef building *Madracis* species are probably of Cretaceous origin (»125,000,000 my ago). In the Caribbean region *Madracis* is known from the middle Eocene to recent, (Budd pers. comm.). However, the fossil record extends only to 15-11 million years ago for extant *M. mirabilis* and *M. decactis* (Budd et al. 1994, 1995) and only to 1.5 million years ago for *M. pharensis* (Budd & Johnson 1999). There is no fossil record for *M. formosa*, *M. senaria* and *M. carmabi*. Whether their absence is an artifact remains uncertain. In any case the modern species of *Madracis* are certainly not older than 12-10 million years and perhaps as young—or younger—than 5 million years. It is conceivable that the species are very recent. During the last Pleistocene glacial episode, which ended between 20,000 and 10,000 years ago, significant changes in sea level greatly affected Caribbean reefs, including those on Curaçao. Local extinctions and/or severe reductions resulted in a recolonization of nearly re-emerged coastal areas with opportunities for occupation of new niches and for potential nascent speciation. I will return to this subject later when I talk about models and mechanisms of speciation in corals and the evidence we have for shallow phylogenies and incomplete reproductive isolation.

The symbiotic dinoflagellate genus *Symbiodinium*

Symbiotic algae are algae that live in close physical contact with another (different) organism. Cyanobacteria, eukaryotic and dinoflagellate algae are examples of algae with a symbiotic life style. Many marine invertebrates, e.g. jelly fish, sponges, zoanthids, gorgonians, foraminifers and bivalves (Langer & Lipps 1994, McNally et al. 1994, Ohno et al. 1995) including most corals, contain interstitial, unicellular phototrophic algal symbionts belonging to the Dinophyceae. These symbionts are also commonly referred to as zooxanthellae, the golden-colored ones. The symbiont genus *Symbiodinium* Freudenthal 1962 is specifically associated with reef building corals and plays an important ecological role throughout oligotrophic, tropical oceans, contributing to coral reef productivity (Muscatine and Porter 1977). Photosynthetic products are translocated from the algal cells, located within cells of the coral polyp, to the animal host with high efficiency (Muscatine et al. 1981). Symbiotic algal photosynthesis also contributes to high calcium carbonate deposition rates of hermatypic corals, thus contributing to the formation of the coral skeleton and the reef framework itself (Goreau 1977). Algal symbiosis in scleractinian corals is probably responsible for the existence of coral reefs as we know them (Stanley and Swart 1995).

Because of their lack of distinctive morphological features, zooxanthellae were initially considered to be a single taxon in which *Symbiodinium microadriaticum* Freudenthal 1962 was, for nearly 20 years, considered a single, pandemic species.

Table 2. List of symbiodinium species, their phylogenetic clade designation, geographic region and host of origin following LaJeuness (2001). Many different taxa belonging to different Symbiodinium clades have been isolated recently from many different hosts (see LaJeunesse 2002).

Symbiont species [a]	Phylogenetic clade	Geographic origin	Host origin
Symbiodinium microadriaticum subsp. *microadriaticum*	A	Caribbean	*Cassiopeia xamachana* (Rhizostmeae)
Symbiodinium microadriaticum subsp. *condylactis* [b]	A	Caribbean	*Condylactis gigantea* (Actinaria)
S. pilosum	A	Caribbean	*Zoanthus sociathus* (Zoantharia)
S. meandrinae	A	Caribbean	*Meandrina meandrites* (Scleractinia)
S. corculorum	A	West Pacific	*Corculum cardissa* (Bivalvia)
S. lincheae	A	Western Atlantic	*Linuche unguiculata* (Coronatae)
S. pulchorum	B	Central Pacific	*Aiptasia pulchella* (Actiniaria)
S. bermudense	B	Western Atlantic	*Aiptasia tagetes* (Actiniaria)
S. goreaui	C	Caribbean	*Rhodactis lucida* (Corallimorpharia)
S. californium	E	East Pacific	*Anthopleura elegantissima* (Actiniaria)
S. kawagutti	F	Central Pacific	*Montipora verrucosa* (Scleractinia)

[a] Cultured isolates
[b] Later referred to as *Symbiodinium cariborum* (Banaszak et al 1993)

Beginning in the 1980s, however, biochemical, physiological, and behavioral studies (Blank & Trench 1985, Blank et al. 1988, Trench 1987) revealed that the genus was highly diverse, and with the advent of PCR and DNA sequencing in the early 1990s, it was clear that *Symbiodinium* contained several species (Rowan & Powers 1991a, b). However, only a few species have been formally described (Table 2)(Trench 1993, reviewed in Rowan 1998).

In most of the coral literature, *Symbiodinium* "species" are simply designated as clades, types, phylotypes or groups—and labeled "A" through "F" including subtypes within these designations (Rowan & Powers 1991a, McNally et al. 1994, Langer & Lipps 1994, Carlos et al. 1999, Baker & Rowan 1997, LaJeunesse 2001,2002). Prior to the present study, nothing was known about the types and distribution of *Symbiodinium* in *Madracis*.

Following the discovery of multiple zooxanthellae "types" or "species", the immediate question was whether or not the associations were host-specific, vertically

inherited and thus illustrative of an obligate co-evolutionary process (see Schoenberg & Trench 1976, Trench 1993); or whether or not the associations were strictly ecologically driven, environmentally acquired and thus illustrative of host-symbiont adaptations that could be adjusted to the local light regime of a given habitat (see Kinzie & Chee 1979). By superimposing the phylogenetic topology of the coral hosts on the phylogenetic topology of the associated symbionts it was possible to show that the associations were not strictly host-specific and did not fit a strict one-to-one correspondence of co-evolution (Rowan & Powers 1991, 1992, McNally et al. 1994). On the contrary, coral species may host a single type of zooxanthellae (Billinghurst et al. 1997) or they may contain several types, varying between individuals of the same species or within one individual of a species in relation to its habitat (Rowan & Knowlton 1995, Rowan et al. 1997, Baker et al. 1997). This in turn raises the question of how corals acquire their zooxanthellae. In some corals they are acquired by repeated environmental capture (Schwarz et al. 1999). However, it is still not clear as to whether a particular coral host preferentially maintains a particular *Symbiodinium* type while purging undesirable ones, or if a particular symbiont type is seasonally or spatially variable. Moreover, most of the aforementioned work has been carried out on only a few species with the emphasis on the genus *Montastraea* so that current generalizations are, in fact, premature. For example, it has become virtually axiomatic (Wood 1999) that the "type(s)" of zooxanthellae present in a coral is/are correlated with light adaptation, i.e., coral individuals living in shallow water have a different type than coral individuals living in deep water. Hard proof of this, however, remains elusive and it has only been investigated in *Montastraea* (Rowan & Knowlton 1995) and *Acropora* (Baker et al. 1997). Obviously a life history strategy that offers preferential flexibility at such a fundamental physiological level has broad implications for local community structure, intra- and inter-specific competitive ability and so forth. It also signals the vulnerability of corals if they lose their zooxanthellae symbionts. This phenomenon is called coral-bleaching (Glynn 1991, Brown & Ogden 1993) and has led to high mortality rates in some corals species but not in others. We have never observed bleaching in *Madracis* in the field.

Key questions and rationale

Because of the ecological importance of *Madracis* it is essential to be able to reliably identify its species in order to understand their evolution and their ecological interactions—with each other, with their symbionts and with other reef residents. While the need for correct identification and a phylogenetic classification seems self-evident, ongoing and seemingly irreconcilable taxonomic problems continue to hinder progress—not just in corals, but in many marine organisms. Quite often, taxa of ecological interest are avoided because no one knows "what-is-what"—or

worse—it is assumed that a common taxon is a single species—when it is not. This taxonomic constraint has become acute in a number of groups but particularly in the marine domain where morphological simplicity and conservatism foil attempts at identification. The advent of molecular data over the past decade has provided new avenues for investigating morphologically "fuzzy" species complexes such as corals. While these methods are no panacea they have provided a means for tackling the problem and the new awareness of the global importance of "biodiversity" is providing opportunities to upgrade outdated taxonomic circumscriptions—many of which were done in the late 19th century. Of broader interest are the understanding of the speciation process in corals and the establishment of both conceptual as well as operational definitions of species—as a unit of biodiversity and conservation.

Much of the research on the evolution of corals is oriented towards the Indo-West Pacific region. Only limited studies have been performed on Caribbean corals with *Montastraea* as the most important example. The present study on *Madracis* is therefore relevant to improving our understanding of more general notions about coral evolution in the Caribbean.

I address the following questions in this thesis.

With respect to the animal component of the coral algal holobiont:

1. What are the phylogenetic relationships among putative species within the coral genus *Madracis*?
2. 2. Is there evidence for cryptic species, sibling species, pseudo-species or reticulate species as assessed using DNA sequence data and a phylogenetic species concept?
3. 3. To what extent do the standard, morphologically defined species of *Madracis* correspond to monophyletic groups based on comparative DNA sequence data?
4. To what extent do quantitative morphometric data from the corallites and the colony habit define morphological species groupings that can be applied in the field?
5. Which morphometric characters (if any) have the most diagnostic power, i.e., give the highest probability of correct identification with the least amount of effort?
6. To what extent can a biological species concept be applied to corals and how should coral reef biologists proceed in the field?

With respect to the algal endosymbiont:

7. Do different species of *Madracis* harbor one or several types of *Symbiodinium*?
8. What is the correlation between zooxanthellae type, morphospecies and/or habitat?
9. Does *Madracis* fit the *Montastraea* paradigm?

Species concepts

There are many species definitions (reviewed by Mayden 1997). Beyond the classical, more or less static, morphological concept, however, most variations center around two central criteria—reproductive isolation and/or the recognition of monophyletic groups that reflect identity by descent. Below I briefly discuss these.

Traditional species-level taxonomies are almost exclusively based on morphological discontinuity—a morphological species concept—according to the classical, Linnaean "type-method" which dates back to the 18th century. In this approach, new specimens are compared against the standard, i.e., the holotype, and assigned accordingly. If sufficient discontinuities are found, then a new species can be described and a new circumscription made based on taxonomic judgment/expertise and opinion. A strict international code of zoological nomenclature provides the rules for the application of names. The main problem with this approach is that there is no objective way to take into account intra- and inter-specific variation or to distinguish homology (=identity by descent) from homoplasy (=identity by convergence). In groups with a large number of species, combined with relatively "low morphology"—typical of many marine organisms including corals—the quality as well as utility of such taxonomies can become marginal. In addition, there is no evolutionary basis for a classification and the groupings remain fundamentally arbitrary, i.e., based solely on taxonomic authority.

The relative simplicity and modular nature of corals has always made them a difficult taxonomic group. Then, with the advent of research diving in the 1960s and 1970s the range of morphological forms discovered—both within habitats and biogeographically—produced a new and even more bewildering array of possibilities that have remained almost intractable using morphological data. While genus and higher-level classifications are generally considered stable, species-level taxonomies are highly questionable in many genera.

With the advent of numerical taxonomy (Sneath & Sokal 1973) and computing in the late 1970s, the comparative approach was made operational in the sense that discrete characters and character-states could be identified and scored on a per-taxon basis, i.e., operational taxonomic units (OTUs). Hierarchical clustering algorithms such as UPGMA (Michener & Sokal 1957) were used, for the first time, to summarize large data sets based on shared, overall similarity. Non-hierarchical, phenetic methods, such as principal component analysis and variants (Sneath and Sokal, 1973), were also used to identify clusters of OTUs based on partitioning of the maximum variance within a suite of overlapping, continuous characters. While both of these approaches provided a major step forward in systematic biology more generally, they still suffered from the lack of an evolutionary context because such methods are unable to separate similarity due to shared ancestry from similarity shared through convergence or parallelism. Still, the utility of phenetic methods

should not be dismissed. They can be very powerful sorting tools, particularly some of the newer multivariate methods, in cases where little convergence has occurred. In such cases the phenetic tree may actually approximate the phylogenetic tree.

While a classification can, in principle, be anything that one wants it to be (e.g., alphabetic, functional, by color or other property of interest in an operational context), the contemporary biological view is that the classification must reflect the evolutionary history of the group. While systematists have more or less subscribed to this view, implicitly—until the last 20 years there was almost no explicit way to apply it.

The "biological species concept" (BSC) (Dobzhansky 1937, Mayr 1942) categorizes species as groups of actually or potentially interbreeding individuals, with boundaries between species defined by barriers to gene flow that have a genetic basis. These barriers to reproduction can be pre- or post-mating isolating mechanisms or they can be ecological, e.g. habitat preference influencing mating probability (Knowlton 2000).

The practical application of a BSC to corals is problematic for several reasons. First, clonal reproduction plays a large role in all corals because they are modular organisms. They grow by clonal propagation of the polyps. Second, reproductive-isolating barriers are apparently weak. Extensive hybridization has now been documented in several species (Miller 1994 [*Platygyra*], Van Oppen et al 2001 [*Acropora*]). The concept of reproductive isolation is further exacerbated by synchronized, broadcast spawning (Harrison et al 1984) across species boundaries and the possibility of ongoing nascent speciation. Not all coral species, after all, are ancient. Although the BSC is heuristically appealing, it's operational applicability remains dubious.

The "phylogenetic species concept" (PSC) (Cracraft 1983, Donoghue, 1985) recognizes species or other taxa on the basis of shared homologous characters that form monophyletic groups. The PSC avoids all reference to reproductive isolation (though it is compatible with the BSC) and focuses instead on shared identity by descent. Phylogenetic based analyses can utilize morphological as well as molecular data sets. However, data sets that have too much or too little variation, and therefore an insufficient number of phylogenetically informative characters (usually but not always the morphological data set), will reduce the resolution of the tree. In corals, morphological data sets are meager and thus not amenable to phylogenetic analysis as compared to molecular data sets. The number of characters in molecular data sets is limited only by the availability of suitable sequences. Highly conserved sequences will not be appropriate at the species level whereas rapidly evolving sequences may present too much variation. Homology, can be established in various ways including

ontogeny, position and outgroup methods (Hillis et al 1996). For DNA sequence data homology is always positional as assessed from the alignment. Subsequent phylogenetic analysis of the data matrix can be carried out using various distance or character-based algorithms, i.e., neighbor joining, maximum parsimony and maximum likelihood.

Coral reproduction

Corals display a wide variety of sexual and asexual reproductive modes. As a modular organisms coral colonies can divide into separate units by partial mortality and fragmentation such that identification of the individual can only be conclusively made using genetic data. In addition, the individual coral animals can asexually reproduce by such varied mechanisms as polyp bail-out (Sammarco 1982) or budding (*sensu* Ayre and Resing 1986), where a single polyp or a larger portion can detach itself from the adult colony and settle again, and asexual production of planulae (Stoddart 1983). Sexual reproductive traits range from gonochoric (dioecious), in which a species maintains separate male and female colonies (thought to be rare), to hermaphroditic species with male and female gametes in the same polyp (most common), and even to sequential hermaphrodites, in which male colonies/polyps are first male and then shift to female or vice versa (Harrison &Wallace 1990).

Fertilization can take place internally or externally. Corals which maintain the zygotes within the polyps until they develop to the planula stage are called brooders; whereas corals that release their eggs and sperm directly into the water column are referred to as broadcast spawners. In many broadcast spawning coral species gametes are simultaneously released as egg/sperm bundles. In principle, this provides opportunities for self-fertilization, particularly in the event of gamete dilution from congeners. However, breeding trials with broadcast spawning corals have shown that fertilization success is low when gamete bundles from the same colony were used (Knowlton et al. 1997). In brooding corals, in contrast, selfing appears to be common (Brazeau et al. 1998). All *Madracis* morphospecies are brooders (Vermeij et al. 2002) but nothing is known about their fertilization or selfing rate since cross fertilization experiments involving corals with a brooding mode of reproduction is extremely difficult.

It is clear that the timing of reproduction can have great influence on the extent of selfing or outbreeding. Species able to release egg/sperm bundles have the potential to outcross or self, depending on whether there is gamete limitation due to dilution. The fact that many coral species, simultaneously release their gametes during certain minutes or hours in the course of a year (Harrison et al 1984, Richmond and Hunter 1990) demonstrates an interesting strategy for insuring fertilization. Such massive spawning events raise the question where barriers to inter-specific reproduction occur. Very subtle differences in timing may be present in which non-self sperm

may have an initial advantage or in which cross-species fertilizations fail in their subsequent development. Species-specific, gamete binding proteins, such as those described for example in echinoderms (Palumbi & Metz 1991), are unknown in corals. Clearly, these issues are important for understanding the processes that promote reproductive isolation in corals.

Speciation in corals

Speciation in the sea presents a number of challenges. It has been argued, for example, that the continuity of the marine environment can, in principle, permit limitless dispersal and, by extension, unlimited gene flow (see Palumbi 1992, 1994). In this view one expects to find fewer species, a lower speciation rate and the presence of species with enormous ranges. Population genetic studies based on allozyme data, especially earlier studies, tended to find low variation and broad monomorphic patterns, which generally supported this view. With the advent of DNA sequence data and microsatellite loci, this view has had to be modified. Despite long periods spent in the pelagic state which facilitate dispersal and gene flow, levels of reproductive isolation are highly variable and very much more influenced by pre- and post-mating barriers than previously thought. This is particularly well-studied in marine mollusks (*Mytilus* for example, Palumbi & Metz 1991, Palumbi & Kessing 1991). More recently, corals have been added to the list (reviewed in Veron 1995).

Corals show a broad range of ecological strategies. High fecundity, high dispersal capabilities, large population sizes, wide distributions, and extreme longevity resulting in largely overlapping generations (Hughes et al. 1992, Palumbi 1994) are displayed by many coral species and have direct effect on their evolution and speciation. In addition, synchronous release of gametes with sympatric congeners—mass spawning (Harrison et al. 1984, Willis et al. 1985, Babcock et al 1986)—creates numerous potential opportunities for hybridization and eventual introgression. Experimental breeding trials with corals confirm that hybridization can occur between sympatric congeners and that it has probably contributed to the evolution of coral species in the genera *Acropora*, *Montipora* and *Platygyra* (Willis et al 1997, Miller and Babcock 1997, Hatta et al. 1999). Although these studies were criticized on the grounds that they may have been laboratory artifacts rather than expressions of natural events in nature, recent molecular studies in the Indo-Pacific region as well as the Caribbean are consistent with hybridization in several species of corals (Odorico and Miller 1997, Hatta et al. 1999, Miller and Benzie 1997, Benzie 1999, van Oppen et al. 2001) including *Madracis* (this Thesis). It may turn out that corals are more like angiosperms than metazoans with respect to weak reproductive isolation and hybridization.

In theory, the key event in the completed speciation process is the formation of two groups of organisms that are reproductively isolated from each other and thus

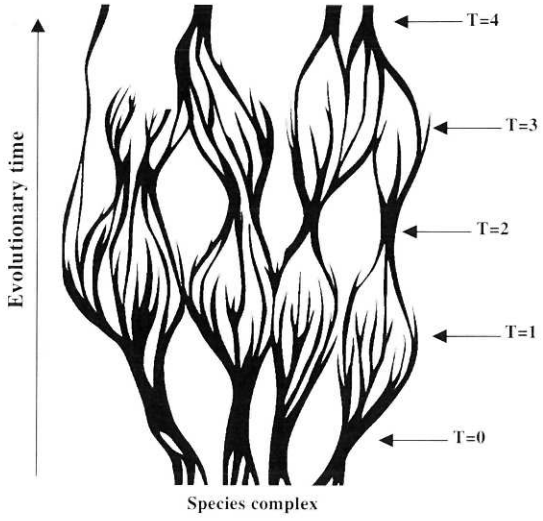

Figure 3. Veron's surface circulation vicariance model for reticulate speciation in corals, redrawn following Veron (1995). The picture represents the evolution of a species complex over time as a result of paleoclimatic cycles of reticulate evolution. Strong surface circulation creates high genetic connectivity resulting in small numbers of well defined species (T=0, 2, 4) while fragmentation of populations due to weak surface circulation results in high numbers of ill-defined species complexes (T=1, 3).

have no gene flow. Many models have been proposed to explain how speciation can take place (Futuyama 1986). Allopatric speciation requires populations to be separated by geographic barriers that restrict and ultimately prevent gene flow. Over time these populations evolve in different directions through natural selection, mutation and genetic drift. Inevitably reproductive isolation barriers develop between geographically isolated populations as a nonadapted by-product of genomic divergence (Avise 1994).

Additional factors that promote reproductive isolation include pre-mating barriers such as local habitat isolation, temporal isolation, behavioral isolation or mechanical isolation; and post-mating barriers such as gamete incompatibility, hybrid inviability or hybrid sterility. All of these factors have been demonstrated for both terrestrial and marine organisms, albeit that the models were originally developed for terrestrial organisms. In corals, however, the picture is far from clear. At the pre-mating level habitat isolation seems the most predominant whereas post-mating factors seems to be relatively weak (Veron 1995). Coral systematists, paleontologists and ecologists are increasingly aware of apparent reticulate species groups for which they do not have good explanations.

Veron (1995) has proposed a model of speciation for corals called surface circulation vicariance. In this model (Fig. 3), populations are intermittently fused together or split apart as a consequence of changing sea level and surface currents (see also Potts 1984). Depending on the scale and timing of the fusion or fission process, corals may speciate to form monophyletic groups with or without reproductive isolation; or they may stay in a paraphyletic state with incomplete lineage sorting. Veron's model is conceptually appealing but remains controversial. However, as more is learned about synchronous, mass spawning, interspecific hybridization based on molecular markers, and peculiarities of flexible life history traits and strategies, it appears that there is considerable support for the model.

Morphological data

Madracis morphospecies are distinguished mostly by their overall colony morphology and the number of primary septa in the corallite skeleton (see inside cover). Typically one is reduced to 3-4 characters. Although taxonomists have sought additional characters (Wells 1973 a,b), most of these are overlapping between morphospecies and, at best, provide only weak diagnostic power. Minimal morphology combined with plasticity is the most difficult situation and common in corals.

Madracis species are usually difficult to identify even by the experienced observer, because the wide variation of growth forms, color morphs and habitat variation (Fenner 1993, Bruno 1997) hinders reliable identification. In addition, nothing is known about the phylogeny of *Madracis* species—an old genus with apparently modern species. The present study uses micro-morphological characters that can not be readily observed in the field by the investigator. These characters are linear measurements of specific landmarks on the coral skeleton and have to be determined specifically for the species under investigation. They are related primarily to the sizes of various corallite architectural features, as well as size and spacing of corallites. In selecting the corallites for measurements care was taken not to select corallites from the tip or edge of the individual colony because the morphology in those areas is highly irregular due to incomplete growth. Septal walls are often very thin and/or not all morphometric characters are sufficiently developed.

Molecular Data

The advantage of molecular data is that it independent of morphology and, for many groups including corals, provides the only way to assess relationships. Within eukaryotic genomes, nuclear ribosomal DNA (rDNA) is organized in long arrays of tandem repeats (Fig 4). The intergenic spacers (IGS) separate the repeats or cistrons. A repeat consists of three genes: the small subunit (SSU or 18S), the 5.8S and large subunit (LSU or 26S/28S). Each gene is separated by an internal transcribed spacer

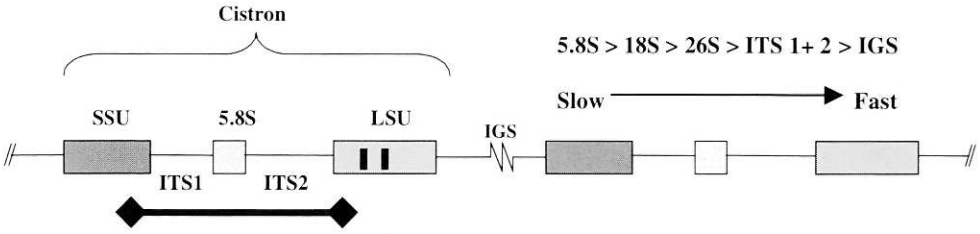

Figure 4. Schematic representation of the nuclear ribosomal DNA cistron. Bold face bars indicate regions used in this thesis. See text for further details.

(ITS1 and ITS2) which are part of the single cistronic transcript. The various regions of the rDNA cistron provide a range of phylogenetic resolution and have been used from the level of populations to kingdoms (Avise 1994). The SSU, 5.8S and LSU genes are the most conserved regions and have been used to unravel deep relationships. Certain secondary structural domains within the genes are also sometimes useful as they exhibit more variation. The ITS and IGS regions are relatively free of evolutionary constraint and have been widely used for phylogenetic studies at the species and subspecies levels (Baldwin et al. 1995) in a wide variety of marine organism including marine algae (Van Oppen et al.1995, Olsen et al. 1998), corallimorpharians (Chen and Miller 1996), and corals (Odorico & Miller 1997, Lopez et al. 1999 Van Oppen et al. 2000) including Madracis (this Thesis).

Although the rDNA repeats are homogenized via processes of concerted evolution (Dover 1982, Arnheim 1983, Schlotterer & Tautz 1994), intra-individual variation due to non-homogenized repeats is potentially problematic and must always be checked for by sequencing several clones per individual. Intra-individual polymorphism is most commonly due to incomplete lineage sorting though hybridization, recent or incomplete speciation, prolonged asexuality or the possibility of cistrons being located at multiple loci on different chromosomes (Quiada et al 1997, Hugall et al. 1999). In corals various levels of ITS variation have been found from little intra-specific variation in *Poritis* (Hunter et al. 1997) to extremely high levels of intra- individual variation in *Acropora* (Van Oppen et al. 2002).

In the present study I utilize the ITS spacers and two domains within the LSU. These regions have been applied to all other studies in corals, which makes cross-comparison possible. A few other genetic markers, nuclear and mitochondrial, have been utilized in corals (Van Oppen et al 1999, Van Oppen et al 2001) but these either show low variability or intractable problems with PCR primer design have made them unsuccessful in *Madracis*. Although it is desirable to use multiple sequences in order to look for consistency and congruence between gene trees and species trees, this is not always possible. Use of mtDNA sequences, such as Cox 1

or D-loop have proven impossible owing to the fact that the areas are either invariable or not present. It appears, for example, that mtDNA in *Acropora* expresses unexpectedly low levels of sequence divergence (Van Oppen et al 1999). It was suggested that cnidarians might have a functional mtDNA repair mechanism unlike higher animals.

Phenetic and phylogenetic analyses

Morphometric data were analyzed in a *phenetic* framework using both principal component and canonical discriminant analysis. These methods differ in that the former makes no *a priori* assumptions about group membership and simply partitions the variance among characters into the fewest number of axes; whereas the latter starts from an initial hypothesis of group membership as defined at the outset and looks for goodness of fit. The main disadvantage of these approaches is that they are not phylogenetic. However, their main advantage is that they provide a way to critically evaluate continuous characters and determine which (if any) provide useful diagnostic power. In the case of corals, the desire to find some level of correlation between analyses based on skeletal morphologies and those based on genetic data is driven by the need to be able to better utilize fossil data—for which no genetic data can be had. As pointed out by Jackson et al (2001), this approach is becoming sufficiently refined as to provide a significant and much welcome addition to unraveling the fossil record of corals and to providing a better understanding of plasticity (Budd 1984, Cuffey and Pachut 1990, Knowlton and Budd 2001).

DNA sequence data was analyzed in a *phylogenetic* framework. Both distance and character-based approaches were used. Neighbor joining (NJ) (Saitou and Nei 1987), maximum parsimony (MP) (Farris 1970, Swofford and Olsen 1990) and maximum likelihood (ML) (Cavalli-Sforza and Edwards 1967, Felsenstein 1981a,b, Kishino and Hasegawa 1989) were variously considered as each has its advantages and disadvantages depending on the type of, quality and quantity of the data as well as the size of the group under study. Although ML methods are generally favored because they are related to a specific underlying, evolutionary model, there are still computational limitations on the size of the datasets that can be analysed. Maximum parsimony on the other hand can handle large datasets. Maximum parsimony methods aim to find the shortest possible tree, i.e the tree with a minimum number of character-state changes that occurred, supporting a particular topology. The disadvantage of this method is that it does not show how much better the tree found is than another tree with the same length or longer. Bootstrapping (Felsenstein 1985) is used as tool to test the stability of a certain tree topology; i.e. to estimate how well the data fit the trees.

To investigate reticulation, polymorphism parsimony was used. Additive sites in the sequence alignment were recoded. Additivity at a site occurs when two site specific nucleotides are present simultaneous in one individual and is indicative for intra- or interspecific hybridization.

Thesis Outline

Phylogenetic relationships within the coral genus *Madracis* are explored in **Chapter 2**. Sampling was restricted to a single location at the Buoy1 site on Curaçao (Netherlands Antilles) where the five commonly recognized morphospecies occur in sympatry. Comparative sequence analysis of rDNA-ITS regions was evaluated at the intra- and inter-individual levels and analyzed in a polymorphism parsimony framework. Results are discussed in relation to various species concepts and to the process of reticulate speciation under the surface circulation vicariance model developed for corals by Veron (1995). With this analysis as a basis, I return to classical morphospecies concepts in **Chapter 3** in which a detailed morphometric analysis of within and between colony variation is explored at the level of the corallite as well as at the level of the colony habit more generally. Data are analyzed in phenetic framework using uni- and multivariate statistical methods. Putative species boundaries based on the quantitative morphometric data analyzed in a phenetic framework of over-similarity are compared with the DNA sequence data analyzed in a phylogenetic framework of identity by descent. A lack of congruence between the topologies of the resulting analysis highlight the problems associated with classical, typological approaches to species identification. The practical reconciliation of the "morphology vs. molecules" dilemma is discussed.

Corals live in photosynthetic symbiosis with endosymbiotic dinoflagellate algae called zooxanthellae. The nature of the symbiosis, the degree to which it is host specific, habitat specific or simply opportunistic is poorly understood because all variations have been observed in various host-symbiont associations among coral reef invertebrates. In particular, a series of studies in the coral *Montastraea annularis* species complex over the past decade has resulted in a virtual paradigm in which symbiont type and distribution is strongly correlated with depth (i.e., light availability). In this paradigm, corals expand their depth ranges by acquisition or inheritance of symbionts adapted to varying light levels. In **Chapter 4**, I test this hypothesis in *Madracis* by comparing phylogenetic relationships among the symbionts against their distribution in coral hosts growing at different depths. The analysis is based on DNA sequence comparisons from the D1 and D2 variable domains of the large subunit of the nuclear rDNA cistron (rDNA-LSU). Results call into question the generality of the paradigm, which is probably an exception rather

than the rule. Although the LSU has been the main region explored in zooxanthellae evolution, a faster marker, showing more variation, has long been needed. In **Chapter 5** I further explore zooxanthellae diversity using variation in the rDNA-ITS regions. The identification of new zooxanthellae, ITS-types was found and results are compared with another recently published studied. Finally, in **Chapter 6** I summarize the overall conclusions about the phylogeny of *Madracis* species and its dinoflagellate symbionts, the problems faced by the "low morphology" problem against reticulate evolution and the possibly "permanent" maintenance of partial or absent reproductive isolation in corals.