HOST SPECIFICITY AND REGIONAL ENDEMICITY IN SYMBIOTIC DINOFLAGELLATES (*SYMBIODINIUM*, DINOPHYTA) ASSOCIATED WITH SEA ANEMENOES IN THE GENUS *AIPTASIA*

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THESIS ABSTRACT

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Recent investigations of coral reef biology have focused on the global biogeography and host specificity of *Symbiodinium*, a diverse group of dinoflagellates that symbiotically associate with many marine organisms, including reef-building corals. Despite this, few studies have investigated the genetic structure of *Symbiodinium* at the population level. One suitable system to investigate *Symbiodinium* population genetics of a single host across a global range is the facultatively symbiotic anemone *Aiptasia*. In order to determined the specificity and population genetic structure of *Symbiodinium* communities associated with *Aiptasia* spp., 356 anemones were sampled from 18 locations throughout the world. *Symbiodinium* diversity was assessed using a variety of

molecular markers that measure diversity from the level of sub-generic clades to populations, including restriction fragment length polymorphisms (RFLPs) of 18S-rDNA, denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer 2 (ITS2) rDNA, flanking region sequences of two microsatellite loci, and allelic variation at six microsatellite loci specific for *Symbiodinium* Clade B. These data revealed that, with the exception of individuals from the Florida Keys, a single phylotype of *Symbiodinium* clade B (ITS2 "type" B1) associates with *Aiptasia* throughout the world. Furthermore, strong population structure was detected across local, regional, and global geographic scales, suggesting limited gene flow among most *Symbiodinium* populations. The high genetic structure of *Symbiodinium* populations and the association with one particular symbiont lineage across large geographic scales suggests strong regional endemism and the existence of specificity in *Aiptasia*-*Symbiodinium* symbioses. This work represents a contribution towards our understanding of the ecology and evolution of cnidarian-*Symbiodinium* endosymbioses.

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CHAPTER 1

LITERATURE REVIEW

I. INTRODUCTION

Dinoflagellates in the genus *Symbiodinium* (Freudenthal 1962) are single-celled eukaryotic microorganism. Members of the genus exclusively form endosymbiotic relationships with other protists or invertebrates such as reef corals, where they acquire access to inorganic carbon, nitrogen and phosphorous from the host. Those chemical elements are then fixed into organic compounds by photosynthesis in the chloroplast of *Symbiodinium*. In exchange, *Symbiodinium* pass over 90% of the newly fixed carbon to their host (Muscatine *et al.* 1981). Thus, this endosymbiosis is regarded as one of the most prominent intracellular association in the sea and underpins the remarkable productivity and biodiversity of coral reef ecosystems worldwide.

However, human-associated events such as global warming events are having significant impacts on coral reef ecosystems. One of the most common and visible threats to corals is referred to as "bleaching", which is the loss of *Symbiodinium* or pigments from the algae (Brown 1987; Glynn 1991). In many cases, if a bleached host does not reacquire its symbionts, death may occur. Thus, protection of coral reef ecosystems from such threats is becoming a topic of interest in conservation biology. In order to better understand the basic biology of this endosymbiosis, there is a need to find a model system of cnidarian-algae symbiosis. While *Symbiodinium* forms relationships with various Cnidaria, Mollusks, Porifera and Protists, sea anemones in the genus *Aiptasia* has been proposed as the model organism to reveal genetic and physiological characteristics

of endosymbioses due to their ease of isolation in the field and culture in the laboratory (Weis *et al.* 2008).

While the genetic diversity of *Symbiodinium* has been well-studied (Coffroth & Santos 2005), little work has focused on the genetics of *Aiptasia*. One possible reason for this focus is that mitochondrial DNA, which serves as a popular molecular marker for revealing genetic diversity in animals, has a relatively slow evolution rate in Anthozoas, which hinders its use in such a context (Shearer *et al.* 2005).

This review aims to summarize research on *Aiptasia*-*Symbiodinium* symbioses over the past decades. In particularly, a brief research background on the genetic diversity of *Symbiodinium* was introduced. Then, this review extends from how specific and flexible is the symbiosis between *Aiptasia* and *Symbiodinium*; to how the endosymbiosis is established, maintained. Additionally, hypotheses concerning bleaching were stated. Finally, this review attempts to provide suggestions for future research on *Aiptasia*-*Symbiodinium* endosymbiosis.

II. THE SPECIFICITY OF THE SYMBIOSIS

Genetic diversity of Symbiodinium *and* Aiptasia:

Although *Symbiodinium* was once regarded as a monotypic genus (Taylor 1974), current understandings come to an agreement that *Symbiodinium* is a heterogeneous group. So far, eight clades of *Symbiodinium* (Clade A through H) have been formally described with molecular approaches such as Restriction Fragment Length Polymorphism (RFLP) analysis of small subunit of nuclear ribosome DNA (SSrDNA) (Rowan & Powers 1991; Carlos *et al.* 1999; LaJeunesse & Trench 2000; LaJeunesse 2001; Pochon *et al.* 2001; reviewed by Coffroth & Santos 2005), with more than one possible species or strain existing in each clade. Relationships between these *Symbiodinium* clades have been inferred from partial large subunit rDNA (LSU rDNA), internal transcribed spacer region 2 (ITS 2) region (Pochon *et al.* 2004), mitochondrial genes (Takabayashi *et al.* 2004) and chloroplast large subunit (23S)-rDNA (Santos *et al.* 2002), all of which produced congruent phylogenies. Recently, fine-scale molecular markers have revealed additional diversity within *Symbiodinium.* For example, LaJeunesee (2001) divided members of each clade into several different "types" by denaturing gradient gel electrophoresis (DGGE) of ITS 2 (LaJeunesse 2001). At the population level, microsatellite loci specific to *Symbiodinium* Clade B have detected even finer-scale genetic differences and specificity between *Symbiodinium* and hosts such as Caribbean octocorals (Santos *et al.* 2004; Pettay & LaJeunesse 2007; Xiang *et al.* 2009).

Thus, additional genetic diversity within *Symbiodinium* will likely be revealed as molecular methodologies improve in the future.

To date, most systematic work on *Aiptasia* has been based on morphological characters. Previous *Aiptasia* studies focused on two abundant species, *A. pulchella* and *A. pallida,* which are geographically separated. *Aiptasia pulchella* is reported to be distributed across the Pacific Ocean, India Ocean and Red Sea, whereas *A. pallida* is distributed throughout the Atlantic Ocean and Caribbean (Oskar 1943, 1952; Cutress 1955). However, current debate concerns whether these two species are synonymous.. Thus, molecular data may prove useful in identifying if and where species boundaries exist between them, which will contribute toward our understanding of specificity between *Symbiodinum* and *Aiptasia,* as well as their co-evolutions.

***Specificity and flexibility of the symbiosis*:**

Specificity, member of same host taxa harbors specific symbionts only, has been reported in many host-*Symbiodinium* symbioses. Examples include: the scyphistoma stage of the jellyfish *Cassiopeia xamachana* selectively phagocytes only particular symbiotic algae (Colley & Trench 1983); the density of *Symbiodinium* from the same host associated with temperate sea anemone *Cereus pedunculatus* reaches higher densities than heterogeneous *Symbiodinium* (from different host) in host cells (Davy *et al.* 1997); the aposymbiotic planulae of the temperate anemone *Anthopleura elegantissima* are only capable of forming an association with fresh algal isolates from a conspecific adult (Banaszak *et al.* 1993; Schwarz *et al.* 1999); and gorgonians such as *Plexaura kuna* and *Pseudoplexaura porosa* harbor members of *Symbiodinium* Clade B after three

months although newly settled polyps naturally acquire *Symbiodinium* Clades A, B and C (Coffroth *et al.* 2001). Similarly, specificity was also described in *Aiptasia* spp. (Schoenberg and Trench 1980a-c). These authors found aposymbiotic *A. tagetes* were more successfully infected by a single taxon of *Symbiodinium* (from same host species) than by heterogenous isolates (from different host), while some *Symbiodinium* were unable to infect individual anemones at all even after six months of inoculation and exposure. Interestingly, algal isolates from identical or closely-related anemones seem to be favored in associations with the *Aiptasia* hosts (Belda-Baillie *et al.* 2002).

Although specificity between symbiotic dinoflagellates and their hosts has been documented in various studies, mixed assemblages can also be established (Banaszak *et al.* 1993; Schwarz *et al.* 1999). For example: *Aiptasia* hosts may associate with algal isolates from tridacnid clams to a limited extent (Belda-Baillie *et al.* 2002). Numerous other marine invertebrates can also host *Symbiodinium* from mixed asemblages under specific, labortory conditions (Schoenberg & Trench 1980a-c; Colley & Trench 1983). However, over extended periods of time, these associations appear to be less stable. While limited, such flexibility in these symbioses may help hosts establish symbioses with new partners over evolutionary time.

In general, there have been more studies reporting specificity than flexibility in associations between *Symbiodinium* and their hosts. Other examples of this specificity include reports between Foraminifera-*Symbiodinium* (Garcia-Cuetos *et al.* 2005), *Madracis mirabilis*-*Symbiodinium* (Diekmann *et al.* 2003) and scleractinian-*Symbiodinium* (LeJeunesse 2004). Future studies will likely report on the existence and extent high specificity in these associations.

III. ESTABLISHMENT AND MAINTENANCE OF SYMBIOTIC RELATIONSHIP

Recognition between Aiptasia *and* Symbiodinium:

Trench *et al.* in 1981 was the first to propose the potential recognition mechanism of symbioses between marine invertebrates and *Symbiodinium* (Trench *et al.* 1981). This mechanism was then illustrated in jellyfish *Cassiopeia xamachana*, which involves selective phagocytosis and persistence of particular *Symbiodinium* lineages (Colley & Trench 1983). Additional work in other algal symbioses has demonstrated that surface molecules on symbiont cells are crucial factors for establishment of symbiotic relationships (Meints & Pardy 1980; Reisser *et al.* 1982). These surface molecules and their carbohydrate groups were subsequently indicated to be involved in cell recognition (Weis & Drickamer 1996). Recent studies on the *Aiptasia*-*Symbiodinium* symbiosis have demonstrated that glycoproteins on the algal cell wall play pivotal roles in the establishment of the association (Lin *et al.* 2000). More detailed information on the glycoprotein-like structure, amino acid sequence and mechanism of how glycoprotein works in the recognition process are needed since their exact roles are still unknown (Lin *et al.* 2000; Belda-Baillie *et al.* 2002).

***Maintenance of Symbiotic Relationship*:**

Within a host cell, *Symbiodinium* are enclosed individually in the symbiosome, a phagosome-derived organelle. This organella provides the algal symbiont protection from herbivores and access to essential inorganic nutrients for photosynthesis. These situations likely contribute to their apparent ecological dominance over other free-living unicellular photosynthetic algae in the tropical reef ecosystem. However, how does *Symbiodinium* survive phagocytosis (when they get in cells of their hosts) and take up essential nutrients across the phagosome membrane for growth and replication?

One possibility involves the use of particular proteins. For example, Rab (a member of the Ras superfamily of monomeric G proteins) family proteins have recently emerged as key regulators of intracellular vesicle trafficking during endocytosis and exocytosis, and several members of this family have been localized to distinct intracellular structures (Pfeffer 2001; Zerial & McBride 2001). Every step of intracellular vesicular transport is thought to be mediated by distinct sets of Rab proteins (Pfeffer 2001; Zerial & McBride 2001). For example, the Rab7 protein is a key regulator of the late endocytic pathway. It is located on late endosomes/lysosomes (Chavrier *et al.* 1990; Meresse *et al.* 1995; Vitelli *et al.* 1997) and regulates intracellular transport from early to late endosomes (Feng *et al.* 1995; Mukhopadhyay *et al.* 1997; Press *et al.* 1998) and from late endosomes to lysosomes (Meresse *et al.* 1995). On the other hand, Rab5 regulates the fusion between clathrin-coated vesicles and early endosomes (Bucci *et al.* 1992; Barbieri *et al.* 1994), and between early endosomes (Gorvel *et al.* 1991). A requirement of Rab5 activity for fusion of phagosomes with endosomes was demonstrated first by Stahl and

co-workers (Alvarez-Dominguez *et al.* 1996) and then by others (Funato *et al.* 1997; Roberts *et al.* 1999). Rab11 mediates endocytic recycling (Zerial & McBride 2001). It is mainly in charge of recycling the membrane of phagosomes to fuse with plasma membrane (Zerial & McBride 2001).

Rab proteins were reported to be crutial factors during establishment of invertebrate-*Symbiodinium* symbioses by Chen *et al.* (2003, 2004, 2005). They cloned and characterized Rab proteins from sea anemone *A. pulchella* harboring *Symbiodinium*. The *Aiptasia* homologue of Rab5, Rab7 and Rab11 (ApRab) proteins contain all the required Rab-specific signature motifs (Chen *et al.* 2003, 2004, 2005). Their research results supported that ApRab7 is located in late endocytic and phagocytic compartments and is able to promote their fusion. Most of the phagosomes containing live *Symbiodinium* did not contain detectable levels of ApRab7, while most phagosomes containing killed or photosynthesis-impaired symbionts were detected by ApRab7 staining. For ApRab5, immunofluorescence study showed that the majority of phagosomes containing live *Symbiodinium* were labeled with ApRab5, while those containing killed or photosynthesis-impaired algae were mostly negative for ApRab5 staining. In all cases, ApRab11 was rarely observed in the phagosomes containing healthy *Symbiodinium.* Overall, these data suggest that live algal symbionts actively retain ApRab5 but exclude ApRab7 and ApRab11 from their phagosomes. By that mechanism, symbionts establish and maintain an endosymbiotic relationship with their hosts and escape destruction by the cell they reside in (Chen *et al.* 2003, 2004, 2005).

IV. METABOLIC INTERCHANGES IN SYMBIOSIS

Metabolic interchanges in algal-invertebrate symbioses have been intensively studied. Generally, symbiont cells release photosynthetic carbon and may also recycle nitrogenous waste from the animal. Glycerol has been identified as a major extracellular product of *Symbiodinium* in numerous studies and the release of glycerol by the algal cells is thought to be stimulated by a "host factor" that might mediate translocation in the intact association (Muscatine 1967; Muscatine *et al.* 1972; Trench 1979; Cook 1983). However, pathways utilized by *Symbiodinium* to synthesize glycerol are unknown (Trench 1979). In addition to glycerol, a variety of other photosynthetic compounds, such as Alanine and a number of other organic acids, are also released by freshly isolated *Symbiodinium* (Trench 1971a), with only those of low molecular weight compounds being released (Trench 1971b). Trench (1993) reported that over half of the carbon fixed by *Symbiodinium* is transferred to the surrounding animal tissues and this appears to give the host enough energy to survive short periods of reduced food supply.

Recycling of nitrogenous waste between *Symbiodinium* and their host makes coral reefs to be flourishing in nutrient poor tropical oceans. Inorganic nitrogen, phosphorus and sulfur are the three most important inorganic nutrients of those recycled, and symbiotic invertebrates have the ability to assimilate dissolved inorganic nutrients from low concentrations in the environment and retain them (Muscatine 1980). Furthermore, only intact algae-invertebrate associations have the ability to uptake these nutrients since

no uptake is detected by aposymbionts, or hosts lacking their algal partners (Muscatine *et al.* 1979).

Studies have suggested that under high amino acid concentrations, uptake of excess animal-derived amino acids and resultant gluconeogenesis will overload the tricarboxylic acid cycle (TCA) cycle and cause metabolic intermediates to be excreted (Gates *et al.* 1995). This indicates that *Symbiodinium* releases photosynthates because their normal metabolism is perturbed by the uptake of animal-derived amino acids (Gates *et al.* 1995). However, Wang and Douglas (1997) found that taurine is a chemical signal that could mediate photosynthate release through a signal cascade. This cascade then switches carbon metabolism from an endogenous fate to export. Given this finding, further studies are needed to address mechanisms for releasing photosynthates.

V. BLEACHING: BREAKDOWN OF SYMBIOSIS

The phenomenon of "bleaching" was first described by Glynn in 1984 among corals in the Pacific Ocean. Bleaching, which typically involving the loss of symbiotic dinoflagellates from a host, is generally deleterious to corals and ultimately the reef community (reviewed by Brown 1997). Bleaching can either be temporary or results in a nearly permanent loss of *Symbiodinium*, the latter of which might lead to death of the host. If the symbionts are only partially lost, the host may recover.

***Causation of bleaching*:**

Bleaching can be induced via multiple means: extremes of temperature (heat shock and cold shock), low and/or high salinity, intense irradiance, prolonged darkness, heavy metals (especially copper and cadmium), pathogenic micro-organisms or a combination of these factors (reviewed by Hoegh-Guldberg 1999; Brown 2000). Among these, temperature is one of the most common reasons for bleaching. Recent large-scale bleaching events on the world's reefs have been attributed to elevated sea water temperature resulting from global warming, often combined with increased solar radiation (Stone *et al.* 1999; Walther *et al.* 2002).

***Adaptive Bleaching Hypothesis*:**

The Adaptive Bleaching Hypothesis (ABH) was first reported by Buddemeier and Fautin in 1993. This hypothesis assumed that 1) different types of *Symbiodinium* have

different responses to environmental parameters like salinity, irradiance and especially temperature; and, 2) bleached hosts might acquire *Symbiodinium* that vary in response to these parameters from the environment. From the time that the ABH was proposed, many studies have focused on testing these hypotheses. Kinzie *et al.* (2001) conducted experimental tests on assumptions underlying the ABH. They found Clade B *Symbiodinium* showed decreasing growth at higher temperature while a Clade C isolate showed increasing growth rate and the responses of Clade A isolates were variable. Additionally, bleached adult hosts can acquire algal symbionts with an apparently dose-dependent relationship between the concentration of *Symbiodinium* and the rate of establishment of the symbiosis (Kinzie *et al.* 2001). These results seem to lend support to the Adaptive Bleaching Hypothesis. However, many of those studies utilized culture *Symbiodinium*, which has been shown to be only a subset of the original population *in hospite* (Santos *et al.* 2001). Therefore, more studies are needed to address the intact symbiosis using natural populations of symbionts.

VI. TEMPERATE VS. TROPICAL SYMBIOSES

Sea anemones with symbiotic algae are distributed in both tropical and temperate oceans. The average biomass or *Symbiodinium* density in temperate anemones is lower than in tropical anemones (Davy *et al.* 1996). In spite of pronounced seasonal variation in light and temperature in temperate regions, *Symbiodinium* densities remain fairly constant on spatial and temporal scales and may even increase during winter conditions for temperate anemones (Dykens & Shick 1984; Bythell *et al.* 1997; Dingman 1998; Squire 2000). *Symbiodinium* densities in the scyphozoan *Cassiopea xamachana* from the Florida Keys were the same in winter and summer (Verde & MaCloskey 1998). However, *Symbiodinium* densities in tropical anemones appear to be more influenced to seasonal differences in light than those from temperate environments (Brown *et al.* 1999; Fitt *et al.* 2000). Additional comparative studies are suggested by Muller-Parker and Davy (2001) to further assess this point. Interestingly, the maximum photosynthetic rates of temperate and tropical *Symbiodinium* are similar (Muller-Parker 1984; Fitt *et al.* 1982), but photosynthetic efficiency in temperate anemones is less than those in tropical anemones due to lower irradiance in temperate regions. Because of the large reduction of light in winter, temperate anemone hosts receive obviously reduced carbon supplies from their *Symbiodinium* (Davy *et al.* 1996). Currently, no clear trends have been detected in the symbiont transmission modes and specificity among anemones in tropical vs. temperate seas. However, past work supports the capacity for symbiont uptake and

persistence in anemones is species-specific (Schoenberg & Trench 1980a-c; also see review by Muller-Parker & Davy 2001).

VII. SUMMARY

The *Aiptasia* -*Symbiodinium* symbiosis is now being proposed as a model system to investigate the endosymbiosis responsible for one of the most productive ecosystems in the world (Weis *et al.* 2008). More and more studies have indicated there is specificity between hosts and *Symbiodinium*. The molecules responsible for this specificity appear to be glycoproteins, although the exact properties of these molecules are still unknown. Rab proteins also appear to play an important role in the symbiosis, particularly in the process of endocytosis. Again, this area also needs more studies.

As a first step in further understanding the *Aiptasia* -*Symbiodinium* symbiosis as a model system, this master study evaluated the specificity and flexibility of the endosymbiosis between *Symbiodinium* and *Aiptasia*. Here, I focus on using a suite of molecular markers toward elucidating the population genetics of *Symbiodinium* from *Aiptasia* spp. in a worldwide scale.

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CHAPTER 2

HOST SPECIFICITY AND REGIONAL ENDEMICITY IN SYMBIOTIC DINOFLAGELLATES (*SYMBIODINIUM*, DINOPHYTA) ASSOCIATED WITH SEA ANEMENOES IN THE GENUS *AIPTASIA*

I. INTRODUCTION

Success of coral reef ecosystems is dependant upon mutualistic symbioses between cnidarians and endosymbiontic dinoflagellates in the genus *Symbiodinium* (Muscatine 1990). *Symbiodinium* spp. provide approximately 90% of their hosts' energetic needs through photosyntic products (Muscatine *et al.* 1981). Although these mutualisms are increasingly threatened globally (reviewed in Hoegh-Guldberg 1999), Cnidarians may alter their symbiotic associations in order to acclimatize to environmental change (Buddemeier & Fautin 1993). Therefore, understanding the diversity of *Symbiodinium* and the degree of specificity versus flexibility in host-symbiont associations is central to the investigation of these important relationships.

Although *Symbiodinium* was once considered to be a single species (Freudenthal 1962, Taylor 1974), a variety of biochemical, physiological, molecular genetic, and ecological approaches have demonstrated that *Symbiodinium* is, in fact, a diverse and heterogeneous group of dinoflagellates (reviewed in Trench 1993; Baker 2003; Coffroth & Santos 2005; Stat *et al.* 2006). To date, eight sub-generic clades of *Symbiodinium* have been described and named A, B, C (Rowan & Powers 1991a), D (Carlos *et al.* 1999), E, F (LaJeunesse & Trench 2000; LaJeunesse 2001), G and H (Pochon *et al.* 2001, 2006). Additionally, each of these sub-generic clades can be further divided into numerous "species", populations, or strains (reviewed in Coffroth & Santos 2005).

The majority of *Symbiodinium* diversity studies have been conducted at the level of clades or cp23S/ITS "types" (e.g., Rowan & Powers 1991a; Carlos *et al.* 1999; LaJeunesse & Trench 2000; LaJeunesse 2001; Pochon *et al.* 2001, 2006; van Oppen *et al.* 2001, 2005; Santos *et al.* 2003a). However, only a few studies have examined the population genetic structure of *Symbiodinium* spp. (i.e., Santos & Coffroth 2003; Santos *et al.* 2004; Kirk *et al.* 2005; Magalon *et al.* 2006; Carlon & Lippe 2008; Howells *et al.* 2009). Considering that populations are the fundamental units of evolution (Futuyma 2005), developing an understanding of the population genetic structure of *Symbiodinium* is essential to discussing how these symbionts and their hosts may respond to global climate change. To date, population level studies on *Symbiodinium* have been focusing on scleractinian, gorgonian and soft coral species. For example, while all *Symbiodinium* inhabiting the gorgonian *Pseudopterogorgia elisabethae* from various Bahamian reefs were cp23S genotype B184 (Santos *et al.* 2003a), microsatellite DNA data indicated significant population differentiation among these B184 *Symbiodinium* when compared between reefs (Santos & Coffroth 2003). In addition, microsatellite-based populations of *Symbiodinium* from the gorgonian *Gorgonia ventalina* were stable over time, regardless of temperature treatment or disease status (Kirk *et al.* 2005). In contrast to these studies, Magalon *et al.* (2006) reported two polymorphic *Symbiodinium* microsatellite loci that showed high levels of within host-colony diversity in the scleractinian *Pocillopora meandrina*. This pattern was interpreted as multiple symbiont genotypes occurring within most *P. meandrina* colonies. Overall these studies suggest that symbiosis between *Symbiodinium* and their host is generally specific at the population genetic level (with more complex situations possible in certain host species). Furthermore, in most cases, a

maximum of two clonal *Symbiodinium* populations of the same clade have been found within one host individual (Santos *et al.* 2004; Carlon & Lippe 2008). Although these previous studies are interesting, they are limited to local and regional geographical ranges; no *Symbiodinium* population genetic data is available across global scale. One potential study system to examine *Symbiodinium* population genetics at a global scale are sea anemones in the genus *Aiptasia*.

Aiptasia spp. have been proposed as model organisms for studying cnidarian-dinoflagellate endosymbiosis, as these anemones occur in subtropical to tropical waters throughout the world and can be maintained and manipulated in the laboratory (e.g., Weis *et al.* 2008). Previous *Aiptasia* studies focused on two abundant species, *A. pulchella* and *A. pallida,* which are geographically separated. *A. pulchella* is distributed across the Pacific Ocean, India Ocean and Red Sea, whereas *A. pallida* is distributed throughout the Atlantic Ocean and Caribbean (Oskar 1943, 1952; Cutress 1955). Although the majority of symbiotic invertebrates acquire *Symbiodinium* from the surrounding environment (i.e., horizontal transmission), *Aiptasia* typically transmit symbionts directly from parent to offspring (i.e., vertical transmission) during an asexual reproductive process known as pedal laceration (Schwarz *et al.* 2002). *Aiptasia* spp. predominantly harbour Clade B *Symbiodinium* throughout the world (Santos *et al.* 2003a; Savage *et al.* 2002; LaJeunesse 2002; LaJeunesse *et al.* 2004), suggesting host-symbiont specificity in this symbiosis. Interestingly, symbioses involving Clades A (either alone or with B) or Clade B has also been reported in *Aiptasia* from the Florida Keys, indicating the possibility of symbiotic flexibility (Santos *et al.* 2003a; Kinzie *et al.* 2001; Goulet *et al.* 2005) in this anemone genus.

Because *Aiptasia* spp. are globally distributed, this study system is suitable for examining how *Symbiodinium* populations are genetically structured across a broad geographic range. Based on the results of previous molecular and population genetic studies summarized above, I hypothesized that 1) *Symbiodinium* associated with *Aiptasia* would exhibit high specificity between host and symbiont at a global scale; 2) *Symbiodinium* populations from different geographic locations are highly structured and localized. In order to test these hypotheses, I characterized the variation and distribution of *Symbiodinium* associated with *Aiptasia* from locations across the world using a suite of approaches: restriction fragment length polymorphisms (RFLPs) of the small subunit ribosome RNA gene (18S rDNA), denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer 2 (ITS2) region of the rDNA, flanking regions from four microsatellite loci, and allelic variation at six microsatellite loci specific for *Symbiodinium* Clade B. My results indicate a high degree of host-symbiont specificity and remarkable population structure throughout most of the global range of *Aiptasia*.

II. MATERIALS AND METHODS

***Collection of the anemone samples,* Symbiodinium *cultures and DNA extractions*:**

Individual *Aiptasia* spp. anemones (n=356) were sampled from 18 localities in eight major geographic areas throughout the world (Fig. 2.1). Individuals were scrapped from the substrate and immediately fixed in 100% ethanol or acetone for molecular analyses. Additionally, 25 *Symbiodinium* Clade B isoclonal cultures (see below) were selected to serve as control DNA for the various analyses conducted in this study (culture details can be found in Table 2.1 and 2.2). *Symbiodinium* cultures were maintained in f/2 media (Guillard & Ryther 1962) at a constant temperature of 29 ºC, irradiance level ~80 µM photons·$m^{-2}s^{-1}$, and a photoperiod of 12:12-h light-dark cycle prior to molecular analysis (Santos *et al.* 2001). Genomic DNA from the anemone samples, which included host and symbiont nucleic acids, as well as from *Symbiodinium* cultures, were extracted using 2× CTAB according to methods of Coffroth *et al.* (1992).

***18S rDNA RFLP*:**

For all *Aiptasia* samples (n=356), *Symbiodinium* small subunit (18S) ribosome DNA was amplified via the polymerase chain reaction (PCR) using the primers ss5 and ss3z (Table 2.3) according to the protocol of Rowan and Powers (1991b). Amplification products were verified by 1% sodium borate (SB) agarose gel electrophoresis (Brody & Kern 2004). Successful amplifications were digested for 3.0 hr with 0.12 U/µL of *Taq*

I (Fermentas Hanover MD, USA) and electrophoresed on 1% SB agarose gels to generate RFLP profiles, according to the protocol of Rowan and Powers (1991b). *Symbiodinium* clades were identified by comparison to restriction digests of cultured standards from Clades A, B and C.

***ITS2-rDNA denaturing gradient gel electrophoresis*:**

The internal transcribed spacer 2 region (ITS 2) of nuclear ribosomal DNA was used to further discriminate molecular "types" of *Symbiodinium* on a representative subset (n=16) of the DNA extracts from field collected populations (sample details provided in Table 2.4) (LaJeunesse 2001, 2002). The ITS2 region was amplified from these DNA extracts for denaturing-gradient gel electrophoresis (DGGE) using primers "ITS 2 clamp" (5' - CGCCCGCCGC GCCCCGCGCC CGTCCCGCCG CCCCCGCCCG GGATCCATAT GCTTAAGTTC AGCGGGT - 3') and "ITSintfor 2" (5' - GAATTGCAGA ACTCCGTG - 3') (LaJeunesse & Trench 2000). PCR was performed under the following conditions: 0.5−1.0 µL DNA, 2.5 µL 10× PCR buffer (Eppendorf), 2.0 µL 25 mM $Mg(OAC)_2$, 2.5 µL 2 mM dNTPs, 0.25 µL 10 µM "ITSintfor2", 0.5 µL 10 µM "ITS2CLAMP", 0.15 µL 5 U/µL *Taq* DNA polymerase, and distilled water to a total volume of 25 µL per reaction. Amplification used the following protocol: initial denaturation 94 ºC, 3 min; 40 cycles of denaturation 94 ºC, 40 s; variable annealing temperature (see below for "touchdown" conditions), 40 s; extension 72 ºC, 30 s; final extension 72 ºC, 10 min. For annealing temperatures, "touchdown" conditions 10 ºC above the final annealing temperature of 52 ºC was used to ensure PCR specificity. The annealing temperature was then decreased by 0.5 ºC after each of the first 20 cycles.

Once the annealing temperature reached 52 ºC, it was maintained at that setting for another 20 cycles.

All PCR amplifications were verified by 1% SB agarose gel electrophoresis prior to DGGE analysis. DGGE gels were poured (following manufactorer's instructions) using 8% polyacrylamide (37.5:1 acrylamide/bisacrylamide ratio), approximately 20 cm long plates, 0.75 mm spacers, and a 45-80% denaturing gradient (100% denaturant contains 7 mol L^{-1} urea and 40% deionized formamide). Prior to loading samples on the DGGE gel, excess denaturant was purged from wells using a micropipette. 20 µL of each PCR reaction was added to 10 µL of xylene cyanol loading dye (pH 7.0) and a total of 10 µl of the combined product was loaded onto each DGGE gel. Runs were performed at 60 °C. The temperature within the buffer chamber was checked prior, during, and after the run at multiple positions to exclude biases caused by incomplete heating. Gels were electrophoresed at 150 V for 10 h (1500 Vh) on a C.B.S. Scientific™ DGGE-1001 model apparatus. All DGGE gels were stained with SYBR Green (Molecular Probes, 10,000× diluted in 1× TAE) for 20 min and photographed under UV light using a digital camera fitted with a SYBR Green filter. To identify symbiont types, the DGGE fingerprint for each sample was compared to ITS2 standards from clonal *Symbiodinium* cultures.

***Sequencing of microsatellite flanking regions*:**

The microsatellite flanking regions from five out of six loci (see below) were sequenced from the *Symbiodinium* of representative *Aiptasia* spp. samples (n=13) and cultures (n=11) (details on these cultures provided in Table 2.2; culture selection was based on Santos *et al.* [2004]). Santos *et al.* (2004) reported that the flanking region

sequence of locus CA6.38 was not variable between *Symbiodinium* from two phylogenetically divergent hosts, therefore, sequence analyses were limited to the flanking regions of the remaining five loci (i.e., CA4.86, Si4, Si8, Si15, and Si34). Details regarding the PCR primer sequences, annealing temperatures, $MgCl_2$ concentrations and references are presented in Table 2.3. PCR reactions for these five loci were performed in 10 μL volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 200 μM dNTP, 0.5 U *Taq* polymerase, 0.60 μM forward primer and reverse primer and 10 ng of template DNA. Thermocycling conditions were as follows: 2 min at 94 °C for initial denaturing, 32−40 cycles of 94 °C for 30 s, a variable annealing temperature (see Table 2.3) for 30 s and 72 °C for 30 s, followed by 5 min at 72 °C for final extension. Amplifications were purified using Montage' PCR Filter Units (Millipore) and DNA sequenced using Big-Dye Terminators and read on a PRISM 3100 Genetic Analyzer (Applied Biosystems). Raw sequence data were assembled using SEQUENCHER 4.7 (Gene Codes) and finished sequences were aligned automatically using CLUSTAL_X (Thompson *et al.* 1997) or manually using SE-AL VERSION 2.0a11 (available at http://tree.bio.ed.ac.uk/software/seal/). All sequences have been deposited in GenBank (Accession nos. XXXXXX-XXXXXX; Table 2.2. Chapter 2 will be submitted to *Molecular Ecology*. Note that accession nos. will be added upon acceptance the manuscript).

Data from the flanking regions of several microsatellite loci were excluded from the analysis for the following reasons. First, attempts to sequence the Si4 locus from numerous *Symbiodinium* samples were unsuccessful. Furthermore, locus Si8 possessed no variation in any of the examined *Symbiodinium* cultures or *Symbiodinium* sample from

Aiptasia. Finally, all variable sites in the flanking regions of locus Si34 were four base pair indels. This suggests that flanking region variability is likely due to differences in the number of microsatellite repeats, indicative of non-phylogenetically informative allelic variation. Thus, loci Si4, Si8, and Si34 were excluded and phylogenetic analyses were conducted based on flanking region sequences from loci CA4.86 and Si15.

Maximum-parsimony (MP) analyses were performed in PAUP4.0b10 (Swofford 2002), with ten additional replicates using stepwise addition to obtain starting trees and Tree-Bisection-Reconnection (TBR) to swap branches. Maximum-likelihood analyses were also performed in PAUP4.0b10 with a GTR+Γ+I model, as recommended by MODELTEST v3.7 based on the Akaike information criterion (AIC) (Posada & Crandall 1998). Heuristic searches were run with ten random-taxon replicates using TBR swapping. All model parameters used fixed values as recommended by MODELTEST v3.7. Branch supports in MP and ML trees were estimated by bootstrap analysis of 1000 replicates in PAUP4.0b10.

***Microsatellite size fragment analysis*:**

Six *Symbiodinium* spp. Clade B microsatellite loci (loci CA4.86 and CA6.38 [Santos & Coffroth 2003] as well as loci Si4, Si8, Si15 and Si34 [Pettay & LaJeunesse 2007]) were used to quantify population genetic differences in 234 *Aiptasia* samples harbouring Clade B *Symbiodinium* in 17 of the 18 sampling locations (*Aiptasia* population WK1 from Florida only contains *Symbiodinium* Clade A). Furthermore, all six microsatellite loci were also screened against 16 clonal *Symbiodinium* cultures, each of which originated from a single cell (details on the cultures can be found in Table 2.1).

Cultures were selected based on Santos and Coffroth (2003) in order to provide DNA from a single genetic entity to serve as control DNA. Finally, to test for background populations of *Symbiodinium* Clade B in Florida *Aiptasia*, the six microsatellite loci were tested on 50 random selected *Aiptasia* individuals from Florida whose RFLP analysis implied the sole presence of *Symbiodinium* Clade A in that host individual.

Sequences of PCR primers, annealing temperatures, and $MgCl_2$ concentrations used in these analyses are presented in Table 2.3. PCR reactions for these six loci were performed in 10 μL volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 200 μM dNTP, 0.5 U *Taq* polymerase, 0.15 μM WellRED D2, D3 or D4 fluorescent-labeled M-13 primer (Sigma-Proligo), 0.30 μM forward primer, 0.15 μM reverse primer and 10 ng of template DNA. Nineteen nucleotides (5'-CACGACGTTG TAAAACGAC-3') were added to the 5' end of reverse primers to allow the incorporation of the M13 fluorescent-labeled primer into PCR products. In all other cases, amplification conditions were identical to those described above for microsatellite flanking regions.

Microsatellite allele size determinations were performed on CEQ-8000 Genetic Analysis System (Beckman Coulter) under the default fragment analysis parameters. Each well contained 4 μL of PCR product, 20 μL sample loading solution (Beckman Coulter) and 0.5 μL 400 base pairs (bp) DNA size ladder (Beckman Coulter). Alleles were scored according to their true allele size by excluding the nineteen 5'-nucleotides of the fluorescent-labled M13 primers.

Genotypes were constructed for the *Symbiodinium* Clade B population of each *Aiptasia* individual using the recovered allele sizes from each of the six microsatellite

loci. These genotypes were tested for linkage equilibrium using the computer program GENETIC DATA ANALYSIS (Lewis & Zaykin 2001). According to the total number of alleles observed for a locus from all samples, allelic frequencies and allelic diversities were calculated separately for each population, as well as across all populations, using the program TOOL FOR POPULATION GENETIC ANALYSIS (TFPGA) V1.3 (Miller 1997). F_{ST} and R_{ST}, which assume infinite-alleles (IAM) and stepwise-mutation (SMM) evolutionary models, respectively, were estimated by using θ and ρ_{ST} respectively under FSTAT V2.9.3.2 (Goudet 2001). The standardization approach of Goodman (1997) was used to make a six-locus measure of R_{ST}. Pairwise tests for *Symbiodinium* sp. clade B population differentiation were also conducted by randomizing genotypes between pairs of populations using FSTAT V2.9.3.2. Multiple simultaneous comparisons were corrected by using sequential Bonferroni corrections (Rice 1989). To graphically describe the relationship between *Symbiodinium* clade B populations in *Aiptasia* anemones, an unweighted pair group method using arithmetic averages (UPGMA) dendrogram was constructed using Nei's minimum genetic distance (Nei 1972) in TFPGA.

III. RESULTS

Symbiodinium *clades associated with* Aiptasia *spp.*:

Symbiodinium 18S rDNA RFLP analysis of *Aiptasia* spp. from 18 locations throughout the world implies that *Symbiodinium* Clade B is widely associated with *Aiptasia* spp. around the globe, including in the Pacific Ocean (i.e., Hawai'i, Australia, Japan, and Mexico), the Indian Ocean (Thailand), the Red Sea, and the western Atlantic Ocean (Bermuda, with the exception of the Florida Keys, see below) (Table 2.4). This result suggested that the symbiosis between *Symbiodinium* and *Aiptasia* host is generally specific.

More complex patterns of symbiotic associations occurred in *Aiptasia* from the Florida Keys (Table 2.4). In contrast to *Aiptasia* spp. from the rest of the world, symbiosis with *Symbiodinium* Clade A was most common in Florida. Furthermore, mixed symbioses of *Symbiodinium* Clades A and B, or more rarely Clades A and C, were also found (Table 2.4); no such mixed symbioses were observed in regions other than the Florida Keys.

Symbiodinium *ITS2-DGGE profiling*:

To further discriminate molecular "types" of *Symbiodinium* within Clade B, PCR-DGGE analysis of the internal transcribed spacer 2 region (ITS2) of nuclear ribosomal RNA genes (LaJeunesse & Trench 2000, LaJeunesse 2002) was employed on a

subset of samples (Table 2.4). All samples examined (n=18) were detected as harbouring only ITS2 type B1 (*sensu* LaJeunesse 2001). Therefore, for ITS2, the symbiont assemblage did not vary among anemones hosting *Symbiodinium* Clade B , at least within the detection limits of DGGE (see Thornhill *et al.* 2006b)

Symbiodinium *microsatellite flanking regions*:

Flanking regions from the *Symbiodinium* Clade B microsatellite loci CA4.86 and Si15 were sequenced from samples of *Aiptasia* spp. throughout the world (n=13). From 359 base pairs recovered from the regions flanking these two loci, no variation (either base pair substitutions or indels) was found, regardless of region sampled. Thus, unique flanking region phylotypes of these two loci from *Symbiodinium* Clade B associates with *Aiptasia* anemones across the entire global range of this host, again indicating specificity in this symbiosis.

In order to confirm that variability exists in the flanking region sequences of other ITS2 "type" B1 *Symbiodinium*, 11 clonal *Symbiodinium* Clade B cultures (of ITS2 "type" B1) were selected for sequencing of microsatellite flanking region loci CA4.86 and Si15 (see table 2.2). The combined dataset consisted of 359 nucleotide positions, 100% of which could be unambiguously aligned. Considerable variation was encountered between the cultured isolates. Specifically, 25 characters (7.0%) were variable, among which 24 (6.7%) were parsimony informative. Consequently, the lack of variation in flanking regions from *Symbiodinium* Clade B hosted by *Aiptasia* cannot be attributed to a lack of potential for polymorphisms in these molecular markers.

To infer phylogenetic relationships among the Clade B *Symbiodinium* of *Aiptasia* and the 11 cutures, an unrooted phylogenetic tree was reconstructed by using MP and ML methods (Fig. 2.2). All *Symbiodinium* Clade B from *Aiptasia* (including field samples and culture FLAp2, the latter of which was isolated from Florida *Aiptasia*) clustered into a single group, with no nucleotide variation between samples. In contrast, the 11 *Symbiodinium* cultured were distributed among six groups. Interestingly, *Symbiodinium* from *Aiptasia* were most closely related to *Symbiodinium* cultured from the gorgonians *Plexaura kuna*, *Gorgonia ventalina* and *Pseudoplexaura porosa*.

***Population genetic structure of* Symbiodinium *associated with Aiptasia* spp.:**

Analyses of six microsatellite loci (i.e., CA4.86, CA6.38, Si4, Si8, Si15, and Si34) from 16 clonal *Symbiodinium* cultures detected only a single allele from each clonal culture (Table 2.1). This is consistent with previous work that reported *Symbiodinium* spp. are haploid (Santos & Coffroth 2003). Therefore, based on results from clonal cultures, it is reasonable to infer that if a single clonal line is harboured by an *Aiptasia* individual, only one allele would be identified per *Symbiodinium* Clade B population *in hospite*.

The six microsatellite loci were examined from a total of 234 *Aiptasia* individuals harbouring *Symbiodinium* Clade B (Table 2.5, detailed results see appendix table). For most *Aiptasia* samples, a single allele was recovered from each *Symbiodinium* Clade B populations (n=180, 76.9% of the colonies). While significant linkage disequilibrium was found in population CK2 (CA6.38/Si15, CA6.38/Si34, Si15/Si34) from the Florida Keys and population RS (CA6.38/Si8, CA6.38/Si15, Si8/Si15) from the Red Sea, this pattern was not found in these loci across all other sampling locations. Thus, I conclude that

these loci are sorting independent of each other. Although locus CA4.86 was found to be monomorphic, regardless of the population sampled, allelic variation was identified in the remaining five loci (Table 2.5). For instance, four different alleles occurred at locus CA6.38, with the frequency of alleles varying significantly by region. The most polymorphic locus was Si34, which included six different alleles. Allele diversity, estimated by heterozygosity (*H*), for each locus ranged from 0 to 0.5. The average *H* for the six loci across all populations was 0.410, indicating high levels of genetic variation in *Symbiodinium* Clade B associated with *Aiptasia* spp. (Table 2.6). Strong subdivision in the *Symbiodinium* Clade B populations of *Aiptasia* spp. was indicated from estimates of population structure estimating by F_{ST} and ρ_{ST} values (Table 2.7).

Symbiodinium Clade B populations associated with *Aiptasia* differed significantly from one another across their global range (Table 2.5). A total of 32 unique genotypes were identified from the 17 geographic localities. Populations from Hawai'i, Florida, Bermuda, and the Red Sea were comprised of multiple genotypes, whereas populations from Mexico, Japan, Thailand, and Australia possessed a single genotype. In most cases, *Aiptasia* harboured site-specific *Symbiodinium* genotypes, indicating that these *Symbiodinium* populations are regionally structured. For example, all *Symbiodinium* genotypes in *Aiptasia* populations from Australia, Bermuda, and Thailand were unique when compared to all other localities. Most populations from the Florida Keys, Red Sea, and Hawai'i were also unique compared to other localities.

Similar results were inferred from the statistical pairwise test (Table 2.8). Of the 136 potential pairwise combinations, 77 were significant (71.3%). Among non-significant comparisons, 97.9% involved populations from Hawai'i, Mexico and Florida. The

remaining non-significant comparisons were between populations in the Red Sea (i.e., RS and EA). In general, results from the pairwise comparisons also supported significant population genetic structure occurred among *Symbiodinium* Clade B populations in *Aiptasia*. Furthermore, the UPGMA dendrogram identified sites sharing the same genotype as grouping together, while geographically proximate sites (i.e., populations RS and EA from Red Sea, populations CK and WK from Florida) also clustered closely together (Fig. 2.3).

Interestingly, the *Symbiodinium* Clade B populations from *Aiptasia* collected at CI1 and WA in Hawai'i as well as at sites in western Mexico and all populations from Japan shared the same genotype (Table 2.5, Fig. 2.3). Along with this, one algal genotype was shared between population CK2 in Florida and population RS in the Red Sea. Thus, while little gene flow at the population level was observed between most geographic regions, some genotypes had wide distributions.

***Occurance of mixed* Symbiodinium *populations*:**

In some cases (n=54 out of 234, 23.1% of hosts), two alleles were recovered in four out of the six microsatellite loci (including CA6.38, Si8, Si15, and Si34). Because *Symbiodinium* is likely haploid (Santos & Coffroth 2003), multiple alleles are indicative of mixed symbiont populations. Regionally, multiple alleles occurred in six of the seventeen sampling localities. For instance, most of the *Aiptasia* from Coconut Island in Hawai'i (21 of 23 from population CI1; 15 of 21 from population CI2) harboured various combinations of two alleles. Two alleles at locus Si34 was also recovered from other *Aiptasia* populations in Hawai'i (e.g., WA). In other localities, the recovery of two alleles

occurred in two of 24 individuals in population CK2 from Florida at loci CA6.38 and Si15, two of 14 individuals in BE population from Bermuda, and seven of 18 individuals in population RS from the Red Sea at loci CA6.38, Si8 and Si15 (Table 2.5). In all cases, a maximum of only two alleles per microsatellite locus was found in a single host individual.

All six microsatellite loci were also tested on 50 random selected *Aiptasia* individuals from Florida whose RFLP analysis indicated the presence of only *Symbiodinium* Clade A. From these, eighteen out of 50 samples (36%) (i.e., 0 of 9 in population WK1, 3 of 13 in population WK2, 6 of 12 in population CK1, and 9 of 10 in population CK2) were detected as also harbouring *Symbiodinium* Clade B (see appendix table). This pattern suggests a frequent incidence of low density or 'cryptic' *Symbiodinium* Clade B (i.e., occurring at a density below the detection limits of 18S rDNA RFLP) in *Aiptasia* anemones from the Florida Keys.

IV. DISCUSSION

Herein, I examined the genetic diversity of *Symbiodinium* from 18 localities across the global range of *Aiptasia* spp. Results from RFLP analysis of 18S rDNA, PCR-DGGE profiling of the ITS2 rDNA, and sequencing of two microsatellite loci flanking regions all indicated a high degree of specificity between *Symbiodinium* and *Aiptasia* spp. Furthermore, microsatellite genotypes from *Symbiodinium* Clade B populations demonstrated that most are regionally specific, with little gene flow between sites or regions. The specificity and regional endemicity found in this study are of particular interest because *Aiptasia* spp. are widely distributed hosts which have been recommended as a model system to study invertebrate-dinoflagellate symbioses (e.g., Weis *et al.* 2008).

***Specificity between* Symbiodinium *and* Aiptasia *spp.*:**

The ability to host multiple and differing genetic and physiological lineages of symbionts, known as symbiotic flexibility, has been hypothesized to be an important adaptation in many symbiotic cnidarians (Buddemeier & Fautin 1993). It is thought that reef-building corals, anemones, and other symbiotic cnidarians that can host several different *Symbiodinium* spp. may be able to acclimatize to changing environmental conditions through a change in their complement of symbionts. Thus, symbiotic change should be particularly pronounced during stress events that result in a decrease in

Symbiodinium density, pigment concentration, or both (a phenomenon known as bleaching; Brown 1987, 1997; Glynn 1991, 1996; Buddemeier & Fautin 1993; Brown *et al.* 1996; Fitt *et al.* 2001). As a result, the degree of flexibility versus stability in the cnidarian-dinoflagellate symbiotic relationship has significant implications for the future success of coral reef environments, particularly in the context of global climate change that has contributed to increasing the frequency and severity of bleaching (Buddemeier & Fautin 1993; Glynn 1991, 1996; Brown *et al.* 1996).

In the present study, results based on RFLP of the 18S rDNA gene, DGGE of the ITS2 region, and sequencing of the flanking regions surrounding microsatellite loci indicate a high degree of specificity between a single phylotype of *Symbiodinium* Clade B and *Aiptasia* spp. throughout the world. In fact, no other *Symbiodinium* clades or phylotypes were found at any of the depths, habitats, or regions sampled, with the sole exception of the Florida Keys. Thus, this specific relationship suggests that when stress events occur, most *Aiptasia* spp. will not be able to acquire alternative *Symbiodinium* phylotypes through either exogenous 'switching' or endogenous 'shuffling' of symbiont species (Baker 2003).

Symbiotic stability and specificity has been reported in a number of previous *Symbiodinium* diversity studies (e.g., Goulet & Coffroth 2003; Santos *et al.* 2004; Thornhill *et al.* 2006a,b). Indeed, a meta-analysis of available *Symbiodinium* diversity data suggests that most hosts (~75% of host species) only harbour a single symbiont clade throughout their entire range (Goulet 2006, 2007; but see Baker & Romanski 2007). Data from this study suggest that most *Aiptasia* spp. also harbour a single clade or phylotype of symbiont, again with the notable exception of hosts from the Florida Keys.

***Global biogeography of Clade B* Symbiodinium *populations associated with* Aiptasia:**

Identical cp23S-rDNA and ITS2-rDNA "types" have been recovered from Indo-Pacific and Atlantic oceanographic basins (e.g., LaJeunesse 2001, 2002, 2005; Santos *et al.* 2001, 2002; LaJeunesse *et al.* 2003, 2004), suggesting widespread dispersal of certain *Symbiodinium* spp. over evolutionary time. My results, based on 18S-rDNA RFLP, ITS2-rDNA DGGE, and microsatellite flanking region sequences, also support long-term dispersal capacity of certain *Symbiodinium* lineages, in this case a specific sub-type of ITS2 "type" B1 (*sensu* LaJeunesse 2001). Despite this, data from six microsatellite loci indicate that *Aiptasia* spp. in most localities harboured regionally endemic *Symbiodinium* Clade B genotypes and populations. Strong population structure was observed across local (e.g., ~30 km between populations CK1 and CK2 in Florida), regional (e.g., ~1,800 km between populations in Florida versus Bermuda), and global (e.g., >14,000 km between Florida and Okinawa, Japan) geographic scales.

Here, significant population structure detected across local to global geographic scales indicates that *Symbiodinium* associated with *Aiptasia* likely have low dispersal capacities over evolutionary time scales (Santos *et al.* 2003b). If this is the case, a lack of symbiont dispersal capacity could pose a considerable impediment to the future success of coral reef ecosystems. Specifically, symbiotic cnidarians likely experience significant *Symbiodinium* population bottlenecks following bleaching events due to the expulsion or death of symbionts. If *Symbiodinium* populations are unable to disperse widely over ecological time scales to replace those lost due to bleaching, the resulting loss of genetic diversity may further exacerbate the damage bleaching events cause to coral reef communities. Therefore, *Symbiodinium* population connectivity should be investigated in

other symbiotic hosts, particularly reef-building corals, to better determine the dispersal and recovery capacity following severe bleaching events.

In contrast to the genetic structure observed across most regions, *Aiptasia* populations from some sites in Hawai'i, Mexico, and Japan shared the same *Symbiodinium* genotype. One potential reason for this shared genotype is oceanic currents driving gene flow between widely separated Pacific locations. Previous studies also proposed that currents affect the population structure of *Symbiodinium* across spatial scales. For instance, Santos *et al.* (2003b) suggested that the similarity of *Symbiodinium* populations in the gorgonian *P. elisabethae* among Bahamas islands was due to currents and tidal flow. Magalon *et al.* (2006) found a significant correlation between the *Pocillopora meandrina* symbiont F_{ST} values and distance matrices, suggesting ocean currents are likely driving population structure in *Symbiodinium* from the Society Archipelago. In the current study, *Aiptasia* populations from Hawai'i, Mexico and Japan may experience gene flow among these Pacific localities. However, genotype sharing did not occur in most of the sampling locations. Given the large geographic distances and potential physical oceanographic barriers (i.e., the East Pacific Barrier; Ekman 1953) between Hawai'i, Mexico and Japan, the possibility of gene flow among those localities may seem unlikely. Alternatively, the observed connectivity may be due to transport via ballast water or some other anthropogenic mechanism rather than natural dispersal. These two hypothesis could possibly also explain the shared genotype between population CK2 in Florida and population RS in the Red Sea.

***Mixed symbiont populations within host individuals*:**

Occurrence of multiple *Symbiodinium* "types" within host individuals has been detected in a number of host species (Goulet 2006 and references within). In previous investigations of *Aiptasia*, up to two different *Symbiodinium* clades (i.e., Clades A and B or Clades A and C) were detected to be simultaneously associated with *Aiptasia* from Florida (e.g., Santos *et al.* 2001, 2003a; Goulet *et al.* 2005). These previous findings are also supported by data from the present study for *Aiptasia* spp. in Florida. However, *Aiptasia* from other regions of the world appeared to be considerably less flexible in their symbioses. One reason for this might be that host mutations and subsequent selective forces on *Aiptasia* in Florida have produced a host species more capable of symbiont change (whether 'switching' or 'shuffling') than those from elsewhere. Symbiotic flexibility in Florida *Aiptasia* may be physiologically advantageous to the host, particularly in response to changing environmental conditions, by enabling changes in the community of symbiotic dinoflagellates (see Buddemeier & Fautin 1993). For *Aiptasia* spp. from other regions of the world, the stable symbiosis observed here may make predictions of performance under stressful conditions less optimistic. However, detailed studies of the performance of various host-symbiont combinations under stressful temperature and light conditions are necessary to validate this conjecture.

Another question that arises from the biogeographic patterns of symbiotic associations in *Aiptasia* spp. is why symbioses in animals from Florida are so different with those from all other locations. One explanation is that *Aiptasia* from Florida are genetically distinct relatively to those from the rest of the world. Since *Aiptasia*'s original description (Gosse 1858), the taxonomy of this genus has undergone considerable

revision (see Daly *et al.* 2003). While the current paradigm of an Atlantic species (*A. pallida*) and an Indo-Pacific species (*A. puchella*) has dominated the recent literature, no molecular genetic studies have been conducted to validate this situation. Consequently, future studies focused on the molecular genetics of the host anemones are warranted. I have preliminarily investigated this topic using inter simple sequence repeats (ISSR) in an attempt to determine the genetic structure of *Aiptasia* from across the world (see conclusions).

Most studies on mixed *Symbiodinium* communities within a host have been conducted using molecular genetic markers that measure *Symbiodinium* diversity at the level of sub-generic clade, phylotype, or approximate "species" (e.g., Coffroth *et al.* 2001; van Oppen *et al.* 2001, 2005; LaJeunesse 2002; Goulet 2006; Thornhill *et al.* 2006a,b; Meiog *et al.* 2007). Few studies have focused on mixed symbiosis using finer-scale population level markers. In my study, only a single ITS2/flanking region phylotype of *Symbiodinium* Clade B was found associated with most *Aiptasia* spp., indicating specificity at higher levels. Despite this, up to two *Symbiodinium* Clade B microsatellite genotypes, indicating two different clonal populations, were found to coexist in a single *Aiptasia* individual. In one instance, a single *Aiptasia* anemone from Florida associated with two different *Symbiodinium* Clade B genotypes as well as (at least) one member of *Symbiodinium* Clade A. Therefore, up to three distinct symbiont populations (two Clade B populations and one or more Clade A populations) were found to simultaneously occur within an individual *Aiptasia* host. This maximum of two populations from a single sub-generic clade being found within an individual host is a finding supported by several previous studies (i.e., Santos *et al.* 2004; Carlon & Lippe 2008; but see Magalon *et al.*

2006). Using *Symbiodinium* microsatellite flanking regions, Santos *et al.* (2004) found that different symbiont "types" were each specific to different Caribbean octocoral species, with a maximum of two clonal symbiont lineages detected within a single host colony. Similar results were also found in the stony coral *Favia fragum*, where no more than two populations of Clade B *Symbiodinium* were detected within a single colony (Carlon & Lippe 2008). This apparent limit of two populations per host may be driven by competition interactions between symbionts, where slower growing strains are displaced by competitive dominants (e.g., Fitt 1985).

Cryptic level background* Symbiodinium *Clade B in* Aiptasia *from Florida:

Low levels *Symbiodinium* Clade B were detected in 18 out of 50 Florida *Aiptasia* individuals whose RFLP profile indicated only the presence of Clade A. These 'cryptic' or background populations of symbionts at low density in *Aiptasia* are analogous to a recent study that reported background levels of *Symbiodinium* Clade D in several symbiotic corals (Mieog *et al.* 2007). Given the highly structured populations in *Symbiodinium* associated with *Aiptasia*, these 'cryptic' symbionts may represent alternative symbiotic combinations via 'shuffling' (*sensu* Baker 2003). However, the presence of low-density symbionts alone provides no indication of the role these symbionts play physiologically in *Aiptasia*. Further study of these background *Symbiodinium* populations is necessary in order to elucidate the significance in physiological contributions to the host.

V. CONCLUSIONS

Here, it was hypothesized that symbioses between *Aiptasia* and *Symbiodinium* would be highly specific and exhibit strong population structure between different geographic locations. My results were generally consistent with these hypotheses. With the exception of Florida, *Aiptasia* spp. associated with a single phylotype of *Symbiodinium* throughout the global range of this host. Analyses of six microsatellite loci further suggested that *Symbiodinium* populations are highly endemic, with the exception of potential population connectivity between sites across the Pacific Ocean (Japan, Hawai'i, and Mexico). The population genetic structure, lack of gene flow, and symbiotic stability observed here has important implications. Future population genetic studies should build upon this work in other species of symbiotic cnidarians, particularly scleractinian corals that form the tropic and structural framework for coral reefs. The generality of the patterns reported here have important considerations for the persistence and successful management of coral reefs in the future.

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SUMMARY

Marine invertebrates and their symbiotic dinoflagellates in the genus *Symbiodinium* have been intensively studied in recent years. However, the degree of specificity and flexibility between partners remains unclear. In this master thesis, a comprehensive review of the symbiosis between *Symbiodinium* and *Aiptasia* was conducted. This work focused on the revealing of population genetics of the symbionts. Specifically, restriction fragment length polymorphism (RFLP) analyses were first utilized to quantify the diversity in symbiont populations from 356 *Aiptasia* individules that were collected from 18 localities worldwide. *Aiptasia* from the Florida Keys were found to host either *Symbiodinium* Clades A, B or mixtures of both A and B simultaneously while *Aiptasia* from all other locations harbored Clade B only. To quantify fine-scale population structure and genetic differences among the symbiont populations, six microsatellite loci specific for *Symbiodinium* Clade B were utilized on 326 individual *Aiptasia*. Strong population structure in Clade B populations was observed since most genotypes were unique to a specific locality. However, no sequence variation was observed in the flanking regions of these loci, suggesting an identical *Symbiodinium* Clade B phylotype associates with *Aiptasia* on a worldwide scale, which implies high specificity in this invertebrate-algal symbiosis. Additionally, I found that 18 out of 50 (36%) Florida *Aiptasia* thought to harbor only Clade A by RFLP analyses also possessed low levels of Clade B symbionts when examined by microsatellite analyses, suggesting

background symbiont populations of a host may escape detection depending on the utilized technique.

Coinciding with the distinction of *Symbiodinium* between Florida and all other locations, preliminary data (using inter simple sequence repeats, ISSR, techniques on nuclear sequences) focusing on the population genetics of the host, *Aiptasia* spp., suggest that Florida *Aiptasia* are genetically distinct from all other localities, implying a high specificity of the symbiosis between *Symbiodinium* and *Aiptasia*. Additionally, the genectic difference of *Aiptasia* from Florida and other localities indicates that this genus is comprised of two "genetic" species. Notably, the distribution of the "genetic" species does not coincide with the range of the morphologically described species *A. pulchella* (Pacific and Indian Oceans and Red Sea) and *A. pallida* (Atlantic Ocean and Caribbean Sea). For this reason, further studies are needed using additional molecular markers to investigate the population structure of the host, *Aiptasia*, which will be important in better understanding the specificity and flexibility of this cnidrian-*Symbiodinium* endosymbiosis. Generating better ISSR markers for quantifying population structure seems time-consuming since optimizing ISSR reaction conditions, cloning and sequencing target fragments, as well as selecting appropriate fragments are all not easily to be done. Compared with ISSR, microsatellite markers may provide more informative population structures although generating microsatellite markers needs considerable efforts as well.

TABLES

Table 2.1. Information on *Symbiodinium* cultures used in analyses of the six microsatellites specific to Clade B. The host from which the culture was isolated, location of isolation, and microsatellite analysis results are included.

Culture name	Host organism	Location	CA4.86	CA6.38	Si4	Si8	Si15	Si34
Ap01	*Aiptasia pulchella*	Okinawa	179	98	129	198	254	252
Zp	*Zoanthus pacificus*	Hawai'i	179	98	129	200	258	252
FLAp2	*A. pallida*	Florida keys	179	100	131	198	258	276
FLAp2 10AB	*A. pallida*	Florida keys	179	100	131	198	258	276
208[a]	*Plexaura kuna*	San Blas Islands, Panama	183	98	131	198	254	244
226[a]	*P. Kuna*	San Blas Islands, Panama	183	98	131	198	254	244
595[a]	*Briareum asbestinum*	Florida keys	191	102	129	200	254	256
1246[a]	*B. asbestinum*	Florida keys	191	102	129	200	254	256
707[a]	*P. Kuna*	San Blas Islands, Panama	191	104	129	200	254	256
1509[a]	*B. asbestinum*	Florida keys	191	104	129	200	254	256
2053[a]	*B. asbestinum*	Florida keys	191	104	129	200	254	256
801	*P. Kuna*	Florida keys	193	98	129	200	246	256
13	*P. Kuna*	Florida keys	193	98	129	200	246	256
206[a]	*P. Kuna*	San Blas Islands, Panama	193	102	129	200	258	260
705[a]	*P. Kuna*	San Blas Islands, Panama	193	102	129	200	258	260
SSPe	*Pseudopterogorgia elisabethae*	Bahamas	193	112	139	224	232	244

[a] Cultures that were from a single dinoflagellate cell.

Table 2.2. GenBank Accession numbers for the flanking regions of two microsatellite loci in *Symbiodinium* populations from *Aiptasia* spp. and algal cultures.

Geographic locations	Host name	Collection sites/Populations	GenBank Accession number	
			CA4.86	Si15
BER6[a]	*Aiptasia* spp.	Walsingham Pond, Bermuda	XXXXXX	XXXXXX
CI2.2 [a]	*Aiptasia* spp.	Coconut Island, Hawai'i	XXXXXX	XXXXXX
CI2.3 [a]	*Aiptasia* spp.	Coconut Island, Hawai'i	XXXXXX	XXXXXX
CK1.8 [a]	*Aiptasia* spp.	Crawl Key, Florida	XXXXXX	XXXXXX
CK2.3 [a]	*Aiptasia* spp.	Crawl Key, Florida	XXXXXX	XXXXXX
Eilat Ap 1 [a]	*Aiptasia* spp.	Red Sea	XXXXXX	XXXXXX
HERAUS1 [a]	*Aiptasia* spp.	Australia	XXXXXX	XXXXXX
MXLP1 [a]	*Aiptasia* spp.	La Paz, Mexico	XXXXXX	XXXXXX
RSAN1 [a]	*Aiptasia* spp.	Red Sea	XXXXXX	XXXXXX
Seso Ap Lite 1 [a]	*Aiptasia* spp.	Sesoko Island, Japan	XXXXXX	XXXXXX
TLAp1 [a]	*Aiptasia* spp.	Thailand	XXXXXX	XXXXXX
Wsk2.4 [a]	*Aiptasia* spp.	West Summerland Key, Florida	XXXXXX	XXXXXX
FLAp2	*Aiptasia* spp.	Long Key, Florida	XXXXXX	XXXXXX
Pk704SymB4	*Plexaura kuna*	San Blas Islands, Panama	XXXXXX	XXXXXX
Gv5.6a	*Gorgonia ventalina*	San Blas Islands, Panama	XXXXXX	XXXXXX
Pp304a	*Pseudoplexaura porosa*		XXXXXX	XXXXXX
SSPe	*Pseudopterogorgia elisabethae*	San Salvador, Bahamas	XXXXXX	XXXXXX
Ba06-146	*Briareum asbestinum?*	Florida Keys	XXXXXX	XXXXXX
Ba06-147	*Briareum asbestinum?*	Florida Keys	XXXXXX	XXXXXX
04-202	—	—	XXXXXX	XXXXXX
04-258	—	—	XXXXXX	XXXXXX
Mf10.14b.2	*Montastrea faveolata*	—	—	XXXXXX
Mf11.5b.1	*Montastrea faveolata*	—	—	XXXXXX

[a]*Symbiodinium* samples are not cultures.

Table 2.3. Sequence information, annealing temperatures and $MgCl_2$ concentrations of *Symbiodinium* Clade B microsatellite primers used in this study.

Primer	Primer sequence (5' to 3')	Study
18S rDNA	Forward (ss5): GGTTGATCCTGCCAGTAGTCATATGCTTG Reverse(ss3Z): AGCACTGCGTCAGTCCGAATAATTCACCGG	Rowan and Powers (1991)
CA4.86[a]	Forward: GCCTTCAATGCAATCACCTT Reverse: GGAATTGGCCATCCCTCTAT	Santos and Coffroth (2003)
CA6.38[b]	Forward: CAAAGAATATTCGGGGGTCA Reverse: AGTTGATACGCCGGATGTGT	Santos and Coffroth (2003)
Si4[c]	Forward: TCGCGATCGAGTCCCATGGTCT Reverse: TGGTTTCCCGTGACATCCCTG	Pettay and LaJeunesse (2007)
Si8[c]	Forward: ACTACAGGCACGACCCACCA Reverse: GCATTCACGCCATCCATCAGTCC	Pettay and LaJeunesse (2007)
Si15[c]	Forward: CTCACCTTGAAATCAGTAGCCA Reverse: CGTAGCTTCTGAAGGTACGACAC	Pettay and LaJeunesse (2007)
Si34[c]	Forward: TGAATGCAGTGAACGCATGG Reverse: ACCTAGTCACCGAAGCACTC	Pettay and LaJeunesse (2007)

[a] 2.5 mM $MgCl_2$, 40 thermal cycles with 50 °C annealing temperatures.

[b] 1.5 mM $MgCl_2$, 40 thermal cycles with 56 °C annealing temperatures.

[c] 2.5 mM $MgCl_2$, 32 thermal cycles with 57 °C annealing temperatures

Table 2.4. Clades (based on 18S-rDNA RFLP) and ITS2 "types" (based on ITS2 DGGE) of *Symbiodinium* associated with *Aiptasia* spp. anemones from throughout the world.

Geographic locations	Collection sites/Populations	18S RFLP profile					ITS2 DGGE profile	
		n	A	B	A+B	A+C	n	ITS2 "type"
Japan	Sesoko Island Dark (SD)	8	–	8	–	–	0	–
	Sesoko Island Light (SL)	7	–	7	–	–	1	B1
	Ishi Ap (IA)	10	–	10	–	–	0	–
	Motobu (MA)	8	–	8	–	–	0	–
	Tadashi Maruyama (TM)	18	–	18	–	–	0	–
Mexico	La Paz (MX)	7	–	7	–	–	1	B1
Hawai'i	Coconut Island (CI) 1	34	–	34	–	–	1	B1
	Coconut Island (CI) 2	21	–	21	–	–	1	B1
	Waikiki Aquarium (WA)	34	–	34	–	–	0	–
Florida	West Summerland Key (WK) 1	16	16	–	–	–	1	B1
	West Summerland Key (WK) 2	44	39	–	4	1	0	–
	Crawl Key (CK) 1	33	20	4	9	–	1	B1
	Crawl Key (CK) 2	37	13	3	21	–	1	B1
Bermuda	Walsingham Pond (BE)	17	–	17	–	–	6	B1
Red Sea	RSAN (RS)	18	–	18	–	–	1	B1
	Eilat Ap (EA)	10	–	10	–	–	0	–
Thailand	Thailand (TL)	8	–	8	–	–	1	B1
Australia	Australia (HS)	26	–	26	–	–	1	B1
Total	18 sites	356	88	233	34	1	16	16

Table 2.5. Genotypic frequencies of six microsatellite loci in *Symbiodinium* Clade B associated with *Aiptasia* spp. from throughout the world.

Genotype						Site																
						Japan					Mexico	Hawai'i		Florida				Bermuda	Red Sea		Thailand	Australia
CA4.86	CA6.38	Si4	Si8	Si15	Si34	SD	SL	IA	MA	TM	MX	CI1	CI2	WA	WK2	CK1	CK2	BE	RS	EA	TL	HS
179	98	129	198	254	253	1.000	1.000	1.000	1.000	1.000	1.000	0.087	–	0.563	–	–	–	–	–	–	–	–
179	98	129	198	258	253/257	–	–	–	–	–	–	0.130	0.048	–	–	–	–	–	–	–	–	–
179	98	129	198/200	258	253/257	–	–	–	–	–	–	0.043	0.095	–	–	–	–	–	–	–	–	–
179	98/100	129	198	258	253/257	–	–	–	–	–	–	0.043	–	–	–	–	–	–	–	–	–	–
179	98/100	129	198	254/258	253/257	–	–	–	–	–	–	0.174	0.048	0.375	–	–	–	–	–	–	–	–
179	98/100	129	198/200	254/258	253/257	–	–	–	–	–	–	0.043	0.048	0.063	–	–	–	–	–	–	–	–
179	100	129	198	258	253/257	–	–	–	–	–	–	0.435	–	–	–	–	–	–	–	–	–	–
179	100	129	198/200	258	253/257	–	–	–	–	–	–	0.043	0.048	–	–	–	–	–	–	–	–	–
179	98	129	198	254	253/257	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–
179	98	129	198	254/258	253	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–
179	98	129	198	254/258	253/257	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–
179	98	129	198/200	258	253	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–
179	98	129	198	258	253	–	–	–	–	–	–	–	0.238	–	–	–	–	–	–	–	–	–
179	98/100	129	198	258	253	–	–	–	–	–	–	–	0.095	–	–	–	–	–	–	–	–	–
179	98/100	129	198	258	257	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–
179	98/100	129	198	254/258	253	–	–	–	–	–	–	–	0.095	–	–	–	–	–	–	–	–	–
179	100	129	198	258	253	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–
179	100	131	198	256	277	–	–	–	–	–	–	–	–	–	0.250	–	–	–	–	–	–	–
179	100	131	198	258	277	–	–	–	–	–	–	–	–	–	0.250	–	0.125	–	–	–	–	–
179	102	131	198	256	269	–	–	–	–	–	–	–	–	–	0.500	–	–	–	–	–	–	–
179	102	131	198	256	273	–	–	–	–	–	–	–	–	–	–	1.000	0.708	–	–	–	–	–
179	100/102	131	198	256/258	273	–	–	–	–	–	–	–	–	–	–	–	0.083	–	–	–	–	–
179	100	129	200	256	257	–	–	–	–	–	–	–	–	–	–	–	0.042	–	0.111	–	–	–
179	100	131	198	258	273	–	–	–	–	–	–	–	–	–	–	–	0.042	–	–	–	–	–
179	104	129	204	256/258	265	–	–	–	–	–	–	–	–	–	–	–	–	0.071	–	–	–	–
179	100/104	129	202/204	254/256	265	–	–	–	–	–	–	–	–	–	–	–	–	0.071	–	–	–	–
179	100	129	202	254	265	–	–	–	–	–	–	–	–	–	–	–	–	0.786	–	–	–	–
179	104	129	204	256	265	–	–	–	–	–	–	–	–	–	–	–	–	0.071	–	–	–	–
179	98	129	202	254	257	–	–	–	–	–	–	–	–	–	–	–	–	–	0.500	1.000	–	–
179	98/100	129	200/202	254/256	257	–	–	–	–	–	–	–	–	–	–	–	–	–	0.389	–	–	–
179	100	129	202	254	257	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.000	–
179	102	131	198	254	253	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.000
n						8	7	10	8	18	7	23	21	16	4	12	24	14	18	10	8	26

Table 2.6. Heterozygosity for six microsatellite loci in *Symbiodinium* Clade B from *Aiptasia* spp. across the world.

populations	Heterozygosity (*H*)					
	CA4.86	CA6.38	Si4	Si8	Si15	Si34
CI1	0.000	0.000	0.000	0.000	0.000	0.000
CI2	0.000	0.278	0.000	0.000	0.000	0.000
WA	0.000	0.000	0.000	0.000	0.000	0.000
MX	0.000	0.000	0.000	0.000	0.000	0.000
WK2	0.000	0.500	0.000	0.000	0.375	0.500
CK1	0.000	0.000	0.000	0.000	0.000	0.000
CK2	0.000	0.351	0.000	0.000	0.298	0.310
BE	0.000	0.153	0.000	0.153	0.153	0.000
RS	0.000	0.298	0.000	0.298	0.298	0.000
EA	0.000	0.000	0.000	0.000	0.000	0.000
TL	0.000	0.000	0.000	0.000	0.000	0.000
HS	0.000	0.000	0.000	0.000	0.000	0.000
SD	0.000	0.000	0.000	0.000	0.000	0.000
SL	0.000	0.000	0.000	0.000	0.000	0.000
IA	0.000	0.000	0.000	0.000	0.000	0.000
MA	0.000	0.000	0.000	0.000	0.000	0.000
TM	0.000	0.000	0.000	0.000	0.000	0.000
Over all populations	0.000	0.607	0.458	0.359	0.412	0.624

Table 2.7. F_{ST} and ρ_{ST} (population differentiation) estimates of *Symbiodinium* Clade B from *Aiptasia* spp. across the global range based on six microsatellite loci.

	F_{ST}	ρ_{ST}
CA4.86	NA[a]	NA[a]
CA6.38	0.844	0.924
Si4	1.000	1.000
Si8	0.919	0.979
Si15	0.816	0.890
Si34	0.920	0.965
Total	0.899	0.9517

[a] locus CA4.86 is not polymorphic.

Table 2.8. *Symbiodinium* Clade B pairwise tests of symbiont population differentiation for *Aiptasia* spp. at 17 sites containing *Symbiodinium* Clade B in the world (site abbreviations, see table 2.1; NS not significant; NA not available; * $P<0.05$).

	CI1	CI2	WA	MX	WK2	CK1	CK2	BE	RS	EA	TL	HS	SD	SL	IA	MA	TM
CI1		NS	NA	NA	NS	NS	NS	NS	NS	NS	NS	NS	NA	NA	NA	NA	NA
CI2			*	*	NS	*	*	NS	NS	*	NS	*	NS	*	*	NS	*
WA				NA	NS	*	*	*	*	*	*	*	NA	NA	NA	NA	NA
MX					NS	*	*	*	*	*	*	*	NA	NA	NA	NA	NA
WK2						*	NS	*	*	NS	NS	NS	NS	NS	NS	NS	*
CK1							NS	*	*	*	*	*	*	*	*	*	*
CK2								*	*	*	*	*	*	*	*	*	*
BE									*	*	*	*	*	*	*	*	*
RS										NS	NS	*	*	NS	*	*	*
EA											*	*	*	*	*	NS	*
TL												*	NS	*	*	*	*
HS													*	*	*	*	*
SD														NA	NA	NA	NA
SL															NA	NA	NA
IA																NA	NA
MA																	NA
TM																	

FIGURES

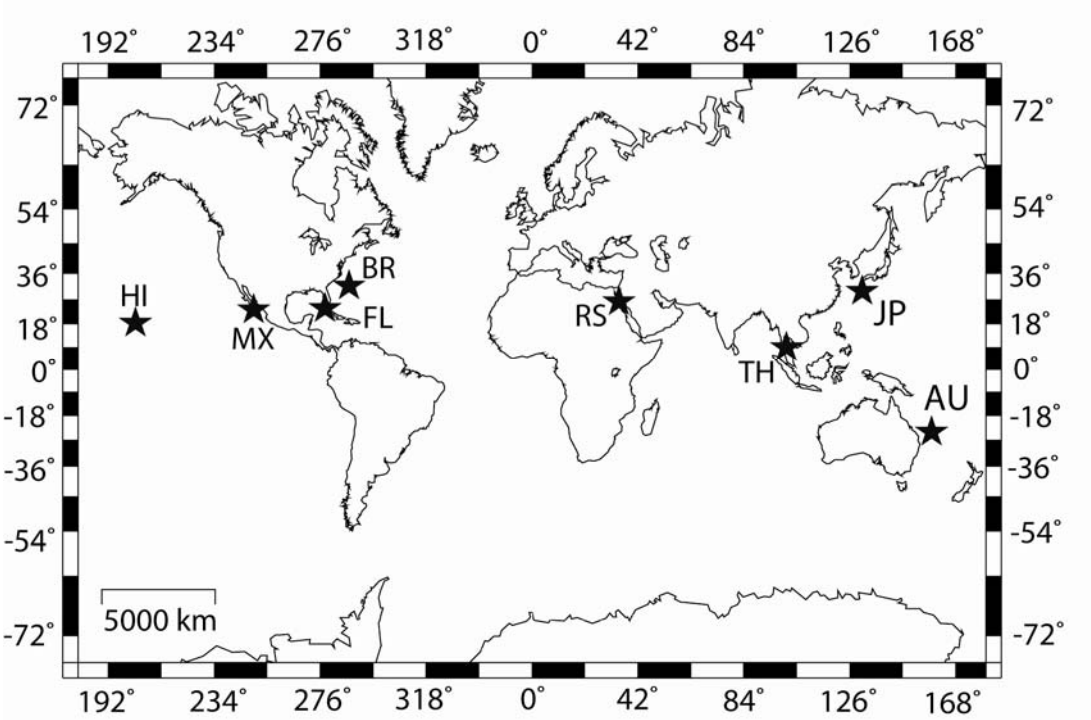

Figure 2.1. Locations of the *Aiptasia* spp. populations collected from eight major geographic localities across the global range of this host. Geographic localities denoted by two-letter abbreviations as follows: HI = Hawai'ian islands; MX = Mexico; FL = Florida Keys; BR = Bermuda; RS = Red Sea; TH = Thailand; JP = Japan and AU = Australia.

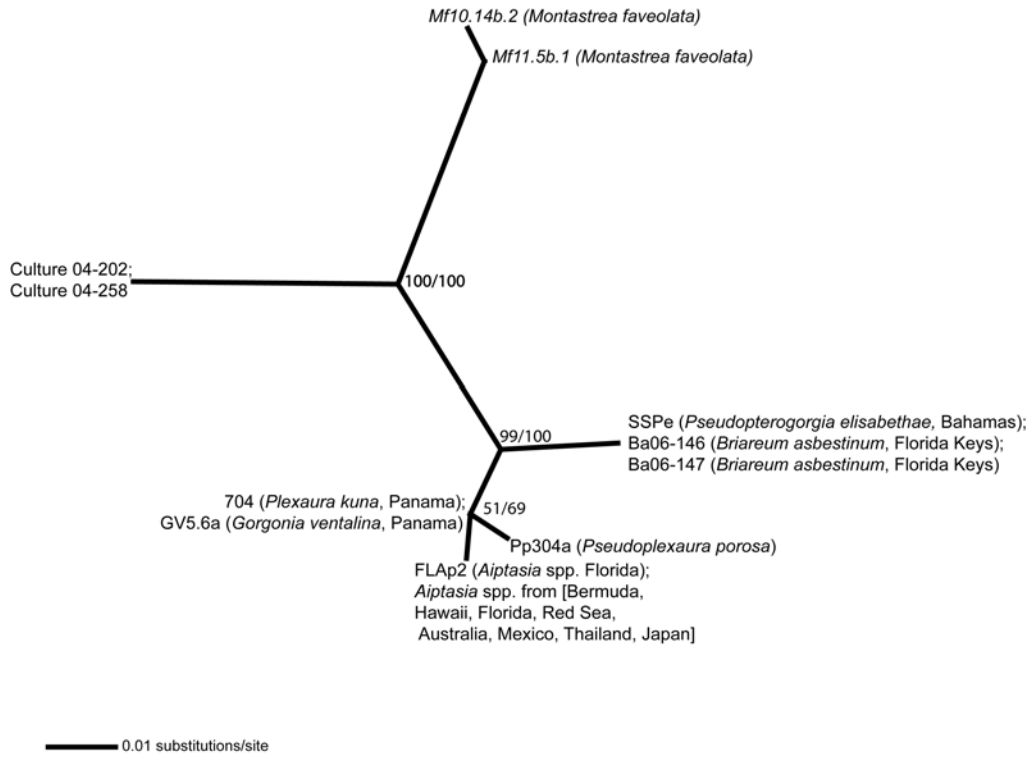

Figure 2.2. Inferred unrooted phylogenetic relationships between *Symbiodinium* Clade B based on concatenated flanking regions of microsatellite loci CA4.86 and Si15. Maximum likelihood (ML) tree (–ln L = 622.68). Numbers before and after slashes are support values based on 1000 bootstrap replications (Parsimony/Likelihood respectively). For locations of *Aiptasia* spp. and cultures, original host name and sample locations of cultures see table 2.

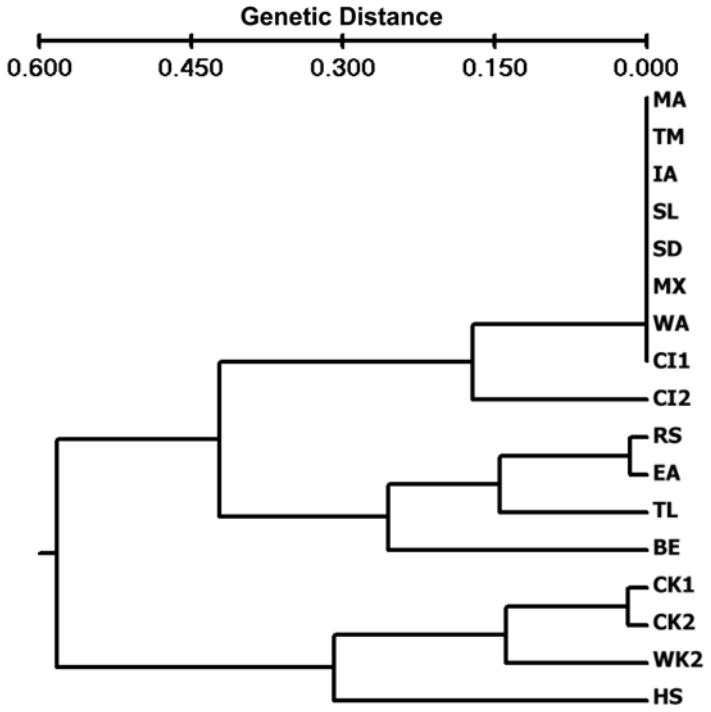

Figure 2.3. Dendrogram by unweighted pair group method using arithmetic averages (UPGMA) depicting relationships between *Symbiodinium* Clade B populations of *Aiptasia* spp. at 17 geographic localities across the global range of the host.

APPENDIX TABLE

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
Wsk2.4	Florida	A+B	179	102	131	198	256	269
Wsk2.7	Florida	A+B	179	100	131	198	256	277
Wsk2.22	Florida	A+B	179	102	131	198	256	269
Wsk2.30	Florida	A	179	100	131	198	256	273
Wsk2.34	Florida	A	179	102	131	198	256	269
Wsk2.44	Florida	A	179	100	131	198	258	277
Wsk2.46	Florida	A+B	179	100	131	198	258	277
CK1.3	Florida	A	179	102	131	198	256	273
CK1.4	Florida	A	179	102	131	198	256	273
CK1.8	Florida	A+B	179	102	131	198	256	273
CK1.9	Florida	A+B	179	102	131	198	256	273
CK1.5	Florida	B	179	102	131	198	256	273
CK1.13	Florida	A+B	179	102	131	198	256	273
CK1.14	Florida	A+B	179	102	131	198	256	273
CK1.15	Florida	A	179	102	131	198	256	273
CK1.16	Florida	B	179	102	131	198	256	273
CK1.18	Florida	A+B	179	102	131	198	256	273
CK1.23	Florida	A+B	179	102	131	198	256	273
CK1.24	Florida	A+B	179	102	131	198	256	273
CK1.25	Florida	A+B	179	102	131	198	256	273
CK1.26	Florida	A	179	102	131	198	256	273
CK1.27	Florida	A+B	179	102	131	198	256	273
CK1.28	Florida	A	179	102	131	198	256	273

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
CK1.33	Florida	B	179	102	131	198	256	273
CK2.1	Florida	A+B	179	102	131	198	256	273
CK2.2	Florida	A+B	179	102	131	198	256	273
CK2.3	Florida	B	179	102	131	198	256	273
CK2.4	Florida	A+B	179	102	131	198	256	273
CK2.5	Florida	A+B	179	100	131	198	258	273
CK2.7	Florida	A+B	179	102	131	198	256	273
CK2.8	Florida	A+B	179	102	131	198	256	273
CK2.9	Florida	A+B	179	102	131	198	256	273
CK2.10	Florida	A+B	179	100	131	198	258	277
CK2.11	Florida	A+B	179	102	131	198	256	273
CK2.12	Florida	A+B	179	102	131	198	256	273
CK2.13	Florida	A+B	179	102	131	198	256	273
CK2.14	Florida	A+B	179	100/102	131	198	256/258	273
CK2.15	Florida	A	179	102	131	198	256	273
CK2.16	Florida	A+B	179	100	131	198	258	277
CK2.17	Florida	A	179	102	131	198	256	273
CK2.18	Florida	A+B	179	102	131	198	256	273
CK2.19	Florida	A	179	102	131	198	256	273
CK2.20	Florida	A+B	179	102	131	198	256	273
CK2.21	Florida	A+B	179	102	131	198	256	273
CK2.22	Florida	A+B	179	102	131	198	256	273
CK2.23	Florida	A+B	179	100/102	131	198	256/258	273
CK2.24	Florida	A	179	102	131	198	256	273
CK2.25	Florida	A+B	179	100	131	198	258	277
CK2.26	Florida	A	179	100	131	198	258	273
CK2.28	Florida	A	179	100/102	131	198	256	273/277
CK2.29	Florida	B	179	102	131	198	256	273

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
CK2.30	Florida	B	179	100	131	198	256	257
CK2.31	Florida	A	179	102	131	198	256	273
CK2.32	Florida	A+B	179	102	131	198	256	273
CK2.33	Florida	A	179	102	131	198	256	273
CK2.34	Florida	A+B	179	102	131	198	256	273
CK2.35	Florida	A	179	100	131	198	258	273
CK2.37	Florida	A	179	102	131	198	256	273
CI1.1	Hawaii	B	179	98/100	129	198	254/258	253/257
CI1.2	Hawaii	B	179	100	129	198	258	253/257
CI1.3	Hawaii	B	179	100	129	198	258	253/257
CI1.4	Hawaii	B	179	98	129	198	258	253/257
CI1.5	Hawaii	B	179	98	129	198	254	253
CI1.6	Hawaii	B	179	98/100	129	198/200	254/258	253/257
CI1.7	Hawaii	B	179	100	129	198	258	253/257
CI1.8	Hawaii	B	179	98/100	129	198	258	253/257
CI1.9	Hawaii	B	179	100	129	198	258	253/257
CI1.10	Hawaii	B	179	98	129	198	258	253/257
CI1.11	Hawaii	B	179	100	129	198	258	253/257
CI1.12	Hawaii	B	179	100	129	198/200	258	253/257
CI1.13	Hawaii	B	179	98/100	129	198	254/258	253/257
CI1.14	Hawaii	B	179	100	129	198	258	253/257
CI1.15	Hawaii	B	179	100	129	198	258	253/257
CI1.16	Hawaii	B	179	100	129	198	258	253/257
CI1.17	Hawaii	B	179	98	129	198/200	258	253/257
CI1.18	Hawaii	B	179	98	129	198	258	253/257
CI1.19	Hawaii	B	179	98/100	129	198	254/258	253/257
CI1.20	Hawaii	B	179	100	129	198	258	253/257
CI1.21	Hawaii	B	179	98/100	129	198	254/258	253/257

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
CI1.22	Hawaii	B	179	100	129	198	258	253/258
CI1.23	Hawaii	B	179	98	129	198	254	253
WA1	Hawaii	B	179	98/100	129	198	254/258	253/257
WA2	Hawaii	B	179	98	129	198	254	253
WA3	Hawaii	B	179	98/100	129	198	254/258	253/257
WA4	Hawaii	B	179	98	129	198	254	253
WA5	Hawaii	B	179	98	129	198	254	253
WA6	Hawaii	B	179	98/100	129	198	254/258	253/257
WA7	Hawaii	B	179	98	129	198	254	253
WA8	Hawaii	B	179	98/100	129	198	254/258	253/257
WA9	Hawaii	B	179	98	129	198	254	253
WA10	Hawaii	B	179	98	129	198	254	253
WA11	Hawaii	B	179	98	129	198	254	253
WA12	Hawaii	B	179	98/100	129	198	254/258	253/257
WA13	Hawaii	B	179	98	129	198	254	253
WA14	Hawaii	B	179	98/100	129	198	254/258	253/257
WA15	Hawaii	B	179	98	129	198	254	253
WA16	Hawaii	B	179	98/100	129	198/200	254/258	253/257
CI2.1	Hawaii	B	179	98/100	129	198/200	254/258	253/257
CI2.2	Hawaii	B	179	100	129	198	258	253
CI2.3	Hawaii	B	179	98	129	198	258	253
CI2.4	Hawaii	B	179	98	129	198	258	253
CI2.5	Hawaii	B	179	98	129	198	254	253/257
CI2.6	Hawaii	B	179	98/100	129	198	258	253
CI2.7	Hawaii	B	179	98	129	198	258	253
CI2.8	Hawaii	B	179	98	129	198	258	253
CI2.9	Hawaii	B	179	98	129	198/200	258	253/257
CI2.10	Hawaii	B	179	98/100	129	198	254/258	253

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
CI2.11	Hawaii	B	179	98	129	198/200	258	253
CI2.12	Hawaii	B	179	98	129	198	254/258	253
CI2.13	Hawaii	B	179	100	129	198/200	258	253/257
CI2.14	Hawaii	B	179	98	129	198/200	258	253/257
CI2.15	Hawaii	B	179	98/100	129	198	254/258	253
CI2.16	Hawaii	B	179	98	129	198	254/258	253/257
CI2.17	Hawaii	B	179	98/100	129	198	258	253
CI2.18	Hawaii	B	179	98/100	129	198	258	257
CI2.19	Hawaii	B	179	98/100	129	198	254/258	253/257
CI2.20	Hawaii	B	179	98	129	198	258	253
CI2.21	Hawaii	B	179	98	129	198	258	253/257
TMAp1	Japan	B	179	98	129	198	254	253
TMAp2	Japan	B	179	98	129	198	254	253
TMAp3	Japan	B	179	98	129	198	254	253
TMAp4	Japan	B	179	98	129	198	254	253
TMAp5	Japan	B	179	98	129	198	254	253
TMAp6	Japan	B	179	98	129	198	254	253
TMAp7	Japan	B	179	98	129	198	254	253
TMAp8	Japan	B	179	98	129	198	254	253
TMAp9	Japan	B	179	98	129	198	254	253
TMAp10	Japan	B	179	98	129	198	254	253
TMAp11	Japan	B	179	98	129	198	254	253
TMAp12	Japan	B	179	98	129	198	254	253
TMAp13	Japan	B	179	98	129	198	254	253
TMAp14	Japan	B	179	98	129	198	254	253
TMAp15	Japan	B	179	98	129	198	254	253
TMAp16	Japan	B	179	98	129	198	254	253
TMAp17	Japan	B	179	98	129	198	254	253

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
TMAp18	Japan	B	179	98	129	198	254	253
Moto Ap1	Japan	B	179	98	129	198	254	253
Moto Ap2	Japan	B	179	98	129	198	254	253
Moto Ap3	Japan	B	179	98	129	198	254	253
Moto Ap4	Japan	B	179	98	129	198	254	253
Moto Ap5	Japan	B	179	98	129	198	254	253
Moto Ap6	Japan	B	179	98	129	198	254	253
Moto Ap7	Japan	B	179	98	129	198	254	253
Moto Ap8	Japan	B	179	98	129	198	254	253
Ishi Ap1	Japan	B	179	98	129	198	254	253
Ishi Ap2	Japan	B	179	98	129	198	254	253
Ishi Ap3	Japan	B	179	98	129	198	254	253
Ishi Ap4	Japan	B	179	98	129	198	254	253
Ishi Ap5	Japan	B	179	98	129	198	254	253
Ishi Ap6	Japan	B	179	98	129	198	254	253
Ishi Ap7	Japan	B	179	98	129	198	254	253
Ishi Ap8	Japan	B	179	98	129	198	254	253
Ishi Ap9	Japan	B	179	98	129	198	254	253
Ishi Ap10	Japan	B	179	98	129	198	254	253
Seso Ap Lite1	Japan	B	179	98	129	198	254	253
Seso Ap Lite2	Japan	B	179	98	129	198	254	253
Seso Ap Lite3	Japan	B	179	98	129	198	254	253
Seso Ap Lite4	Japan	B	179	98	129	198	254	253
Seso Ap Lite5	Japan	B	179	98	129	198	254	253
Seso Ap Lite6	Japan	B	179	98	129	198	254	253
Seso Ap Lite7	Japan	B	179	98	129	198	254	253
Seso Ap Dark1	Japan	B	179	98	129	198	254	253
Seso Ap Dark2	Japan	B	179	98	129	198	254	253

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
Seso Ap Dark3	Japan	B	179	98	129	198	254	253
Seso Ap Dark4	Japan	B	179	98	129	198	254	253
Seso Ap Dark5	Japan	B	179	98	129	198	254	253
Seso Ap Dark6	Japan	B	179	98	129	198	254	253
Seso Ap Dark7	Japan	B	179	98	129	198	254	253
Seso Ap Dark8	Japan	B	179	98	129	198	254	253
BER3	Bermuda	B	179	100	129	202	254	265
BER5	Bermuda	B	179	100	129	202	254	265
BER6	Bermuda	B	179	100	129	202	254	265
BER9	Bermuda	B	179	100	129	202	254	265
BER10	Bermuda	B	179	100/104	129	202/204	254/256	265
BER11	Bermuda	B	179	100	129	202	254	265
BER12	Bermuda	B	179	100	129	202	254	265
BER13	Bermuda	B	179	100	129	202	254	265
BER14	Bermuda	B	179	104	129	204	256	265
BER15	Bermuda	B	179	100	129	202	254	265
BER16	Bermuda	B	179	100	129	202	254	265
BER17	Bermuda	B	179	100	129	202	254	265
BER18	Bermuda	B	179	104	129	204	256/258	265
BER19	Bermuda	B	179	100	129	202	254	265
HERAUS1	Australia	B	179	102	131	198	254	253
HERAUS2	Australia	B	179	102	131	198	254	253
HERAUS3	Australia	B	179	102	131	198	254	253
HERAUS4	Australia	B	179	102	131	198	254	253
HERAUS5	Australia	B	179	102	131	198	254	253
HERAUS6	Australia	B	179	102	131	198	254	253
HERAUS7	Australia	B	179	102	131	198	254	253
HERAUS8	Australia	B	179	102	131	198	254	253

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
HERAUS9	Australia	B	179	102	131	198	254	253
HERAUS10	Australia	B	179	102	131	198	254	253
HERAUS11	Australia	B	179	102	131	198	254	253
HERAUS12	Australia	B	179	102	131	198	254	253
HERAUS13	Australia	B	179	102	131	198	254	253
HERAUS14	Australia	B	179	102	131	198	254	253
HERAUS15	Australia	B	179	102	131	198	254	253
HERAUS16	Australia	B	179	102	131	198	254	253
HERAUS17	Australia	B	179	102	131	198	254	253
HERAUS18	Australia	B	179	102	131	198	254	253
HERAUS19	Australia	B	179	102	131	198	254	253
HERAUS20	Australia	B	179	102	131	198	254	253
HERAUS21	Australia	B	179	102	131	198	254	253
HERAUS22	Australia	B	179	102	131	198	254	253
HERAUS23	Australia	B	179	102	131	198	254	253
HERAUS24	Australia	B	179	102	131	198	254	253
HERAUS25	Australia	B	179	102	131	198	254	253
HERAUS26	Australia	B	179	102	131	198	254	253
RSAN1	Red Sea	B	179	98	129	202	254	257
RSAN2	Red Sea	B	179	98	129	202	254	257
RSAN3	Red Sea	B	179	98	129	202	254	257
RSAN4	Red Sea	B	179	98	129	202	254	257
RSAN5	Red Sea	B	179	98/100	129	200/202	254/256	257
RSAN6	Red Sea	B	179	98	129	202	254	257
RSAN7	Red Sea	B	179	98	129	202	254	257
RSAN8	Red Sea	B	179	98/100	129	200/202	254/256	257
RSAN9	Red Sea	B	179	98/100	129	200/202	254/256	257
RSAN10	Red Sea	B	179	98	129	202	254	257

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
RSAN11	Red Sea	B	179	98/100	129	200/202	254/256	257
RSAN13	Red Sea	B	179	98	129	202	254	257
RSAN14	Red Sea	B	179	98	129	202	254	257
RSAN15	Red Sea	B	179	100	129	200	256	257
RSAN16	Red Sea	B	179	98/100	129	200/202	254/256	257
RSAN17	Red Sea	B	179	98/100	129	200/202	254/256	257
RSAN18	Red Sea	B	179	100	129	200	256	257
RSAN21	Red Sea	B	179	98/100	129	200/202	254/256	257
Eilat Ap1	Red Sea	B	179	98	129	202	254	257
Eilat Ap2	Red Sea	B	179	98	129	202	254	257
Eilat Ap3	Red Sea	B	179	98	129	202	254	257
Eilat Ap4	Red Sea	B	179	98	129	202	254	257
Eilat Ap5	Red Sea	B	179	98	129	202	254	257
Eilat Ap6	Red Sea	B	179	98	129	202	254	257
Eilat Ap7	Red Sea	B	179	98	129	202	254	257
Eilat Ap8	Red Sea	B	179	98	129	202	254	257
Eilat Ap9	Red Sea	B	179	98	129	202	254	257
Eilat Ap10	Red Sea	B	179	98	129	202	254	257
MXLP1	Mexico	B	179	98	129	198	254	253
MXLP2	Mexico	B	179	98	129	198	254	253
MXLP3	Mexico	B	179	98	129	198	254	253
MXLP4	Mexico	B	179	98	129	198	254	253
MXLP11	Mexico	B	179	98	129	198	254	253
MXLP12	Mexico	B	179	98	129	198	254	253
MXLP13	Mexico	B	179	98	129	198	254	253
TLAp1	Thailand	B	179	100	129	202	254	257
TLAp2	Thailand	B	179	100	129	202	254	257
TLAp3	Thailand	B	179	100	129	202	254	257

Sample	Location	18S rDNA RFLP	CA4.86	CA6.38	Si4	Si8	Si15	Si34
TLAp4	Thailand	B	179	100	129	202	254	257
TLAp5	Thailand	B	179	100	129	202	254	257
TLAp6	Thailand	B	179	100	129	202	254	257
TLAp7	Thailand	B	179	100	129	202	254	257
TLAp8	Thailand	B	179	100	129	202	254	257

Note: sample name abbreviations and sample locations refer to figure 2.1 and table 2.4.