Madracis auretenra (Scleractinia: Pocilloporidae) – testing our knowledge of systematics, biology and connectivity in the western North Atlantic

by

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ABSTRACT

The systematic biology of the Caribbean scleractinian coral *Madracis auretenra* Locke, Weil and Coates 2007 is presented. This includes a new species description and brief taxonomic history, an explanation for the name *Madracis mirabilis* (Duchassaing and Michelotti 1860), a biological and bibliographic revision of *M. auretenra,* and information on this coral's genetic diversity and phylogeography in the greater Caribbean region. *Madracis auretenra* is described for a common, shallow-water, zooxanthellate coral, previously mis-identified as *M. mirabilis*. *Madracis mirabilis* is a subjective junior synonym for the deep-water species *Madracis myriaster* and according to the International Rules of Zoological Nomenclature, the name *M. mirabilis* cannot be used for another coral species. The problematic taxonomic history of this coral is presented as a perspective to explain what the binomen *M. mirabilis* refers to, in order to make this information easy to understand and accept by the community of coral researchers. To ensure earlier work on the species is recognized, a biological and bibliographic review of *M. auretenra* incorporates literature from more than 170 publications on the species, when it was incorrectly identified as *M. asperula*, *M. mirabilis*, or *M. mirabilis sensu* Wells 1973 during the years 1959-2008. Conspecificity with *M. auretenra* has been verified by authors of 97 *M. mirabilis* publications; unverified studies are noted. The review includes an extensive description of *M. auretenra* with new information on colony branch diameter and information on the distribution, ecology, physiology, molecular and experimental research of this common Caribbean species. To inform conservation management of Bermuda's geographically isolated high latitude reef system, assessments of genetic diversity and connectivity were made for the hermaphroditic, brooding coral *M. auretenra.* Patterns of genetic structure and evolutionary history for the coral in Bermuda, the Florida Keys and Puerto Rico are elucidated using the

nuclear intron *SRP54*. Twenty distinct nDNA haplotypes were determined from a trimmed alignment of 219 bp. Nucleotide and haplotypic diversity in Bermuda exceeded that of Florida and Puerto Rico, suggesting the island could be a coral refugium. Significant population structure was suggested to exist between Bermuda, Florida and Puerto Rico ($F_{st} = 0.153$, $p < 0.001$; $F_{ct} = 0.141$, $p < 0.05$). However, a shared historical connection between regions is evident in phylogenetic reconstructions. Distinct *SRP54* haplotypes for Bermuda and Puerto Rico support the recent division of these populations. Geographically shared phylogenetic clades for some Bermuda and Florida haplotypes indicate that geographic isolation my be broken periodically by gene flow to Bermuda from Florida via dispersal of coral planulae or settled, rafted individuals caught in Gulf Stream cyclonic eddies. This rare dispersal is predicted to occur too infrequently to sustain Bermuda coral populations indicating that conservation efforts of Bermudian coral species should be focused locally.

RESÚMEN

Se presenta la biología sistemática del coral escleratinido *Madracis auretenra* Locke, Weil y Coates 2007 del Caribe. Se incluye además la descripción de una nueva especie, una breve historia taxonómica, la explicación para el nuevo nombre y la invalidez de *Madracis mirabilis* (Duchassaing and Michelotti 1860), una revisión biológica y bibliográfica de *M. auretenra* e información sobre la diversidad genética y filogeografía en la región del Caribe. *Madracis auretenra* es un coral zooxantelado común de aguas llanas y profundidades intermedias, incorrectamente identificado previamente como *Madracis mirabilis*. *Madracis mirabilis* es un sinónimo subjetivo menor para la especie de aguas profundas *Madracis myriaster* y que de acuerdo con las Reglas internacionales de nomenclatura zoológica, este nombre no puede ser usado para otra especie de coral. La problemática historia taxonómica de este coral es presentada desde la perspectiva de explicar que sugiere el nombre binomial *M. mirabilis,* con la intensión de que esta información sea fácil de entender y aceptar por la comunidad de investigadores. Para asegurar que trabajos previos de la especie sean reconocidos, una revisión biológica y bibliográfica de *M. auretenra* incorpora literatura de más de 170 publicaciones de la especie de cuando era incorrectamente identificada como *M. asperula*, *M. mirabilis*, o *M. mirabilis sensu* Wells (1973) entre los años 1959-2008. La conspecificidad con *M. auretenra* ha sido verificada por 97 autores de publicaciones sobre *M. mirabilis*; estudios sin verificación taxonómica son señalados. La revisión incluye una descripción extensa de *M. auretenra* con información nueva del diámetro de las ramas coloniales e información sobre la distribución, ecología, fisiología, estudios moleculares y experimentales de esta especie común para el Caribe. Para mejorar el manejo y conservación de los arrecifes de latitudes altas y geográficamente semi-aislados de Bermuda, se hizo una evaluación de la diversidad genética y la conectividad para el coral

hermafrodita y planulador *M. auretenra.* Patrones de estructura genética e historia evolutiva son clarificados para el coral en Bermuda, los Cayos de la Florida y Puerto Rico usando el intrón nuclear *SRP54.* Veinte haplotipos distintos de nDNA fueron caracterizados usando alineamientos recortados de 219 pb. La diversidad de nucleótidos y haplotipos en Bermuda excede la observada en la Florida y Puerto Rico, sugiriendo a la isla como un refugio coralino. Se sugiere también que existe una estructura poblacional significativa entre Bermuda, Florida y Puerto Rico (F_{st} = 0.153, $p < 0.001$; F_{ct} = 0.141, $p < 0.05$). Sin embargo una conexión histórica es evidente en la reconstrucción filogenética. Haplotipos *SRP54* distintivos para Bermuda y Puerto Rico afirman la división reciente de estas poblaciones. Clados filogenéticos geográficamente compartidos por algunos haplotipos de Bermuda y Florida, indican que el aislamiento geográfico puede ser interrumpido periódicamente por el flujo de genes a Bermuda de la Florida a través de la dispersión de plánulas coralinas, establecidas o atrapadas en giros a mesoescala ciclónicas de la corriente del golfo. Esta dispersión infrecuente se prevee que ocurra muy infrecuentemente para mantener las poblaciones coralinas de Bermuda, indicando que los esfuerzos de conservación de los corales de Bermuda deben enfocarse localmente.

For Ancil,

Perhaps not the doctor you had in mind

"In Pulvere Vinces"

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1. Introduction: the taxonomic status and future of reef-building corals in the Caribbean region

An uncertain future faces coral reef ecosystems worldwide and forecasts of temperature and sea level rise and ocean acidification are grim. Anthropogenically exacerbated global climate change may escalate the effects of the many human and natural disturbances already impacting coral reef ecosystems. As the human population continues to grow it is supposed that over–fishing and excessive input of sediment and nutrients via land development will act synergistically with the natural disturbances of hurricanes, bleaching and disease to tip the balance of these fragile systems to the point of no recovery (Hughes 1994, Gardner et al. 2003, Pandolfi et al. 2003, 2005, Nystrom 2006).

The scleractinian corals, which are the structural species that underpin the majority of all other reef species associations, have suffered frequent and extensive mortality from the direct and indirect effects of such disturbances (Hughes 1994, Pandolfi et al. 2003, Rogers et al. 1991, Wilkinson and Souter 2008). In fact, coral decline is so severe and widespread that nearly one third of the world's coral species are now considered threatened (Carpenter et al. 2008). This degenerative trajectory is exceedingly evident within the greater Caribbean (The Greater Caribbean is defined herein as the region encompassing the islands of the Caribbean Sea, the mainland countries of Central and South America that border this sea in the south and the areas of SW Florida and Bermuda.) (Pandolfi et al. 2005, Gardner et al. 2003) with reported declines in coral coverage (Hughes 1994, Wilkinson and Souter 2008) and increased prevalence of diseases (Weil et al. 2006, Weil and Croquer 2009) and bleaching (Miller et al. 2006, Manzello et al. 2007) (Wilkinson and Souter 2008). To combat the current and impending threats to corals and the reef ecosystems they foster, recent emphasis has been placed on research that could inform coral preservation and conservation through proactive reef management. To implement

appropriate management strategies it is necessary to have an understanding of species distributions and the genetic connections between populations, at the very least.

Within the Caribbean, molecular techniques have been used to aid in inferring the degree and patterns of genetic connections among populations and to estimate levels of intra-specific genetic variation. The latter is supposed to provide an indication of how well coral populations might cope with disturbance, or resilience. It is also significant to the recognition of inter-species boundaries and thus to determinations of species distributions and genetic connections. Understanding these attributes of individual coral species and their populations are crucial to determining spatial scales for management programs that are relevant to different species, communities and Caribbean locations.

1.1 Diversity and taxonomy of corals of the greater Caribbean

Documenting the diversity and characters of coral species and their distributions is a cumulative effort of many regional coral scientists, representing many different research specialities. Within the greater Caribbean, a total of 27 genera and 60 to 70 species of scleractinian coral are known to occupy shallow-water (1-70 m) reef ecosystems. Between the years of 1955 and 1985 there were only six new species descriptions for the region (Wells, 1973a, 1973b), in contrast to 125 new descriptions in the Indo-Pacific over the same time period. Since the 1973 descriptions of John W. Wells, only three new species of shallow-water scleractinians have been described from the Caribbean (Zlartarski 1990, Vermeij et al. 2003, Locke et al. 2007). This declining rate in new species records may be some indication of a good or complete understanding of diversity and distribution of shallow-water coral species in the Caribbean.

1.1.1 Problems in coral taxonomy

Even though there is a long and extensive historical record of studies on scleractinian diversity (Linneaus 1758, Forsskål 1775, Lamarck 1801), the taxonomy and relationships of the group remain largely unresolved (Daly 2007, Huang et al. 2009). Classification of scleractinian corals continues to rely heavily upon qualitative morphological descriptors, such as thick versus thin branches. In some cases, these differences could be described using more rigorous analyses of variation but usually they have not. These problems of identification are compounded by a numerical deficiency of visible morphological characters and high levels of ecophenotypic plasticity. Ecologically induced variation in hard corals occurs across the range of spatial scales, so that even conspecific individuals located adjacent to each other but experiencing slightly different conditions, such as in degrees of shading, may be very different colors (for example). It is also possible that hybridization between congeneric species contributes to fuzzy species boundaries (Willis et al. 1997), as has been extensively investigated within Caribbean Acroporidae (van Oppen et al. 2000, Vollmer and Palumbi 2002) and less so in other Caribbean species (Veron 1995). Therefore, describing local or regional species diversity of corals can be difficult, especially when this is undertaken in the field. Also stemming from fully field-based studies of diversity is the absence of relevant reference materials by which verifications of species identities might, eventually, be made.

Additional issues in coral taxonomy arise from poor communication among coral scientists, who represent many different areas of research and who have different primary goals. A full understanding of the principles of taxonomy and recommended practices is not an underlying aspect of education in the practice of many of these areas of research. The Code of Zoological Nomenclature (ICZN 1999), a set of taxonomic rules governing zoological species classification

was set in place, for good reason, in 1905, but many practitioners, both occasional and frequent, of taxonomy either are not familiar or choose to defy these standards-of-practice. Veron's (2000) treatment of Scleractinia is a recent example of this. Descriptions of new zooxanthellate coral species and revisions of higher taxa and families, within these volumes (Veron 2000) did not follow the rules of the International Code of Zoological Nomenclature; accordingly and justifiably the validity of these have been questioned (Daly et al. 2007) but retroactively correcting the situation will be (and has already been – almost a decade) a time consuming process. Given the numerous substantive issues of coral taxonomy (ecophenotypic plasticity, hybridization, limited numbers of visible diagnostic characters; enough samples and sound statistics); all coral researchers must try to be good practicing taxonomists. This means adhering to the standards and recommendations of the ICZN and taking the simple steps of documenting their identification protocols and retaining reference materials that can allow verification of hypotheses of the identity of an individual. A case in point and one focus of this dissertation, the history of the coral species *Madracis auretenra* Locke, Weil and Coates 2007 is an example of a taxonomic saga, determined by many lapses in good taxonomic practice, and an example of the time and effort that is required to retrieve valuable information about this species.

1.2 Scleractinian molecular research and connectivity

In any study that involves one or a set of named subjects, it is imperative to be able to consistently recognize these and to understand any outstanding taxonomic issues. Nonetheless, in coral research a thoroughly considered identification seems to have lost its importance with the advent of molecular work. This is very problematic if public genetic databases, such as the National Center for Biotechnology Information (NCBI), are to serve their intended purpose of

information sharing. An incorrect name attached to molecular data can mislead any subsequent analyses by individuals who did not collect the data.

Recent studies and reviews indicate the importance of molecular techniques for elucidating species boundaries (Knowlton et al. 1992, Weil 1992, Weil and Knowlton 1994, Hunter et al., 1997, Lopez et al. 1999, Medina et al., 1999, Diekmann et al. 2001, Wolstenholme et al. 2003, Avise 2004), phylogenetic relationships (Fukami et al 2008, Nunes et al. 2008, Huang et al. 2009), and levels of intra-specific genetic diversity and population connectivity (Ridgway et al. 2001, Ridgway 2002, Takabayashi et al. 2003, Mackenzie et al. 2004, Baums et al. 2005, Vollmer and Palumbi 2007) within the Scleractinia. Even so, molecular markers informative to these issues are very limited and molecular techniques have yet to be developed for many groups within the Scleractinia (Lopez, 1999, Huang et al 2009). This deficiency continues to be a limitation for coral researchers.

Within the coral genome both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) markers have been investigated, however the anthozoans examined thus far differ from other marine invertebrates and vertebrates in that their mtDNA evolves at a much slower rate than nDNA (Shearer et al. 2002, McFadden et al. 2004). The faster rate of mutation accumulation in nDNA provides a better data resource for phylogeographic studies and phylogenetic studies of intra-specific taxa (Hellberg 2007, Concepcion et al. 2008). Common nuclear markers currently used to address species level relationships in corals are microsatellites and the introns minicollagen, calmodulin, Pax-C, and the multi-copy ribosomal internal transcribed spacer region (ITS) (Table 1.1).

Table 1.1. Summary of scleractinian coral population genetic studies noting molecular marker utilized and location of study. Abbreviations: Msats, microsatellites; Mini-C, minicollagen; ITS, internal transcribed spacer; Cal, calmodulin; AFLP, amplified fragment length polymorphisms (after Vollmer and Palumbi 2007).

Species	Marker	Location	Reference
Indo-Pacific			
Acropora cuneata	Allozymes	GBR and Lord Howe	1
	Allozymes	GBR	2
Acropora cytherea	Allozymes	GBR	2
Acropora hyacinthus	Allozymes	GBR	2
Acropora millepora	Allozymes	GBR	2
Acropora nasuta	Msats;intron (Mini-C, cnox2)	GBR	3
Acropora palifera	Allozymes	GBR	2, 4
Acropora valida	Allozymes	GBR and Lord Howe	1
	Allozymes	GBR	2
Goniastrea aspera	Allozymes	Japan	5
Goniastrea australensis	Allozymes	GBR and Lord Howe	6
Plesiastrea versipora	ITS rDNA	GBR and Japan	7
Pocillopora damicornis	Allozymes	GBR and Lord Howe	1, 6
	Allozymes	GBR	2, 4, 8
	Allozymes	Lord Howe	9
	Allozymes	Western Australia	10
	Allozymes	Japan	11
Pocillopora verrucosa	ITS rDNA	South Africa	12
Pocillopora meandrina	Msats	South Pacific	13
Mycedium elephantotus	Allozymes	Taiwan	14
Seriatopora hystrix	Allozymes	GBR and Lord Howe	1
	Msats	Red Sea	15
	Msats	NW Australia	16
	Msats	GBR	17
Stylophora pistillata	Allozymes	GBR and Lord Howe	1
	Allozymes	GBR	2
	ITS rDNA	GBR and Japan	18
	ITS rDNA	Red Sea	19
Greater Caribbean			
Acropora cervicornis	MtDNA; intron (Mini-C, Cal, PaxC)	Caribbean wide	20
Acropora palmata	Msats	Caribbean wide	21
Agaricia agaricities	AFLP	Bahamas and Florida	22
Diploria strigosa	AFLP	Gulf of Mexico, FGB	23
Madracis decactis	AFLP	Gulf of Mexico, FGB	23
Montastraea annularis	MtDNA; AFLP; ITS	Bahamas and Panama	24
Montastraea faveolata	MtDNA; AFLP; ITS	Bahamas and Panama	24
Montastraea franksi	MtDNA; AFLP; ITS	Bahamas and Panama	24
Montastraea cavernosa	AFLP	Gulf of Mexico, FGB	23

References: (1) Ayre and Hughes 2004; (2) Ayre and Hughes 2000; (3) Mackenzie et al. 2004; (4) Benzie et al. 1995; (5) Nishikawa and Sakai 2003;(6) Miller and Ayer 2008; (7) Rodriguez-Lanetty and Hoegh-Guldberg 2002; (8) Ayre et al. 1997; (9) Miller and Ayre 2004; (10) Stoddart 1984; (11) Adjeroud and Tsuchiya 199); (12) Ridgway et al. 2001; (13) Magalon et al. 2005; (14) Dai et al. 2000; (15) Maier et al. 2005; (16) Underwood et al. 2007; (17) van Oppen et al.2008; (18) Takabayashi et al. 2003; (19) Zvuloni et al. 2008; (20) Vollmer and Palumbi 2007; (21) Baums et al. 2005; (22) Brazeau et al. 2005; (23) Atchison et al. 2008; (24) Fukami et al. 2004.

A more recently applied molecular marker with possible relevance for population-level studies of coral species is a single copy intron in the nuclear gene encoding the signal recognition particle 54 (*SRP54*) (Concepcion et al. 2008). The *SRP54* is the subunit of the signal recognition particle which binds to newly synthesized proteins and aids in their transfer to the endoplasmic reticulum (Egea et al. 2005). Using a single copy gene like *SRP54* in studies of population diversity and relationships eliminates the need for extensive cloning of individuals, a requirement of the popular, but problematic, population marker ITS. As a result, investigations using *SRP54* should be less time and resource consuming than the ITS gene, while yielding data of the same relevance to population questions.

To date, studies of population connectivity in scleractinian corals have focused on the Pacific and Indian Oceans (Ayre & Hughes 2004, 2000; Magalon et al. 2005; Ridgway 2005, 2001, Rodriguez-Lanetty & Hoegh-Guldberg 2002, Takabayashi et al. 2003; van Oppen et al. 2008, Underwood et al. 2007, Zvuloni et al. 2008 [Red Sea]). Only five studies have been conducted on the coral populations of the greater Caribbean and the Gulf of Mexico (Baums et al. 2005, Brazeau et al. 2005, Fukami et al. 2004, Vollmer and Palumbi 2007, Atchison et al. 2008 [Gulf of Mexico]). Together these studies have applied molecular markers to a total of seven genera and 16 species in the Pacific and five genera and nine species in the greater Caribbean (Table 1.1). Allozyme electrophoresis was initially employed for studies of population connectivity but the greater resolution and ease of repeatability provided by recent DNA molecular techniques allows rapid advances in this area of study. Even so, to date a limited number of markers have been applied at the population level (Table 1.1).

For population connectivity studies, the use of genetic markers and the kinds of analyses employed to discern relationships among populations only enable inference of what may be

occurring among them. Larval dispersal tracks are difficult to record directly and thus indirect data are used to document the potential results of these tracks and to infer dispersal patterns. The best estimates of the real patterns may eventually come from a combination of these indirect methods with direct larval tracking – which may well involve applying other molecular techniques, such as tagging.

In 1997, Roberts presented the opinion that Caribbean marine populations were open populations and exchanged gene flow regularly, with upstream sources fueling more resilient populations downstream. Simply put, dispersive larval stages of coral reef inhabitants are passively distributed by currents and, therefore, surface current patterns should reveal routes of larval transport and patterns of population connectivity (Roberts 1997). In light of the precarious future predicted for reefs worldwide, there has been an increase in the demand for local and regional reef management programs and this has provided a new impetus for studying larval dispersal and reef connections.

Notably, since this landmark paper by Roberts (1997), scales of larval connectivity have been determined to be much smaller than anticipated (Cowan et al. 2007, Steneck et al. 2009) for most species. Thus far, admittedly limited, population connectivity research conducted on Caribbean scleractinians, using genetic methods, has indicated that populations are closed, or not connected, at distances greater than 500 km (Vollmer and Palumbi 2007). These indications of the absence of long distance dispersal may render some geographically separated, high latitude oceanic reefs such as Bermuda even more isolated than we suspected, and this would significantly alter population recovery and management priorities for these populations. Such isolated populations are expected to be genetically depauperate and less resilient to disturbance (Ayre & Hughes 2004; Miller and Ayre 2008; van Oppen and Gates 2006).

1.3 Coral reefs of Bermuda

The islands of Bermuda are home to the Atlantic Oceans most northern occurring coral reef ecosystem, at a geographical position of 32º 20'N, 64º 45'W. The nearest source populations for Bermudian corals are over 1000 km away in the Caribbean and Florida. Hypotheses of the oceanic formation and persistent high latitude location of Bermuda indicate it always has been isolated from major continental landmasses.

The surrounding Sargasso Sea and warm Gulf Stream today provide Bermuda with a subtropical climate and surface sea water with temperatures ranging between 18ºC and 30ºC thus allowing the survival of tropical marine species at such a high latitude. The shallow-water marine fauna of Bermuda exhibit low endemism (2.4 %) and represent a reduced species assemblage of nearby greater Caribbean regions (Sterrer 1986, 1998). It is supposed that the islands' marine benthic fauna originated by dispersal from Continental US or Caribbean island sources (Logan 1988, Sterrer 1986).

The initial oceanic formation of the Bermuda Rise ~110 million years ago (Ma) and a subsequent event 33 Ma produced a cluster of extinct volcanoes or seamounts dominated by the Bermuda pedestal. The Bermuda pedestal, which rises above sea level, is the largest of four related seamounts occupying the Bermuda Rise. Arranged in a northeast trending line the seamounts are Plantagenet Bank (also know as Argus), Challenger Bank, Bermuda, and Bowditch seamount (Vogt and Jung 2007). The three submerged seamounts are the nearest known areas to Bermuda that possess corals outside of the Caribbean and US coastline. The seamounts are greater than 60 m (200 feet) below sea level, approximately 38 km^2, and as far as 39 km from the island.

The shallow-water coral reefs of Bermuda occupy approximately 550 km^2 of the Bermuda pedestal and are concentrated to the northwest of the islands of Bermuda. The islands cover ~7 % of the pedestal with the remaining 93 % covered by coral reef and soft bottom benthic communities. The coral reef ecosystem consists of a broad shallow terrace, patch reefs in lagoonal and inshore areas, a rim reef at the descending edge of the platform and deeper reefs on the descending slope (Logan 1988).

Accounts of the number of shallow-water zooxanthellate scleractinian corals inhabiting the reef systems of Bermuda have fluctuated over the years. The earliest records of corals from Bermuda are comprised of various lists: Jones (1869), nine species; Dana (1872), 10 species; Quelch (1886), 23 species; and Heilprin (1888), 25 species known, 19 species obtained. In 1903, Verrill did an extensive study of Bermuda corals recording 20 species from the area. The most recent coral species census of the islands documented by Cairns, den Hartog & Arneson (Sterrer, 1986) included a listing of 20 zooxanthellate coral species representing 10 families. This publication has served as a standard for many reef studies in the area, however, the species account was based on past records and an extensive field survey was not conducted (Sterrer pers. comm.). Other, recent, records of extant shallow-water zooxanthellate coral species distribution in Bermuda are often misleading and based on inaccurate accounts (see Veron, 2000). Veron (2000) published 25 species of zooxanthellate corals as occurring in Bermuda; the following of which are unsubstantiated: *Diploria clivosa; Eusmilia fastigiata; Isophyllastrea rigida* (listed as *Isophyllia rigida); Manicina areolata; Mycetophyllia lamarckiana*; and the occurrence of *Astrangia poculata* is highly questionable. Based upon the reference collection of the Bermuda Museum of Natural History (BMNH) and personal observations, a total of 20 species of scleractinian corals are currently well-documented as occurring in Bermuda (Table 1.2). The

presence of the solitary, zooxanthellate and azooxanthellate *Astrangia poculata* would increase this number to 21 species; but its occurrence has been objectively confirmed. *Siderastrea siderea* has been reported from Bermudian reefs, however, this is strongly questioned (W Sterrer pers. comm., SR Smith pers. obs.). Venn et al (2009) reported *Madracis carmabi* and Frade (2009) reported *Madracis senaria* and *Madracis formosa* from Bermuda, however, there is no material (pictures, specimens, detailed written records, export permits, CITES permits, etc.) to substantiate these record (Venn pers. comm.). Microscopic observations of material deposited within the BMNH and listed as *Porites furcata* and *Porites divaricata* indicate these specimens may represent ecological variants of *Porites porites* (Weil pers. obs., Locke pers. obs.).

Among the 20 validated zooxanthellate scleractinian species recorded from Bermuda only 9 families and 13 genera are represented compared to 14 families and 27 known genera in the Caribbean. The published literature suggests that brooding larvae seems to dominate as a reproductive mode for the coral species present in Bermuda. However, the reproductive strategies of the Bermuda coral species have not been studied in detail and most are generalized from accounts of Caribbean corals.

Table 1.2. Shallow-water zooxanthellate scleractinian coral species currently documented as occurring in Bermuda.

Species	Author
Family Agariciidae	
Agaricia fragilis	Dana, 1846
Family Astrocoeniidae	
Stephanocoenia intersepta	Lamarck, 1816
Family Faviidae	
Favia fragum	(Esper, 1795)
Diploria strigosa	(Dana, 1846)
Diploria labyrinthiformis	(Linnaeus, 1758)
Montastraea cavernosa	Linnaeus, 1767
Montastraea franksi	(Gregory, 1895)
Family Meandrinidae	
Meandrina meandrites	(Linnaeus, 1758)
Dichocoenia stokesi	Milne Edwards & Haime, 1848
Family Mussidae	
Isophyllia sinuosa	(Ellis & Solander 1786)
Scolymia cubensis	(Milne Edwards & Haime, 1849)
Family Oculinidae	
Oculina diffusa	(Lamarck 1816)
Oculina varicosa	(Lesueur 1821)
Oculina valenciennesi	Milne Edwards & Haime, 1850
Family Pocilloporidae	
Madracis decactis	(Lyman, 1859)
Madracis auretenra	Locke Weil and Coates, 2007
Family Poritidae	
Porites porites	(Pallas, 1766)
Porites astreoides	Lamarck, 1816
Family Siderastreidae	
Siderastrea radians	(Pallas, 1766)
*Siderastrea siderea**	(Ellis and Solander, 1786)

* Current distribution uncertain.

Bermuda depends upon its coral reef ecosystems as does any other nation within the coral reef latitudes, for tourism, fishing, and most importantly as a protective barrier to island erosion. Bermuda's marine systems are considered well-protected especially since the establishment of the North and South Shore coral reef preserves in the early 1960's. In fact, all corals on the Bermuda platform have been afforded some level of protection since the adoption of the 1972 Fisheries Act. However, these are passive and somewhat "accidental" measures. To date, an overall plan for monitoring, conservation and management does not exist for any of Bermuda's marine habitats. Considering the rapid decline of coral reef ecosystems in particular, this is alarming. If Bermuda corals are truly isolated this further justifies focus on an effective, local, management plan. In contrast, if Bermuda reefs depend heavily upon Caribbean sources for genetic variability and recruitment, then a more active role in protecting the populations of the entire Caribbean is required.

Within the geographic area chosen for sampling herein, the coral populations from Bermuda are expected to be genetically distinct from Caribbean conspecifics due to the island's geographic isolation and high latitude location. The corals populating Bermuda likely originated in the Caribbean and were transported via the Gulf Stream; however actual data supporting this dispersal hypothesis are rare (Park and Ó Foighil 2000, Bilewitch 2006, Bilewitch et al. submitted).

Management programs for Bermudian coral reef environments would benefit immeasurably from an improved understanding of the degree of connectivity between its coral reefs and reefs in the greater Caribbean. Similarly, better management of Caribbean reefs would clearly be depicted as more than just a local issue.

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2. A newly documented species of *Madracis* (Scleractinia: Pocilloporidae) from the Caribbean

Locke, JM, E Weil and KA Coates. 2007. A newly documented species of *Madracis* (Scleractinia: Pocilliporidae) from the Caribbean. Proceedings of the Biological Society of Washington. 120:214-226.

2.1 Abstract

Madracis auretenra, new species, is described for a common, shallow-water, zooxanthellate coral species found throughout the greater Caribbean. This new species is distinguished from other species of the genus by a thin branched, dendritic morphology and depth distribution of 1-60 m. Other characteristics include: non-living basal branch portions; a fairly smooth coenosteum; a distinct line of coenosteal spines centrally located between adjacent corallites; no visible secondary septa in corallites; and closely spaced corallites. Individuals of this taxon have been incorrectly referred to *Madracis mirabilis* (Duchassaing & Michelotti 1860), which is a deep-water species and which is synonymous with *Madracis myriaster* (Milne-Edwards & Haime 1849), in several publications subsequent to 1973. Herein, a brief explanation of the taxonomic confusion surrounding *M. mirabilis* and the undescribed species is provided along with a complete description of this new species of *Madracis*. Records of the new species are confirmed for Puerto Rico, Curaçao, Grenada, and Bermuda. Authors of many recent studies on "*Madracis mirabilis* sensu Wells" will need to reconsider and reconfirm the identities of their study organisms.

2.2 Introduction

Coral reef ecosystems are the current focus of many marine biologists and ecologists, including large research consortia, and are of great interest to the larger public that is extremely concerned about global climate change. Studies of scleractinian biodiversity, genetics, toxicology and disease are providing valuable data that are informing preservation and conservation of these systems. Unfortunately, the importance of sound α-taxonomy (species determination) as the foundation of much of this research is not fully understood or appreciated. Accurate explanations of anthozoan biology and regional biodiversity patterns are highly dependent upon correct and consistent taxonomy (Daly & den Hartog 2004).

Scleractinian corals are considered to be taxonomically problematic in that species are difficult to distinguish if one attempts to adhere to a strict definition of a biological species (genetic independence) or rely on morphology alone (Willis 1990, Knowlton 2001). Morphological variation is exhibited within individuals and species and there is an overall lack of documentation of this intraspecific variability for widely accepted species. Veron (1995) has suggested that some of the difficulties in recognizing morphological boundaries between species may stem from reticulate evolution (hybridization) among species. However, some taxonomic problems in this group, as well as in other groups of invertebrates, have originated through simply failing to apply basic taxonomic practices, such as referring to original species descriptions and type material.

The current and common use and referral of the name *Madracis mirabilis* (Duchassaing & Michelotti 1860) (*sensu* Wells 1973a) to what is, in fact, an undescribed species is an instructive example of the importance of rigorous application of the best taxonomic methods. Type material of *M. mirabilis* has been confirmed to be *Madracis myriaster* (Milne-Edwards & Haime 1849) a

deeper occurring azooxanthellate species (Cairns 1979, Table 2.1). The shallow-water, zooxanthellate, thin-branched coral that is widely distributed in the Caribbean region and that regularly is incorrectly referred to *M. mirabilis* (see Veron 2000 for example; note that the species authority for *M. mirabilis* cited in Veron is incorrect) has never been formally described or named. As it is nomenclaturally preoccupied, the name *M. mirabilis* is unavailable for this or any other species (ICZN 1999). Herein, one widely distributed, thin-branched, common, shallow-water species of *Madracis* is described and named; resolving, at least, some of the confusion between deep, azooxanthellate, and shallow, zooxanthellate, species of *Madracis* in the Caribbean.

Table 2.1. Statistical mean and standard deviation ($\bar{x}$, *SD*) reported for six morphological characters for the "type" of *S. mirabilis*, and specimens of *M. myriaster* and *M. auretenra*. The results of this preliminary study confirm statistically significant differences between *M. myriaster* and *M. auretenra* (*ANOVA*, $p<0.001$). Results of Holm-Sidak pairwise comparisons are denoted by lowercase letters each of which indicates a statistically different group. Characters indicated in bold distinguish the two species.

	Madracis myriaster			*Madracis auretenra*		
	MZUT 358	USNM 79719	USNM 79726	PR1	PR2	BDA1
Diameter (mm)						
Corallite	1.55(0.09)a	1.54(0.09)a	1.54(0.13)a	1.32(0.13)b	1.31(0.14)b	1.49(0.13)c
Columella	0.8(0.10)a	0.85(0.07)a	0.76(0.11)a	0.64(0.14)b	0.56(0.15)b,c	0.51(0.09)c
Length (mm)						
Primary Septa	0.42(0.06)a	0.42(0.06)a	0.38(0.04)b	0.35(0.07)b	0.37(0.09)b	0.49(0.06)c
Neighbor Distance (mm)						
Minimum	1.2(0.29)a	1.24(0.26)a	1.15(0.23)a	0.73(0.18)b	0.46(0.13)c	0.38(0.22)c
Maximum	5.10(1.32)a	4.54(1.26)a	3.65(0.86)b	1.51(0.28)c	1.51(0.22)c	0.94(0.24)d
Density (cm^{-2})						
Corallite	7.70(1.75)a	8.01(1.12)a	11.83(1.46)b	21.33(1.10)c	22.09(2.25)c	21.29(3.12)c

Note: PR1=Paratype USNM XP1X; PR2 and BDA1= other material

2.2.1 Brief taxonomic review of *Madracis* and *M. mirabilis*

The original descriptions of two, indeed the same, rather similar genera of scleractinian coral, *Axhelia* and *Madracis*, were presented in a single publication of Milne-Edwards and Haime (1849). The type species of these genera were designated *Axhelia myriaster* and *Madracis asperula*, respectively. Pourtalès (1871) revised and synonymized the two genera, designating *Madracis* as the senior synonym; however, his later actions (Pourtalès 1874; mentioned below) with regard to *Stylophora mirabilis* suggest he subsequently reconsidered this decision. In accord with this possibility, Vaughan (1901) synonymized *Madracis* Milne-Edwards & Haime 1849 with *Axhelia* Milne-Edwards & Haime 1849 and transferred *M. asperula* to *Axhelia*. Vaughan and Wells (1943) returned both *A. myriaster* and *Axhelia asperula* to *Madracis* in a reversal of Vaughan's (1901) action, and *Axhelia* became, in practice, the junior synonym.

According to the current understanding of the genus, *Madracis* Milne-Edwards & Haime, 1849 is a common taxon in temperate and tropical waters from the Atlantic and Caribbean through the Pacific to the Indian Ocean and Red Sea (Cairns 1999, Veron 2000, Vermeij et al. 2003a). Excluding the newly described species there are currently 15 valid and extant azooxanthellate and zooxanthellate species of *Madracis* worldwide (Cairns 1999, Vermeij et al. 2003a). Ten of these species are reported as azooxanthellate; *Madracis pharensis* (Heller 1868) and *M. asperula* are reported both with and without zooxanthellae. The only zooxanthellate species from the Pacific and Indian Oceans is the laminar and encrusting *Madracis kirbyi* Veron & Pichon 1976 (Cairns 1999). Within the greater Caribbean, seven extant zooxanthellate *Madracis* are recognized (Cairns 1999, Vermeij et al. 2003a, 2003b). Taxonomic debate continues within the genus regarding the species status of *Madracis decactis* (Lyman 1859), *M.*

pharensis and *Madracis formosa* Wells 1973b (Fenner 1993, Diekmann et al. 2001, see Vermeij et al. 2003b).

Madracis mirabilis was first described from St. Thomas, Lesser Antilles, as *Stylophora mirabilis* Duchassaing & Michelotti 1860, and a specimen, now considered by some as a paralectotype, was deposited at the Museo Zoologia Università, Turin, Italy. Rossi (1959) assigned holotype status to the specimen in Turin, believing it to be the only material deposited by the authors. Recently, it has been related that specimens or fragments of specimens from the Duchassaing & Michelotti collection were donated by Michelotti to the Museum of Florence. The specimen of *S. mirabilis* in Florence has catalogue number MZUF 63, and is accompanied by the notes “St. Thomas” and "fragment of the specimen represented in the original plate". Accordingly, the curators in Florence have designated the specimen in Florence the lectotype and the specimen in Turin paralectotype (see Volpi & Benvenuti 2003). Additional information about the original specimen indicates that some fragments may also be located in museums in Florence, Paris, London and at Harvard University. Neither the original description of the species, nor the information with the deposited specimens, included a collection depth.

In 1874, Pourtalès placed *S. mirabilis* within the genus *Axohelia* Milne-Edwards and Haime 1849 (sic, misspelled for *Axhelia* Milne-Edwards and Haime 1849); subsequently reporting it in 1880 from a depth range of 336-1572 feet. Vaughan (1901) synonymized the deep-water species *Axhelia schrammi* (Pourtalès 1874) with *A. mirabilis*, and reported *A. mirabilis* from a depth of 258 feet. In the same year Verrill (1901) indicated that *A. schrammi* and *Axohelia myriaster* Milne-Edwards & Haime 1849 were the same. The combination of these actions (Verrill 1901, Vaughan 1901) have the effect of synonymizing *A. mirabilis* with *A. myriaster*, with the latter being the senior synonym.

Vaughan and Wells (1943) reinvigorated use of the name *Madracis mirabilis*, unfortunately without discussion of the earlier history and events surrounding this taxon and without justifying their action (S. D. Cairns pers. comm.). The record of *Madracis mirabilis* in Vaughan and Wells (1943) is of the specimen reported by Vaughan (1901) from 258 feet, and up to that time *M. mirabilis* had been reported only as a deep-water coral, occurring over a depth range of 258-1572 feet.

Subsequently, specimens of shallow-water *Madracis* were identified as the deep-water azooxanthellate taxa *M. mirabilis* and *M. asperula* (Goreau 1959, Lewis 1960, 1965, Roos 1964, also see Cairns 2000), possibly because these were the only existing descriptions of branching species of *Madracis*. These authors did not provide descriptions of their material and based on the literature alone it is not possible to determine what species they may have had. Goreau and Wells (1967) seem to be the first to specifically list *M. mirabilis* (= *M. myriaster*) as a shallow-water inhabitant. In this publication (Goreau and Wells 1967), *M. mirabilis* is reported as previously recorded from Jamaica as *M. asperula* from a depth range of 1-60 meters (1-180 feet), and to be very common. Goreau and Wells (1967) provided no description of their material and did not substantiate their identification of it as *M. mirabilis* in any particular way, for example by comparison to the originally deposited specimen. *Madracis asperula* is a deep-water, azooxanthellate species (100 m) (Cairns 2000, Vermeij et al. 2003b), which has extremely slender branches (3 mm in Wells 1973a; 1.7 mm in Cairns 2000; and 1.7 mm, J. M. Locke pers. obs., USNM specimens 99046, 99048 and 45507), so that it should be readily distinguished from the shallow-water, zooxanthellate, branching forms of the genus, even in the field.

Wells (1973a) presented an artificial key of *Madracis* species in which he keyed shallow-water, thick-branched (6-10 mm) *Madracis* specimens as *M. mirabilis*. Of the other keyed

species in Wells (1973a), branch diameter is only reported for *M. asperula* (slender, 3 mm) and *M. formosa* (thick, 15 mm). Wells (1973a) also included *M. myriaster* (to which *M. mirabilis* is a junior synonym) in his key as a deep-water azooxanthellate species. Since this publication (Wells 1973a), most literature on Caribbean corals has referred the name *M. mirabilis* to common, branched, shallow-water (1-60 m) corals that monopolize large reef areas in some habitats. In fact, this species (or group of species–see Discussion) remains undescribed. Nonetheless, it has become a common experimental taxon for numerous coral reef-related studies and the name *M. mirabilis* has become deeply entrenched within the literature for shallow, thinly-branched zooxanthellate species of the genus.

Cairns (1979) raised the problems with the taxonomy and the use of the name *M. mirabilis* in his work on deep-water Scleractinia. He examined the type material of *M. mirabilis* held in Turin (MZUT 358) and found, in confirmation of Vaughan (1901), that it was the same morphological species as *M. myriaster*, a striate, deep-water, azooxanthellate species. (Note: this type specimen has been referred to as holotype [Cairns 1979:28, 29], syntype [Cairns 1979:plate1, fig 4] and now as paralectotype [Volpi & Benvenuti 2003:L. Levi pers.comm.]). Cairns (1979) considered *Stylophora mirabilis* a junior synonym of *M. myriaster* and also stated that the common, shallow-water nonstriate species, known today as *M. mirabilis sensu* Wells 1973, required a new name. More than 25 years, and many specific studies, later *Madracis mirabilis sensu* Wells, 1973, remains undescribed and without a legitimate name.

Morphometric analysis of colony and corallite characters among a type specimen of *M. mirabilis*, *M. myriaster* material, and new material of a shallow-water zooxanthellate species that could be identified as *M. mirabilis sensu* Wells 1973 (Table 2.1) have corroborated Cairns'

(1979) prediction. A new species is described herein, for this shallow-water, thin-branched form of *Madracis* that is found throughout the Caribbean region and in Bermuda.

2.3 Materials and Methods

An overall, general description of each freshly collected specimen was made; the specimens were bleached, rinsed with fresh water and dried, prior to morphometric measurements.

Material of other species examined were *Madracis myriaster* from the Smithsonian Institution Museum of Natural History (USNM), USNM 79719, USNM 79726 and *Stylophora mirabilis* from the Museo Zoologia Università de Torino (MZUT) MZUT 358 (image provided by L. Levi).

All material was examined and photographed using an Olympus SZ410 stereomicroscope with analog camera and "Snappy 4.0" image capture. Images of individual corallites were taken using a Scopetronix "Max view Plus" system with a Canon S45 digital camera and captured with Canon ZoomBrowser® Ex 4.1 remote capture. Measurements were taken using SigmaScan Pro® 5.0.0 (SPSS Inc., 2002). Ten corallites were measured for each specimen and four characters were measured per corallite; corallite diameter; columella base diameter; length of primary septa; and width of primary septa. The distance from inside corallite wall to closest inside corallite wall; distance from wall to farthest neighboring corallite; and diameter of branches and density of corallites cm^{-2} were also recorded for each specimen. All measurements were taken at least 1 centimeter from branch tips. For each trait measured, the mean and standard deviation were calculated using SigmaStat® 3.0 (SPSS Inc. 2002) (Table 2.1).

SigmaStat 3.0 (SPSS Inc. 2002) was used to confirm differences between *M. myriaster* and the new species described herein. A one-way analysis of variance (ANOVA) was done for

specimens for six morphometric characters among *Stylophora mirabilis* MZUT 358, specimens of *M. myriaster* and specimens of the new species (Table 2.1). Statistically significant differences were further analyzed using Holm-Sidak *post hoc* tests for multiple pairwise comparisons. For distance from inside corallite wall to farthest neighboring corallite wall values were log transformed (Table 2.1).

2.4 Results

Family Pocilloporidae Gray, 1842

Genus *Madracis* Milne Edwards & Haime 1849

2.4.1 Diagnosis (after Cairns 1979, 1999)

Colonial, extratentacular budding producing massive or ramose coralla. Coenosteum costate or spinose. Septa arranged in groups of six, eight, or ten, but rarely in more than two cycles. Columella styliform. Paliform lobes often present on first cycle of septa.

2.4.2 Type-species

Madracis asperula Milne Edwards & Haime 1849, by monotypy.

2.4.3 Remarks

A synonymy of the genus is presented in Vaughan (1901) and Vaughan and Wells (1943). We know of no substantial revisions since these two.

2.4.4 New species and synonymy

Madracis auretenra, new species

Figs. 2.1-2.5

Madracis asperula.--- Lewis, 1960:1133, 1139, 1140, figs. 9-11.--- Roos, 1964:7, pls. 4b, 6b. *Madracis mirabilis*.--- Werding & Erhardt, 1976:49, pl.4, fig.1.--- Colin, 1978:212 (color), 214, 215.--- Cairns, 1982:274, fig. 120e.--- Lewis & Snelgrove, 1990:268, figs. 1a-c.--- Bruno & Edwards, 1997:2179, figs. 1a,b.--- Grotolli-Everett & Wellington, 1997:292, fig. 1.--- Veron, 2000:20, 21 (color).--- Humann & Deloach, 2002:103 (color).

2.4.5 Holotype

Four branches from one colony, two of which are fused, USNM 1098754, dry skeletal specimen, collected Jan, 2006 by JML.

2.4.6 Type locality

Media Luna SW fore reef of barrier reef, La Parguera, Puerto Rico. 17°56’086 N, 67°03’010 W. Colony 17 cm in height and 46 by 30 cm in diameter. Depth 11.5 m.

2.4.7 Paratypes

Five colony branches, USNM 1098755 Cayo Laurel W, patch reef (3-5 m), La Parguera, Puerto Rico, 17°56’496 N, 67°04’034 W; USNM 1098756 near Chubb Head SW, patch reef (6 m), Bermuda 32°15’074 N, 64°58’613 W; USNM 1098757 Aquarium reef, fringing reef (10-20 m), Curaçao 12°05’039 N, 68°53’693 W; USNM 1098758 Flamingo reef, fringing reef (10 m),

Grenada 12°05’517 N, 61°45’544 W; and Bermuda Aquarium Museum and Zoo, BAMZ 2006 251 016, Tynes Bay, patch reef (8 m), Bermuda 32°18’461 N, 64°46’569 W.

2.4.8 Other material examined

Two branches from separate colonies from Cayo Laurel W, patch reef (3-5 m), La Parguera, Puerto Rico, 17°56’496 N, 67°04’034 W; one colony branch collected Tynes Bay, patch reef (7 m), Bermuda 32°18’461 N, 64°46’569 W , two colony branches from Aquarium reef, fringing reef (10-20 m), Curaçao and two colony branches from Flamingo reef, fringing reef (10 m), Grenada, coll. EW.

2.4.9 Description

Colony of several separate, thin, short to elongate branches, originating centrally and radiating upward and outward (Fig. 2.1). Occasional fusion between branches. Basal portions of colony branches often dead (Fig. 2.2). Healthy colony color most often pale yellow to golden brown; zooxanthellate with Clade B zooxanthellae (Bermuda) (L. Holland, 2006, pers. comm.). Branch length from dead basal skeleton to live tip from 2.0-6.1 cm (*n*=31, $\bar{x}$=4.4, *SD*=1.0). Branches thin in comparison to other species of *Madracis*, diameters from 4.9-10.1 mm (*n*=60, $\bar{x}$ =7.4, *SD*=1.3). Branches circular in cross-section. Branches often bifurcate into secondary and rarely tertiary branches (Fig. 2.2). Corallites round to slightly oval (Fig. 2.3). Corallite diameter 0.9-2.3 mm (*n*=364, $\bar{x}$=1.5, *SD*=0.2). Corallites with 10 prominent, primary septa; no secondary septa observed. Very rarely, larger corallites, diameters 1.6-2.3 mm (*n*=8, $\bar{x}$=1.9, *SD*=0.3), with 16 septa. Length of primary septa 0.2-0.7 mm (*n*=262, *X*=0.4, *SD*=0.08). Width of primary septa 0.07-0.2 mm (*n*=262, $\bar{x}$=0.1, *SD*=0.03). With intracolony variation in corallites. Septa connect to

a central columella; columella flat, or with slight central bump sunken within corallite, or protruding to a point matching height of extended septa (Fig. 2.3). No observed intracolony

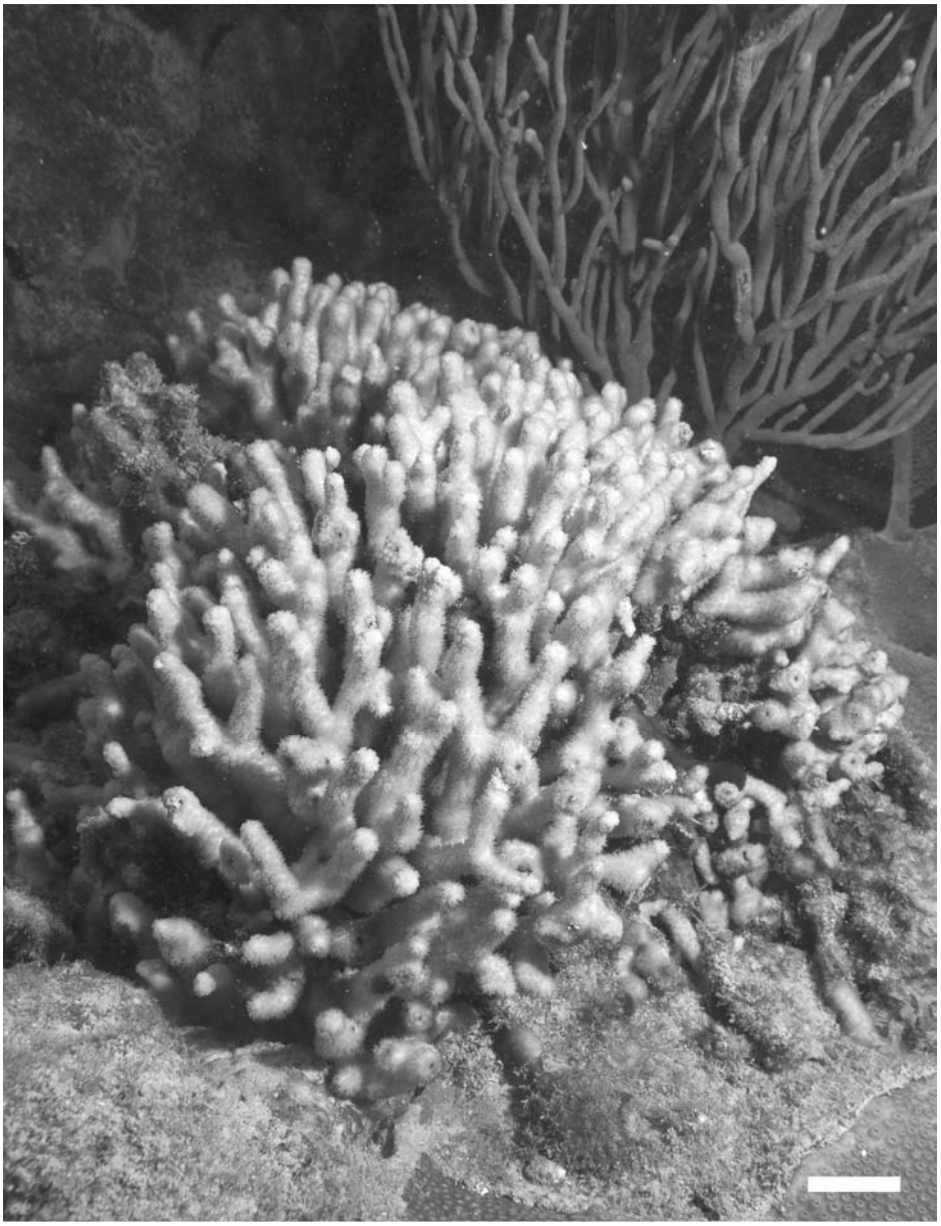

Fig. 2.1. Live solitary colony of *Madracis auretenra* holotype in situ at Media Luna SW Puerto Rico, depth 11.5 m. Scale 2 cm. Photo credit Hector Ruiz.

distribution pattern of columella type. Small spines on primary septa occasional. Distance from inside wall of corallite to nearest neighboring corallite inside wall 0.2-1.1 mm (*n*=130, $\bar{x}$=0.6, *SD*=0.15) and distance to furthest neighboring corallite 0.7-2.6 mm (*n*=130, $\bar{x}$=1.3, *SD*=0.4). Corallite density 16-36 corallites cm^{-2} (*n*=65, $\bar{x}$=26, *SD*=5). Corallites flush with coenosteum or raised; often primary septa project above coenosteum. Coenosteum slightly granular, often with distinct lines of intercorallite spines; spines forming five-sided corallite boundaries (Figs. 2.3 & 2.4).

Fig. 2.2. *Madracis auretenra* holotype, from Media Luna SW, Puerto Rico, illustrating the normal branching morphology, strong secondary and short tertiary branches and dead basal branch portions. Scale 2 cm.

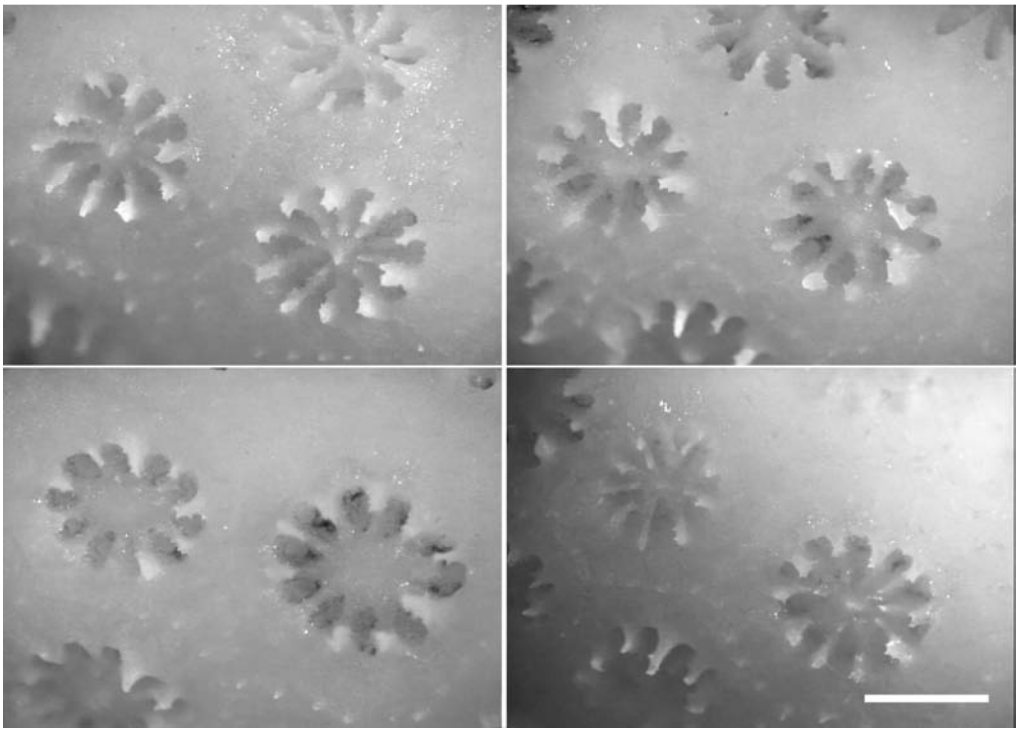

Fig. 2.3. Representation of intra-colony corallite variation in one branch of the holotype. Note differing columella morphology and boundary spines between corallites. Scale 1 mm.

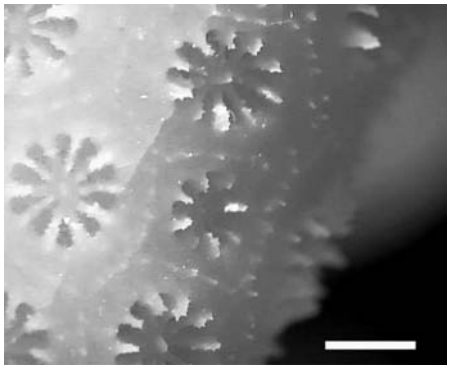

Fig. 2.4. Coenosteum of *Madracis auretenra* exhibiting boundary spines between corallites. Scale 1 mm.

2.4.10 Etymology

The species is named from Latin to represent its thin, golden branched appearance, *aureus*, of the color of gold, *tenuis*, thin, *ramus*, branch.

2.4.11 Taxonomic remarks

Madracis auretenra n. sp. represents the seventh described, extant, species of the genus in the Caribbean region (Table 2.2). The new species differs from known, extant *Madracis* species by a combination of the following characters: its thin (4.9-10.1 mm), usually elongate, branches; decamerally arranged septa; linear spines on the coenosteum; presence of zooxanthellae; and depth range. Other shallow-water, branching species of *Madracis* possessing zooxanthellae and corallites with ten septa are *M. decactis* and the more recently described *M. carmabi* Vermeij, Diekmann & Bak 2003, but both of these have blunt or lobed branches of a wider diameter (12.5 -26 mm) than found in *M. auretenra*. Similar species to *M. auretenra*, which also have thin branches, but occur in deeper water, are azooxanthellate *M. myriaster*, which possesses a striate coenosteum and widely-spaced corallites, and *M. asperula*, the colonies of which are small and delicate with extremely slender branches (1.7 mm-3 mm). (Cairns [2000] stated the largest colony of *M. asperula* he examined was 4 cm in height with an attachment base of 3.5 cm.)

2.4.12 Habitat and distribution

Inhabits mostly intermediate water depths (5-15 m) but can be found from 1-60 m. Known from Atlantic and greater Caribbean regions: Bermuda, Curaçao, Grenada and Puerto Rico.

Colonies of *M. auretenra* may be distributed as large fields or be solitary (Figs. 2.1 & 2.5), possibly attributable to asexual or sexual modes of propagation, respectively.

2.4.13 Other remarks

A partial 18S ribosomal RNA gene sequence is available for *M. auretenra*, (labeled as *M. mirabilis*) from GenBank under accession number AY950684.

Table 2.2. Zooxanthellate *Madracis* species with known distributions in the Atlantic (+ indicates species which may also be azooxanthellate). * Mistakenly called by the name *M. mirabilis*.

Species	Distribution
Madracis asperula[+] Milne-Edwards & Haime, 1849	West and East Atlantic
Madracis decactis (Lyman, 1859)	West and East Atlantic
Madracis carmabi Vermeij, Diekmann & Bak, 2003	West Atlantic
Madracis formosa Wells, 1973a	West Atlantic
Madracis auretenra new species*	West Atlantic
Madracis pharensis[+] (Heller, 1868)	West and East Atlantic
Madracis senaria Wells, 1973b	West Atlantic

Fig. 2.5. Colonial field of *Madracis auretenra* located on the south side of Mona Island, Puerto Rico. Depth 20 m. Scale 10 cm. Photo credit: Hector Ruiz

2.5 Discussion

Madracis auretenra may continue to carry the common name applied to it, "yellow pencil coral", throughout the greater Caribbean region – which was not a name ever given to the true *M. mirabilis* (a junior synonym of *M. myriaster*). Following the synonymy of *M. mirabilis* with *M. myriaster*, this common name was however applied to *M. myriaster* in the species database of the Convention on International Trade in Endangered Species (CITES) (UNEP-WCMC 2006) [http://www.unep-wcmc.org/isdb/CITES/Taxonomy/tax-species-result.cfm?displaylanguage=eng&Genus=Madracis&Species=myriaster&source=animals&Country=]. *Madracis myriaster* is commonly referred to as "striate finger coral" (Cairns 2000, Cairns et al. 2002).

The presence of a striate coenosteum in the paralectotype of *M. mirabilis*, which is absent in *M. auretenra*, and significant differences in five corallite characters (corallite diameter,

columella base diameter, near and far distances between corallites and density of corallites cm^{-2}) provide evidence that the type of *M. mirabilis* is different from *M. auretenra* (Cairns 1979, Table 2.1). The corallite character, primary septal length, was significantly different within each species but not between *M. myriaster* and *M. auretenra* (Table 2.1). The formalized synonymy of *M. mirabilis* with *M. myriaster* (Cairns 1979) renders the name *M. mirabilis* unavailable for the zooxanthellate, shallow-water, thin-branched species of *Madracis* found in the Caribbean region.

Considering the numerous (>125) studies (pers. obs.) that have experimented with or referred to "*M. mirabilis sensu* Wells" as a shallow-water taxon, clarification of the taxonomy of this coral may be considered a nuisance to some who are considering issues of coral reef preservation and conservation. However, advocating and retaining this unsupported taxonomy – to suit individual and immediate needs - has many negative implications.

Increasing interest in deep-water, azooxanthellate species and in the differences in physiology between azooxanthellate and zooxanthellate groups is sure to bring attention to the true *M. mirabilis* (= *M. myriaster*). Thus, confusion of shallow and deep-water taxa is a looming problem.

As a result of Cairns' (1979) investigations, the only mention of *M. mirabilis* within the species database of the Convention on International Trade in Endangered Species (CITES) is as a synonym for *M. myriaster*. Thus the new species, which has previously been called "M. mirabilis", is not in that list, and not afforded any of the protection that being listed provides.

At this point it is not possible to have any confidence that the numerous studies referring to "*M. mirabilis*", as indicated above, have all considered one and the same species given that: previously there has been no detailed and specific reference for the identification of the species; and very few authors provide comprehensive descriptions of their specimens. Nonetheless,

limited molecular and reproductive data (Diekmann et al. 2001, Vermeij et al. 2004, 2003b) suggest that within shallow coral reef habitats of the Caribbean, there is only one thin-branched *Madracis* taxon or "yellow pencil coral". Thus, studies subsequent to 1973 referring to the shallow-water, thin-branched *Madracis* species misidentified as ***M. mirabilis***, have some probability (greater than 0) of having considered the newly described ***M. auretenra***. However, the only way of confirming this is if voucher specimens from the original studies have been kept or recorded (as high resolution photographs, for example). The synonymy provided herein, lists only citations which include complete descriptions (none) or images that can be identified as the new species. We can only hope that some authors now will undertake to confirm their identifications and that they will consistently adopt the best practice of keeping and safely storing taxonomic reference materials. We also hope to encourage reference to primary taxonomic literature and taxonomic experts, and to discourage a total reliance on handbooks, guidebooks and brief keys. The last three are invaluable resources, but they are the starting – not end – points for a species identification.

2.6 Acknowledgements

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3. A Perspective on the taxonomic history and status of *Madracis mirabilis*

3.1 Abstract

The primary objective of this perspective is to explain simply what the binomen *Madracis mirabilis* (Duchassaing and Michelotti 1860) refers to, in order to make this information easy to understand and accept by the community of coral researchers. A straightforward statement of the status of this name is: **"*Madracis mirabilis* (Duchassaing and Michelotti 1860) is a subjective junior synonym for the deep-water species *Madracis myriaster* (Milne-Edwards and Haime 1849)"**. Such a status indicates that, in the well-considered opinion of at least one expert, the material on which the description of *M. mirabilis* was based is of the same species as *M. myriaster*. This status also means that, according to the rules of the International Code on Zoological Nomenclature, the name *Madracis mirabilis* cannot be used for another coral species. A short history of these two names follows, including their original descriptions and a subsequent, revised description of *M. myriaster*.

3.2 Perspective

For years spanning 1967-2008 most researchers have applied the name *M. mirabilis* to common shallow-water, zooxanthellate, branched coral forms found in the greater Caribbean region. In fact, most of these references were to an undescribed species, which has recently been described and named *Madracis auretenra* Locke, Weil and Coates 2007 (Chapter 2, herein). *Madracis auretenra* is a common and wide spread species and consequently the name *M. mirabilis* has been used incorrectly in as many as 175 publications (J.M. Locke pers. obs.; Chapter 4, herein). Forty-one years of using the name *M. mirabilis* to refer to this coral form has

made this behaviour so entrenched and the name so familiar that there is hesitation to adopt a taxonomically correct name and also doubts about the usefulness of such a change. Nonetheless, during those years there were other taxonomic studies that pointed out the incorrect application of the name and the synonymy of *M. mirabilis* to *M. myriaster*.

The common name for *M. myriaster* is striate finger coral. The common names yellow pencil coral, small finger coral and branching coral (Cairns et al.2002, Humann and DeLoach 2002) were only associated with *M. mirabilis* when this name was misused for *M. auretenra* and remain good common names for this coral species.

3.2.1 A short history of *Madracis mirabilis* (Duchassaing and Michelotti 1860)

Type material is some or all of the specimens the original describer(s) of a species examined, and on which they based their description. In the case of *M. mirabilis* the type material now is spread among a few museums in Italy (Fig. 3.1), France, England, and perhaps the United States (see Cairns 1979, Locke et al. 2007). Vaughan (1901) provides information that the situation is similar for type specimens of other species described from the Caribbean in the mid-1880s. The original description of *M. mirabilis*, translated below, although short, was informative. Published considerations of *M. mirabilis* were made by Pourtalès (1874, 1880), Vaughan (1901), Verrill (1901), Cairns (1979), Cairns et al. (2002), Hunter (1993), and Locke et al. (2007). These authors came to the conclusion that *M. mirabilis*, as described by Duchassaing and Michelotti (1860), was indistinguishable from *M. myriaster* (Fig. 3.2).

Figure 3.1. Type material for *Stylophora mirabilis* deposited at the Museo Zoologia Università, Turin, Italy. Scale bar 2 cm. Inset of coenosteal striations and granules characteristic of species. Scale bar 1 cm.

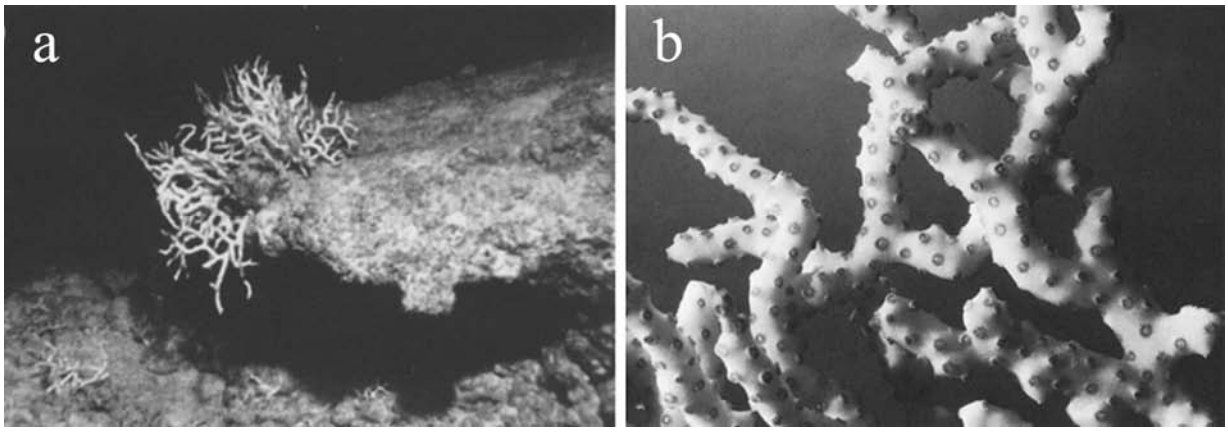

Figure 3.2. *Madracis myriaster* in 196 m depth, Castle Roads, Bermuda (Fricke and Meischner 1985) (a) A fan of *Madracis myriaster* along edges of carbonate rocks. (b) Close up of *Madracis myriaster* branch morphology. Scales not provided in original publication. Reproduced courtesy of Springer Science and Business Media and the authors.

When two differently named species are deemed to be the same, their names are synonymized, with the name of the older described species becoming the name used for the species, or the senior synonym (ICZN 1999, Articles 23.1 - 23.3). In this case, ***M. myriaster*** is the senior synonym. ***Madracis mirabilis,*** as a junior subjective synonym (ICZN 1999, Article 61.3.1), is not a valid name for the species (Verrill 1901; Cairns 1979; Locke et al. 2007). Even though it is considered invalid, the binomen ***M. mirabilis*** remains unavailable for any other coral because it is associated with a description and type material and is a potentially valid name for the species of coral thus represented. If subsequent revisers reverse the subjective opinion of synonymy then ***M. mirabilis*** would be the valid name for the original material. As Cairns (1979) strongly indicates, the history of these two names is not straightforward, but knowing these details are crucial to understanding correct taxonomic usage (see Cairns 1979, Locke et al. 2007).

3.2.2 Original descriptions

3.2.2.1 Madracis myriaster

Milne Edwards and Haime 1849, p. 69

"Genre 10. ***Axhelia.---*** Surface entièrement couverte de stries subgranuleuses. Columelle compacte. Pas de palis. Cloisons débordantes. ***Oculina myriaster,*** Valenciennes, collect. du Museum" [Surface entirely covered with subgranular striations. Compact columella. No pali. Septa raised.]

This is the description of both the new genus ***Axhelia*** and the type species for the genus ***Axhelia myriaster***. The object of the type is/are specimens initially identified as ***Oculina***

myriaster by Valenciennes and held, at that time, in the Museum in Paris. The type of *A. myriaster* could not be found at the Museum Nationale d'Histoire Naturelle, Paris in 1975 and is presumed lost (see Cairns 1979, p. 29). The new genus *Madracis* was also described in the paper by Milne Edwards and Haime (1849). Later, the genera *Axhelia* and *Madracis* were synonymized by Pourtalès (1871) who designated *Madracis* the senior synonym (Cairns 1979, p. 28).

The original description of *M. mirabilis* as *Stylophora mirabilis* Duchassaing and Michelotti, 1860 was published just over a decade later than that of *M. myriaster*. Pourtalès (1874) placed *S. mirabilis* within the genus *Axohelia* Milne-Edwards and Haime, 1849 (sic, misspelled for *Axhelia* Milne-Edwards and Haime, 1849); however in an earlier work (Pourtalès 1871) he had already made *Axhelia* a junior synonym of *Madracis*.

3.2.2.2. Madracis mirabilis

Duchassaing and Michelotti 1860, p.338, plate IX, figures 6, 7.

Stylophora mirabilis nobis.

Espèce flabelliforme à rameaux inégaux non coalescents: calices irrégulièrement placés, à bords élevés: surface sillonnée et glabre. … St. Thomas." [Flabelliform species with unequal, non-coalescent branches: calices irregularly placed, raised: surface striated and smooth.]

The original description of *M. mirabilis* mentions important characteristics of the species, including branching morphology and a striate coenosteum, and also includes illustrations. In 1901 Vaughan commented on the species *Axhelia mirabilis*, and in his explanation of the plates clearly noted "the surface of the coenchyma is covered with elongate granules, which show a decided tendency to be arranged in striae" (Vaughan 1901, p. 319) (Fig. 3.1).

These original descriptions of both *M. myriaster* and *M. mirabilis* are brief, but mention characteristics significant to recognizing the similarity of these taxa. Neither their antiquity nor their length negate an ongoing value in taxonomic investigations.

The original description of *M. myriaster* included no locality information, but a year later Milne Edwards and Haime (1850) mention “mer des Indes” [Indian Ocean]. As a result both Pourtalès (1880) and Vaughan (1901) chose to use the name *M. mirabilis* for deep water corals (> 43 fathoms) found in the Caribbean, which were indistinguishable from *M. myriaster* [Vaughan 1901, p. 295 “I can discover no criterional characters from the description or figure of *Ax. myriaster* by which the West Indian species can be separated from it.”]. Thus *M. mirabilis* as reported for the Caribbean by Vaughan (1901) had the same coenosteal features and branching pattern as *M. myriaster*. Cairns (1979, 2000) has recently indicated that there are no verified records of *M. myriaster* from the Indian Ocean and that it is a Caribbean species. The source of the mistaken locality of the original material remains unknown to us, but the effects of this apparent mistake are clear in taxonomic studies of Caribbean coral species.

Notably, in Vaughan and Wells (1943) all of *Madracis asperula* Milne Edwards and Haime, 1849, *M. mirabilis*, and *M. myriaster* are reported from the Caribbean: *Madracis asperula*, Puerto Rico, depth 15 ¼ fathoms; *M. mirabilis*, Puerto Rico depth 43 fathoms; and *M. myriaster*, “West Indies”, no depth given. These authors thus implied two modifications from Vaughan (1901), 1) *M. myriaster* does occur in the Caribbean and 2) it is distinguishable from *M. mirabilis*, but no explanations for these new ideas were provided in the later publication.

3.2.3 “Madracis mirabilis” after 1967

Wells (1973) is the obvious trigger point for widespread misidentification of a shallow-water species occurring in the Caribbean as *M. mirabilis*, nonetheless, the first unambiguous use

of the name *M. mirabilis* for a shallow-water coral was in 1967 (Goreau and Wells 1967). This usage was foreshadowed as early as 1959 when the same species was being misidentified as *M. asperula* (Goreau 1959, Lewis 1960, Roos 1964, and see Locke et al. 2007). The misidentification of the shallow-water species as *M. asperula* may have originated from Wells (1956) systematic study of scleractinian families and genera, which was a modification of Vaughan and Wells (1943). In Wells (1956) the examples of *Madracis* listed (p. F372) are *M. asperula* from 15 m in Puerto Rico and *M. mirabilis* from Puerto Rico (no depth given). Vaughan is credited as the source of the illustrations of both these species (Wells 1956, P. F373, Fig. 263, 4a, b). Indeed, these are the same, with slight modifications, as those found in Vaughan (1901, Plate 1, Fig. 3, 4) and in Vaughan and Wells (1943, Plate 5, Fig. 8, 8a). However, in both Vaughan (1901) and Vaughan and Wells (1943) it is clearly stated that the specimen illustrated as *M. asperula* was collected from a depth of 15¼ "fms", not 15 m. It seems that Wells (1956) confused the units of depth when he reported *M. asperula* from 15 meters rather than 15 fathoms. Subsequently, *M. asperula* was often reported as a shallow-water species until this usage was replaced by *M. mirabilis* (Goreau and Wells 1967, Wells 1973). Whereas Vaughan provided original illustrations of *M. mirabilis* and *M. asperula*, the source of the illustration of *M. myriaster* in Vaughan and Wells (1943) is credited to Milne Edwards and Haime [no date given]. Thus it seems neither Vaughan nor Wells had collected specimens from the Caribbean which they recognized as *M. myriaster*, but had some other basis for reporting its occurrence. In their publication they do not say how these two species could be distinguished.

Goreau and Wells (1967) and Wells (1973) applied the name *M. mirabilis* to a species of *Madracis* that clearly was not the same as *M. myriaster*. In his continued use of the name *M. mirabilis*, Wells (1973) most likely was simply following Vaughan's tutelage. He seems to have

recognized the correct identity of *M. myriaster* (see Wells 1973, key) but not its synonymy with *M. mirabilis*, thus creating "*M. mirabilis sensu* Wells 1973" (as coined by Cairns 1979 as a means of temporary reference) for an undescribed shallow-water species. Locke et al. (2007) provided a complete description for the undescribed species as *Madracis auretenra* (also see synonymy in Locke et al. 2007; Chapter 2, herein) (Figs. 3.3 and 3.4).

In conclusion, there were a number of events that contributed to this confusing taxonomic situation, including: missing and misunderstood information about geographic distribution, simple errors in reporting depth records, limited use of original literature and type specimens to confirm species identities, and, in more recent years, the status and funding of taxonomic research.

Figure 3.3. *Madracis auretenra* holotype, from Media Luna SW, Puerto Rico (Locke et al. 2007). Scale bar 2 cm. Reproduced courtesy of the Proceedings of the Biological Society of Washington.

Figure 3.4. *Madracis auretenra*, the yellow pencil coral, from an inshore patch reef, Bermuda, 5 meters.

3.2.4 Description of *Madracis myriaster*

Madracis myriaster is, so far, a relatively easy to distinguish species, among those species of *Madracis* found in the Caribbean. The most complete, recent descriptions of *M. myriaster* are found in Cairns (1979, 2000), where complete synonymies of *M. myriaster* are also provided and explained. Distinguishing and diagnostic characteristics are noted in the following description.

Madracis myriaster after Cairns 1979, p. 27; 2000, pp 41-43; J.M. Locke personal observation (Figs. 3.1 and 3.2)

= *Madracis mirabilis* (Duchassaing and Michelotti 1860)

Non "*Madracis mirabilis sensu* Wells 1973" (after Cairns 1979)

Colonies broad and bushy with irregular branching in one plane. Large colonies can be up to 40 cm in height and 4 cm in basal branch diameter. Basal branch firmly attached by an encrusting base, bearing calices. Branch diameters of main, basal branch 25 mm; mid-colony branch portions 5-6 mm; and terminal branches 3-4 mm. Corallites bear 10 highly exsert primary septa, secondary septa absent. Corallites may be flush or raised on mounds. Calices widely separated at base (one to three calicular diameters) and close-set at branch tips (¼ to ½ calicular diameter). Coenosteum prominently striate on the encrusting base and basal branches; striae changing gradually to close-set, large, rounded granules along medium diameter branches toward branch tips. Living coral is an intense pinkish-orange with yellowish or white polyps. *M. myriaster* is a deep-water, azooxanthellate species.

Personal observations are based on reference material of *M. myriaster* and type material of *M. mirabilis* (Locke et al. 2007).

3.3 Following standards – ICZN

Standards for naming and classifying organisms compiled as the International Code on Zoological Nomenclature by the International Commission on Zoological Nomenclature have been available since 1905; these were most recently revised in 1999 (http://www.iczn.org/iczn). The fundamental aim of the Code is to provide the maximum universality and continuity in the scientific names of animals, compatible with the freedom of scientists to classify animals according to taxonomic judgments (ICZN 1999).

The Code has certain underlying principles one of which is the Principal of Priority, used to determine which names are valid. The Principal of Priority may be set aside under certain circumstances when its application would be destructive of stability or universality (ICZN 1999). In the case of the binomen ***M. mirabilis*** there have been a series of publications, from 1874 to 2007, pointing out the synonymy of this name with ***M. myriaster*** and the name ***M. mirabilis*** is still available for a well-described and typified deep-water species. Thus a continuation of the practice of using this name for another species has much more potential for future confusion than does the forward step taken by Locke et al. (2007) of describing and renaming a mistakenly identified species.

Starting points for avoiding such confusion are improved taxonomic practices such as: observing type material; referring to original descriptions; assuming individual responsibility for the names that are applied to one's specimens; retaining voucher specimens (if at all possible); and retaining such material in ways that will allow acquisition of additional character data (for example, if molecular methods are not used in initial species identifications, keeping material so that this could be possible).

3.4 Acknowledgments

It is my honour to thank Lisa Levi formally of Museo Regionale di Scienze Naturali, Torino for providing photos of ***Stylophora mirabilis***, and Steve Cairns and Alison Green for providing valuable literature. This perspective was greatly improved by valued discussions with Kathryn Coates. Appreciation is also expressed to Hans Fricke and Dieter Meischner along with Springer and Business Media and the Proceedings of the Biological Society of Washington for permission to use figures of ***Madracis myriaster*** and ***Madracis auretenra*** respectively.

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4. *Madracis auretenra* – Biologic and Bibliographic Review

4.1 Abstract

This biologic and bibliographic review of the scleractinian coral species *Madracis auretenra* incorporates literature published on the species when it was incorrectly identified and named *Madracis asperula* Milne Edwards and Haime 1849, *Madracis mirabilis* (Duchassaing and Michelotti 1860) or *Madracis mirabilis sensu* Wells 1973 between the years 1959 and 2008. It includes more than 170 publications. Conspecificity with *M. auretenra* has been verified by the authors of these studies for the majority of publications; those not so verified are noted. An extensive description of *Madracis auretenra* includes new information on branch diameter and information about the distribution, ecology, physiology, reproduction, molecular and experimental research of this common Caribbean species.

4.2 Introduction

The taxonomic (non) status of the Caribbean shallow-water *Madracis* species commonly referred to as the yellow pencil coral since 1967, was a surprise to the majority of coral researchers. The lack of a description and other taxonomic issues with this coral called in to question what species many researchers were actually working with. The first step to rectifying this complex of problems was describing and naming the yellow pencil coral (Chapter 2, herein), now called *Madracis auretenra* Locke, Weil and Coates 2007.

The common and widely distributed scleractinian coral species *M. auretenra* had been extensively researched within the Caribbean, briefly as *Madracis asperula* (1959-1968) and more prominently as *Madracis mirabilis* (1967-2008) between the years of 1959 and 2008. The

two corals whose names were misused are actually azooxanthellate species known to occur at deeper depths, but which became confused with shallow-water species (see Cairns 1979, 2000 and Locke et al. 2007). Subsequent to a comprehensive revision by Cairns (1979) of deep-water scleractinians of the Caribbean, some authors named their shallow-water specimens as "*M. mirabilis sensu* Wells 1973", which Cairns clearly indicated was not the same species as *M. mirabilis* (Duchassaing and Michelotti 1860). Cairns (2000) provided a revised description, including a synonymy and figures, for *M. asperula,* and although its depth range may overlap with *M. auretenra,* it is azooxanthellate and possesses a much smaller and more delicate colony framework. *Madracis auretenra* (= "*M. mirabilis sensu* Wells 1973"), which is not known to inhabit the deep bathymetric range of *M. asperula* or *M. myriaster,* is a shallower water species, possessing zooxanthellae; and it has the apt common name, yellow-pencil coral (Fig. 4.1). *Madracis mirabilis* is a subjective junior synonym for *Madracis myriaster* (Milne-Edwards and Haime 1849) and *M. myriaster* is a Western Atlantic, deep-water species that does not have zooxanthellae (See Chapter 3, herein: Fig. 3.2). A thoroughly researched synonymy for *M. myriaster* up to 1979 is provided by Cairns (1979); a number of other authors, both before Cairns and more recently, recognized the morphological identity of *M. mirabilis* to *M. myriaster* (Pourtalès 1874; 1880, Vaughan 1901, Verrill 1901, Cairns 1979, Hunter 1993, Cairns et al. 2002, Locke et al. 2007).

For previously published studies, a straightforward change of name from *M. mirabilis* to *M. auretenra* would seem the obvious approach. However, because no species description was used as the basis of identification of *Madracis mirabilis* for the years 1967-2008 we cannot be certain what species were being studied or reported. Communication with authors did reveal that many based their coral identification on Wells' (1973) key. Even though the species name *M. mirabilis*

is invalid, this short dichotomous key does separate *M. auretenra* from other *Madracis* species and where Wells' (1973) key was used to identify *M. mirabilis*, these studies were referring to *M. auretenra* (see Hunter 1993, Cairns 1999, Vermeij et al. 2001, 2002, 2003, 2004, Trapido-Rosenthal et al. 2005). In fact, the frequency and broad distribution of *M. auretenra* within the Caribbean has led to its study and employment in many areas of coral reef research.

To minimize the confusion and accelerate the period of adopting and accepting this taxonomic revision a comprehensive biologic and bibliographic review of the literature since 1959 is presented here.

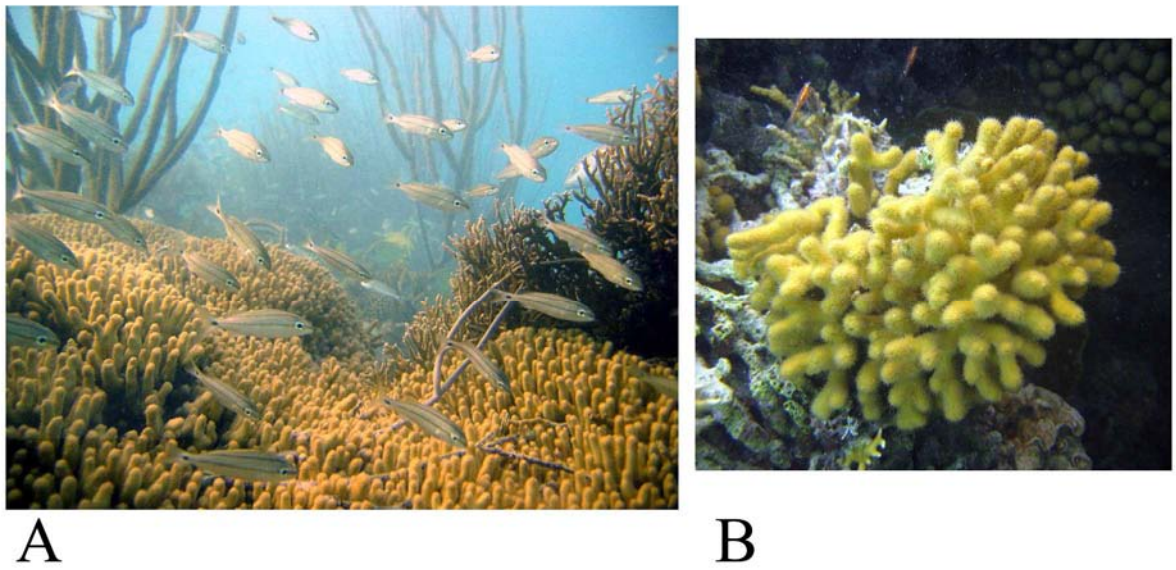


Figure 4.1. The common, shallow-water coral *Madracis auretenra* inhabiting Bermuda's inshore lagoonal areas. A. Aggregation at Shelly Bay Shoals with small adult grunts (tomtates) at 4.5 m. B. Isolated colony close-up at a Hogg Fish Crescent patch reef, depth 3.3 m.

4.3 Methods

Computerized literature searches and visual searches of literature cited sections of relevant publications were made to locate primary references published since 1959 and using the species names *M. asperula* and *M. mirabilis*. The first or principal authors of the publications were contacted by electronic mail for confirmation of the identity of their study material as *M. auretenra*, *M. myriaster* or another species. They looked for evidence of the distinguishing morphological features that became apparent once *M. auretenra* had been described (Locke et al. 2007). In cases where contact with the principal author could not be made, collaborating authors were contacted and or figures within the publication were used for validation of species identities. All authors were asked if the source of their specimen identification information was from memory, photographs, reference specimens, and/or another source. Publications stating that species identification was based on Wells (1973) (i.e. *M. mirabilis sensu* Wells, 1973) were considered validated as *M. auretenra* and not *M. myriaster*. Publications of Bermudian shallow-water *M. mirabilis* were also confirmed as *Madracis auretenra* based upon current shallow-water distribution records for the island and personal observations.

Publications included within the review may briefly mention or may focus entirely on the coral species *Madracis auretenra* (but named *M. mirabilis*), including investigations of its biology, ecology, importance as a micro-habitat, use as an experimental organism and records of its distribution.

Additions to the initial description of *M. auretenra* incorporated information gleaned from validated publications. An amendment to previously reported metrics for the colony skeletal trait of branch diameter is made based on measurements of Florida Keys specimens which were taken on high resolution in-situ photographs using Sigma Scan Pro® 5.0.0 (SPSS Inc. 2002).

Surveys of *M. auretenra* were conducted in the Florida Keys by Erich Bartels (Mote TRL) as part of the Florida Reef Resilience Program 2008 annual reef surveys, in partnership with the Nature Conservancy and in Bermuda (2007-2008) in collaboration with Sarah Manual and Kathy Coates of Bermuda Government Department of Conservation Services.

4.4 Addition to *Madracis auretenra* description

The information included in the review of *Madracis auretenra* is the result of information from publications citing *M. mirabilis* but now validated as *M. auretenra*. The species as listed within the cited publications will therefore be *M. mirabilis* and not *M. auretenra,* but the latter name will be used herein.

4.4.1 Synonymy *Madracis auretenra* Locke Weil and Coates, 2007

See Table 4.1

Madracis asperula.--- Goreau, 1959:70.--- Lewis, 1960:1133, 1139, 1140, figs 9-11.--- Roos, 1964:7, pls. 4b, 6b.--- Lewis et al., 1968.--- Huston, 1985:21.--- Mallela and Perry, 2007:132.

Madracis decactis f. *mirabilis*.--- Fenner, 1988:136-140 listed.

Madracis mirabilis.--- Goreau & Wells,1967: 446.--- Livingston and Thompson, 1971:789,792.--- Porter, 1972:89-116.--- Goreau and Goreau, 1973:399-464.--- Lang, 1973:272.---- Wells, 1973:18, 19 (key), 56 (appendix).--- Scatterday, 1974:86, 98, 99, 103, fig.11.--- Lewis & Price,

1975:529,530,532,534, pl. 1b; 1976:80, 83 fig. 3d..--- Bak, 1976, pl. 1e.--- Werding & Erhardt, 1976:49, pl.4, fig.1.--- Bonem & Stanley, 1977:175-181.--- Hunter, 1977(MSc thesis).--- Colin, 1978:212 (color), 214, 215.--- Dryer & Logan, 1978:399-425, fig. 6.--- Luckhurst & Luckhurst, 1978:1395-1397.--- Cairns, 1979:21.--- Rogers, 1979:345.--- Bak and Luckhurst, 1980:147, 149, fig. 2.--- Bak & Criens, 1982:222, figs 1a.b.--- Cairns, 1982:274, fig. 120e.--- Logan, 1984:131-138.--- Rogers et al., 1984:73.--- Solbakken et al., 1984:150-153.--- Logan, 1985:63-68, fig. 2.--- Hendler & Littman, 1986:31,32, fig.1c.--- Coates and Jackson, 1987:365, fig.1f.--- Kensley & Snelgrove, 1987:186.--- Logan, 1988:12, 54, 57 (appendix).--- Porter & Targett, 1988:233.--- Snelgrove & Lewis, 1989:249-257.--- Lewis & Snelgrove, 1990:267-272, figs. 1a-c.--- Fenner, 1991:721-723.--- Ferrier, 1991:183-187.--- Fenner, 1993a:1100-1104.--- Fenner, 1993b:14.--- Hunter, 1993 (PhD dissertation).--- Bruno, 1995 (MSc thesis).--- Bruno & Edwards, 1997:2177-2190, figs. 1a,b.--- Bruno, 1998:169-181.--- Bruno & Edmunds, 1998:187-195.--- Cook et al. 1994:157-165.--- Duffy, 1996:564, 572.--- Hunter & Jones, 1996:249, 251, 253.--- Grotolli-Everett & Wellington, 1997:292, fig. 1.--- Fenner, 1998:19-26.--- Guzman, 1998:75-80.--- Guzman and Guevara, 1998a:893-916; 1998b:601-623.--- Leichter et al., 1998.--- Branton et al., 1999:675-682.--- Cairns, 1999:36.--- Duffy & Macdonald, 1999:284.--- Fenner, 1999:147.--- Gates & Edmunds, 1999:36.--- Guzman and Guevara, 1999: 659-675.--- Hawkins et al., 1999:894.--- Kelty, 2000 (PhD dissertation).--- Mills, 2000 (PhD dissertation).--- Shyka, 2000 (MSc thesis).--- Veron, 2000:20-21, figs. 1-5 (color) (species author incorrectly listed as (Lyman, 1859)).--- Cairns et al., 2002.--- Diekmann et al., 2001:221-233.--- Guzman and Guevara, 2001:53-66.--- Vermeij et al., 2001:87-90.--- Diekmann et al., 2002:221-232.--- Humann & Deloach, 2002:103 (color).--- Meesters et al., 2002:237.--- Owen et al., 2002:623-632.--- Savage et al., 2002:19.--- Vermeij, 2002 (PhD dissertation).--- Vermeij and Bak,

2002:105-116.--- Vermeij et al., 2002:423-429.--- Diekmann, 2003 (PhD dissertation).--- Diekmann et al., 2003:29-33, fig.1b.--- de Putron, 2003 (PhD dissertation).--- Owen et al., 2003:542, 543.--- Vermeij and Bak, 2003:725-744.--- Vermeij et al., 2003:75-84.--- Weil 2003:328.--- Mallela et al., 2004:305-307, figs. 8, 9.--- Mills et al., 2004:311-323.--- Vermeij et al., 2004:206-214.--- Trapido-Rosenthal et al., 2005:3-6.--- Holland, 2006:92, 94, 97, 135, 137-139, 160,167, 170, 180, 204.--- Leichter and Genovese, 2006.--- MacDonald et al., 2006.--- Edmunds, 2007:784.--- Downs & Downs, 2007:47-57.--- Elahi & Edmunds, 2007:20-28.--- Loram et al., 2007:260, 263, 265.--- Mallela and Perry, 2007:132, 137, 138.--- Banks et al., 2008:207.--- Fukami et al., 2008:3-5, (Supporting Information).

The synonymy is complete to date and incorporates publications listed within the synonymy and original description of ***M. auretenra*** (Locke et al. 2007). A more recent synonymy and description for the azooxanthellate deep-water species ***M. asperula*** can be found in Cairns (2000). Cairns (1982) anomalously proposed synonymizing zooxanthellate shallow-water *Madracis* species misidentified as ***M. asperula*** and ***M. mirabilis*** both as ***M. mirabilis*** (Duchassaing and Michelotti 1860). It is not clear why he did this, even to the author himself (SD Cairns pers. comm.).

Table 4.1. Publications of *Madracis mirabilis* now verified (by authors) as *Madracis auretenra*, including publications of *Madracis asperula*. Criteria used for author validation are listed as well as the context of research conducted on the coral species and the location of the study for distribution purposes. Brief mention of the species in publications is noted as "mentioned" or "listed". Abbreviations for author validation criteria are: CVP, cite validated publication; JL, validated by Dr. Judith Lang; JML, personal validation by Jan M. Locke of Bermuda studies based upon knowledge of species presence; M, memory/recollection; P, reference photo; PP, publication photo; RS, reference specimens; SW, sensu Wells, 1973; Note a: supplementary information notes probably *M. auretenra*; Note b: identified by TF Goreau, validated by Judith Lang. Publications originally identified as *Madracis asperula* are noted by *.

Publication	Validation Criteria	Research Context	Location
Bak, 1976	PP	Ecology	Curaçao
Bak & Criens, 1982	PP	Ecology	Curaçao
Bak and Luckhurst, 1980	M	Ecology, Disturbance	Curaçao
Banks et al., 2008	P	Distribution	Florida
Bonem and Stanley, 1977	M, P	Distribution, Ecology	Discovery Bay, Jamaica
Branton et al., 1999	JML	Experimental	Bermuda
Brewer and Hubbard, 2006	P, RS	Paleobiology	Domican Republic
Bruno, 1995	M	Phenotypic plasticity	Discovery Bay, Jamaica
Bruno, 1998	M	Ecology	Discovery Bay, Jamaica
Bruno and Edmunds, 1998	M	Phenotypic plasticity	Discovery Bay, Jamaica
Bruno and Edmunds, 1997	M, PP	Phenotypic plasticity	Discovery Bay, Jamaica
Cairns, 1979	RS	Mentioned	
Cairns, 1982	PP, RS	Distribution	Carrie Bow Cay, Belize
Cairns, 1999	SW	Listed	
Cairns et al., 2002	RS	Listed	
Coates and Jackson, 1987	PP	Mentioned	
Colin, 1978	JML	Field Guide	Caribbean
Cook et al., 1994	M, P	Feeding Physiology	Bermuda
Diekmann, 2003	M, PP	Ecology	Curaçao
Diekmann et al., 2003	M	Molecular, Zooxanthellae	Curaçao
Diekmann et al., 2002	M	Molecular, Zooxanthellae	Curaçao
Diekmann et al., 2001	M	Molecular, Species Boundaries	Curaçao
Downs and Downs, 2007	JML	Experimental	Bermuda
Dryer and Logan, 1978	M	Ecology	Bermuda

Table 4.1. Continued

Publication	Validation Criteria	Research Context	Location
Duffy, 1996	M, RS	Micro Habitat	Carrie Bow Cay, Belize
Duffy and Macdonald, 1999	M, RS	Micro Habitat	Carrie Bow Cay, Belize
Edmunds, 2007	CVP	Mentioned	
Elahi and Edmunds, 2007	P	Physiology	Columbus Park, Jamaica
Fenner, 1999	M	Distribution	Cozumel, Belize
Fenner, 1998	M	Distribution	St. Lucia
Fenner, 1993a	M	Taxonomy	Cozumel, Jamaica, St. Lucia, Roatan, Cayman Islands
Fenner, 1993b	M	Distribution	Cayman Brac, Little Cayman, Roatan
Fenner, 1991	M	Disturbance	Cozumel, Mexico
Fenner, 1988	M	Distribution	Cozumel, Mexico
Ferrier, 1991	JML	Feeding Physiology	Bermuda
Fukami et al., 2008	Note a	Phylogeny	Bocas del Toro, Panama
Gates and Edmunds, 1999	CVP	Mentioned	
Goreau, 1959*	JL	Ecology	Jamaica
Goreau and Wells, 1967	JL	Distribution	Jamaica
Goreau and Goreau, 1973	JL	Distribution	Jamaica
Grotolli-Everett and Wellington, 1997	PP	Distribution, Disturbance	Florida Keys
Guzman, 1998	M	Distribution	Cayo Cochinos, Honduras
Guzman and Guevara, 2001	M	Distribution	Bocas del Toro, Panama
Guzman and Guevara, 1999	M	Distribution	Bocas del Toro, Panama
Guzman and Guevara, 1998a	M	Distribution	Bocas del Toro, Panama
Guzman and Guevara, 1998b	M	Distribution	Bocas del Toro, Panama
Hawkins et al., 1999	M	Disturbance	Bonaire
Hendler and Littman, 1986	M, PP	Micro Habitat	Carrie Bow Cay, Belize
Holland, 2006	M, RS	Zooxanthellae	Bermuda
Humann and Deloach, 2002	P	Field Guide	
Hunter and Jones, 1996	M	Distribution	Grand Cayman
Hunter, 1977	M	Distribution	Bardados
Hunter, 1993	M, RS	Taxonomy, Ecology	Cayman Islands
Kelty, 2000	JML	Physiology	Bermuda

Table 4.1. Continued

Publication	Validation Criteria	Research Context	Location
Kensley and Snelgrove, 1987	M	Micro Habitat	Bardados
Lang, 1973	M	Ecology	Jamaica
Leichter and Genovese, 2006	M, P	Physiology	Discovery Bay, Jamaica
Leichter et al., 1998	M, P	Physiology	Florida Keys
Lewis, 1960*	PP	Ecology	Barbados
Lewis et al., 1968*	CVP	Physiology	Barbados
Lewis and Snelgrove, 1990	M	Micro Habitat, Ecology	Barbados
Lewis and Price, 1975	M	Feeding Physiology	Barbados
Lewis and Price, 1976	M	Feeding Physiology	Barbados
Livingston and Thompson, 1971	Note b	Experimental	Discovery Bay and Kingston, Jamaica
Logan, 1988	M	Distribution	Bermuda
Logan, 1985	M	Immunology	Bermuda
Logan, 1984	M	Ecology	Bermuda
Loram et al., 2007	M	Methods, Zooxanthellae	Bermuda
Luckhurst and Luckhurst, 1978	M	Micro Habitat	Curaçao
MacDonald et al., 2006	M, RS	Micro Habitat	Carrie Bow Cay, Belize
Mallela and Perry, 2007	M	Distribution	Rio Bueno, Jamaica
Mallela et al., 2004	M	Ecology, Distribution	Rio Bueno, Jamaica
Meesters et al., 2002	CVP	Mentioned	
Mills, 2000	JML	Ecology	Bermuda
Mills et al., 2004	JML	Feeding Physiology	Bermuda
Owen et al., 2003	JML	Experimental	Bermuda
Owen et al., 2002	JML	Experimental	Bermuda
Porter, 1972	M	Distribution	Panama
Porter and Targett, 1988	M	Ecology	St. Croix, U.S. Virgin Islands
de Putron, 2003	JML	Reproduction	Bermuda
Rogers, 1979	P	Listed	La Parguera, Puerto Rico
Rogers et al., 1984	P	Recruitment	St. Croix, U.S. Virgin Islands
Roos, 1964*	PP	Distribution	Curaçao
Savage et al., 2002	JML	Zooxanthellae	Bermuda
Scatterday, 1974	PP	Ecology and Paleobiology	Bonaire

Table 4.1. Continued

Publication	Validation Criteria	Research Context	Location
Shyka, 2000	JML	Ecology	Bermuda
Solbakken et al., 1984	JML	Experimental	Bermuda
Snelgrove and Lewis, 1989	M	Ecology	Barbados
Trapido-Rosenthal et al., 2005	SW, JML	Experimental, Zooxanthellae	Bermuda
Vermeij, 2002	SW	Ecology	Curaçao
Vermeij et al., 2004	SW	Reproduction	Curaçao
Vermeij et al., 2003	SW	Reproduction	Curaçao
Vermeij and Bak, 2003	SW	Ecology	Curaçao
Vermeij and Bak, 2002	SW	Ecology	Curaçao
Vermeij et al., 2002	SW	Ecology	Curaçao
Vermeij et al., 2001	SW	Ecology	Curaçao
Veron, 2000	PP	Identification, Distribution	
Weil, 2003	RS	Distribution	Venezuela
Wells, 1973	JL	Distribution	Jamaica
Werding and Erhardt, 1976	PP	Ecology	Chengue Bay, Columbia

4.4.2 Publications of *Madracis mirabilis* not validated as *Madracis auretenra*

The following list of publications regarding *M. mirabilis* have not been validated as references to *M. auretenra* for reasons including: no current author contact information, no reply from authors to correspondence, authors have replied but are currently referring to reference specimens before validating or, in one instance, non-agreement. Without validation the following publications can not be confidently included within the above synonymy of *M. auretenra*.

See Table 4.2

Madracis mirabilis species dubia.--- Bak, 1973; 1974; 1976; 1978.--- Bak and van Eys, 1975.--- Bak and Elgershuizen, 1976.--- Elgershuizen and de Kruijf, 1976.--- Chassaing et al., 1978.--- van den Hoek et al., 1978.--- Bak and Engel, 1979.---Ducklow and Mitchell, 1979.---Frydl, 1979.--- Muscatine et al., 1979.--- Schoenburg and Trench, 1980.--- Bak et al., 1982.--- Highsmith, 1982.--- Stearn 1982.--- van't Hof, 1982.--- Burns, 1985.--- Hughes, 1985; 1989.--- Jaap, 1985.--- Schindler, 1985.--- Scott, 1985.--- Mah and Stearn, 1986.--- de Ruyter va Steveninck and Bak, 1986.--- Tomascik and Saunders, 1987.--- Kobluk and Lysenko, 1987; 1992. --- Delvoye, 1988.--- Jordan Dahlgren, 1988.--- Sebens and Miles, 1988.--- Wilkerson et al., 1988.---Budd et al. 1989.--- Rowan and Powers, 1991.--- Sebens and Johnson, 1991.--- Budd et al., 1994.--- Johnson et al., 1995.--- Sebens et al., 1996; 1997; 1998.--- van Veghel et al., 1996.--- Aronson and Precht, 1997.--- Greenstein and Pandolfi, 1997.--- Pandolfi and Geenstein, 1997.--- van Veghel, 1997.--- Aerts, 1998.--- Bak et al., 1998.--- Budd & Petersen, 1998.--- Budd & Johnson, 1999.--- Hughes and Connell, 1999.--- Steiner, 1999; 2003.--- Nagelkerken et al., 2000.--- Levy et al., 2001.--- Pandolfi, 2001.--- Pandolfi and Jackson, 2001.--- Bohnsack et al., 2002--- Hoetjes et al., 2002.--- Pandolfi et al., 2002.--- Petrichtcheva et al., 2002.--- Feingold et al., 2003.--- Greenstein and Pandolfi, 2003.--- Kaandorp et al., 2003.--- Klaus and Budd, 2003.--- Maier et al., 2003.--- Myer et al., 2003.--- Richelle-Maurer et al., 2003.--- Kaandorp et al., 2005.--- O'Farrell and Day, 2005.--- Aronson and Precht, 2006.--- Croquer et al., 2006.--- Kruszyjski et al., 2007.--- Banks et al., 2008.--- Chen et al., 2008.---Frade et al., 2008.--- Houlbrèque and Ferrier-Pagès, 2008.--- Maliao et al., 2008.--- Somerfield et al., 2008.--- Frade, 2009.

Table 4.2. Publications of *Madracis mirabilis* not yet validated by authors as *Madracis auretenra*. The reason for lack of validation is indicated as well as the context of research conducted on the coral species and the location of the study. Brief mention of the species in publications is noted as "mentioned" or "listed". Abbreviations for lack of validation are: E, no current contact information, NA, not in agreement; NC, not contacted; NR, no reply to correspondence for validation; RR, still referring to reference specimens. Publications mentioning *M. asperula* noted by *.

Publication	Lack of Validation	Research Context	Location
Aerts, 1998	E	Ecology	Curaçao
Aronson and Precht, 2006	NR	Ecology	
Aronson and Precht, 1997	NR	Distribution	Channel Cay Shoal, Belize
Bak, 1978	NR	Ecology	Curaçao
Bak, 1976	NR	Ecology	Curaçao
Bak, 1974	NR	Ecology	Curaçao
Bak, 1973	NR	Ecology	Curaçao
Bak et al., 1998	NR	Ecology	Curaçao
Bak et al., 1982	NR	Ecology	Curaçao
Bak and Engel, 1979	NR	Recruitment	Curaçao, Bonaire
Bak and Elgershuizen, 1976	NR	Experimental	Curaçao
Bak and Van Eys, 1975	NR	Ecology	Curaçao, Bonaire
Bohnsack et al. 2002	RR	Distribution	Looe Key, Florida
Bouchon et al., 2004	RR	Status, Distribution	St. Vincent and Grenadines
Bries et al., 2004	E	Disturbance	Bonaire and Curaçao
Budd and Johnson, 1999	RR	Paleobiology	Dominican Republic, Jamaica, Curaçao
Budd et al., 1998	RR	Paleobiology	Trinidad
Budd et al., 1994	RR	Paleobiology	Dominican Republic
Budd et al., 1989	RR	Mentioned	
Burns, 1985	E	Distribution	South Florida
Chassaing et al., 1978	E	Distribution	French Antilles
Chen et al., 2008	NR	mtDNA genome	Bocas del Toro, Panama
Cróquer et al., 2006	NR	Disease	Los Roques, Venezuela
Delvoye, 1988	NR	Reproduction	
Ducklow and Mitchell, 1979	E	Mentioned	
Elgershuizen and de Kruijf, 1976	E	Experimental	Curaçao
Feingold et al., 2003	RR	Distribution	Abacos, Bahamas
Frade, 2009	NA	Molecular	

Table 4.2. Continued

Publication	Lack of Validation	Research Context	Location
Frade et al., 2008	NA	Molecular, Zooxanthellae	Curaçao
Frydl, 1979	E	Predation	Barbados
Greenstein and Pandolfi, 2003	NR	Paleobiology	Florida Keys
Greenstein and Pandolfi, 1997	NR	Mentioned	Florida Keys
Highsmith, 1982	E	Mentioned	
Hoetjes et al., 2002	NR	Status, Distribution	Eastern Caribbean
Houlbrèque and Ferrier-Pagès, 2008	NR	Heterotrophy	
Hughes, 1989	NR	Listed	
Hughes, 1985	NR	Recruitment	Rio Bueno, Jamaica
Hughes and Connell, 1999	NR	Disturbance	Jamaica
Huston, 1985*	NC	Mentioned	
Jaap, 1985	NR	Bleaching	Florida Keys
Johnson et al., 1995	RR	Listed	
Jordan Dahlgren, 1988	E	Distribution	Cozumel, Mexico
Kaandorp et al., 2005	E	Ecology	Curaçao
Kaandorp et al., 2003	E	Ecology	Curaçao
Klaus and Budd, 2003	RR	Paleobiology	Domincan Republic
Kobluk and Lysenko, 1992	E	Disturbance	Bonaire
Kobluk and Lysenko, 1987	E	Ecology	Bonaire
Kruszyjski et al., 2007	NR	Growth modeling	Curaçao
Levy et al., 2001	NC	Mentioned	
Mah and Stearn, 1986	E	Disturbance	Barbados
Maier et al., 2003	E	Ecology, Physiology	Curaçao
Maliao et al., 2008	NR	Ecology	Florida Keys
Meyer et al., 2003	NR	Mentioned	Curaçao
Muscatine et al., 1979	NC	Physiology, Zooxanthellae	Discovery Bay, Jamaica
Nagelkerken et al., 2000	NR	Ecology	Curaçao
O'Farrell and Day, 2005	E	Bleaching	Tobago
Pandolfi, 2001	NR	Paleobiology	Barbados, Curaçao, Columbia
Pandolfi et al., 2002	NR	Paleobiology	Barbados
Pandolfi and Jackson, 2001	NR	Paleobiology	Curaçao
Pandolfi and Greenstein, 1997	NR	Paleobiology	Florida Keys

Table 4.2. Continued.

Publication	Lack of Validation	Research Context	Location
Petrichtcheva et al., 2002	NR	Experimental	Nenguange Bay, Columbia
Richelle-Maurer et al., 2003	NR	Experimental	Curaçao
Rowan and Powers, 1991	NR	Molecular, Zooxanthellae	St. Croix, U.S. Virgin Islands
de Ruyter van Steveninck and Bak, 1986	NR	Ecology	Curaçao
Schindler, 1985	E	Ecology	Jamaica
Schoenberg and Trench, 1980	E	Molecular, Zooxanthellae	
Scott, 1985	E	Habitat	Jamaica
Sebens et al., 1998	NR	Ecology	Discovery Bay, Jamaica
Sebens et al., 1997	NR	Ecology	Discovery Bay, Jamaica
Sebens et al., 1996	NR	Ecology	Discovery Bay, Jamaica
Sebens and Johnson, 1991	NR	Mentioned, Prey Capture	
Sebens and Miles, 1988	NR	Experimental	Discovery Bay, Jamaica
Somerfield et al., 2008	NR	Ecology	Florida Keys
Stearn, 1982	E	Mentioned	
Steiner, 1999	NR	Distribution	South Caicos, Turks & Caicos
Steiner, 2003	NR	Distribution	Dominica
Tomascik and Saunders, 1987	NR	Ecology	Barbados
van den Hoek et al., 1978	E	Distribution	Curaçao
van't Hof, 1982	E	Field Guide	Bonaire
van Veghel, 1997	E	Distribution	Bonaire and Curaçao
van Veghel et al., 1996	E	Ecology	Curaçao
Wilkerson et al., 1988	E	Zoozanthellae	Discovery Bay, Jamaica

4.4.3 Material examined

One hundred published accounts of *M. asperula* and *M. mirabilis* as zooxanthellate, shallow-water corals between the years 1959 and 2008, now validated by authors as *M. auretenra.*

High resolution photographs of thirteen colonies from the Florida Keys taken by E. Bartels; two from Long Key (24º 45.258’ N, 80º 45.562’ W), three from Duck Key (24º 41.116’ N, 80º 55.315’), two from Key West (24º 29.974’ N, 81º 44.147’ W) and six from Key Largo (24º 56.119’ N, 80º 29.202’ W and 25º 01.792’ N, 80º 21.612’ W).

4.4.4 Addition to morphological description

Colonies of *M. auretenra* may display a wider branch diameter which may be more oval then circular in cross-section, than that originally described (J. Bruno pers. comm., J. M. Locke pers. obs.) and some colonies may possess blunt branch tips (Fig. 4.2) (Cairns 1982, Bruno 1995, J.M. Locke pers. obs.). The branch diameter originally reported for *M. auretenra* was 4.9 – 10.1 mm (n = 60, $\bar{x}$ = 7.4, SD = 1.3) (Locke et al. 2007). Branch diameter data of Florida specimens ranged from 16.3-28.9 mm (n = 40, $\bar{x}$ = 23.8, SD = 3.12). The range difference may be indicative of ecophenotypic plasticity. Combining these diameters with those of the original description yields a branch diameter range of 4.9 – 28.9 mm (n = 100, $\bar{x}$ = 13.9, SD = 8.3) for the species. *Madracis auretenra* differs from *Madracis decactis* (Lyman 1859) and *Madracis carmabi* Vermeij, Diekmann and Bak 2003, which share a decameral septal arrangement, by its distinct colony morphology and coenosteal pattern. *Madracis decactis* colonies are nodular to submassive and *M. carmabi* displays more numerous branch bifurcations from a broader basal portion. The latter species is considered to have originated through hybridization of *M. decactis* and *Madracis formosa* Wells 1973.

Figure 4.2. *Madracis auretenra* colony exhibiting wider branch diameter and blunt branch tips. The colony is located in Florida's Middle Keys off Long Key at 13.4 m. Scale equals 10 cm.

4.4.5 Geographic range and distribution

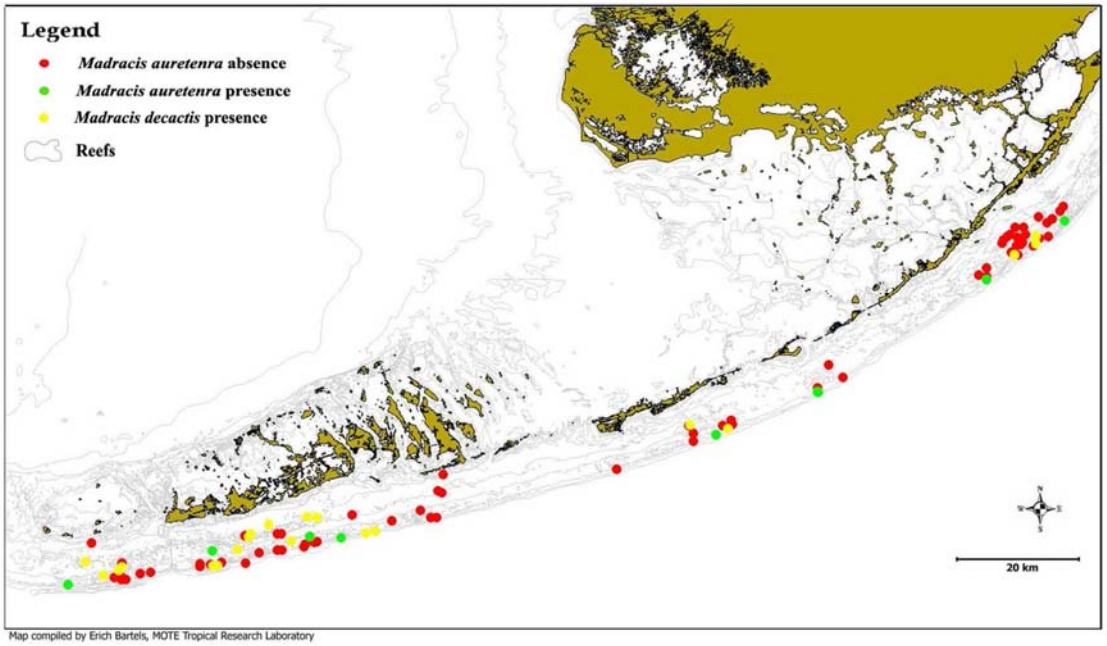
Madracis auretenra has previously been reported as occurring in Bermuda, Curaçao, Grenada and Puerto Rico. Now the species is also known from Barbados, Belize, Bonaire, Cayman Islands, Columbia, Dominican Republic, Florida, Honduras, Jamaica, Mexico, Panama, St. Croix, St. Lucia, Trinidad and Venezuela (Table 4.1). The most northern known habitats for the species are Delray Beach, Palm Beach County, Florida (K. Banks pers. comm.) (26° 26.580'N, 80° 02.801'W) (Fig. 4.3), in the continental US and the oceanic island of Bermuda. Within Florida this species is not recorded from the Biscayne Bay area (Lirman et al. 2003). *Madracis auretenra* is also not known to occur in Brazil (which is not Caribbean).

Figure 4.3. *Madracis auretenra* aggregation located offshore at Delray Beach, Palm Beach County, Florida. One of the most northern habitats for the species, in the continental US. Depth 19 m. Photo credit: Kenneth Banks, September 12, 2007.

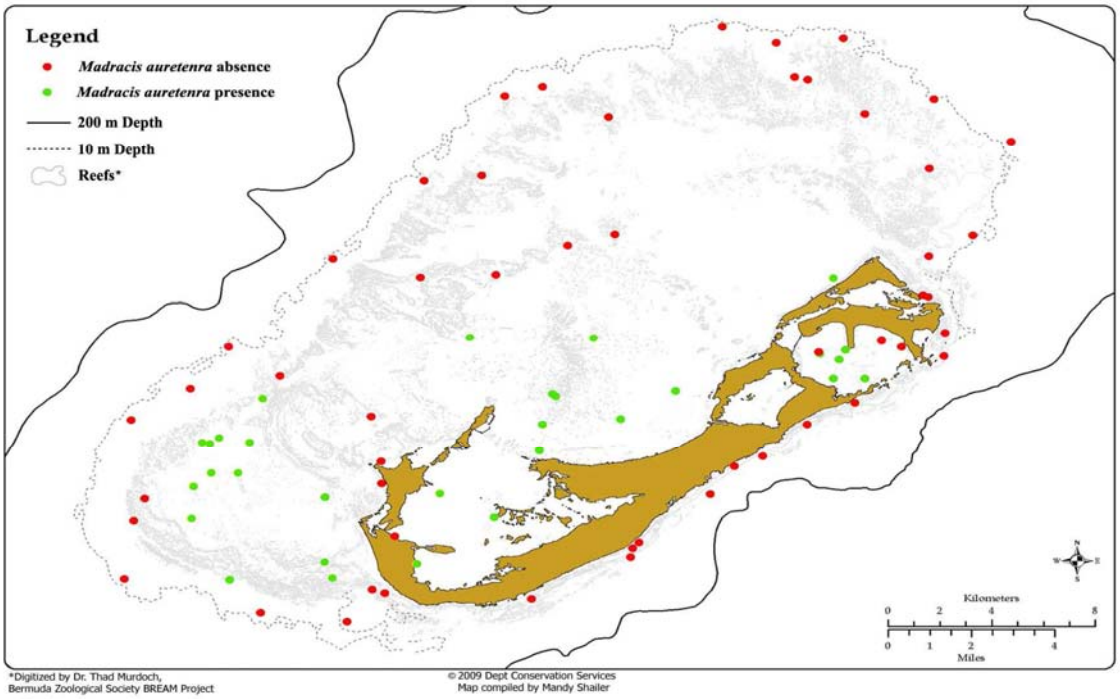
Within shallow reef environments of the greater Caribbean *M. auretenra* is found between the depths of 1-60 m, although deeper occurrences have been recorded (E. Weil pers. comm.). *Madracis auretenra* is an erect and branching coral that appears pale to bright yellow in color *in situ*. Colonies are often found as either small isolated heads or dense colonial fields, referred to as aggregations (Fig. 4.1). Branches of colonies are physically connected by a common skeleton but not by tissue (Bruno and Edmunds 1997, Bruno 1998). *Madracis auretenra* is known to display a range of variation in its community structure. In Jamaica, *M. auretenra* forms hemispherical aggregations of thin cylindrical branches up to 2 m in diameter in forereef areas, and larger aggregated colonies >5 m in diameter in backreef and lagoonal areas. The aggregated colonies have thickened branches with flattened tips (Bruno 1995, Bruno and Edmunds 1997). Whereas, in Barbados colony morphology may differ slightly with small, isolated, hemispherical heads (~20 cm in diameter) possessing short, robust, widely separated branches (covered by 50

to 80% live tissue in shallow-water) or large monospecific continuous beds (aggregations) of long, thin, tightly spaced branches (20 to 30% live tissue) in deeper water that are ten's of meters in width and up to 0.5 m in vertical thickness (Kensley and Snelgrove 1987, Lewis and Snelgrove 1990). In Bermuda, ***M. auretenra*** dominates inshore patch reefs prone to high sedimentation and morphological variation is noticeable although the flattened branch tips (***sensu*** Bruno 1995) are not as pronounced (J.M. Locke pers. obs.).

Madracis auretenra often occurs in isolated patches where it is found. Reef surveys of the Florida reef tract and the islands of Bermuda during 2007 and 2008 provide evidence of this patchy distribution. In 2008, a total of ninety reef sites were surveyed in search of ***M. auretenra*** from Key Largo to Key West, covering an approximate area of 973 km^2. Of the ninety survey sites in Florida only eight were found to have ***M. auretenra*** present (Fig. 4.4a) (data provided by Erich Bartels (Mote TRL) and Florida Reef Relief Program in collaboration with the Nature Conservancy). It should also be noted that within the Florida Keys ***Madracis decactis*** and ***M. auretenra*** are not always found together (Fig 4.4a). This data provides new information on the distribution of this species in the Florida Keys. Reef habitat surveys in Bermuda included random sites across the entire platform. ***Madracis auretenra*** presence appeared to be concentrated among inshore lagoonal patch reefs although there were a few occurrences in off shore areas. The species was absent from survey sites on Bermuda's south shore and on the outer northern rim of the island, both of which are generally higher energy locations (data provided by Sarah Manual and Kathryn Coates, Bermuda Government Department of Conservation Services) (Fig. 4.4b).



A



B

Figure 4.4. Geographic Information System representation of *Madracis auretenra* reef surveys. A. The Florida Keys and B. Bermuda Islands. *Madracis auretenra* presence denoted by green dots, absence by red dots, and presence of *M. decactis* in Florida represented by yellow dots. Credit for GIS representation is noted beneath each map.

4.4.6 General biology and ecology

Several aspects of the general biology and ecology of *M. auretenra* have been studied over the years and are summarized accordingly. In a number of instances the species was selected as a model species because of its abundance in a variety of environments within the Caribbean and also due to its relative robustness to experimental handling (Bruno and Edmunds 1997, Leichter and Genovese 2006, Elahi and Edmunds 2007).

Madracis auretenra displays a noticeably distinct overall colony structure and biology that sets it apart from other branching species of the genus. Colonies grow by apical extension of the branches and as a branch extends the tissue at the base of the branch recedes exposing the basal portion which becomes colonized by sponges and algae (Bruno and Edmunds 1997). Each branch is therefore a functionally separate colony of physiologically integrated polyps (Connell 1973, Bruno and Edmunds 1997, 1998). Fragments usually break apart from the colony at basal tissue-free areas which may be the weakest point of the skeleton. This breakage rarely causes a tissue wound reducing the species susceptibility to disease (Bak and Criens 1982, Bruno 1998).

In *M. auretenra* the age of coral tissue appears to affect calcification rate (Elahi and Edmunds 2007). Elahi and Edmunds studied the plasticity of calcification in the coral and noted that the “proximal modules” (polyps) of the species are subject to physiological senescence and young tip fragments calcified faster than more basal, older branches.

4.4.6.1 Phenotypic plasticity

Madracis auretenra is known to display ecophenotypic plasticity (Bruno and Edmunds 1997). Bruno and Edmunds noted that corallite traits and branch diameters could be significantly affected by single or synergistic environmental factors. In 1998, these authors determined that

this variability represented an important adaptation of ***M. auretenra*** to different rates of water flow. They discovered that colony branch spacing was generally inversely proportionate to the rate of water movement and that colonies with the greatest branch spacing also had the highest rates of respiration. Branch density was also related to water flow (Bruno 1995, Bruno and Edmunds, 1998) and in forereef environments branch density increased with water flow (more, closely spaced branches instead of fewer, widely spaced branches in low flow environments), whereas in lagoonal environments branch diameter (but not density) was greater near high flow micro-environments. In addition, Bruno and Edmunds (1997) used DNA fingerprinting to determine eight of ten morpho-genotypes in ***M. auretenra*** demonstrating that both genotype and environment act together to determine morphology in the species.

The investigations of Bruno and Edmunds into the phenotypic plasticity of ***M. auretenra*** are by far the most extensive studies to date into environmentally driven morphological variation for any coral species and aid tremendously in our understanding of the adaptive capacity possessed by scleractinian corals.

4.4.6.2 Micro-Habitat

The inter-branch spaces of ***M. auretenra*** colonies provide valuable micro-habitat for other marine invertebrates and for small vertebrates on the reef. In Barbados, Kensley and Snelgrove (1987) and Lewis and Snelgrove (1990) documented several cryptofaunal crustaceans inhabiting the spaces and crevices between the corals branches, including isopods, decapods, amphipods, tanaids and copepods. In Carrie Bow Cay, Belize numerous species of brittlestars are known to live between the branches (Hendler and Littman 1986). Several demosponge species, ***Xestospongia*** cf. ***subtriangularis, Hyattella intestinalis, Xestospongia proxima, Lissodendoryx***

cf.*strongylata* and *Oceanapia* sp. grow among both dead and live basal branches of *M. auretenra* (Duffy 1996, Duffy and Macdonald 1999, MacDonald et al. 2006). Inhabiting these sponges are several (~36) species belonging to the Alpheid snapping shrimp genus *Synalpheus* (Duffy 1996, Duffy and Macdonald 1999, MacDonald et al. 2006). *Madracis auretenra* is also a recognized habitat and shelter for juvenile and small adult fishes (Luckhurst and Luckhurst 1976). Post larval and juvenile fish species *Eupomacentrus parititus* (bicolor damselfish) and *Muraena miliaris* (now *Gymnothorax miliaris*, an eel) take temporary shelter in the corals branches, whereas, *Lythrypnus mowbrayi* (goby), *Starksia atlantica* (blenny), *Pseudogramma bermudensis* (reef bass) and *Risor ruber* (blenny) may be more permanent residents; the last mostly within attached sponges (Luckhurst and Luckhurst, 1976).

4.4.6.3 Disturbance

The branched morphology and lack of host connective tissue between the primary branches of *M. auretenra* causes colonies to be fragile and more susceptible to invertebrate (*Diadema antillarum*) and fish predation (Bak 1976, Grotolli-Everett and Wellington 1997) and physical disturbance (storm and wave surge) (Bak 1976, Bak and Luckhurst 1980, Fenner 1991, Bruno 1998, Hawkins et al. 1999). Besides limiting the vertical distribution of *M. auretenra* (Grotolli-Everett and Wellington 1997), constant damage to colonies by predation may result in more stunted colonies with reduced branch length and the blunt branch tips often observed in this species.

A positive effect of being fragile and these physical and biological disturbances is the increased potential for asexual dispersal and colonization, including in the areas damaged by disturbances.

4.4.6.4 Reproduction and recruitment

Madracis auretenra reproduces both asexually by fragmentation (Bak and Criens 1982, Bruno 1998) and sexually as a hermaphroditic brooder that releases planulae (de Putron 2003, Vermeij et al. 2003, 2004).

It has been suggested that asexual reproduction is the more common mode of reproduction in *M. auretenra* (Bak, 1976). Bruno (1998) determined that a single 1 m^2 aggregation of *M. auretenra* broken apart completely had the potential to yield > 4000 fragments or asexual propagules; a tremendous amount of dispersal and recolonization capability. The dispersal of asexual fragments may be limited to distances < 20 cm and is not considered to be related to size, however survivorship of fragments is higher in forereef areas than in sediment laden lagoonal areas (Bruno 1998). Distance traveled must depend to some degree on the physical force acting as the dispersive agent.

Vermeij and colleagues (2003, 2004) have studied *Madracis* sexual reproduction in detail and determined that the regulation of planulation is dominated by yearly temperature cycle and not lunar cycle and that the timing of gamete maturation is positively correlated with seawater temperature. Oocyte development begins in June and precedes that of the spermaries in late August. In *M. auretenra* spermaries have been reported as more abundant than oocytes (Vermeij et al. 2004). Sperm release has been observed from colonies of *M auretenra* in the field (Vermeij et al. 2003, 2004). In Curaçao, planulae are released between March and December, with a maximum from September to November (Vermeij et al. 2003). Histological sections reveal mature gametes within tissues over maximum annual seawater temperatures during August and September in Bermuda (de Putron 2003) and August to November in Curaçao (Vermeij et al 2004). This species produces lower numbers of planulae than other *Madracis* species, excluding

Madracis senaria, however the number of planulae produced is not related to colony size (Vermeij et al. 2003). *Madracis auretenra* planulae are spheroid, contain more yolk than other *Madracis* species, and have been observed with a brown ring of zooxanthellae at the oral end (Vermeij et al. 2003, 2004). Neither embryos nor planulae have been observed in histological sections of reproducing ***M. auretenra*** (de Putron 2003, Vermeij et al. 2004; 8,000 histological sections examined). Vermeij et al. (2004) attribute this absence to immediate embryo release upon fertilization and coined the term "quick release" as a more appropriate term than brooder to describe the sexual reproductive mode in this species. Therefore, all the embryological development, with the associated risks and potential high mortalities, occurs in the plankton, causing one to ponder the adaptive advantage of the proposed "quick release" strategy. Planulae of *Madracis* species (including ***M. auretenra***) have been observed to explore the benthos 16-24 hours after parental release, often resuming swimming for small distances (< 0.50 m) (Vermeij et al. 2003).

Studies in Jamaica, the US Virgin Islands and the Florida Keys focusing on recruitment in ***M. auretenra*** have determined that larval recruitment is rare for this species (Hughes 1985, Bruno and Edmunds 1997, Bruno 1998, Rogers et al. 1984).

4.4.6.5 Feeding Physiology and Behaviour

Research on the feeding physiology of ***M. auretenra*** includes studies of the behaviour and the process of chemical uptake and of the significance of feeding on the symbiotic relationship of host and zooxanthellae. *Madracis auretenra* can be considered both autotrophic, by way photosynthesis of their symbiotic zooxanthellae and heterotrophic via suspension feeding by the coral polyps. Lewis and Price (1975, 1976) determined that ***M. auretenra*** was different from the

majority of coral species in that it expanded its polyps both day and night. It feeds by directly using its tentacles, and rarely employs mucus nets or strips. Tentacles inflate into pronounced bulbs heavily armed with nematocysts and directly capture brine shrimp, transferring them to the stomodeum by tentacle movement, under laboratory conditions. The patterns of ciliary currents, thought to be involved in the feeding process, have also been studied in detail for this species (Lewis and Price 1975, 1976).

Experimental investigations of nitrogen supply and limitation of zooxanthellae in ***M. auretenra*** conducted by Cook and colleagues (1994) showed that coral host feeding was an important factor in supplying nitrogen for zooxanthellae and that there was temporal variability in nitrogen availability at field sites where ***M. auretenra*** was found. This species is known to ingest small particulate matter (Lewis and Price 1975, Mills et al. 2004). However, studies of particulate ingestion show a lack of nitrogen enrichment by both host and zooxanthellae (Mills et al. 2004). The uptake of dissolved inorganic and organic nitrogen has been recorded for ***M. auretenra*** (Ferrier 1991, Shyka 2000, Mills et al. 2004). ***Madracis auretenra*** was one of four species used by Ferrier (1991) to provide the first definitive proof of net uptake of dissolved free amino acids in corals which may be important in the acquisition and retention of nitrogen for corals. It was proposed by Mills et al. (2004) that ***M. auretenra*** may be adapted to feeding on zooplankton or taking up dissolved inorganic matter but not utilizing suspended particulate matter or particulate matter deposited on its surface as a source of nitrogen. Leichter et al. (1998) and Leichter and Genovese (2006) have indirectly linked the effects of internal tidal bores carrying plankton rich sub-surface waters to increased growth rate in ***M. auretenra***. Coral specimens increased in skeletal weight, volume and the number of branches and there were significant effects of depth and initial coral weight on coral growth (Leichter et al. 1998).

However, there were many environmental variables at play that may affect the growth of the species in this case.

4.4.6.6 Immunology

Intra-specific immunological response using tissue grafts shared between different individuals of ***M. auretenra*** are known to result in complete tissue fusion (Logan 1985). In inter –specific interaction studies, the species was found to be one of the least aggressive scleractinian corals (Lang 1973). Of the ~40 coral species used in the study, ***M. auretenra*** only displayed aggressive behaviour toward ***Stephanocoenia spp.***, but was dominated by all other coral species within the study.

4.4.6.7 Experimental

Madracis auretenra, including its symbiotic zooxanthellae, has been utilized as a test species in several ecotoxicology studies. Research includes investigations into the presence of trace elements in the coral skeleton (Livingston and Thompson 1971) and of the immediate and long-term effects of hydrocarbons on corals (Solbakken et al. 1984). This species and it's zooxanthellae has been used to demonstrate the negative impact of Irgarol 51, an antifoulant, exposure on zooxanthellae photosynthesis (Owen et al. 2002, 2003) and on host-cell health and the associated mechanisms of Irgarol toxicity (Downs and Downs 2007).

Examination of the effects of elevated temperatures on zooxanthellar nitric oxide synthase (NOS) activity in ***M. auretenra*** indicated that coral bleaching may be a stress response initiated by the symbionts rather than by the coral host (Trapido-Rosenthal et al. 2005). Other experimental investigations include immunological evidence of a small heat shock/α crystalline protein in ***M. auretenra*** provided for the first time by Branton et al. (1999).

4.4.6.8 Zooxanthellae molecular studies

Madracis auretenra is known to host Clade B *Symbiodinium sp.* or zooxanthellae in Bermuda, Curaçao and Bonaire (Diekmann et al. 2002, 2003, Savage et al. 2002, Holland 2006, Loram et al. 2007). Molecular study of zooxanthellae in *M. auretenra* using the rDNA internal transcribed spacer region (ITS) revealed that the species is distinct from other *Madracis* species in that it possesses type B13 zooxanthellae, whereas all other species within the genus are know to harbour type B7 (Diekmann et al. 2003), thus *Symbiodinium* type B13 is specific for *Madracis auretenra*. The host specificity of *M. auretenra* with its type B13 symbionts may suggest that it is reproductively isolated from congenerics (Diekmann et al. 2003). However, in a Bermuda specimen, symbiodinium type B7 has also been documented from *M. auretenra* (Holland 2006).

An experimental comparison of methods used to explore the incidence of mixed *Symbiodinium* infections within coral hosts conducted by Loram et al. (2007) found that *M. auretenra* may bear a second clade (Clade A) at an extremely low abundance (one cell to 500-60 000 cells). The method for this determination, quantitative real time PCR, is known to be more sensitive than those previously used to elucidate the presence of dominant Clade B. It was noted however by the authors that this finding should be taken with caution as contamination may have played a role in the presence of the rare clade and that further investigation was warranted.

4.4.6.9 Madracis auretenra classification and taxonomy using molecular data

Molecular investigations of species boundaries within the genus *Madracis* using the rDNA internal transcribed spacer region (ITS) suggested that the species *M. auretenra* is a monophyletic genetic species and is reproductively isolated from other *Madracis* species

(Diekmann et al. 2001). *Madracis auretenra* has also been included in phylogenetic studies of evolutionary relationships of the Scleractinia (Fukami et al. 2008). That research was based on two mitochondrial genes, cytochrome oxidase I and cytochrome b and two nuclear genes, β-tubulin and rDNA - ITS including 5.8S. These authors note that their *M. mirabilis* were probably *M. auretenra* and the samples were collected from Bocas del Toro, Panama, an area where the presence of *M. auretenra* and not *M. mirabilis* (= *M. myriaster*) has been confirmed (H. Guzman pers. comm.).

4.4.7 NCBI accession numbers

Following are the National Center for Biotechnology Information (NCBI) GenBank accession numbers for sequences of coral species and associated zooxanthellae incorrectly associated with *M. mirabilis*. The specimens associated with these sequences have been verified by their authors as or from *M. auretenra.* Nonetheless, in the GenBank database the name *M. mirabilis* remains associated with these accession numbers until amended, if that is possible.

Note: It should not be assumed that the sequences in themselves have been verified, but only that the species sequenced was *M. auretenra* (or from *M. auretenra* in the case of zooxanthellae sequences).

Diekmann et al. 2001, rDNA-ITS, host: AF251847, AF251848, AF251849, AF251850, AF251851, AF251852, AF251853, AF251854, AF251855, AF251856, AF251857, AF251858, AF251859, AF251860.

Fukami et al. 2004, host: COI: AB441226-7, Cytb: AB441311-2, β-tubulin: AB441391, rDNA-ITS: AB441412.

Diekmann et al. 2002, RFLP, zooxanthellae: AF331858, AF331859, AF331860, AF331861, AF331862, AF331863, AF331864, AF331865, AF331866.

Diekmann et al. 2003, rDNA-ITS, zooxanthellae: AF458597, AF458595, AF458596.

Unpublished (BDA) 18S rRNA host: AY950684

4.5 Discussion

The zooxanthellate scleractinian coral ***M. auretenra*** is one of the best studied Caribbean coral species and it is therefore very problematic that the extensive research of its biology and ecology preceded the description and naming of the species. This review provides a compilation documenting studies in which the species was misidentified and misnamed as either ***M. asperula*** or ***M. mirabilis***. The purpose is to ensure the information gained from these studies is incorporated into future research of ***M. auretenra***, and appropriately used in comparative studies (e.g. See Chapter 5).

The publications that are listed in this revision as unvalidated ***M. mirabilis*** references more than likely studied ***M. auretenra*** and not ***M. mirabilis***. Reasons for proposing this include: 1.there are no known shallow-water zooxanthellate ***Madracis*** species that were even remotely similar to the yellow pencil coral; and 2. several “unvalidated” publications share authors and report the same species distribution as validated publications. However, without author verification of what species was studied, doubt remains and these publications are omitted from the revision of ***M. auretenra*** in the absence of other indications (e.g. photographs or reference specimens). Any

reference to these unvalidated publications should be done so with caution because, in fact, no valid species name can be ascribed to their *M. mirabilis*.

4.5.1 *Madracis auretenra* investigations – then and now

The research conducted by Bruno (1995) and Bruno and Edmunds (1997, 1998) on the morphological variation and phenotypic plasticity of *M. auretenra* is extremely important to the understanding of the taxonomy of this species. Within the original description of *M. auretenra* we used the terminology “thin branched species”, whereas this species may display thin and thicker branched forms, as colonies will change morphology when transplanted into a different environment (J. Bruno pers. comm.). Bruno and Edmunds’ (1997, 1998) studies provide strong evidence that these are environmental morphotypes within *M. auretenra* and not a complex of sibling species. The thick diameters of terminal branches reported by Bruno (1995) and Bruno and Edmunds (1997, 1998) correspond to more basal branch diameters in other more thin branched colonies and this may be due to loss of the terminal branches through predation or breakage or the presence of these colonies in higher energy environments which lead them to be more robust, with shorter, wider diameter branches. The ecophenotypic plasticity of *M. auretenra* should be considered when dealing with the taxonomy and identification of this species.

Even with the extensive knowledge we have of this species, several aspects of its distribution and biology remain incompletely known. Confirmed records of the species are lacking for several Caribbean locations including Aruba, the Bahamas, the British Virgin Islands, Costa Rica, Cuba, Dominica, the Dry Tortugas, The Gulf of Mexico, Haiti and Navassa Island, Turks and Caicos, Venezuela, and the majority of the islands comprising the Lesser Antilles.

Where *M. auretenra* is found it is known to inhabit both, clear, high energy forereef areas and backreef and lagoonal areas high in sediment, the latter of which it seems to prefer and do well in quite possibly because of its branched morphology. This morphology and the capability of this coral to adapt its skeletal structure to differing environmental regimes are clear indications of the species' versatility and resilience.

The resilience of *M. auretenra* is also evident in its apparent lowered susceptibility to disease and bleaching. The coral is rarely if ever mentioned in reports of these reef disturbances and stressors (however see unvalidated Table 4.2). Research into the apparent disease resistance of this coral species may provide valuable insight into how and why other species are more affected.

Several pieces of information about reproduction, distribution and recruitment suggest that sexual reproduction may be very limited in *M. auretenra*. Colonies are known to be hermaphroditic and presumed to brood larvae, if only for a very short time. However, although the release of gametes (sperm) has been observed (Vermeij et al. 2003, 2004), little is known about the fertilization of oocytes in this species, or the stages of embryogenesis before planular release from the parent. Studies also have not investigated the possibility of the production of asexual planulae in *M. auretenra*; which has been noted in Pacific *Pocillopora damicornis* (Stoddart 1983), a fellow member of the Family Pocilloporidae. This may have interesting implications for studies of population genetic diversity and connectivity, as would any information on the pelagic larval duration of *M. auretenra* planulae. Estimates of planulae longevity would be extremely helpful to determining temporal dispersal limitations for this species and would aid to inform hypotheses of population connectivity.

4.5.2 Issues of retaining an invalid name

Molecular investigations which result in gene sequence data of scleractinian corals are increasing and the importance of proper taxonomy in these investigations is imperative to limiting confusion especially regarding shared databases such as NCBI's GenBank. The taxonomic correction and new name *M. auretenra* has been slow to infiltrate the area of molecular biology and many studies since the 2007 species description still use the invalid name *M. mirabilis* (i.e. Frade et al. 2008, Chen et al. 2008, Frade 2009). In some cases, researchers are simply citing mistaken references; however, other researchers merely continue to use the invalid name in their more recent work. This appears to be based on ignorance or defiance of taxonomic standards. Even so, it is baffling that various editors do not respond appropriately. The recent publication of the entire mitochondrial genome for *M. mirabilis* (Chen et al. 2008) is most likely of *M. auretenra*. As for other studies mentioned here, *M. auretenra* is known to occur in Bocas del Toro, Panama, where specimens for the study were collected, whereas the deep habitat where *M. myriaster* (= *M. mirabilis*) is found, does not exist at Bocas del Toro. The area has been extensively surveyed by Guzman and Guevara (see Table 4.1) and the presence of *M. auretenra* not *M. mirabilis* has been validated (H. Guzman pers. comm.). The NCBI GenBank remains to be updated to mirror the validated molecular publications.

Another issue raised when *M. auretenra* was described, and it was explained why the name *M. mirabilis* could not be used for this species, was the potential for confusing deep and shallow-water *Madracis* species. In fact, this problem has now been realized by Frade (2009) who uses the names *M. mirabilis* and *M. myriaster* (which are synonymous) to refer to different species inhabiting shallow and deep waters. Since the author is well aware of the new species description

and synonymy of *M. mirabilis* with *M. myriaster*, what he has reported is incomprehensible and will unfortunately add more confusion to an issue that realistically has been resolved.

It may be necessary for a time to simplify references to *M. auretenra* in publications and therefore for this reason I offer the following advice to authors concerning documentation. The name “yellow pencil coral” seems to have only been applied to this coral and as such remains a very useful, familiar, communication tool. Reference to this common name would be acceptable as would the notation *Madracis auretenra* (= *Madracis mirabilis sensu* Wells, 1973). Other forms of notation that are appropriate to use in this case would be, *M. auretenra* (≠ to *M. mirabilis* (Duchassaing and Michelotti 1860)) or *M. auretenra* (≠ *M. myriaster*). The notation *M. auretenra* (= *M. mirabilis*) is not correct and should be avoided as it implies the species *M. auretenra* is equivalent to an azooxanthellate deep-water species synonymous or equal to *M. myriaster*. I am pleased to report that the number of publications using the valid name of *M. auretenra* is increasing exponentially.

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5. Genetic diversity and phylogeography of the scleractinian coral *Madracis auretenra* in the western North Atlantic

5.1 Abstract

Assessing the level of genetic diversity and connectivity for Bermuda island populations of scleractinian corals provides important preliminary information for conservation management of the islands' geographically isolated high latitude reef system. Patterns of genetic structure and evolutionary history for *Madracis auretenra* in Bermuda, Florida and Puerto Rico are elucidated using the nuclear intron *SRP54*. Among 87 individuals 20 distinct nDNA haplotypes were determined from a trimmed alignment of 219 bp. Bermuda possesses nucleotide and haplotypic diversity that exceeds that of Florida and Puerto Rico populations of *M. auretenra*, suggesting the island could be a coral refugium. Bermuda populations are genetically structured from those of neighboring sampled regions (F_{st} = 0.153, p<0.001; F_{ct} = 0.141, p<0.05), but not so isolated that the genetic diversity of its coral populations is reduced. Bermuda's genetic diversity may be attributed to either the local diversification of initial colonists to form unique haplotypes, connectivity with an unsampled population also possessing the haplotypes, or these unique haplotypes are relicts and were once present but have since been extirpated in the other areas sampled. A shared historical connection between the three geographic regions is evident in phylogenetic reconstructions that reveal three monophyletic clades, one of which is distinctly Bermudian. Distinct *SRP54* haplotypes and phylogenetic clades are identified for both Bermuda and Puerto Rico, supporting the recent division of these populations. Geographically shared phylogenetic clades for some Bermuda and Florida haplotypes indicate that geographic isolation may be broken periodically by gene flow to Bermuda from Florida via dispersal of coral planulae or settled, rafted individuals caught in Gulf Stream cyclonic eddies. Transport of larvae from

outside regions is predicted to occur too infrequently to sustain Bermuda marine populations. This study presents additional information regarding the connections between marine populations of the geographically isolated oceanic islands of Bermuda to greater Caribbean Floridian and Puerto Rican conspecifics indicating that conservation efforts of Bermudian coral species should be focused locally.

5.2 Introduction

Conservation of Caribbean coral reef populations requires knowledge of species genetic diversity and the implementation of practices that help maintain this diversity, as far as possible. For whole communities some estimates of diversity are based on numbers of species present in a given area; within species, assessments of diversity are usually based on genetic variation. For most of the Caribbean region, it is accepted that assessments of the numbers of scleractinian coral species are fairly accurate and that we can recognize changes in this diversity. However, for these same species, levels of intraspecies and intrapopulation genetic variation are largely unknown.

Reproductive exchange between populations is hypothesized to underlie the singular evolutionary pathway of a species and also to maintain the genetic diversity of individual populations. Nonetheless, levels and pathways of connectivity and, in fact, the degree to which diversity is maintained via connectivity between marine populations is poorly understood (Cowen et al. 2006, 2007, Steneck et al. 2009). Approximately 66 species of scleractinian coral live in the Caribbean region and of these 12 may be considered important reef builders. So far within the greater Caribbean, only four genetic studies, investigating a total of six coral species, have recorded levels of recent but not historical genetic connections between scleractinian

populations (Baums et al. 2005, Brazeau et al. 2005, Fukami et al. 2004, Vollmer and Palumbi 2007). Thus it appears that studies documenting both genetic diversity and recent and historical connectivity are vital to implementing proper management strategies for populations in most of the larger Caribbean region. Indirect inferences of genetic connectivity can be made from assessments of genetic diversity and structure of coral populations.

5.2.1 Genetic diversity

As an essential element for evolutionary change, genetic diversity is a strong predictor of an organism's ability to adapt to its environment (Frankham et al. 2002). Theoretically, high levels of intraspecific genetic diversity are considered advantageous to population fitness whereas loss of genetic diversity is related to inbreeding which reduces population fitness (Reed and Frankham 2003).

Intraspecific measures of genetic diversity account for the variation found within genes or regions of a species genome and are represented by different base pair sequences or haplotypes (alleles). The frequencies of these different sequences, referred to as the gene or haplotype diversity (previously referred to as heterozygosity in allozyme studies), allow for comparison of genetic diversity among populations. A more informative measure of genetic diversity is nucleotide diversity which takes into account the number of base pair differences between sequences and sequence length (Page and Holmes 1998). The greater the genetic diversity (haplotype and nucleotide diversity nearer to 1) within species of a population the greater the chance the species may adapt to change ('more tools in the toolbox').

Low levels of genetic diversity have been documented for populations of brooding and broadcast spawning scleractinian corals of isolated high latitude Pacific reefs using allozyme

data (Ayre and Hughes 2004, Miller and Ayre 2004, 2008). Compared to larger populations of the same species in neighboring locations, the isolated populations had lower levels of heterozygosity and allelic diversity. With lowered genetic variability, population fitness may be reduced as measured by lowered reproductive ability (Keller and Waller 2002) due to, for example, lower resistance to disease resulting in partial or total colony mortality. Very high levels of variation may be required for reef species to have the inherent capacities to adapt to the current period of rapid global climate change and increasing anthropogenic stressors (van Oppen and Gates 2006). However, studies directly linking levels of genetic variability to how reef species will cope with this impending stress are non-existent and claims are purely hypothetical and generalized from theoretical studies of other organisms.

5.2.2 Connectivity

In marine populations connectivity can be defined as the exchange of individuals among geographically separated sub-populations that comprise a metapopulation (Cowen et al. 2007). Theoretical studies suggest that population connectivity plays a fundamental role in maintaining intraspecific genetic diversity and resiliency of populations (Hastings and Harris 1994, Botsford et al. 2001, Cowen et al. 2007).

Connectivity in corals is dependent on the dispersal of individual larvae, propagules or gametes within and between populations and the survival of those individuals to reproductive maturity. Reduced genetic diversity resulting from gene flow cessation has been found in small, isolated populations of endangered land animals (Hedrick, 1992) and this may be generalized to rare gene flow to distant high latitude reefs. However, reduced gene flow has been suggested but not proven as a reason for reduced genetic variability in these coral populations (Ayer and

Hughes 2004, Miller and Ayre 2004, 2008). Factors limiting connectivity for coral species of isolated reefs include pelagic larval (or gamete) duration, distance between reefs and mechanisms of dispersal - the most common being surface currents.

The aforementioned Caribbean coral connectivity studies collectively recommend that, within the greater Caribbean, gene flow appears to be restricted over distances of 500 km for all of the few species that have been investigated. The most northern coral reefs that are considered as part of the greater Caribbean region are around the islands of Bermuda, located over 1000 km from reefs which would be possible sources of *ex-populo* diversity. Other than introduced diversity, *in-situ* mutations would be another source of genetic diversification for the relatively small Bermuda populations. In fact, there are no data on genetic variability of the resident reef-forming coral species in Bermuda, or on their similarity to Caribbean populations; a few data for single genes have been studied for genetic diversity of some soft corals in Bermuda (Bilewitch 2006, Bilewitch et al. submitted). Bermuda's isolation may prove to be a long term risk to the survival of its reef ecosystems should some of the predicted results of global climate change ensue, such as rise in sea level and temperature, and changes in surface currents. Elucidating levels and pathways of connectivity are crucial to evaluating the sustainability of Bermuda coral species, within various models of climate change, and are fundamental to management efforts.

5.2.3 Bermuda – location and history

Bermuda is an oceanic island cluster, which originated in isolation and has remained isolated from other land masses for its entire history (~110 Ma). Its current climate is strongly influenced by the surrounding Sargasso Sea, so that it has a subtropical climate even though it is located at a high northern latitude (approximately 32°18 N, 64°46 W). This combination of

geological history, location and climactic influences has created a sub-tropical island cluster that is remote and partially isolated from other populations of tropical and subtropical marine neritic and shallow-water benthic species. The areal extent of reef forming, zooxanthellate corals in Bermuda is 550 km^2, located primarily in a narrow rim at the seaward edge of the Bermuda platform, extending from just below the sea surface to 70 m. The islands' pioneer benthic marine populations must have traversed great distances to colonize Bermuda's marine habitats. Presently, the nearest source populations for such dispersal are the southeastern continental US and the Caribbean islands, and both are separated from Bermuda by deep open ocean (~4000 m), at distances of over 1000 km. Despite these great disjunctions of suitable habitat, Bermuda harbours most of the same species as these nearest neighbours (Bermuda has a few marine species that are currently known only as endemics – a killifish, a few species of polychaetes and clitellates and an abundance of cave invertebrates, but no other native marine species that are not also found in the Caribbean or US) and there must have been demographic connections at some point in history (Logan 1988, Sterrer 1986, Sterrer et al. 2004, Hellberg 2007).

The widely held belief that the marine fauna of Bermuda originated by dispersal from the Caribbean in the Gulf Stream seems logical (Logan 1988, Sterrer 1986, Sterrer et al. 2004, Mitton et al. 1989, Park and Ó Foighil 2000), but the original and ongoing connectivity via this major boundary current has been sporadically investigated for only a few, frequently atypical, marine species. The Gulf Stream, which is a culmination of the Florida Current and the Antilles Current, presently circulates to the northwest of Bermuda. As the nearest known surface circulation it may serve as an agent of dispersal to positions near Bermuda, from southern Florida and the Bahamas (Jackson 1986, Park and Ó Foighil 2000). Jackson (1986) determined the distance of about 1500 km represented a minimum of 21 to 30 days of drift time for passive

pelagic transport of larvae or rafted propagules. Whereas, Schultz and Cowen (1994) estimated minimum travel time (Florida to Bermuda) to be ~43-47 days using various transport models. Mesoscale, cyclonic eddies from the Gulf Stream are known to approach the Bermuda Platform and may reduce or even cross the distance from the Gulf Stream proper to the platform (Parker 1971, Hogg et al. 1978, Olsen 1991). These cold core eddies are shed on average 22-35 times per year (Hogg and John 1995) and may entrain either warm water larvae that are present in the surface waters of the Gulf Stream (The Ring Group 1981, Schultz and Cowen 1994) or rafting objects that coral larvae have settled upon (Jackson 1986, Jokiel 1984) and transport these to Bermuda.

5.2.4 Molecular evidence of Bermudian marine species connections

The few, existing, molecular studies of population structure and genetic variation of Bermuda populations of marine species provide varying conclusions on their genetic diversity in comparison with Florida and Caribbean conspecifics. These differences may be linked to species pelagic larval duration as species with longer larval stages and competency periods may survive for as long as the time needed to make the journey to Bermuda (Park and Ó Foighil 2000). Analyses of allozyme, mitochondrial DNA (mtDNA) and nuclear bindin (sperm recognition protein) in the spiny lobster, *Panulirus argus,* (Hateley and Sleeter 1993; Silberman et al. 1994) and the sea urchin, *Lytechinus variegatus* (Zigler and Lessios 2004) indicated no genetic subdivisions between Bermudian populations and those of Florida and the Caribbean proper. These species have longer larval durations, as long as 6-12 months in the spiny lobster (Silberman et al. 1994). Allozyme based studies of the queen conch, *Strombus gigas,* were interpreted as showing Bermudian populations were isolated from a relatively homogeneous

Caribbean gene pool; Florida was not included in the study (Mitton et al., 1989). The mtDNA work of Ó Foighil and Jozefowicz (1999) and Park and Ó Foighil (2000) indicated that, although populations of the direct-developing bivalve *Lasaea* in Bermuda and Florida were genetically similar, these populations were distinct and that overall genetic diversity was higher in the Bermuda populations. Similarly, recent study of the gorgonian ***Briareum asbestinum*** using the rDNA internal transcribed spacer regions determined genetic diversity was higher within a few Bermuda individuals than those of Florida and the Bahamas (Bilewitch 2006, Bilewitch et al. submitted). To date, there are no similar published studies of Bermudian scleractinian populations, and the status of their relationships with probable source populations on reef systems in Florida and the Caribbean are limited to the presence in both of many of the same species - indicating at least a deep historical connection.

5.2.5 Scleractinian species in Bermuda

Shallow-water zooxanthellate coral species diversity in Bermuda is significantly lower than that of the Caribbean, with 13 genera and 20 species recorded in comparison to 27 genera and some 66 species in the Caribbean. Even though Bermuda presents a relatively small (in area) reef system it is difficult to verify all species reports, including some very recent ones (Frade 2009, Venn et al. 2009) most particularly because representative specimens of the putative species have not been kept. Nonetheless, what Bermuda lacks in numbers of coral species is made up for in overall species health and, apparently, disease resistance (although there is a high prevalence of black band and yellow blotch disease) (Weil et al. 2002, Weil and Cróquer 2005, 2009). Improved species health could be the result of broad seasonal temperature changes in Bermuda

waters which may affect the level of epizootic occurrence and reduce bleaching events (Weil and Cróquer 2009).

One such resilient coral, common to greater Caribbean waters, is the zooxanthellate, shallow-water coral *Madracis auretenra* Locke, Weil and Coates 2007. This branching coral is a dominant species within Bermuda's inshore waters where it is known to form extensive patch reefs that provide essential fish habitat. *Madracis auretenra* displays both asexual reproduction by fragmentation and sexual reproduction as a hermaphroditic brooder in Bermuda and the Caribbean (de Putron 2003, Vermeij et al. 2004). Mature gametes are present during periods of maximum annual seawater temperatures, about August and September in Bermuda (de Putron 2003) and August to November in Curaçao (Vermeij et al. 2004).The species is known for its "quick release" of planulae after fertilization, however because neither embryos nor planulae have been observed in histological sections of reproductive *M. auretenra* (Delvoye 1988, de Putron 2003, Vermeij et al. 2003, 2004) the duration of the brooding stage is unknown but assumed to be extremely short. Vermeij et al. (2003) report that planulae of *Madracis* species may begin to explore the bottom 16-24 hours after release from the parent colony.

Although it is generally accepted that brooding coral species exhibit limited dispersal capabilities (Underwood et al. 2007, van Oppen et al. 2008) there are demonstrated exceptions to this generalization (Ayre and Hughes 2004, Underwood et al. 2007). As such, the dispersal capabilities, duration and environmental resilience, of many brooding species remain unknown. The majority of shallow-water zooxanthellate corals in Bermuda are brooding species (55 %), which is common for isolated, oceanic islands (Ayre and Hughes 2004). Therefore in order to gain perspective on the overall reproductive connectivity of Bermuda's coral populations this study investigates a species with this reproductive strategy.

In the present study, the hypervariable nuclear intron, signal recognition particle 54 (*SRP54*) is evaluated within a number of populations of *M. auretenra* to assess genetic diversity and structure and provide an indirect inference of larval dispersal among the greater Caribbean regions of Bermuda, Florida and Puerto Rico. The gene history of these regions is also investigated based on molecular phylogenetic reconstruction of *SRP54* nDNA haplotypes.

The main objective of the study is to gain insight into phylogenetic patterns and population structure within and between the three study regions, with particular interest in the identification of upstream sources of genetic diversity for the Bermudian populations. This information will be useful in assessing the dependency of Bermuda's corals on outside source populations and aid in the design and implementation of appropriate strategies for management of Bermuda's coral reef ecosystems.

5.3 Materials and Methods

5.3.1 Sample collection

Colonies of *Madracis auretenra* were sampled from Bermuda, the Florida reef tract and southwestern Puerto Rico (Fig. 5.1). Bermuda: On the Bermuda platform at least ten samples were collected from each of four areas: Castle Harbour (11); Shelly Bay Shoals / Tynes Bay Channel (10); Hogfish Crescent (12); and the southwestern platform area (11). The greatest distance between Bermuda collecting sites was 15 km (Castle Harbour to the west end sites) with the least distance being 1-3 km (Hogfish Crescent to Shelly Bay Shoals/Tynes Bay Channel) (Fig. 5.2a). Florida Keys: In Florida 32 samples were collected from the Upper (6), Middle (13) and Lower (13) Keys. Within the Florida Keys the Upper Keys sites were approximately 50 km from those in the Middle Keys and 150 km from the Lower Keys sites (Fig. 5.2b). The distance

between Middle and Lower Keys sites was 90 km. Puerto Rico: In La Parguera, southwestern Puerto Rico, 11 samples were collected from Laurel and Media Luna. These two reefs are less than 2 km apart and in some analyses data were pooled (Table 5.1). The approximate distances separating the three collecting regions were 1710 km between Bermuda and Florida's Upper Keys and 2700 km between Bermuda and SW Puerto Rico. The distance between Florida and SW Puerto Rico is approximately 1650 km. Distance measurements follow ocean pathways (prevailing surface currents).

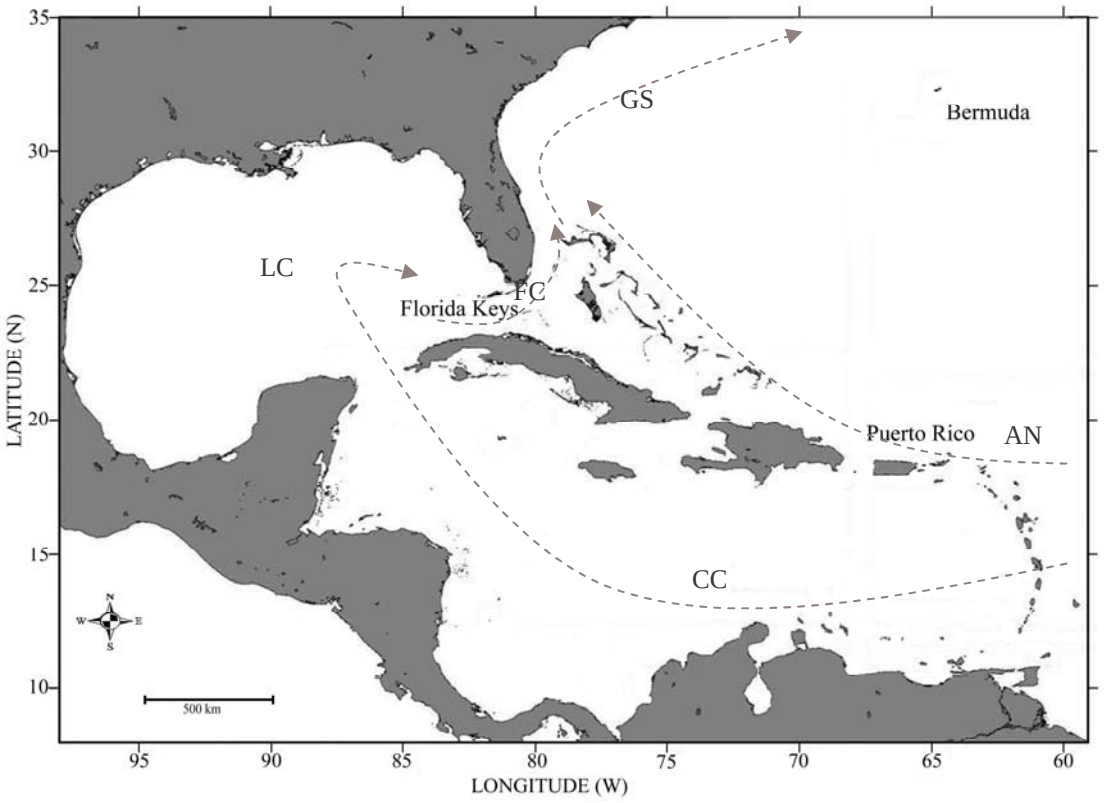

Figure 5.1. The greater Caribbean area indicating the three regions where *Madracis auretenra* was sampled: Bermuda; The Florida Keys; and SW Puerto Rico. Major surface currents are represented by AN: Antilles Current; CC: Caribbean Current; LC: Loop Current; FC: Florida Current; and GS: Gulf Stream.

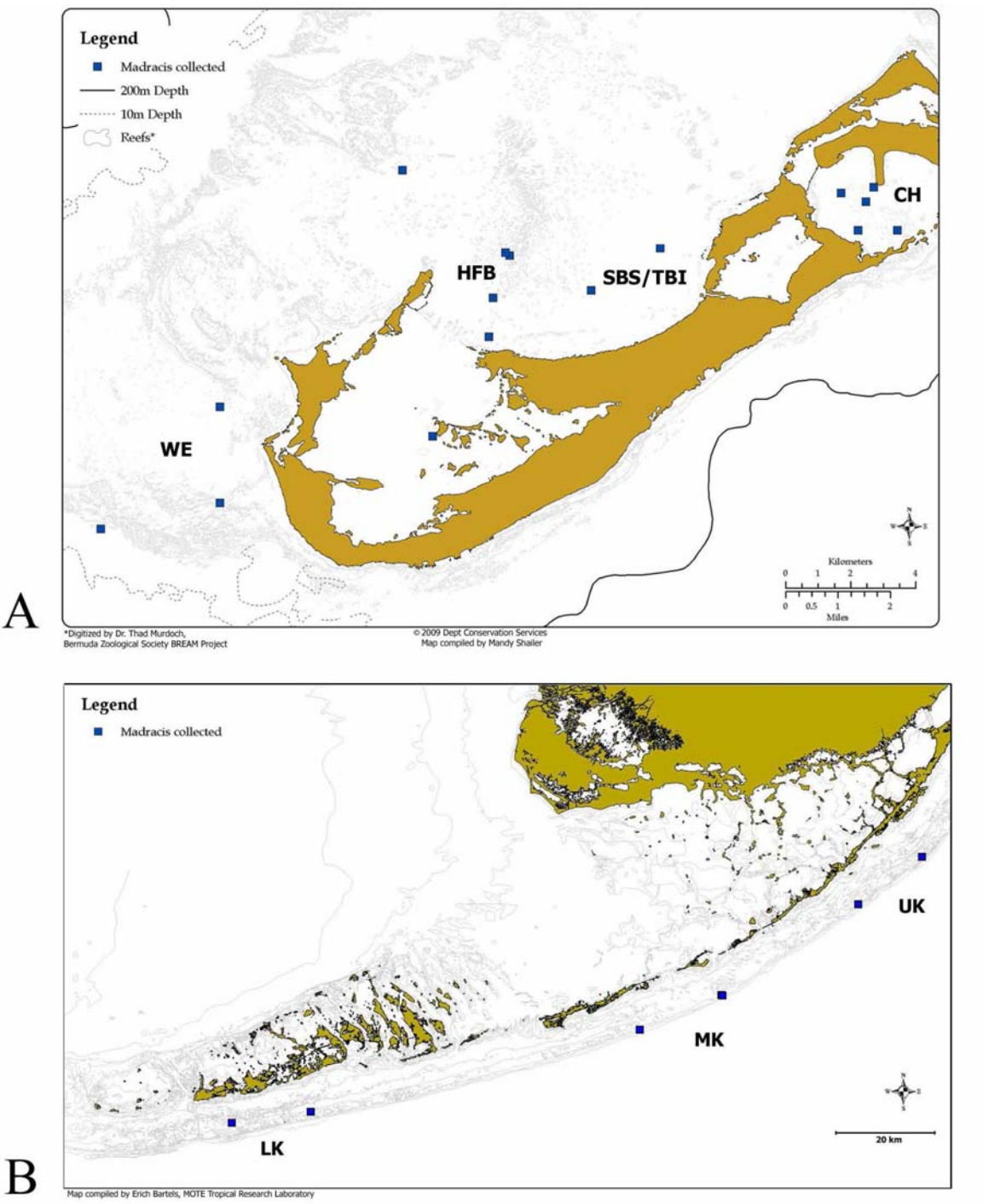

Figure 5.2. Geographic Information System representation of *M. auretenra* regional collection sites. A. Bermuda (CH: Castle Harbour, SBS/TBI: Shelly Bay Shoals / Tynes Bay Channel, HFB: Hogfish Crescent, WE: West End. B. The Florida Keys (LK: Lower Keys, MK: Middle Keys, UP: Upper Keys. C. La Parguera, SW Puerto Rico (L: Laurel, ML: Media Luna). Collection sites are represented by squares.

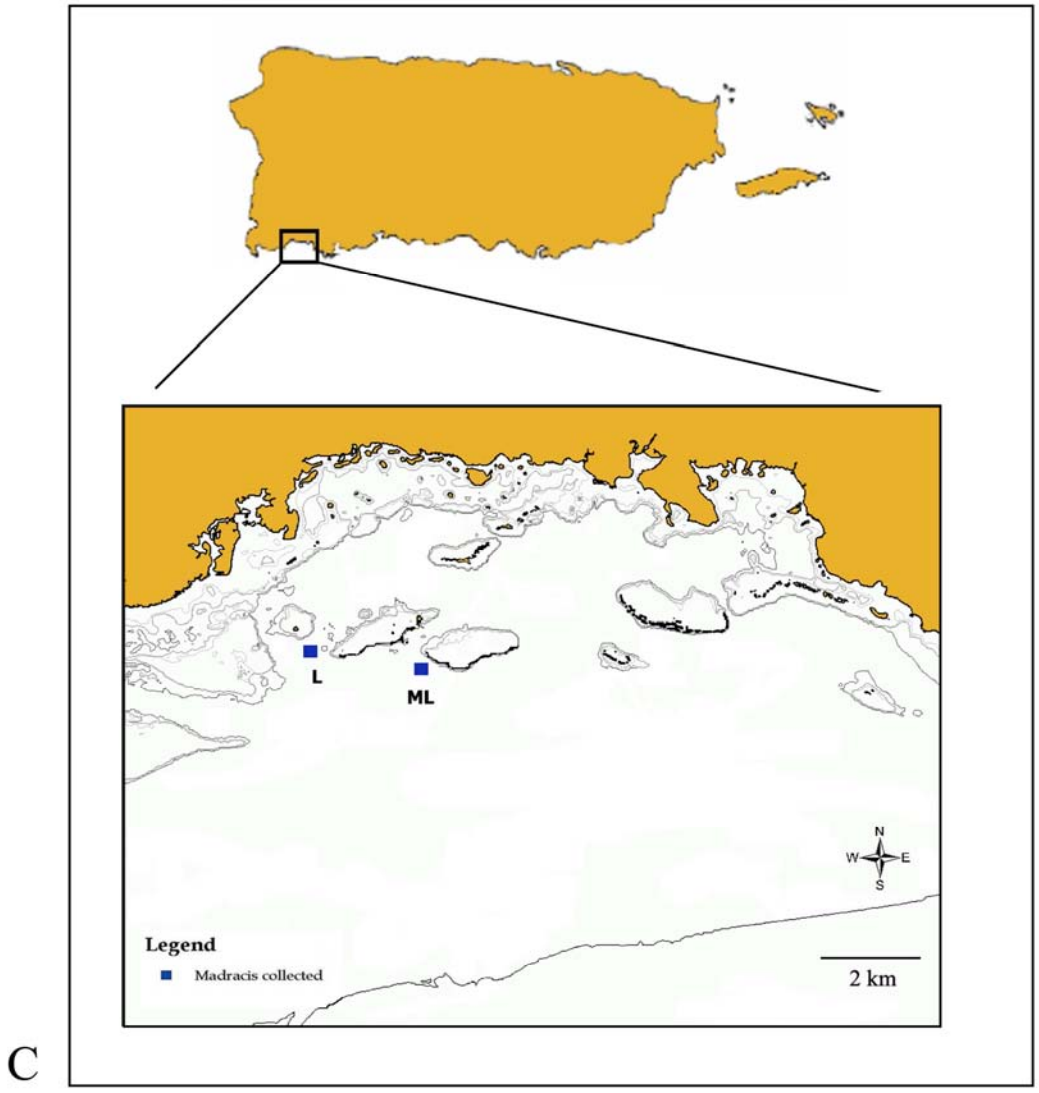

Figure 5.2. Continued

Coral colonies were sampled at least 3 m apart to reduce collection of asexual clone mates. Each sampled colony was photographed for reference purposes. Branch tips, not exceeding 2 cm in length, were excised from colonies. Following collection, samples were either frozen in liquid nitrogen or preserved in 95% ethanol prior to DNA extraction.

Table 5.1. Collection information for regional *M. auretenra* samples listing site, coordinates, depth and haplotype designation for each colony. Homozygotes are represented by a single number.

Regional Samples	Site	Coordinates		Depth (m)	Haplotype
Bermuda					
M2	Castle Harbour	32 21.086	64 41.892	4.6	15 and 1
M5		32 21.086	64 41.892	5.2	15 and 10
M6		32 20.469	64 40.787	4.6	9
M7		32 20.469	64 40.787	5.8	13 and 20
M8		32 20.469	64 40.787	5.8	11 and 15
M10		32 20.469	64 40.787	4.6	6 and 5
CH32		32 20.940	64 41.410	7.6	15 and 1
CH33		32 20.470	64 41.558	6.1	1
CH34		32 20.940	64 41.410	6.7	1
CH35		32 21.185	64 41.255	5.2	11 and 1
CH36		32 21.185	64 41.255	8.2	1 and 20
SBS 1	Shelly Bay Shoals	32 20.163	64 45.439	3.4	15 and 7
SBS 2		32 20.163	64 45.439	2.7	16 and 1
SBS 3		32 20.163	64 45.439	3.7	20
SBS 4		32 20.163	64 45.439	1.8	1 and 20
SBS 5		32 20.163	64 45.439	4.0	15 and 20
TBI 6	Tynes Bay Channel	32 19.462	64 46.788	5.8	20
TBI 7		32 19.462	64 46.788	6.4	1
TBI 8		32 19.462	64 46.788	7.3	12 and 1
TBI 9		32 19.462	64 46.788	7.0	9
TBI10		32 19.462	64 46.788	5.8	11
DB1	Diving Board Island	32 17.035	64 49.898	3.0	5 and 9
HB1	Hogfish Crescent	32 18.691	64 48.791	4.6	1 and 20
HB5		32 18.691	64 48.791	4.0	20
HFB11		32 19.338	64 48.717	3.0	15 and 9
HFB15		32 19.338	64 48.717	3.0	8 and 9
HFB17		32 19.338	64 48.717	2.1	9
HFB18		32 19.338	64 48.717	2.1	20
HFB19		32 19.338	64 48.717	4.3	11 and 15
HFB20		32 19.338	64 48.717	3.4	2 and 20
HFB30		32 20.085	64 48.473	5.5	1 and 20
HFB31		32 20.038	64 48.378	7.6	19 and 20
KAC1		32 21.462	64 50.495	7.6	11 and 1
WE21	West End Offshore	32 15.919	64 54.060	4.9	15 and 20
WE22		32 15.919	64 54.060	5.5	15 and 9
WE23		32 15.919	64 54.060	5.8	20
WE24		32 15.919	64 54.060	4.9	14
WE25		32 15.919	64 54.060	3.0	1
SOM26	West End Inshore	32 17.521	64 54.060	3.4	7
SOM27		32 17.521	64 54.060	5.2	11
SOM28		32 17.521	64 54.060	3.0	11 and 7
SOM29		32 17.521	64 54.060	4.6	11 and 15
WE37	West End Offshore	32 15.482	64 56.394	3.0	3 and 20
WE38		32 15.482	64 56.394	3.0	1 and 20

Table 5.1. Continued

Regional Samples	Site	Coordinates		Depth	Haplotype
Florida	Lower Keys				
FL11	Maryland Shoals	24 31.308	81 31.709	7.3	11 and 15
FL12		24 31.308	81 31.709	8.0	11 and 15
FL13		24 31.308	81 31.709	6.4	11 and 15
FL14		24 31.308	81 31.709	7.3	11 and 15
FL15		24 31.308	81 31.709	7.6	11 and 15
FL16		24 31.308	81 31.709	8.0	11 and 15
FL17		24 31.308	81 31.709	7.6	11 and 15
FL18		24 31.308	81 31.709	8.5	11 and 15
FL19		24 31.308	81 31.709	7.6	11 and 15
FL20		24 31.308	81 31.709	6.7	11 and 15
FL21		24 31.308	81 31.709	6.1	20
FL27	Key West	24 29.974	81 44.147	8.5	1 and 9
FL28		24 29.974	81 44.147	8.5	11 and 15
	Middle Keys				
FL2	Long Key Offshore	24 45.154	80 45.473	13.7	14
FL3		24 45.154	80 45.473	13.4	14
FL4		24 45.154	80 45.473	13.4	11 and 15
FL5		24 45.154	80 45.473	12.8	14
FL6		24 45.154	80 45.473	12.8	14
FL7		24 45.154	80 45.473	12.8	14
FL8		24 45.154	80 45.473	13.4	14
FL10		24 45.154	80 45.473	13.7	15
FL22	Long Key	24 45.258	80 45.562	8.8	11 and 15
FL23		24 45.258	80 45.562	8.8	9
FL24	Duck Key	24 41.116	80 55.315	16.0	11 and 15
FL25		24 41.116	80 55.315	15.8	1 and 9
FL26		24 41.116	80 55.315	16.5	1 and 11
	Upper Keys				
FL29	Key Largo	24 56.119	80 29.202	7.6	15 and 9
FL30		24 56.119	80 29.202	7.6	15 and 9
FL31		24 56.119	80 29.202	7.6	15 and 9
FL32		25 01.792	80.21.610	11.0	9
FL33		25 01.792	80.21.610	11.0	9
FL34		25 01.792	80.21.610	11.0	9
SW Puerto Rico					
P1	Laurel	17 56.496	67 04.034	7.6	17 and 20
P2		17 56.496	67 04.034	7.0	9 and 4
P3	Media Luna	17 56.086	67 03.010	12.1	9 and 4
P4		17 56.086	67 03.010	10.0	18 and 20
P5		17 56.086	67 03.010	11.2	9 and 4
P6		17 56.086	67 03.010	11.0	9 and 4
P7		17 56.086	67 03.010	11.0	9 and 4
P8		17 56.086	67 03.010	10.0	9 and 4
P9		17 56.086	67 03.010	12.1	9 and 4
P10		17 56.086	67 03.010	11.5	9 and 4
P11		17 56.086	67 03.010	12.5	9 and 4

5.3.2 DNA isolation and sequencing

For DNA extraction a hexadecyl trimethyl ammonium bromide (CTAB) buffer, proteinase K (20mg/ml) and chloroform extraction was modified from Winnepenninckx et al. (1993). Using a sterile mortar and pestle, a small piece of coral (~0.3 cm^2) was broken apart from the collected preserved sample. The coral fragment was placed in 600 µl of CTAB (1.4M NaCl, 20mM EDTA, 100mM Tris-HCl pH 8.0, 2% CTAB, 0.2% 2-mercaptoethanol) on ice. Proteinase K was added to a final concentration of 250ug/ml. The digestion was vortexed and incubated at 65ºC for 1.5-2 hours, with vortexing every 30 minutes, until the homogenate was clear. An equal volume of chloroform was added and the sample was vortexed, inverted by hand for 5 minutes, vortexed again and spun at 15,000 rpm for 20 minutes. The aqueous layer of the sample was transferred, precipitated in 1 ml of ice-cold 95% ethanol at -70ºC for 45 minutes, and then pelleted by centrifugation (13,200 rpm) at 4ºC for 30 minutes. The supernatant was discarded and DNA pellets were dried in a vacuum centrifuge at 45ºC. Dried pellets were dissolved in 20 µl of TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA) at 37ºC for 30 minutes.

Genomic DNA was amplified using *SRP54* degenerative primers SRP54f and SRP54r (Jarman et al. 2002). The PCR reaction volume was 25 µl and consisted of 1 µl template DNA, 5.0 µl of 5X Colorless GoTaq® Flexi Buffer, 3.0 µl of 25mM $MgCl_2$, 0.5 µl dNTP's (0.2 mM each in reaction), 0.65 µl of each primer (10mM), 1.25 U GoTaq® Hot Start Polymerase and deionized sterile water to volume. PCR amplification was conducted on a Biometra T-Gradient thermocycler as follows: 95ºC for 2 min (activation of polymerase); 95ºC for 30s; 60ºC for 30s, and 72ºC for 60s (35 cycles); followed by a final extension of 72ºC for 5 min. PCR products were verified by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

PCR products for individual samples were purified and sequenced at a commercial facility (High-Throughput Genomics Unit, University of Washington, Seattle, Washington, USA). Initial sequences of the *SRP54* locus of *M. auretenra* samples revealed the presence of heterozygosity. Heterozygote nuclear alleles were observed as double peaks in direct sequences. To determine alleles present for known heterozygotes, PCR products were purified using the Wizard® SV Gel and PCR Clean-UP System (Promega), ligated into pGEM®-T Easy vectors (Promega) and screened for successful *SRP54* inserts as per the manufacturer's instructions. For each coral sample 10-20 positive (white) colonies were sampled using sterile toothpicks and suspended in 20 µl of sterile distilled water. Individual cell suspensions were boiled at 99ºC for 5 minutes. One microliter of boiled cell suspension was added to the PCR mixture (total volume 25 µl) and amplified using the primary PCR profile (both listed above). PCR amplification was performed in a separate room from cloning work. Clones were sequenced until both copies of each sequence were found and these were compared with initial direct sequences to verify the identity of putative alleles and to ensure that multiple copies did not exist.

Following the sequencing of cloned PCR products, four samples appeared to display three alleles. To increase confidence in allelic identity by reducing heteroduplex products, a reconditioning PCR of 3 cycles was employed on these samples (Thompson et al. 2002, Acinas et al. 2005, Concepcion et al. 2008, Lenz & Becker, 2008). PCR products were reconditioned by 10-fold dilution in sterile distilled water and re-amplification of 1 µl of this dilution in fresh PCR mixture (same reagent concentrations as original PCR reaction), with the PCR profile: 95ºC for 2 min (activation of polymerase); 95ºC for 30s; 60ºC for 30s, and 72ºC for 60s (3 cycles); followed by a final extension of 72ºC for 5 min. Resulting PCR products were purified, cloned and sequenced as above.

5.3.3 Sequence analysis

Forward and reverse sequence chromatograms were viewed, assembled and edited in GeneiousPro 4.6 (Drummond et al. 2009). Sequences were aligned using ClustalW 1.82 (Thompson et al. 1994) within GeneiousPro. *Pocillopora damicornis* (GenBank accession number EU006859) was included in the alignment as the outgroup taxon. The alignment was adjusted manually with MacClade 4.05 (Maddison and Maddison 2002) and identical sequences were collapsed. The *SRP54* sequences were checked through BLAST searches (www.ncbi.nlm.nih.gov) to ensure sequences did not originate from zooxanthellae or other contaminating organisms.

Uncorrected pair-wise distances of haplotype sequences were calculated using DAMBE 5.0.8 (Xia and Xie 2001). Haplotype and nucleotide diversity were calculated with ARLEQUIN 3.1 (Excoffier et al. 2005). When calculated for DNA sequences, heterozygosity is often referred to as gene or haplotype diversity and although informative, measures of genetic variation that take into account the actual number of base changes between sequences, rather than just similarities or differences between sequences, are more informative (Page and Holmes 1998). To test for deviation from neutrality Tajima's D (Tajima 1989) and Fu's F statistic (F_s) (Fu 1997) were implemented in ARLEQUIN 3.1. Significantly low statistics for both neutrality tests can indicate non-neutral evolution (Tajima, 1989).

To assess if the molecular data contained phylogenetic information, two approaches were taken. First, a permutation-tail probability (PTP) test (Archie 1989) was conducted to ensure non-random phylogenetic signal was present in the alignment using 1000 permutation replicates and 10 random additions in PAUP*4.0b10 (Swofford 2002). Secondly, the level of substitution saturation was assessed using DAMBE. Substitution saturation for the *SRP54* haplotype

alignment, both including and excluding the outgroup, was based on transitions and transversions plotted against genetic distance.

To infer the relationship between *SRP54* nDNA haplotypes of *M. auretenra* a parsimony network was constructed with TCS 1.21 (Clement et al. 2000) using default settings, with insertion-deletions (indels or gaps) set as a 5th nucleotide state. Branch connections between sequences were tested for both 90% and 95% connection limits. Any loops in the result were unambiguously resolved through comparison of sequences (*sensu* Templeton et al. 1992). By not incorporating historical information parsimony networks show only the current haplotype situation and not how it came to be.

5.3.4 Molecular phylogenetic analysis

Historical patterns of gene flow were inferred from phylogenetic analyses on the basis of maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). Gaps were treated as a 5th nucleotide state in MP analyses. Using PAUP*4.0b10, a heuristic search with 100 random addition repetitions was used for both MP and ML with 1000 bootstrap replicates. For ML and BI the model of sequence evolution was determined by hierarchical likelihood ratio tests using the Akaike Information Criterion (AIC) in ModelTest 3.7 (Posada and Crandall 1998) and jModeltest 0.1.1 (Posada 2008, Guindon and Gascuel 2003), respectively. Both analyses were conducted using the Hasegawa-Kishino-Yano plus Gamma model (HKY+G) (Hasegawa et al. 1985). Bayesian analysis was conducted with MrBayes 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck, 2003). Four parallel chains of 500 000 generations were run and trees were sampled every 100 generations, with 1250 “burn in trees” excluded from the

consensus tree. The average standard deviation of split frequencies after 500 000 generations was 0.0076352. Posterior probabilities were based on a consensus of 7458 trees.

5.3.5 Population genetic analysis

To assess regional genetic variation and population structure, haplotypes were grouped into three regions: Bermuda, Florida and Puerto Rico. To test for structure within each region, samples were grouped and analyzed by sampling site. Bermuda sites were: Castle Harbour, Shelly Bay/Tynes Bay Channel, Hogfish Crescent, and the west end. Florida reef tract sites were: Upper, Middle, and Lower Keys. The Puerto Rico sample size was small; therefore Media Luna and Laurel were analyzed both independently and as pooled data for southwestern Puerto Rico. Population genetic structure was estimated using analysis of molecular variance (AMOVA, Excoffier et al. 1992) in ARLEQUIN 3.1. To evaluate population subdivision, an AMOVA was first run for pooled regional data without incorporating site information, secondly a hierarchical AMOVA was used to estimate genetic differentiation among regions (F_{ct}), among sites within regions (F_{sc}) and among all sampled sites (F_{st}). AMOVA analysis was also conducted for Bermuda and Florida excluding Puerto Rico sites. Pair-wise comparisons of Fixation Index (F-statistic) measures (ARLEQUIN 3.1) were utilized to discern structure between regional sites. Wright's measure of population subdivision (F_{st}) is a measure of shared genetic variation between groups. Fst is the most inclusive measure of population substructure and is useful for examining overall genetic divergences among populations. An Fst of 0 implies complete mixing (no subdivision or structure), while an Fst of 1 implies no shared genetic variation or complete isolation (extreme subdivision or structure). Fst is difficult to interpret because it is influenced by

past and present gene flow, however, it can still provide a useful estimate of population similarity (Neigel 2002).

5.4 Results

5.4.1 *Madracis auretenra SRP54* nDNA

Amplifications of *SRP54* nuclear DNA in 87 *M. auretenra* colonies yielded 146 sequences between 204 bp and 219 bp in length, all of which could be unambiguously aligned. In total, 65 nucleotide sites were variable with 33 sites being parsimoniously informative. The Akaike Information Criterion (AIC) in jModeltest chose HKY+G as the best fit model of sequence evolution, with base frequencies A = 0.3334, C = 0.1301, G = 0.1862, T = 0.3502 and a transition/transversion (Ti/Tv) ratio of 1.4410. Among individuals 20 distinct nDNA haplotypes were determined from a trimmed alignment of 219 bp (Appendix 5.1). Haplotype presence differed among regions with 17 different haplotypes occurring in Bermuda, 6 in Florida and 5 in SW Puerto Rico. Unique haplotypes were found in Bermuda (11) and Puerto Rico (3) but not in Florida. Two haplotypes (9 and 20) were found in all three regions. Among the twenty haplotype sequences, haplotype 20, displayed a prominent 10 bp insertion, and haplotype 19 displayed a unique 7 bp deletion (Appendix 5.1). The 87 coral samples yielded a total of 28 homozygotes (32%) and 59 heterozygotes (68%). These overall percentages were similar to those found specifically within each of Bermuda (34% and 65%) and Florida (37% and 62%). The level of heterozygosity in SW Puerto Rico was 100%; however this could simply reflect a small sample size (n = 11). All homozygous haplotypes other than haplotype 14 also occurred in a heterozygous state. Results from both cloning of heterozygotes and reconditioned PCR revealed that no more than 2 haplotypes were found within any one individual. This affirmed that the

SRP54 locus exists as a single copy in *M. auretenra*, as indicated for other hexa- and octocorallians by Concepcion et al. (2008).

5.4.2 Data properties

Overall nucleotide diversity for the *SRP54* nuclear intron in *M. auretenra* samples was 0.060 +/- 0.031. Nucleotide diversity was highest in Bermuda populations at 0.072 +/- 0.036 compared to Florida 0.033 +/- 0.017 and Puerto Rico populations 0.039 +/- 0.021. Haplotype diversity was consistent with this trend (Table 5.2). Tests of neutrality using Tajima's D and Fu's F_s did not indicate significant deviation from neutrality for *SRP54* in the sampled populations of *M. auretenra* (Table 5.2) so that the neutral hypothesis may explain the DNA polymorphism present. Pair-wise sequence divergence values between haplotypes are shown in Table 5.3. The maximum uncorrected distance (*p*) between *M. auretenra* haplotypes was 0.098 (9.8%). A pair-wise difference of zero was detected for 2 haplotype pairs, in both cases the sequences differed by an indel at one site.

Table 5.2. Site information for *M. auretenra SRP54* nDNA locus. Haplotype number, sample number (n), haplotype diversity (*h*), nucleotide diversity (π), Tajima's D and Fu's F_s. Neutrality tests were not significant.

Region	Population	# of haplotypes	n	*h* (±SD)	π (±SD)	D	F_s
Bermuda	Castle Harbour	9	11	0.8655 (0.0561)	0.0579 (0.0305)	-0.063	3.11
	Shelly/Tynes Bay	8	10	0.8762 (0.0595)	0.0798(0.0421)	0.805	4.023
	HogFish Cresent	9	12	0.8762 (0.0457)	0.0780 (0.0404)	0.419	5.473
	West End	8	11	0.8971 (0.0421)	0.0786 (0.0411)	1.007	5.079
	Overall	**17**	**44**	**0.8697 (0.0190)**	**0.0720 (0.0360)**		
Florida	Lower Keys	5	13	0.6333 (0.0582)	0.0244 (0.0136)	-1.355	5.164
	Middle Keys	6	13	0.8301 (0.0544)	0.0444 (0.0239)	-0.407	5.429
	Upper Keys	2	6	0.5000 (0.1283)	0.0193 (0.0121)	1.459	6.203
	Overall	**6**	**32**	**0.7745 (0.0301)**	**0.0328 (0.0174)**		
SW Puerto Rico	Cayo Laurel	4	2	1.0000 (0.1768)	0.0879 (0.0593)	0.085	1.068
	Media Luna	4	9	0.6340 (0.0685)	0.0301 (0.0167)	-0.898	6.747
	Overall	**5**	**11**	**0.6840 (0.0632)**	**0.0394 (0.0211)**		

Table 5.3. Pair-wise uncorrected distances between *SRP54* haplotypes (Hap) found in 87 individuals of *M. auretenra*. Maximum distance between any two sequences, among all pair-wise comparisons, is indicated in boldface.

	Hap 1	Hap 2	Hap 3	Hap 4	Hap 5	Hap 6	Hap 7	Hap 8	Hap 9	Hap 10	Hap 11	Hap 12	Hap 13	Hap 14	Hap 15	Hap 16	Hap 17	Hap 18	Hap 19	Hap 20
Hap 1	-																			
Hap 2	0.005	-																		
Hap 3	0.005	0.010	-																	
Hap 4	0.005	0.010	0.010	-																
Hap 5	0.058	0.063	0.063	0.063	-															
Hap 6	0.058	0.063	0.063	0.063	0.015	-														
Hap 7	0.058	0.063	0.063	0.058	0.049	0.049	-													
Hap 8	0.034	0.039	0.039	0.039	0.049	0.044	0.044	-												
Hap 9	0.019	0.024	0.024	0.024	0.039	0.039	0.049	0.034	-											
Hap 10	0.020	0.024	0.024	0.024	0.039	0.039	0.049	0.034	0.000	-										
Hap 11	0.044	0.049	0.049	0.049	0.054	0.054	0.064	0.049	0.025	0.025	-									
Hap 12	0.045	0.050	0.050	0.050	0.054	0.054	0.064	0.050	0.025	0.025	0.000	-								
Hap 13	0.049	0.054	0.054	0.054	0.059	0.059	0.069	0.054	0.030	0.030	0.005	0.005	-							
Hap 14	0.039	0.044	0.044	0.044	0.049	0.049	0.059	0.044	0.020	0.020	0.005	0.005	0.010	-						
Hap 15	0.039	0.044	0.044	0.044	0.049	0.049	0.059	0.044	0.020	0.020	0.015	0.015	0.020	0.010	-					
Hap 16	0.044	0.049	0.049	0.049	0.054	0.054	0.064	0.049	0.025	0.025	0.020	0.020	0.025	0.015	0.005	-				
Hap 17	0.044	0.049	0.049	0.049	0.054	0.054	0.064	0.049	0.025	0.025	0.020	0.020	0.025	0.015	0.015	0.020	-			
Hap 18	0.049	0.054	0.054	0.054	0.059	0.059	0.069	0.054	0.030	0.030	0.025	0.025	0.029	0.020	0.020	0.025	0.005	-		
Hap 19	0.050	0.055	0.055	0.055	0.055	0.055	0.070	0.050	0.030	0.030	0.036	0.036	0.041	0.030	0.030	0.036	0.036	0.041	-	
Hap 20	0.078	0.083	0.083	0.083	0.093	0.093	**0.098**	0.078	0.068	0.069	0.084	0.085	0.089	0.079	0.079	0.084	0.074	0.079	0.086	-

A PTP test indicated that the length of the parsimony trees was significantly skewed (p=0.001) compared to 1000 permuted simulations, indicating nonrandom phylogenetic signal in the aligned dataset. The number of transitions and transversions between sequence pairs were plotted against genetic distance to test substitution saturation (Fig. 5.3) (Xia et al. 2003). The index of substitution saturation for the *SRP54* sequences was lower than the critical index value indicating the phylogenetic signal between haplotypes is defensible, however some phylogenetic signal may be obscured between the ingroup and outgroup (Fig. 5.3b) where there is much more intervening site by site substitutions. The data quality tests suggest that there is evidence that the sequences are suitable for inferring relationships among the taxa.

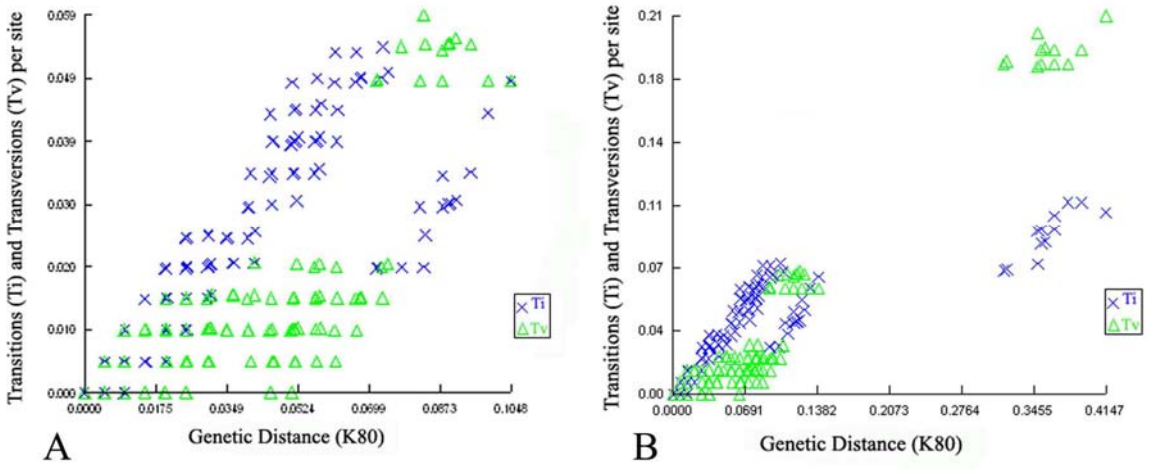

Figure 5.3. Test for substitution saturation for *SRP54* sequences, based on transitions (Ti, blue x's) and transversions (Tv, green triangles) plotted against Kimura 2-parameter distance (K80). A. Ingroup only B. Ingroup and outgroup.

5.4.3 Haplotype networks

Statistical parsimony analysis (TCS), using a 95% connection limit for the *SRP54* sequence data set resulted in three haplotype networks and four haplotypes without connections (Fig. 5.4a). The inferred ancestral haplotypes for the two main networks were haplotypes 1 and 11. Haplotypes 1 and 11 were represented within Florida and Bermuda samples with the remaining haplotype network (5) consisting of two haplotypes (5 and 6) only found in Bermuda. The four solitary haplotypes 7, 8, 19 and 20 showed the greatest mutational differences and were unique to Bermuda with the exception of haplotype 20, which was found within all three regions and also had the highest frequency of all haplotypes. A confidence limit of 90% reduced the number of haplotype networks to one, with haplotype 11 inferred as the most ancestral (Fig. 5.4b). Haplotypes 7, 19 and 20 remained unconnected to the network, the number of mutational differences necessary for connection to the network being 12 bp (from haplotype 9), 15 bp (from haplotype 14) and 26 bp (from haplotype 9) for each unconnected haplotype respectively. All peripheral unique haplotypes were Bermudian with the exception of haplotypes 4, 17 and 18, which were distinct to Puerto Rico. Geographical partitioning was not evident in the connected network nor in individual higher frequency haplotypes. Thus the networks indicate “ongoing” gene flow between the three regions, when diploid individuals are considered.

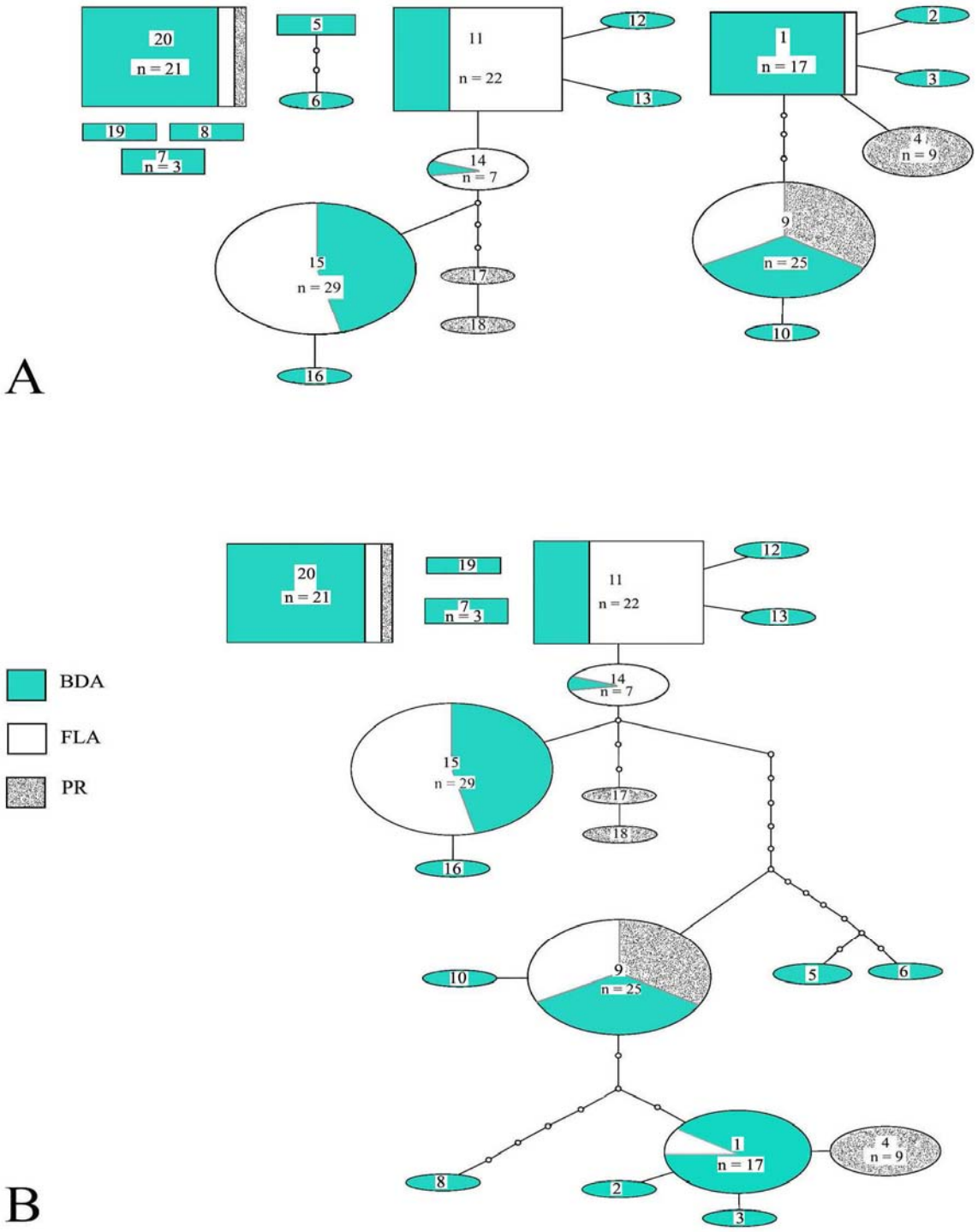

Figure 5.4. TCS networks of Bermuda, Florida and Puerto Rico *M. auretenra SRP54* haplotypes using A) 95% connectivity level and B) 90% connectivity level. Sampled haplotypes are indicated by colored ovals or rectangles, each with haplotype designation numeral; missing or unsampled haplotypes are depicted by circles. Rectangles indicate the haplotype with the highest ancestral probability. Each branch indicates a single mutational difference. Oval size is proportional to observed haplotype frequency; the number of times the haplotype was obtained is given by "n=" when greater than one. Haplotypes are coded according to geographical region, see legend.

5.4.3 Molecular phylogenetic results

All the methods used to infer phylogenetic relationships within this study yielded similar topologies for the *SRP54* intron data. Maximum parsimony analyses produced four most-parsimonious trees, each with a length of 138 steps (CI:0.877, RI:0.838, RC:0.735) (Fig. 5.5). Within these four gene trees three distinct clades were recognized, with haplotype 20 consistently placed as basal to all other haplotypes (bootstrap support 82%). The three clades were Clade I (Haps 1-4); boot strap support of 92%; Clade II (Haps 5-7 or 8); bootstrap support of 46%, with the exception of one tree (Fig. 5.5c); and Clade III (Haps 11-19); bootstrap support of 63%. The four trees varied in the placement of Clade II and three haplotypes (8, 9 and 10) (Fig. 5.5). In two of four trees Clade I was sister to Clade II (Fig. 5.5b and d), whereas in the two remaining trees it was sister to Clades II and III (Fig. 5.5a and c). A subclade of Clade II, consisting of Bermudian haplotypes 5 and 6, had the highest bootstrap support at 94%, Clade I was also strongly supported (92%) as was the subclade of Puerto Rican haplotypes 17 and 18 (87%). Clade III excluding haplotype 19 had 76% bootstrap support. Figure 5.5d shows two clades arising directly from the basal lineage (one includes Clades I, II and haplotypes 9 and 10 and the other is represented solely by Clade III). One basal haplotype for each of these two super clades are haplotypes now only found in Bermuda, a second for Clade II is found in all three regions.

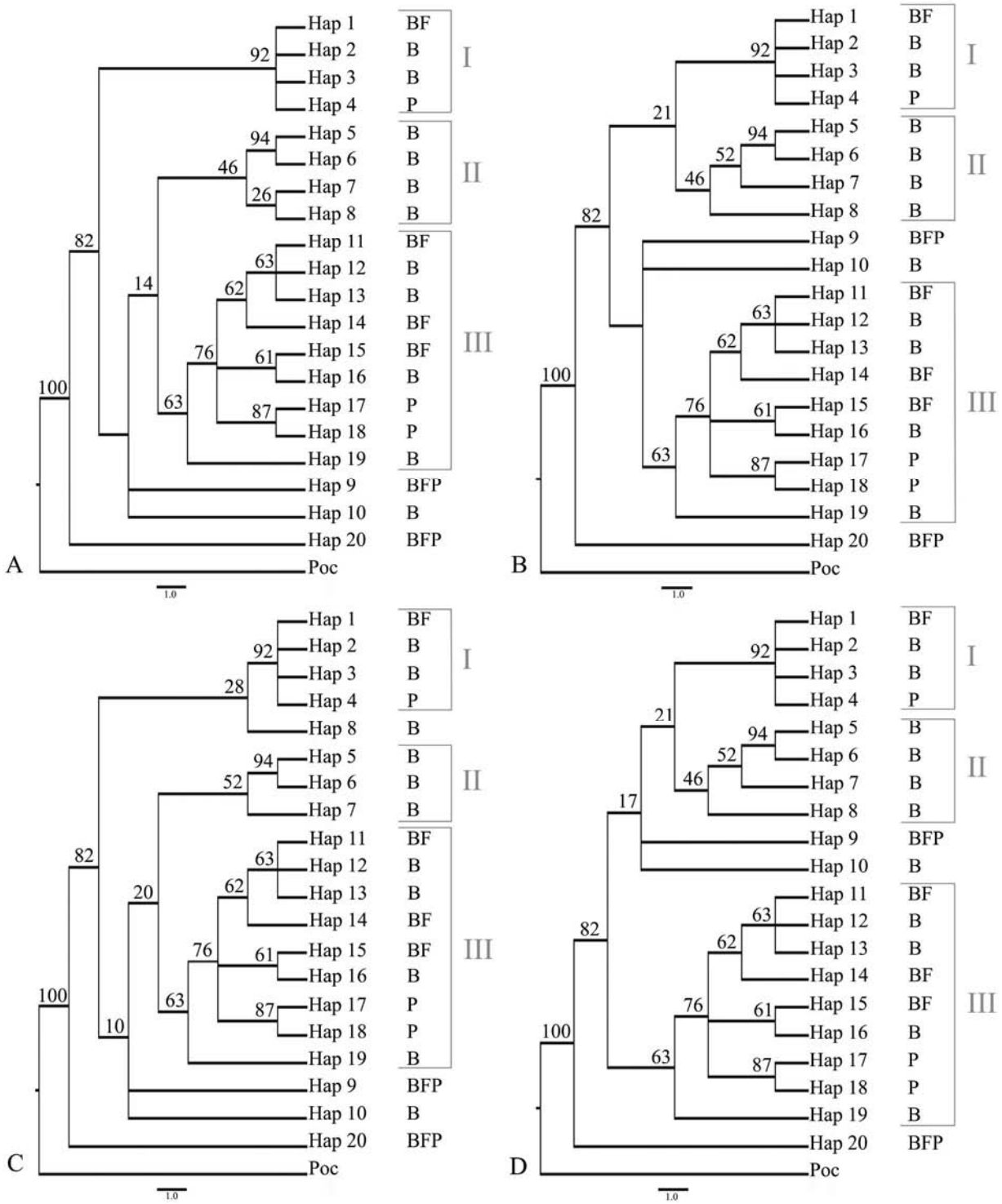

Figure 5.5. Four most-parsimonious trees produced by heuristic search with gaps treated as 5th nucleotide. Numbers above branches indicate bootstrap support as a percentage of 1000 replicates. Regional occurrence of each haplotype is noted in the left column as: BFP, Bermuda, Florida and Puerto Rico; BF, Bermuda, Florida; B, Bermuda; P, Puerto Rico. Total length=138 steps, CI=0.877, RC=0.735. Roman numerals represent clades of interest.

The topologies of ML and BI analyses were almost identical with slight variation in the position of four haplotype groups. Three distinct monophyletic clades were present (Figs. 5.6a and b). Clade I was highly supported in both ML (90%) and BI (96%) analyses. Clade II showed low support in the ML analysis (40%) but better support (73%) in BI analysis. Both methods identified haplotype 20 as the sister lineage to Clade II. Clade III had lower support values 32% (ML) and 49% (BI) but with the exclusion of haplotype 19 support increased to 43% and 59% respectively. Clade II was sister to Clade III in the ML analysis but this grouping had no consistent support in the BI analysis, where Clade II was sister to Clade I. The Bermudian pairing of haplotypes 5 and 6 was highly supported in both ML and BI analyses (86% and 98%) respectively. The BI analysis did provide more resolution than other analyses, resolving relationships within Clade I and of haplotypes 9 and 10.

In all analyses, Clade II represented a distinct geographic assemblage of haplotypes unique to Bermuda, with a sister common to all three regions (haplotype 20) and an optimized nodal character state of all locations (BFP). Clade II included haplotypes 5, 6 and 7 in all analyses and haplotype 8 in all but one. The two remaining clades, Clades I and III were not geographically unique, each including haplotypes from all three regions. Interestingly, in all analyses a haplotype lineage unique to Bermuda (haplotype 19) originates basal to Clade III (Figs. 5.5 and 5.6). It should be noted that MP and BI showed high nodal support for the clade including Puerto Rico haplotypes 17 and 18 (MP:87; BI:90). Bayesian Inference resolved haplotypes 17 and 18 as a basal subclade of Clade III, possibly indicating that the derived Florida and Bermuda haplotypes in Clade II originated from Caribbean haplotypes (Puerto Rico). In all other analyses this node was unresolved.

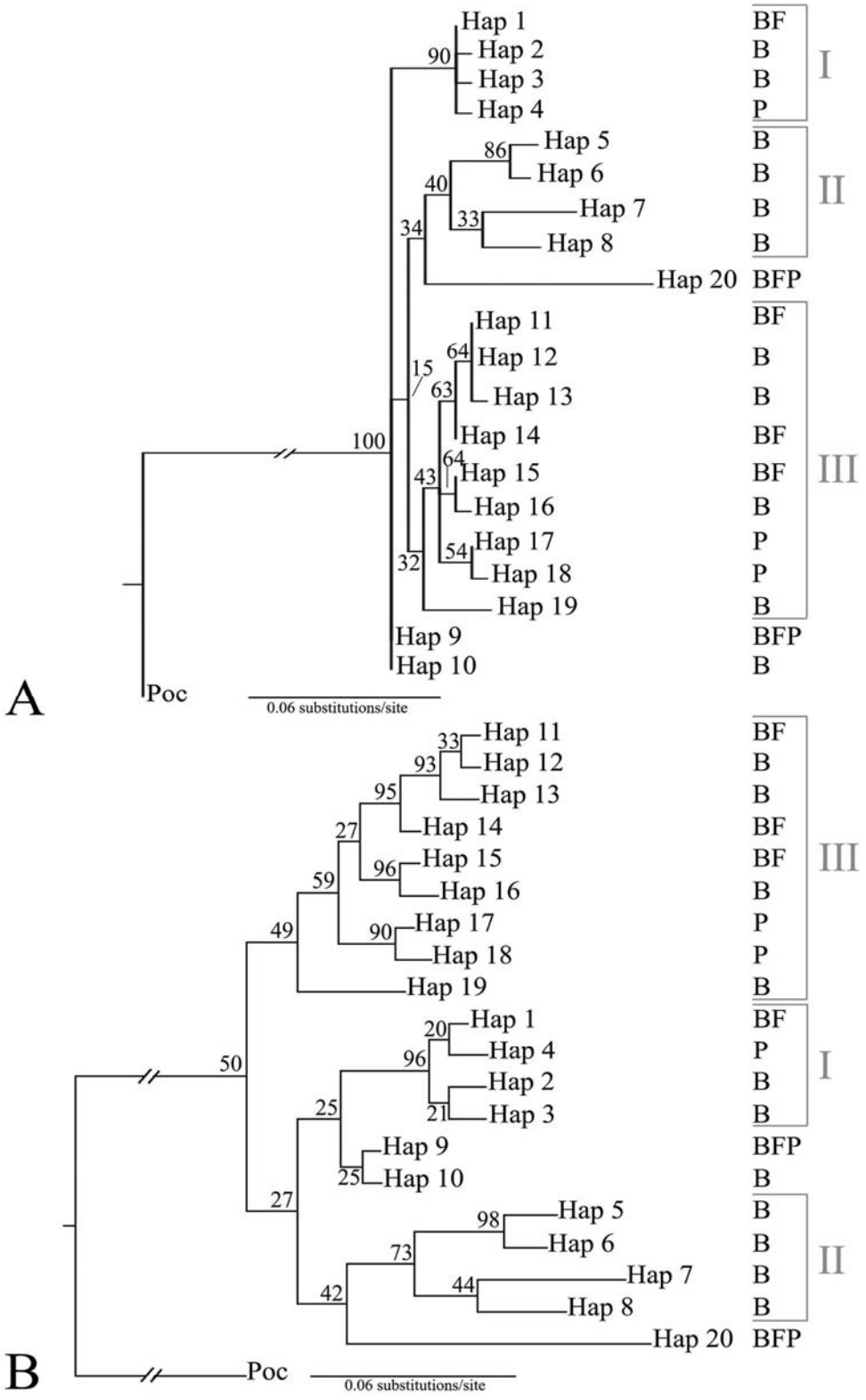

Figure 5.6. Likelihood-based trees using HKY + G model of evolution. A) Maximum Likelihood heuristic search using 100 random additions. Outgroup Poc original branch length 0.2506 subs/site. B) Bayesian likelihood search using 500 000 generations sampled every 100 generations. Outgroup Poc original branch length 0.4345 subs/site. Branch labels in A. indicate bootstrap support from 1000 replicates and in B. indicate posterior probabilities calculated from a consensus of 7458 trees. Roman numerals represent clades of interest.

An obvious difference between the tree topologies produced by the three different phylogenetic analyses was the positioning of haplotype 20. In the MP analysis haplotype 20 occupied a position more derived than that of the outgroup but basal to all clades and ungrouped haplotypes. Bootstrap support for this topology was 87%. However, in ML and BI analyses, haplotype 20 was instead basal to Clade II, with which it formed a monophyletic clade. (This difference in topology for haplotype 20 between MP and ML/BI analyses may be attributed to the latter two analyses treatment of gaps as missing data rather than as a 5^{th} state as in MP.) The 10 bp insertion only present in haplotype 20 would not have been evaluated in the ML/BI analyses and this has a clear influence on tree structure. This influence was very evident when the MP analysis was run on the same data set with gaps treated as missing rather than as a 5^{th} state. The analysis resulted in a strict consensus tree topology similar to ML and BI with haplotype 20 closer to Clades I and II rather than basal to all haplotypes (Appendix 5.2). This emphasizes the possible effect of including gaps in analyses rather than dismissing them as uninformative.

5.4.4 Population genetic results

Madracis auretenra exhibited significant population structure across the three sampled regions of the greater Caribbean in the ***SRP54*** data set. Analysis of molecular variation (AMOVA) for the three sampled regions detected significant regional genetic structure between Bermuda and Florida (F_{st}: 0.13617 $p<0.0001$) and Bermuda and Puerto Rico (F_{st}: 0.05888 $p<0.05$), with Florida and Puerto Rico exhibiting the highest level of regional genetic structure (F_{st}: 0.32975 $p<0.0001$). AMOVA incorporating regional sites revealed significant genetic structure between regions (F_{ct}: 0.141 $p<0.05$) and between overall sites sampled (F_{st}: 0.153 $p<0.001$) but no significant structure between sites within regions (F_{sc}: 0.013, PR sites

independent; F_{sc}: 0.014, PR sites pooled) (Table 5.4). The significance of the results was the same if PR-sampled sites were treated independently or pooled as a SW Puerto Rico site. When Puerto Rico sites were excluded, AMOVA of Bermuda and Florida sampled sites yielded low but significant structure between regions (F_{ct}: 0.133 p<0.05) and between sampled sites (F_{st}: 0.143 p<0.05), but not between sites within regions.

Table 5.4. AMOVA results showing levels of genetic structure between regional sites (F_{st}), between sites within regions (F_{sc}), and between regions (F_{ct}) for the nDNA SRP54 locus in *M. auretenra*. Table entitled Bermuda and Florida samples only does not include samples from Puerto Rico within the analysis. Significant values are denoted by * *P* <0.05 and ** *P*<0.001; statistical probabilities derived from 10,100 permutations.

Source of variation	df	SS	Variance components	Percentage of variation	F_{st}	F_{sc}	F_{ct}
Among regions	2	99.604	0.96286	14.14			0.141*
Among sites w/in regions	6	41.720	0.07675	1.13		0.013	
Within sites	137	790.580	5.77066	84.73	0.153**		
Total	145	931.904	6.81027				

*P < 0.05, **P<0.001

Bermuda and Florida samples only

Source of variation	df	SS	Variance components	Percentage of variation	F_{st}	F_{sc}	F_{ct}
Among regions	1	63.98	0.93572	13.32			0.133*
Among sites w/in regions	5	36.142	0.06955	0.99		0.011	
Within sites	117	705.886	6.03321	85.7	0.143*		
Total	123	806.008	7.04029				

*P < 0.05

Pair-wise comparisons of fixation indices among sampled sites within regions showed differing levels of genetic structure. Within Bermuda, the four sampled sites exhibited no

population structure, indicating that the ***M. auretenra*** sampled from these sites are not reproductively isolated from one another and readily exchange genes; they are panmictic. However, all Bermuda populations exhibited significant genetic structure from Florida Lower Key populations. Bermuda populations were not significantly structured from Florida Upper and Middle Key populations, with one exception, the Hogfish Crescent population was significantly structured from the Middle Keys population (0.135821 $p<0.05$). Within Florida populations no significant population structure was detected for the Lower and Middle Keys, however the Upper Keys population was significantly structured from both Lower and Middle Keys with respective F_{st} values of 0.30654 ($p<0.05$) and 0.12524 ($p<0.05$). No significant population structure was detected between the 2 sampled Puerto Rico sites. When analyzed as two different Puerto Rico sites, Laurel reef (n = 2) exhibited significant structure from both Bermuda and Florida regional sites, with highest values determined for the Middle, Lower and Upper Keys. This should be interpreted with caution. Media Luna reef was significantly structured from the Lower Keys but no other sampled sites. When samples from the two Puerto Rico sites were pooled, significant structure was evident between Puerto Rico and the Florida Keys, but no significant structure was detected with Bermuda. Overall, the highest population structure values were found between Florida's Lower Keys and all Bermuda samples (F_{st}: 0.18736 – 0.25117) and Puerto Rico (Laurel F_{st}: 0.50311; Media Luna F_{st}: 0.36644; and PR pooled F_{st}: 0.44202) sites (Table 5.5).

Table 5.5. Pairwise F_{st} values between regional sites for the SRP54 nDNA intron in *M. auretenra*. With Puerto Rico sites separated into two sites and with Puerto Rico sites pooled. Significant values are denoted by * *P* <0.05 and ** *P*<0.001; statistical probabilities derived from 10,100 permutations. Values in bold denote differences found between unpooled and pooled Puerto Rico results.

Pairwise Fst between regional sites								
	1	2	3	4	5	6	7	8
1. Castle Harbour								
2. Shelly/Tynes Bay	-0.00966							
3. HogFish Beacon	0.02124	-0.04602						
4. West End	-0.01232	-0.05730	-0.02346					
5. Lower Keys	0.19401**	0.25117**	0.29891**	0.18736*				
6. Middle Keys	0.05649	0.08437	0.13581*	0.04078	0.00992			
7. Upper Keys	0.01429	0.09867	0.11148	0.07756	0.30654*	0.12524*		
8. Laurel	0.07363*	0.11549*	0.10886*	0.13763*	0.50311**	0.32418**	0.18225*	
9. Media Luna	-0.07033	-0.15505	-0.14548	-0.12703	0.36644*	0.09433	0.12374	0.04623

*P < 0.05, **P<0.001

Pairwise Fst between regional sites with PR sites pooled							
	1	2	3	4	5	6	7
1. Castle Harbour							
2. Shelly/Tynes Bay	-0.00966						
3. HogFish Beacon	0.02124	-0.04602					
4. West End	-0.01232	-0.05730	-0.02346				
5. Lower Keys	0.19401**	0.25117**	0.29891**	0.18736*			
6. Middle Keys	0.05649	0.08437	0.13581*	0.04078	0.00992		
7. Upper Keys	0.01429	0.09867	0.11148	0.07756	0.30654*	0.12524*	
8. ML/L	0.04596	0.0723	0.07033	**0.09663***	**0.44202****	**0.27047****	**0.12953***

*P < 0.05, **P<0.001

5.5 Discussion

The *SRP54* genetic data imply that *M. auretenra* populations within Bermuda are genetically structured from and possess greater nucleotide and haplotypic diversity than those of Florida and Puerto Rico. The level of genetic diversity in Bermuda populations contradicts the predictions / hypotheses of lower levels of diversity being associated with geographically isolated reefs (Ayre and Hughes 2004, Miller and Ayre 2004, 2008), in particular those near geographic range limits. The high level of genetic diversity in *M. auretenra* is suggestive of

resilience within the Bermuda population. In other words, although geographically isolated and genetically structured from neighboring conspecifics, Bermuda's population of *M. auretenra* may possess an increased probability of survivorship during environmental or biological changes. Phylogenetic analyses indicate patterns of haplotypes shared across the greater Caribbean sampled sites, plus diversification locally in Bermuda populations. Isolated islands, those with few colonists, have been theorized to experience *in situ* diversification based on low (founding) diversity (Paulay 1994). However, it may also be the case that these differences in levels of genetic diversity are the result of the Puerto Rico and Florida populations already having experienced genetic "weeding".

5.5.1 Genetic Diversity

5.5.1.1 Colonization and diversification

Quaternary climatic oscillations, including Pleistocene sea level fluctuations and subsequent postglacial colonizations (Benzie 1999, Hewitt 2004, Sterrer et al. 2004), may have contributed to *M. auretenra*'s genetic diversification in Bermuda. Sea level changes drastically altered the amount of available habitat for the islands' marine species with levels reaching a maximum of 22 m higher than (400 000 years ago) (Hearty et al. 1999) and a minimum of 120 m lower than (18 000 years ago) present day levels (Sterrer et al. 2004). Ten of 20 *SRP54* nDNA haplotypes are unique to Bermuda and either the initial colonizers of Bermuda diversified to form these haplotypes or these unique Bermuda halotypes are relicts and were once present but have since been extirpated in the other areas sampled. Oceanic island biotas often include relict taxa (Paulay 1994). The regionally shared Haplotype 20 considered by parsimony analysis (Figs 5.4 and 5.5) to be basal (and disconnected from the haplotype network), may be such a relict

taxa, as may be Haplotype 19. Haplotype 20 is rare in Florida and Puerto Rico but quite prevalent in Bermuda samples and is likely to represent a conserved ancient lineage.

The difference in depth of collection for the three regions is interesting and may provide a clue to these differences in genetic diversity (Table 5.1). There is also the possibility that Bermuda's genetic diversity is derived from an unsampled region (i.e. the Bahamas) within the western Caribbean, although this is not supported by the presence of unique haplotypes in the other regions sampled herein. Other Bermudian marine connectivity studies in Bermuda have demonstrated that marine invertebrate genetic diversity exceeds that of Florida and also revealed the presence of unique Bermudian haplotypes (Ó Foighil and Jozefowicz 1999, Park and Ó Foighil 2000, Bilewitch 2006, Bilewitch et al. submitted) and alleles (Mitton et al.1989). Overall, it at least seems conclusive that genetic flow to Bermuda is one-way.

The lower genetic diversity found in the Florida Keys points to a few plausible explanations/hypotheses. It is clear from current surveys (E. Bartels pers. comm., Locke pers. obs.) that ***M. auretenra*** is somewhat uncommon, even within the Sanctuary Preservation Areas (SPA's) of the Florida Keys National Marine Sanctuary (FKNMS), and populations may be smaller than in Bermuda. Quite possibly ***M. auretenra*** has always been an uncommon coral within the Florida Keys with low genetic diversity or it may be in decline. In general, ***M. auretenra*** colonies sampled in Florida occurred deeper (6.1 m – 16.5 m) than those in Bermuda (1.8 m – 7.6) (Table 5.1). Although not directly observed, a contributing factor may be predation by corallivorous parrotfish and/or pufferfish which has been reported to restrict the vertical distribution of ***M. auretenra*** to depths below 13 m at Conch Reef in Florida's Upper Keys (Grotolli-Everett and Wellington 1997). So that this species may be strongly constrained by available habitat and subsequently has smaller, isolated populations, low reproductive success (sexual) and resulting low genetic diversity.

Puerto Rico was not extensively sampled; therefore the haplotype diversity reported for Puerto Rico is preliminary and suggestive. Even with a low sample number and small sample area (Fig. 5.2c) genetic diversity for Puerto Rico is as high as that for Florida (Table 5.2). Depths of collection in Puerto Rico (7.0 m – 12.5 m) were also greater than those of Bermuda. There are no studies on predation comparable to those of Florida, to my knowledge. Depth range differences between regions (is more than likely related to available light levels at depth but) may be correlated with particular haplotype frequencies, although there was no correlation between depth and haplotype in Bermuda. Given the northern location of Bermuda, I suggest that depth distribution differences among the locations is more likely related to light quality at depth.

The levels of genetic variation found within Bermuda *M. auretenra* populations suggest that this northwestern Atlantic island may be an important coral refuge of high conservation value as opposed to a downstream dependent of Caribbean diversity. It has recently been suggested that more genetically diverse coral populations could contribute to the repopulation of conspecifics by transplantation to threatened or degraded reefs elsewhere, thereby increasing coral resilience to future disturbance in the affected populations (Shearer et al. 2009).

5.5.1.2 Genetic diversity in Bermuda – origins and future

Bermuda's lower species diversity may allow western North Atlantic lineages to thrive by enabling species to escape severe competitive interactions that impact individuals in other higher diversity parts of their geographic range (Park and Ó Foighil 2000). Another factor that may contribute to Bermuda as an ideal site for maintained diversity is the general health of its reef ecosystem. Although affected by natural and anthropogenic disturbance in the last 100 years (Flood et al. 2005), the scale of this disturbance has been lower than that experienced by neighboring coral reef populations of Florida and the Caribbean. It has been suggested that

Bermuda reefs receive less impact by hurricanes and storms than Caribbean reefs (Wilkinson and Souter 2008) and in this respect Bermuda has a significant advantage over the majority of Caribbean islands in that it lacks abundant topsoil and freshwater rivers. Therefore, Bermuda's coral reef ecosystem receives minimal terrestrial siltation during storms and hurricanes compared to other larger islands with freshwater outflows. Bermuda is also under different environmental regimes (in particular lower surface seawater temperatures and greater seasonality) than countries in the northern and southern Caribbean and both of these environmental aspects may contribute to the decreased prevalence of disease and bleaching in the islands' corals (Weil and Cróquer 2005, 2009). The widespread and severe bleaching event of 2005 which drastically impacted Caribbean reefs including those of Puerto Rico and Florida was minimal on Bermuda reefs (Manzello et al. 2007, Weil and Croquer 2005, 2009, Wilkinson and Souter 2008, Locke pers. obs.). Considering the rise in sea temperature predicted to result from global climate change, and that Bermuda's water temperature is generally cooler than that of the Caribbean, the island's waters may well remain within the temperature limit for healthy coral for the foreseeable future.

5.5.2 Genetic Connections

Populations of ***M. auretenra*** within Bermuda display no noticeable genetic subdivision and are considered panmictic, indicating connectivity potential at distances of <16 km. However, there is significant population structure among the three greater Caribbean regions of Bermuda, Florida and Puerto Rico (Table 5.4) signifying a lack of or very limited connectivity over a distance of 1500 km. It appears that evidence is gradually accumulating for locally retained larvae of reef species and short 50-100 km larval dispersal distances (Cowen et al. 2007). The four existing studies of Caribbean coral connectivity indicate gene flow is commonly restricted

over distances of 500 km (Baums et al. 2005, Brazeau et al. 2005, Fukami et al. 2004, Vollmer and Palumbi 2007). Within the study reported here the three geographic regions sampled are separated by ocean distances of approximately 1600 – 1900 km so that some of the indications of absence of significant structure between sites are unexpected.

Phylogenetic gene trees show two ancient lineages (9 and 20) shared between Bermuda, Florida and Puerto Rico indicating that the regions were historically connected (Figs 5.5 and 5.6). These trees also support the hypothesis that these connections may no longer be in existence; more derived clades are geographically distinct with the exception of a few Bermudian and Floridian haplotypes. Bermuda and Florida share four recently derived haplotypes (Figs 5.5 and 5.6); suggesting that isolation between the two regions may periodically be broken. Further evidence for these connections is found in low levels of genetic structure and pair-wise comparisons of population subdivision between Florida's Middle and Upper Keys with certain sites in Bermuda (Table 5.5). All Florida haplotypes are found in Bermuda but not vice versa indicating either the diversification of Bermuda's population or extirpation of these haplotypes in Florida. The occurrence of unique haplotypes in Bermuda and in Puerto Rico, which form two geographically distinct clades further indicates that these two regions are not connected with each other or providing genetic variability to Florida. Even though pair-wise comparisons of F_{st} show no significant population subdivision between Bermuda and Puerto Rico when Puerto Rico sites are pooled, this could result from the small sample size, as can be seen by the clear differences in haplotypes found in each location. It should also be noted that this fixation index does not take historical pattern into account. Bermuda and Puerto Rico share two relict haplotypes (20 and 9) and the distribution of more derived haplotypes suggests the connection between the two regions no longer exists.

The historical patterns represented by all gene trees also show the unique Bermuda haplotype 19 as a sister lineage to Clade III (containing Bermuda, Florida and Puerto Rico haplotypes) suggesting this might also be a relict. Haplotype 19 is rare in this study – 1) perhaps easily lost or 2) difficult to detect in small samples. Park and O Foighil (2000) also found distinct Bermudian haplotypes with deep origins in the bivalve *Laseaea* implying dispersal against present surface currents. The authors ruled out the possibility based on genetic differences of island and mainland populations. The Florida current has apparently been a persistent feature of the North Atlantic Gyre (Lynch-Stieglitz et al. 1999, Duplessy 1999) and combined with the lack of certain Bermuda haplotypes in Florida provides evidence against the hypothesis of counter current gene flow. An unstudied population or the extinction of other ancient haplotypes may also have contributed to the ancestral pattern observed.

Measures of F_{st} and their correlation to genetic structure and connectivity within and between populations appear to vary across studies (species and markers). For studies of Caribbean corals using different molecular markers, significant genetic structure was hypothesized for populations with F_{st} values ranging from 0.041 – 0.235. Baums et al. (2005) suggested that an F_{st} of 0.041 (based on microsatellite data from Acropora palmata) indicated significant genetic structure. Whereas, Vollmer and Palumbi (2007) rely on much higher levels (F_{st}: 0.235) of population structure based on mtDNA and nDNA sequences of *Acropora cervicornis*, to indicate low gene flow across the Caribbean. And in a study of mtDNA in terrestrial vertebrates F_{st} values less than 0.05 were considered to indicate high levels of gene flow (Epps et al. 2005) and no population structure. Variation among markers and species make inter-study comparisons difficult and all assessments of levels of closure relative not absolute.

5.5.3 Mechanisms of dispersal

Caribbean connectivity studies have investigated broadcast spawning species: *Acropora cervicornis* and *Acropora palmata* (also known to primarily reproduce asexually), *Montastraea annularis, Montastraea faveolata* and *Montastraea franksi* and the single hermaphroditic brooder *Agaricia agaricites.* Different molecular markers were employed in each study. *Madracis auretenra* studied here is reported to be a sexual reproducing brooder. In all these species, propagules (gametes, larvae or pre-settlement juveniles (planulae)) are assumed to be the primary dispersive stage. In the region of study and between the regions, the Gulf Stream is the surface current most likely (now) to carry propagules to Bermuda.

The Gulf Stream is estimated to have existed (at least) since the rise of the Central American Isthmus, 3.5 million years ago (ma.) (Burton et al. 1997). The closure of the inter-American seaway established today's general pattern of ocean circulation in the Atlantic. The formation of the Caribbean current (Budd 2000) and the Antilles Current, possibly at this time, would have provided surface transport between Puerto Rico and Florida. The Quaternary position of the Gulf Stream is predicted to have been affected by Pleistocene glaciers, which forced it to the South and into a more west-east orientation than today (Keffer et al. 1988, Duplessy 1999), bringing it closer to Bermuda. Changes in the position of the Gulf Stream would impact Bermudian connections. The infrequent cold-core rings known to separate from the Gulf Stream and travel near Bermuda (The Ring Group 1981) are mechanisms that could deliver *M. auretenra* propagules from Florida to Bermuda.

As mentioned previously, some Caribbean coral species seem to show restricted gene connectivity at distances greater than 500 km, where there are average surface flow rates, not like the Gulf Stream. The Upper Florida Keys are separated from Bermuda by more than 1700 km of deep ocean (the Bahamas supplying the only reef habitat along the way, still ~1500 km

away), a definite record for coral population genetic connectivity in the greater Caribbean thus far. The presence of unique haplotypes in Bermuda absent from Florida suggests that this dispersal is in the direction of Gulf Stream flow.

At peak ***M. auretenra*** reproductive times Gulf Stream current speeds are approaching maxima and the core flow shifts slightly southward, towards Bermuda (Frankignoul et al. 2001). Jackson (1986) determined a 30 day maximum, and Schultz and Cowen (1994) a 97 day, maximum transport time in the Gulf Stream between Florida and closest approach to Bermuda.

In general, planulae of ***Madracis*** species start to explore the bottom 16-24 hours after release, but often resume swimming for small distances (<0.50 m) (Vermeij et al. 2003). Planulae of ***M. auretenra*** are known to have high yolk content and zooxanthellae (Vermeij et al. 2003), both of which would be advantageous to long distance dispersal.

Chances of dispersal may be increased by rafting of settled propagules (Jackson 1984); Pacific corals are known to settle and raft great distances on natural and man made objects (Jokiel 1984). Introduction of benthic marine species to Bermuda on fouled ships bottoms has been proposed (Clarke and Downey 1992). This is an interesting hypothesis considering the number of ships that have wrecked on Bermuda's reefs, and introduction of benthic marine species on fouled ship hulls may be a possibility. While rafting on natural substrata may have been occurring for millennia, human-mediated introductions exist on a temporal scale of 400-500 years ago and not on evolutionary time scales, however they may explain the origins of Bermuda's recent unique haplotypes.

For the majority of coral species, pelagic larval duration (PLD) has not been studied in detail and we can only speculate on any species' dispersal potential. Although brooding species are generally assumed to exhibit limited dispersal capabilities, brooding corals have occasionally dispersed long distances (Underwood et al. 2007). The Pacific brooder ***Pocillopora damicornis***

can remain competent to settle >130 days after parental release (Richmond 1987) and although laboratory experiments are known to show longer durations before settlement (Richmond 1987) conditions could be very similar to passive pelagic transport.

5.5.4 Bermuda populations

Dispersal from neighboring greater Caribbean source populations was historically important for Bermuda's initial colonization and quite possibly its current genetic diversity, however more recently these populations have become divergent in Bermuda and transport of colonizing propagules is predicted to occur too infrequently to sustain Bermudian marine populations. Therefore, the results of this study indicate a reliance on local recruitment and thus local management of corals in Bermuda.

Bermuda populations of ***M. auretenra*** appear to be panmictic and self seeding, reducing the dependence of the island on rare disperal from outside source populations; reassuring considering the degraded state of some greater Caribbean reefs and the fact that the Caribbean has largest proportion of corals in high extinction risk categories (Carpenter et al. 2008). On the other hand, this independence may prove precarious if a major disturbance results in the loss of the adult coral population or a reduction in the fecundity of the Bermudian population. After severe or localized disturbances, it has been proposed that recruitment from external sources is likely to be extremely important for recovery (van Oppen et al. 2008). This study reveals that larvae from greater Caribbean sources (i.e. Florida) would not be effective in the repopulation of the islands' coral species under present conditions and in that case, Bermuda's reef systems may be at risk unless an unsampled population (i.e. Bahamas) provides an upstream source of coral larvae.

Bermuda's reliance on its coral reef ecosystems both economically and as a protective barrier to island erosion enforces the need for safeguarding the islands coral populations. Protection has been afforded to corals across the Bermuda platform since the adoption of the 1972 Fisheries Act. However, besides this "no-take" level of protection an overall management plan for the coral reef habitats of the island does not exist. The evidence provided within this genetic study of *M. auretenra* has determined that Bermuda corals are diverse, isolated, and self-sufficient. These findings combined with Bermuda's potential as a coral refugium further justify focus on an effective local management plan for the islands' coral population.

5.5.5 Future research

Further substantiation of Bermudian scleractinian genetic diversity and genetic connections among conspecifics requires more extensive sampling of potential neighboring source populations. Future investigations should extend to other species of differing reproductive modes. Considering the high level of genetic diversity found herein for Bermuda's *M. auretenra* population and also for previous studies of the islands marine invertebrates (see Introduction) it would be interesting to establish if this diversity extends to even more Bermudian marine species, potentially verifying Bermuda's high conservation value as a marine refuge. Studies of finer-scaled structure among Bermuda reefs would be also advantageous to local management efforts.

Molecular data often reflect only a small portion of an organism's genome and therefore can illustrate a relatively narrow portion of the evolutionary history within that species. This study is based on the findings of a single molecular marker in a single species. With this in mind, more molecular markers need to be employed to effectively resolve intra-specific diversity and

population connections, no small task considering the deficiency of informative markers for corals.

Herein, the utility of the single copy *SRP54* nuclear intron has been demonstrated for population and phylogeography research of scleractinian corals providing an additional marker for coral studies as previously determined by Concepcion et al. (2008). To date this is the first population connectivity study to use the *SRP54* nuclear intron in scleractinian corals. Considering this, a greater *SRP54* database upon which to make comparisons across species and geographic locations is necessary. Of the five existing molecular studies of population connectivity in the Caribbean, only two or three incorporate the same molecular markers allowing for effective comparison of resultant data. In order to make any headway with connectivity studies, collaboration and communication is necessary.

The conclusions reached in this preliminary study of the *SRP54* nuclear intron in *M. auretenra* populations may change with increased sampling and understanding of coral molecular biology, phylogeny and population structure. However, for now they provide us with indirect evidence of Bermuda's coral population history, resilience and level of genetic connectivity with nearby conspecifics populations. Of course direct studies of coral demography, larval duration and dispersal are always a hope for future investigations of scleractinian population connectivity.

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Appendix 5.1. DNA alignment of SRP54 haplotype sequences used in phylogenetic analysis. Numbers in boldface indicate nucleotide position in alignment.

Hap 1	T G A A C T G A A G C T T G A G G A C A A T G A A G A A C T	**30**
Hap 2	. . . . .	
Hap 3	. . . . .	
Hap 4	. . . . .	
Hap 5	. . . . .	
Hap 6	. . . . .	
Hap 7	. . . . .	
Hap 8	. . . . .	
Hap 9	. . . G	
Hap 10	. . . . .	
Hap 11	. . . . .	
Hap 12	. . . . .	
Hap 13	. . . . .	
Hap 14	. . . . .	
Hap 15	. . . . .	
Hap 16	. . . . .	
Hap 17	. . . . .	
Hap 18	. . . . . C	
Hap 19	. . . . .	
Hap 20	. . . . .	
Poc	? ?	

Hap 1	T A T T G A C A A A C T T A A A C A T G G T G A G A A A T C	**60**
Hap 2	. . . . .	
Hap 3	. . . . .	
Hap 4	. . . . .	
Hap 5	. . . . .	
Hap 6	. . . . .	
Hap 7	. . . . .	
Hap 8	. . . . .	
Hap 9	. . . . .	
Hap 10	. . . . .	
Hap 11	. . . . .	
Hap 12	. . . . .	
Hap 13	. . . . .	
Hap 14	. . . . .	
Hap 15	. . . . .	
Hap 16	. . . . .	
Hap 17	. . . . .	
Hap 18	. . . . .	
Hap 19	. . . . .	
Hap 20	. . . . .	
Poc	? R . . . . M . .	

Appendix 5.1. Continued

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Hap 1   A T T T A C G G A T G G G A G A A T T A C T C T T C T A T T  90
Hap 2   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 3   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 4   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 5   . . . . . . . . . . . . . . . . . . . . . . A . . . . . .
Hap 6   . . . . . A . . . . . . . . . . . . . . . . G . . . . . .
Hap 7   . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 8   . . . . . A . . . . . . . . . . . . . . . . G . . . . . .
Hap 9   . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 10  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 11  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 12  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 13  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 14  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 15  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 16  . . . . . A . . . . . . . . . . . . . . . . . . . . . . .
Hap 17  . . . . G . A . . . . . . . . . . . . . . . . . . . . . .
Hap 18  . . . . G . A . . . . . . . . . . . . . . . . . . . . . .
Hap 19  . . . . . A . . - - - - - - - - G . . . . . C . . . . . .
Hap 20  . . . . G - A . . . T . . . . . T . . . . . . . . . G . .
Poc     C . A A . . A . . . A . T . . - - . G . . A T A . . . . . .

Hap 1   T - - - - - - - - - - - T G A A A G G A T G T G A T C - T A C 120
Hap 2   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 3   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 4   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 5   . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .
Hap 6   . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .
Hap 7   . . . . . . . . . . . . . . . . . . . . . . A . . G . . A . . .
Hap 8   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 9   . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 10  . . . . . . . . . . . - . . . . . . . . . . C A . . . . . .
Hap 11  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 12  . . . . . . . . . . . - . . . . . . . . . . C A . . . . . .
Hap 13  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 14  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 15  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 16  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 17  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 18  . . . . . . . . . . . . . . . . . . . . . . C A . . . . . .
Hap 19  . . . . . . . . . . . . . . . . . . . . C . . C A . . . . . .
Hap 20  . A A A C T A A G T T . . . . T . . . C . . . . . .
Poc     . . . A C C A G R T T . T . . . . . C A A . . . . . .
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Appendix 5.1. Continued.

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Hap 1    T G T T T A A A T T T A A T G G A T G T T G G T G G C A A A T  150
Hap 2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 3    . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . .
Hap 4    . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . .
Hap 5    . . . . . . . . . . . . . . . . . . . . . . A . . . T . . . .
Hap 6    . A . . . . . . . . . . . . . . . . . . . . A . . . T . . . .
Hap 7    . . . . . . . . . . . . . . . . T C . . . . A . . . . . . . .
Hap 8    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 9    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 10   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 11   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 12   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 13   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 14   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 15   . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . .
Hap 16   . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . .
Hap 17   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 18   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 19   . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . .
Hap 20   . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . .
Poc      . . G . C . T . G . . . . A - - - - - - - - - - - - . . . .

Hap 1    T C A T T T A A C T G T T G T A A A G T A G T A A A G T G C  180
Hap 2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 3    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 4    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 5    . . . C . . . . . . A . . . . . . . . . . . . . . . . . . . .
Hap 6    . . . C . . . . . . A . . . . . . . . . . . . . . . . . . . .
Hap 7    . . . . . . . . . . A . . A . . . . . . . . . . . . . . . . .
Hap 8    . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . .
Hap 9    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 10   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Hap 11   . . . . . A . . . . A C . . . G . . A . . - - - . . . . . .
Hap 12   . . . . . A . . . . A C . . . G . . A . . - - - . . . . . .
Hap 13   C . . . . A . . . . A C . . . G . . A . . - - - . . . . . .
Hap 14   . . . . . A . . . . A C . . . G . . . . . - - - . . . . . .
Hap 15   . . . . . . . . . . A C . . . G . . . . . - - - . . . . . .
Hap 16   . . . . . . . . . . A C . . . G . . . . . - - - . . . . . .
Hap 17   . A . . . . . . . . A C . . . G . . . . . - - - . . . . . .
Hap 18   . A . . . . . . . . A C . . . G . . . . . - - - . . . . . .
Hap 19   . . . . . . . . . . A C . . . . . . . . . . . . . . . . . .
Hap 20   . . . . . . . . . . T A A C . . . . . . . . . . . . . . . .
Poc      . A . . G . . . G . . - - . C . . T T C . - - - - T . . . S
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Appendix 5-1. Continued

Hap 1	A T T - C A T T T C T T T T T T - A T T A G G T G T T T T C	**210**
Hap 2	. . . . . C	
Hap 3	. . . .	
Hap 4	. . . .	
Hap 5	. . A . . . . C	
Hap 6	. . A . . . . C	
Hap 7	. . A . . . . C . . . . T	
Hap 8	. . A . . . . C . . . . T	
Hap 9	. . . .	
Hap 10	. . . .	
Hap 11	. . . T	
Hap 12	. . . T	
Hap 13	. . . T	
Hap 14	. . . T	
Hap 15	. . . T	
Hap 16	. . . T	
Hap 17	. . . T . . . . T	
Hap 18	. . . T . . . . T	
Hap 19	. . . T	
Hap 20	. . A - . . . . . C . . . . . T	
Poc	C C . . A . . C . T . . A . . . A T G	

Hap 1	A G T T T G C G A	**219**
Hap 2	. . . . .	
Hap 3	. . . . .	
Hap 4	. . . . .	
Hap 5	. . . . . A . . .	
Hap 6	. . . . . A . . .	
Hap 7	. . . . . A . . .	
Hap 8	. . . . . A . . .	
Hap 9	. . . . . A . . .	
Hap 10	. . . . . A . . .	
Hap 11	. . . . . A . . .	
Hap 12	. . . . . A . . .	
Hap 13	. . . . . A . . .	
Hap 14	. . . . . A . . .	
Hap 15	. . . . . A . . .	
Hap 16	. . . . . A . . .	
Hap 17	. . . . . A . . .	
Hap 18	. . . . . A . . .	
Hap 19	. . . . . A . . .	
Hap 20	. . . . . A . . T	
Poc	. . . . . A - - -	

Appendix 5.2. Maximum parsimony strict consensus gene tree of *M. auretenra SRP54* haplotypes produced by a heuristic search with gaps treated as missing data.