Morphological and genetic variation in the Caribbean species of the hydrocoral genus *Millepora*

By

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ABSTRACT

Millepora, an important structural component of tropical coral reefs, is a ubiquitous hydrocoral genus in the shallow water reefs of the Caribbean and is traditionally thought to consist of four species: *M. alcicornis* Linnaeus 1758, *M. complanata* Lamarck 1816, *M. striata* Duchassaing & Michelotti 1864 and *M. squarrosa* Lamark 1816. Intermediate forms and high phenotypic variability oftentimes hinder the correct identification of colonies. A multicharacter approach based on morphology and DNA sequences was used to evaluate taxonomic differences among Caribbean *Millepora* species. Samples of *M. alcicornis*, *M. complanata* and *M. squarrosa* were collected from the La Parguera reef system in southwest Puerto Rico; samples from *M. striata* were collected at Bocas del Toro, Panama. Morphological traits of gastropore and dactylopore diameter, distances among gastropores, among dactylopores and from gastropore to the nearest dactylopore were compared between *M. complanata* and *M. alcicornis*. High intraspecific variability and overlap among the morphotypes was observed; thus, the traits were more powerful delimiting species when used in conjunction than when used alone. A portion of the Cytochrome Oxidase Subunit I (COI) gene was used to examine the genetic differences among the four putative species. High levels of haplotypic diversity (H_d=0.94) were observed and the most common haplotypes were shared by *M. alcicornis*, *M. complanata* and *M. striata*. Sequence divergence ranged from 0-3% among colonies identified as *M. alcicornis*, *M. complanata* and *M. striata*, contrasting with the 25% divergence observed among these species and *M. squarrosa*. Bayesian analysis of the genealogy of *Millepora* resulted in paraphyletic clades suggesting that only two species of *Millepora* are present in the Caribbean: *M. squarrosa* and the species currently described as *M. complanata*, *M. alcicornis* and *M. striata*. The lack of

congruence between the molecular and morphometric results indicates an uncoupling of morphological and molecular evolution in the genus *Millepora*.

RESUMEN

Poco se conoce sobre la biología del género *Millepora,* el cual es un componente importante de los arrecifes coralinos. *Millepora* es frecuente en los arrecifes someros del Caribe, y tradicionalmente consta de cuatro especies: *M. alcicornis* Linnaeus 1758, *M. complanata* Lamarck 1816, *M. striata* Duchassaing & Michelotti 1864 y *M. squarrosa* Lamark 1816. La gran abundancia de formas intermedias y la alta variabilidad fenotípica dificulta identificar correctamente las especies. Se utilizó un método multivariado, basado en la morfología y secuencias de ADN, para caracterizar y distinguir entre las especies de *Millepora* en el Caribe. Muestras de *M. alcicornis*, *M. complanata* y *M. squarrosa* fueron colectadas en los arrecifes de La Parguera en el Suroeste de Puerto Rico, M. striata fue colectada en Bocas del Toro, Panamá. El diámetro de los gastroporos y dactiloporos, la distancia entre gastroporos y entre dactiloporos y la distancia entre gastroporos al dactiloporo más cercano fueron los caracteres taxonómicos utilizados. Estos caracteres fueron mas poderosos en la delimitación de las especies cuando se usaron en conjunto que usados por separado; sin embargo se observó alta variabilidad entre las medidas, algunas solapando entre diferentes morfotipos. Una porción del gen COI fue usado para examinar las diferencias genéticas entre las cuatro especies. Se observó un 94% de diversidad haplotípica para el gen COI y los haplotipos más comunes fueron encontrados en todas las especies. La divergencia entre secuencias varió de 0-3% entre las colonias identificadas como *M. alcicornis*, *M. complanata* y *M. striata*, contrastando con el 25% de divergencia de *M. squarrosa*. La genealogía Bayesiana agrupó las especies en grupos para-

filéticos, los cuales no concuerdan con la morfología. Los resultados obtenidos sugieren la existencia de dos especies de *Millepora* en el Caribe: *M. squarrosa* y una especie morfológicamente muy variable compuesta por *M. complanata*, *M. alcicornis* y *M. striata*.

To my family…

To true friends....

"Nothing in biology makes sense except in the light of evolution"
Theodosius Dobzhansky

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1. INTRODUCTION

The hydrocoral genus *Millepora* consists of 17 species distinguished by their growth form (Boshma 1948; Cairns 1999). The genus is globally distributed in warm waters (Cairns 1999; Lewis 2006), where it can dominate the shallow turbulent environments forming dense reef rims (Lewis 1989; Edmunds 1999). *Millepora* is an important component of tropical coral reefs; it provides shelter and habitat to a large number of species and contributes to the complexity of the carbonate structure (Lewis 1989). Despite their abundance, geographical distribution and geological importance, the milleporids have seldom received attention in coral reef studies (Lewis 1989).

The few published records of *Millepora* show that members of the genus are among the first cnidarians to lose their zooxanthellate symbionts during bleaching events (Glynn 1993; Paulay 1999; Marshall and Baird 2000). Existing information also suggests that milleporids are superior space competitors over scleractinian corals and gorgonians under disturbance conditions (Wahle 1980; Edmunds 1999) and are immune to predation by the starfish *Acanthaster planci* (Lewis 1989). Nevertheless, information about the reproductive biology, growth, recruitment and mortality of milleporids is limited. This lack of information is partly caused by the high degree of morphological variation within the genus, which renders the identification of each species difficult. Therefore, a correct classification of the species would fill a fundamental gap in studies of the biology, ecology and evolution of the genus.

The taxonomic status of the various *Millepora* species has been controversial for more than three and a half centuries (Boshma 1948, Manchenko 1993). Efforts to identify each species were based on morphological characters of the corallum (the calcium carbonate skeleton): presence or absence of ampullae (receptacles bearing the sexual medusa), texture of

the colony and number of gastropores and dactylopores (Boshma 1948; deWeerdt 1984; Razak and Hoeksema 2003). However, due to their great morphological variability, disagreement exists on the species classification based on those traits (deWeerdt 1984; Lewis 1989; Amaral et al. 2002). Some recent studies showed that growth forms of what were thought to be different species belong to a single species (Manchenko 1993), while other studies suggest the opposite conclusion (Amaral et al. 1997; Meroz-Fine et al. 2003). Accepted valid species are those described by Boshma (1948) and later revisited by Cairns (1999); however, the taxonomic review of the genus is still in progress. New forms of milleporids are still being described and proposed as new species (Amaral et al. 2002; Amaral et al. 2008), while new revisions continues to study the extent of morphological variation and the validity of the taxonomical characters in *Millepora* (Razak and Hoeksema 2003).

Even though controversy persists around the genus *Millepora*, little has been done to unravel its taxonomic ambiguity by using multi-character approaches including molecular techniques. When several seemingly independent lines of evidence (e.g. molecular, behavioral, and morphological) consistently distinguish taxa from the same habitat, a strong argument can be made for the presence of different species (e.g. in the scleractinian species complex *Montastraea*; Weil and Knowlton 1994). The application of molecular markers, for example, has completely redefined our understanding of the relationships of scleractinian corals (Fukami et al. 2004b; Fukami et al. 2008; Nunes et al. 2008). Use of molecular tools has been rudimentary in milleporid research; the few available studies focus on species from Vietnam, the Red Sea and Brazil. Thus, virtually nothing is known about the genetic polymorphism and divergence within and among the *Millepora* in the Caribbean.

The purpose of this paper is to combine the morphologically-based *Millepora* classification scheme with molecular information to investigate whether different morphotypes represent genetically isolated entities and delineate the *Millepora* species present in Caribbean waters. Five morphological traits were used in the taxonomical description of the genus and were compared among the main morphotypes of *M. alcicornis* and *M. complanata*, the most widely distributed species in the Caribbean. Second, DNA divergence was estimated among the four recognized species of the Caribbean (*M. alcicornis*, *M. complanata*, *M. squarrosa* and *M. striata*), using a portion of the mitochondrial gene Cytochrome Oxidase subunit I (COI). The genealogical relationships among species were studied to reveal any association among morphotypes and the mitochondrial haplotypes. Lastly, the role of phenotypic plasticity, divergence with gene flow, hybridization and incomplete lineage sorting were considered to explain the low divergence but high morphological and genetic variation found in the Caribbean species of *Millepora*.

1.1. Literature Review

The morphological variability observed in *Millepora* is a major obstacle to species identification and generates uncertainty around the studies regarding the biology of the genus. Before 1898, scientists described new species based exclusively on the growth form of the colony, making subsequent identifications subjective (Boshma 1948). In 1898, Hickson studied the growth forms of the described species of *Millepora* and concluded that the various morphotypes were manifestations of the extreme variability of *M. alcicornis* when exposed to different environments (as cited in Boshma 1948).

Boshma (1948) was convinced of Hickson's hypothesis until he observed different coexisting forms of *Millepora* and colonies of one morphotype overgrowing colonies of another.

Based on the growth form of the corallum and field observations, 13 valid species were recognized by Boshma (1948). Boshma (1948) noted that the taxonomic confusion could not be fully resolved based on the growth form of the colonies and that the existence of cryptic species was possible.

From 1948 to 1966, Boshma made many contributions to the systematics of *Millepora*, but several problems remained (Lewis 1989). With the exception of the experiments of deWeerdt in the Caribbean during the early 80's (deWeerdt 1981, deWeerdt 1984), milleporids received little attention beyond incidental observations (Lewis 1989). Lewis (1989) gathered information about the ecology of *Millepora* in an effort to inspire interest in the genus. Recent advances in computationally intensive statistics (e.g. canonical discriminant function analysis) allow us to rigorously assess the importance of these morphological characters in the *Millepora* taxonomy.

According to Lewis (1989), the genus *Millepora* is important to reef geology because milleporids are significant framework builders, with a pantropical distribution and a depth range from <1 m to about 40 meters. Ecologically, *Millepora* is a voracious plankton feeder, consuming up to 8 prey/cm^2/day (Lewis 1992), and an aggressive competitor for reef substrate (Wahle 1980; Edmunds 1999).

Manchenko et al. (1993) compared the electrophoretic profiles of 21 loci in three colony forms of *Millepora* from South Vietnam in the first attempt to resolve the taxonomic problem of the milleporids with molecular tools. The "plate-like" morph, *M. platyphylla,* was found to be genetically distinct from the "branch-like" *M. intricata* and "comb-like" *M. dichotoma* (Manchenko et al. 1993). *Millepora intricata* and *M. dichotoma* were genetically similar, suggesting that they represent growth forms of a single species. According to Manchenko et al.

(1993), the morphological similarity of the growth forms of *Millepora* reflect real phylogenetic proximity and indicate a recent species split.

In the Red Sea, Vago et al. (1994) studied the morphology of *M. dichotoma* and determined that *Millepora* exhibited four major morphotypes: encrusting, delicate lace-like, leaf-like blades and robust box-work. Robust plate forms are founded in turbulent waters, delicate leafy and branched forms occur in calmer waters, and encrusting forms occupy all depths. It was suggested that the encrusting form is always the initial mode of growth of *M. dichotoma*, followed by upward, branching growth (Vago et al. 1994).

Meroz-Fine and colleagues (2003) examined the four major morphotypes of *M. dichotoma* reported by Vago et al. (1994) in the Gulf of Elat, in the Red Sea. The growing forms of *M. dichotoma* were grouped in two categories: branching (delicate lace-like and leaf-like blades) and encrusting (encrusting and robust box- work). The abundance of colonies in the reef, colony size, morphological plasticity and nematocyst size were compared among branching and encrusting morphs. In addition, the genetic patterns of the hydrozoan host and its zooxanthellae were analyzed. The colony size and abundance of the branching and encrusting morphs differed significantly among the study locations in the Gulf of Elat. Differences in growth plasticity, growth rates and the size of the nematocyst capsule were evident between the branching and encrusting morphs (Meroz-Fine et al. 2003). The ribosomal ITS region of the branching and encrusting morphs varied in size, resulting in a significant divergence among the two morphotypes of *M. dichotoma*. For the zooxanthellae, RFLP analysis of the small subunit ribosomal RNA suggested the presence of different genetic lineages in each of the two morphotypes of *M. dichotoma*. Based on the ecological, biological and molecular data, Meroz-Fine et al. (2003) suggested that the two morphs of *M. dichotoma* were separate species.

In Brazil, three species of *Millepora* have been reported: *M. alcicornis*, *M. nitida* and *M. braziliensis*. The last two of these, however, are considered synonyms of *M. alcicornis* (Amaral et al. 1997), however it is not known if *M. alcicornis* from the Caribbean were included in the study. Since some of the skeletal characters of *M. alcicornis* overlap with the Caribbean *M. complanata*, the distinction of Brazilian and Caribbean forms of these two species becomes ambiguous. The taxonomic confusion has grown since *M. braziliensis* was also been reported as a synonym of *M. squarrosa* by Amaral et al. (2002). To clarify if *M. braziliensis* was a real species or a morph of *M. alcicornis* or *M. squarrosa*, Amaral et al. (1997) compared the allozyme variation of the first two species. Though there was some morphological overlap, large amounts of variation in colony shape were found in both species. The level of genetic similarity between *M. braziliensis* and *M. alcicornis* was very low; seven of the 12 allozyme loci studied were diagnostic for each species, demonstrating that there was not a significant amount of gene flow between species. Amaral et al. (1997) concluded that, regardless of the morphological similarities between *M. alcicornis* and *M. braziliensis,* these were two different species.

Later, Amaral et al. (2002) studied the morphological characters of the three Brazilian ecotypes of *Millepora,* focusing on the diameter of the gastropores and dactylopores. An ANOVA test of the diameters of the gastropores and dactylopores among the species showed considerable intra-specific variability and some inter-specific variation. Based on those characters, the authors proposed that the species present in Brazil were *M. alcicornis*, *M. braziliensis*, *M. nitida* and an undescribed species. The unknown species was described as *M. laboreli* based on the morphological characters and the ecology of the hydrocoral (Amaral et al. 2008).

The few taxonomical studies on the Caribbean *Millepora* highlight the high degree of skeletal polymorphism encountered in the genus. The described species for the Caribbean are *M. alcicornis*, *M. complanata*, *M. squarrosa* and *M. striata*. Martinez-Estalella (1982) compared the skeletal traits of the Caribbean species, and concluded that the distinctions between species were uncertain due to the high morphological variability among the colonies. The same difficulties were encountered by deWeerdt (1981, 1984), as the colony shape and the skeletal characteristics overlapped among species.

DeWeerdt (1981) observed a zonation pattern with depth for the different morphotypes; branched colonies were more abundant in deeper areas ($\geq$5 m), box-work *Millepora* dominated extremely shallow locations (less than 0.5 m), whereas all forms were observed in shallow, high energy environments (from 0 to 5 m). Reciprocal transplantation of the morphotypes revealed that colony shape was influenced by the environment; blade-like morphologies transplanted to deeper areas developed branches, while deep-branched colonies developed thicker or blade-like branches when transplanted to shallow areas (deWeerdt 1981). The author observed that encrusted *Millepora* grew branches or blades when transplanted to the deep area (deWeerdt 1981). It was also reported that most of the colonies described as the box-work species *M. squarrosa* developed blades when transplanted, suggesting some morphological overlap between *M. squarrosa* and *M. complanata*.

DeWeerdt (1984) found that neither growth form nor skeletal characters alone could be used to identify species. She studied the taxonomical characters of the species and recognized that size and density of the gastropores and dactylopores could be used to identify the species when considered in conjunction with the growth form of the colony (1984). Due to the shape overlap of the honey-combed/box-work structure most colonies identified as *M. squarrosa* were

instead *M. complanata*. *Millepora complanata* and *M. alcicornis* were very difficult to distinguish by their skeletal characters or colony shape, suggesting they were very closely related species if not morphological variants of the same species (deWeerdt 1984).

2. MATERIALS AND METHODS

2.1 Species description

Six species of *Millepora* are described from the western tropical Atlantic: *M. alcicornis* Linnaeus 1758, *M. complanata* Lamarck 1816, *M. squarrosa* Lamarck 1816, *M. striata* Duchassaing & Michelotti 1864, *M. nitida* Verrill 1868 and *M. braziliensis* Verrill 1868, the last two restricted to Brazil. The latest taxonomic validation of the Caribbean species was conducted by deWeerdt (1984) based on the size and density of the cavities left in the skeleton by the hydrozoan's gastrozooids (gastropores) and dactylozooids (dactylopores). The species in this study included *Millepora alcicornis*, *M. complanata*, *M. squarrosa* and *M. striata* (Table1, Fig.1).

Millepora alcicornis is abundant in the Caribbean, Bermuda, Brazil and West Africa, primarily at less exposed sites of reefs and lagoons (deWeerdt 1984; Lewis 2006). Colonies are branching; branches may be very delicate or coarse and largely united into plate-like constructions and flattened at the growing edges. The corallum grows mostly upright, but it may also encrust gorgonians (e.g. *Gorgonia ventalina*, *Eunicae flexuosa*), other sessile organisms and hard substrates. The surface of the colony is rather smooth and even. According to deWeerdt (1984), the gastropore size ranges from 0.15 to 0.30 mm while the dactylopores are smaller (0.06-0.17 mm).

Millepora complanata inhabits the surf zone and reef flats where its blades grow perpendicular to the direction of the current. Despite its habitat specificity, *M. complanata* is

common throughout the Caribbean (deWeerdt 1984; Lewis 2006). The colonies are formed by simple plates growing from a common base or may create complex honey-combed structures of interconnected plates. In places with strong wave action, colonies may remain as a large encrusting base. The surface of the colonies varies from smooth to rough. The gastropores are large (0.22 to 0.36 mm), and dactylopores are variable in size (0.12-0.24 mm).

Millepora squarrosa is distributed throughout the Caribbean and Brazil (deWeerdt 1984; deWeerdt 1990). The colonies consist of irregular, heavy-connected, thick masses with smooth rounded edges. In older colonies, the growth form can become more plate-like, and the growing edges sharpening, but the plates remaining connected along their whole length, resulting in box-work structure. The surface is smooth but made irregular by the presence of crests and tubercles. Gastropore size varies from 0.20 mm to 0.30 mm, and the dactylopores are very small (0.07-0.15 mm).

Millepora striata is reported from Panama, Colombia, Venezuela and Guadeloupe (deWeerdt 1984). Colonies are formed by loosely-connected plates of very sharp edges with a strong tendency to divide along the upper edge. Longitudinal folds make the surface very uneven. Gastropores grow to sizes from 0.15 to 0.25 mm and dactylopores vary from 0.08 to 0.18 mm.

2.2 Sampling Methods

Colonies were classified as *M. alcicornis*, *M. complanata* or *M. squarrosa* using the most recent taxonomic descriptions by deWeerdt (1984) for the Caribbean species. Collections of samples took place in the front reefs of Turrumote, Media Luna, Enrique, Pelotas and Margarita in the La Parguera reef system off the southwestern coast of Puerto Rico (Fig. 2) between 1 and 15 meters, from August 2005 to August 2007. Samples from *M. squarrosa* were collected at

Media Luna and Turrumote reefs at depths of 15 meters.

Millepora alcicornis was classified into two morphotypes (Fig. 3); free growth branching and encrusted on gorgonians. De Weerdt (1984) and Lewis (1989) identified *Millepora* growing on gorgonians as *M. alcicornis*; however, encrusting milleporids develop as branched or bladed when transplanted to deeper areas (deWeerdt 1981). In addition, colonies of both *M. alcicornis* and *M. complanata* have been observed attacking and overgrowing gorgonians (Wahle 1980). Following the general consensus for classification by growth form, *Millepora* overgrowing octocoral colonies was identified as *M alcicornis.* However, the growth form was treated as a distinct morphotype to reduce the range of variation reported for *M. alcicornis*.

. *Millepora squarrosa,* was relatively common in Puerto Rico (deWeerdt 1984; deWeerdt 1990), especially before the 2005 bleaching event (Weil and Yoshioka, pers. comm.) but only three colonies were found in Turrumote, Media Luna and Margarita. Due to the small size of the colonies, tissue was collected only for genetic analysis. *Millepora striata* has not been reported for Puerto Rico, but four specimens were obtained from Bocas del Toro, Panama in October 2007 and used in the genetic analysis.

Due to the high phenotypic plasticity, ten colonies of the most representative morphotypes of *M. alcicornis* branched, *M. alcicornis* encrusted, and *M. complanata* were sampled (N total = 30). Selected colonies were spaced at least 5 m from each other to minimize the collection of clones. Two fragments of each colony were collected and preserved in 100% ethanol for micro-morphological and genetic analyses. In addition, two whole colonies from each morph were collected for estimation of character variation at different positions (base, center and tips) within the colony.

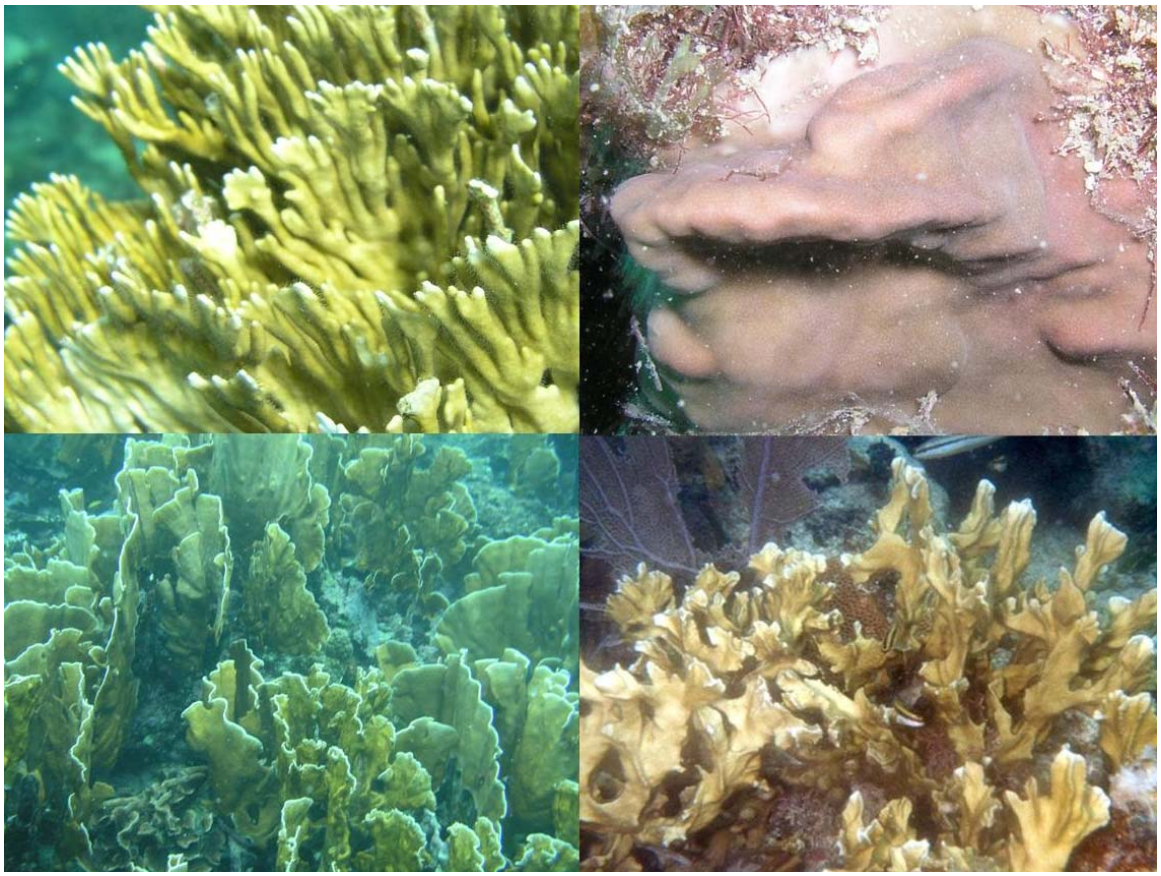

Figure 1. Caribbean species of *Millepora*. Clockwise from the upper left: *M. alcicornis*, *M. squarrosa*, *M. striata,* and *M. complanata.*

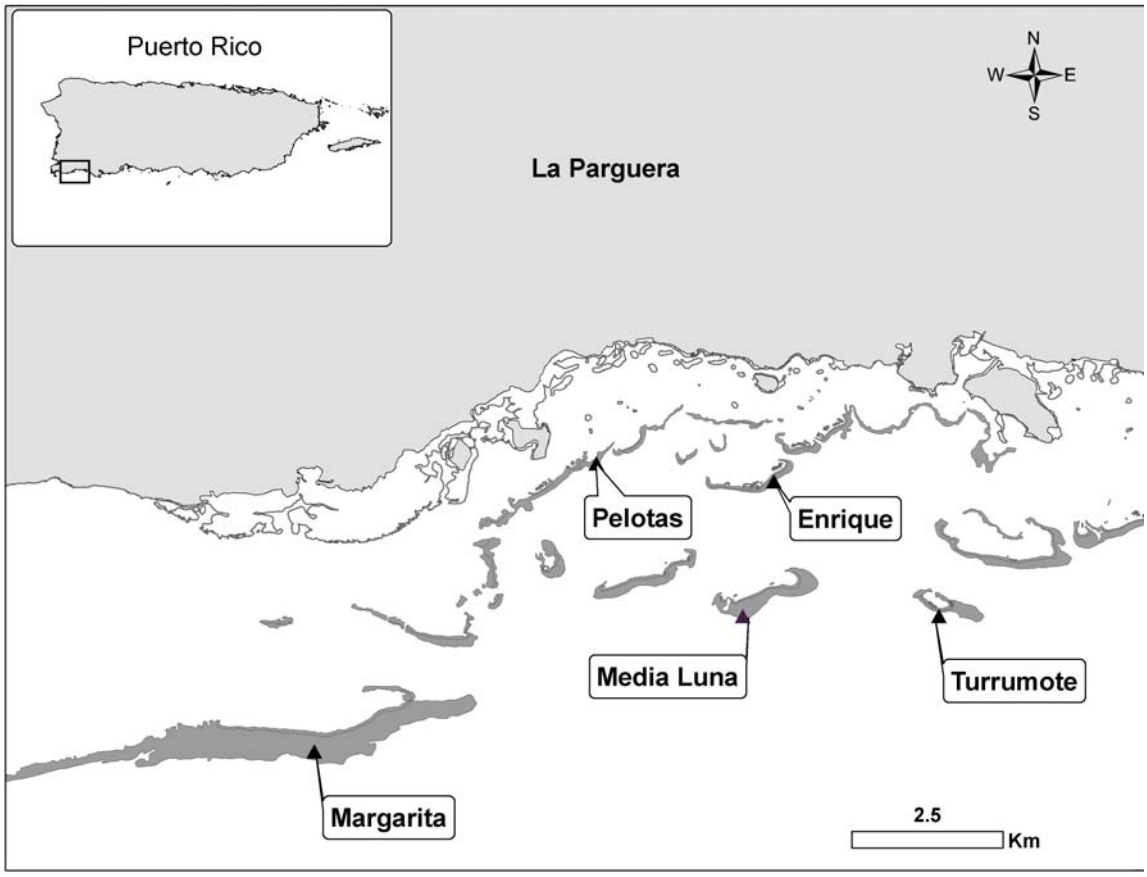

Figure 2: The reef system of La Parguera, southwestern Puerto Rico. All colonies were sampled from the front reef of the Pelotas, Enrique, Media Luna, Turrumote and Margarita reefs (Shearer and Nemeth unpublished Data).

Figure 3. Morphotypes of *M. alcicornis*. Left: *M. alcicornis* free grow branching, Right: *M.alcicornis* encrusted on gorgonians.

Table 1. List of the samples used for the morphological and genetic analysis: Name and morphotype, reef, zone within the reef and depth (in meters) in which the colony was found

Sample	Morphotype	Reef	Zone	Depth	Morphology	Genetics
Mc123	*M. complanata*	Enrique	frontreef	1	yes	yes
McC1	*M. complanata*	Turrumote	frontreef	1	yes	yes
McE3	*M. complanata*	Enrique	frontreef	1	yes	yes
McE5	*M. complanata*	Enrique	frontreef	1	yes	yes
McE7	*M. complanata*	Enrique	frontreef	1	yes	yes
McE8	*M. complanata*	Enrique	frontreef	1	yes	yes
McMg1	*M. complanata*	Margarita	frontreef	2	yes	yes
McMg3	*M. complanata*	Margarita	frontreef	2	yes	yes
McMg6	*M. complanata*	Margarita	frontreef	2	yes	yes
McMg9	*M. complanata*	Margarita	frontreef	2	yes	yes
MabCo2	*M. alcicornis* branched	Turrumote	frontreef	1	yes	yes
MabMg1	*M .alcicornis* branched	Margarita	frontreef	2	yes	yes
MabMg2	*M. alcicornis* branched	Margarita	frontreef	2	yes	yes
MabML1	*M. alcicornis* branched	Media Luna	frontreef	8	yes	yes
MabT1	*M. alcicornis* branched	Turrumote	frontreef	7	yes	yes
MabT22	*M. alcicornis* branched	Turrumote	frontreef	7	yes	yes
MabT23	*M. alcicornis* branched	Turrumote	frontreef	7	yes	yes
MabT24	*M. alcicornis* branched	Turrumote	frontreef	7	yes	yes
MabT25	*M. alcicornis* branched	Turrumote	frontreef	7	yes	yes
MabT26	*M. alcicornis* branched	Turrumote	frontreef	7	yes	yes
MaeLP10	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeLP2	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeLP3	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeLP4	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeLP5	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeLP7	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeLP9	*M. alcicornis* encrusted	Pelotas	frontreef	8	yes	yes
MaeMg1	*M. alcicornis* encrusted	Margarita	frontreef	5	yes	yes
MaeML1	*M. alcicornis* encrusted	Media Luna	frontreef	8	yes	yes
MaeT15	*M. alcicornis* encrusted	Turrumote	frontreef	7	yes	yes
MsqML2	*M. squarrosa*	Media Luna	frontreef	11	no	yes
MsqT2	*M. squarrosa*	Turrumote	frontreef	14	no	yes
MS2	*M. striata*	Panama	**	**	no	yes
MS3	*M. striata*	Panama	**	**	no	yes
MS4	*M. striata*	Panama	**	**	no	yes
MS5	*M. striata*	Panama	**	**	no	yes

2.3 Morphological variability

A total of 30 colonies were examined. Ten colonies of each morphotype were classified as: *M. alcicornis* branched (Mab, n=10), *M. alcicornis* encrusting (Mae, n=10) and *M. complanata* (Mc, n=10). A piece of the colony was used for DNA extraction, and the rest of the colony was cleaned with a solution of 5 % sodium hypochlorite, dried, and analyzed under a dissecting microscope (Amaral et al. 2002). Five skeletal characters that have been used extensively in previous *Millepora* studies were selected for the morphometric analysis: 1) diameter of the dactylopores, 2) diameter gastropores (Amaral et al. 2002, deWeerdt 1984), 3) distance between the gastropores (Razak and Hoeksema 2003), 4) the distance between the dactylopores, and 5) the distance from gastropore to nearest dactylopore (Fig 4, Table 2).

Micro-morphological analyses consisted of two stages; first, the variability among the skeletal traits was compared within the colony and then the skeletal traits were compared among colonies of the same morphotype and among colonies of different morphotype. To study variation within the colony, two colonies of each morphotype were examined. Thirty individual measurements of the following traits were taken from the base (b, n=30), the center (c, n=30) and the tip (t, n=30) of the colony: diameter of gastropores and dactylopores, distances among gastropores and among dactylopores, and distance from gastropore to nearest dactylopore (Table 2). Measures were taken from the center of the colony (the less variable position) and 30 measurements performed for each trait: diameter of gastropores and dactylopores, distances among gastropores and among dactylopores, and distance from gastropore to nearest dactylopore (Table 2).

Table 2. Summary of the traits measured to quantify morphological variability within the colonies (Intra-colony variation) and among the colonies (Inter-colony variation).

		Measurements				
		Intra-colony variation			Inter-colonyvariation	
Morphological traits	Code	position	colony	morphotype	colony	morphotype
Gastropore diameter	G	30	120	240	30	300
Dactylopore diameter	D	30	120	240	30	300
Distances among dactylopores	D-D	30	120	240	30	300
Distances from gastropore to nearest dactylopore	G-D	30	120	240	30	300
Distances among gastropores	G-G	30	120	240	30	300

Photographs of the traits were catalogued digitally at 3x magnification with an Olympus C-5050 camera system attached to an Olympus SZH-10 dissecting microscope. The selected traits were measured using SigmaScan Pro Software (SPSS Inc.) after calibration with a slide of 10 μm accuracy.

Parametric and non-parametric (when data did not meet the assumptions of equal variances and normality) one-way ANOVA were used to compare the individual traits within colonies and among colonies of the same morph and across morphs/species. An *a posteriori* Tukey's test of means or medians was used to identify the colonies or species that exhibited traits significantly different.

A Discriminant Function Analysis (DFA) was used to test the utility of the five morphological characters to distinguish the species as identified during the collection. The statistical analyses were performed in InfoStat version 2004 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina), SigmaStat (SPSS Inc., Chicago, Illinois, USA) and JMP version 5.0.1 (SAS Institute Inc., Cary, NC).

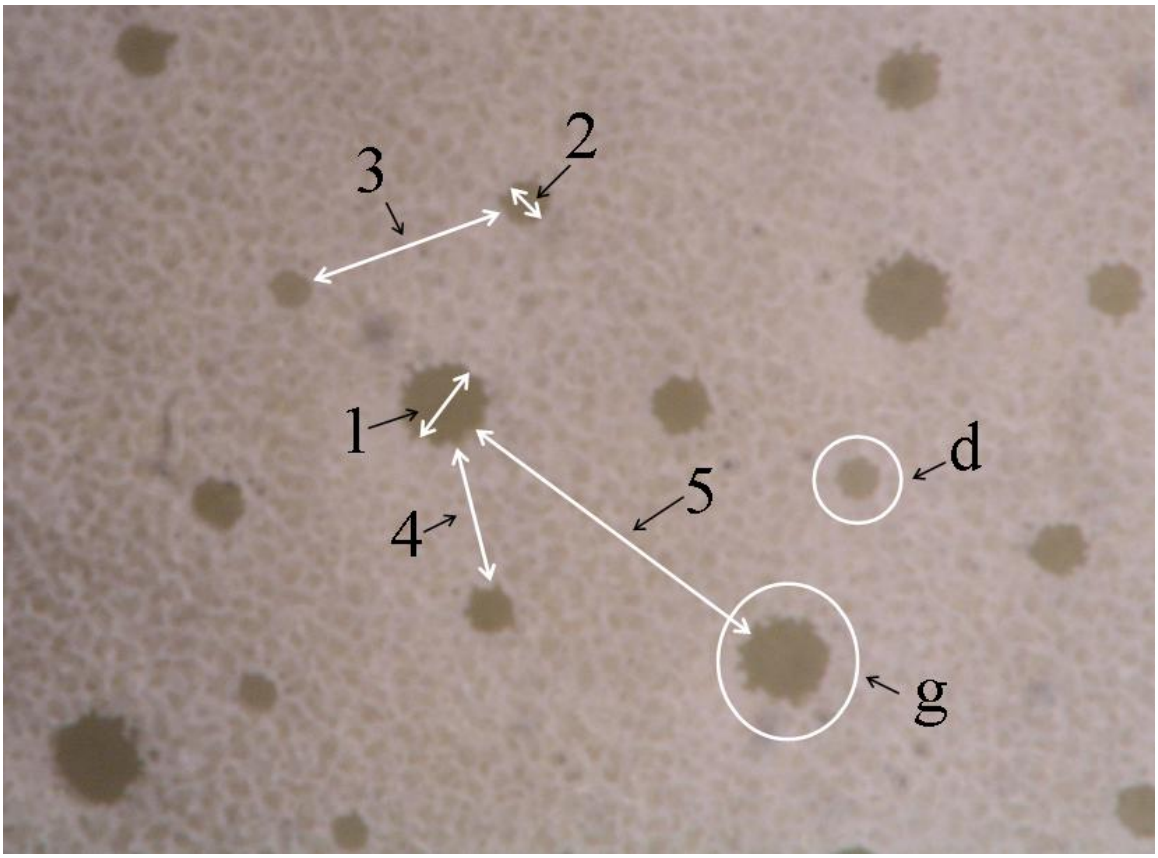

Figure 4: Measured traits of coralla in *M. alcicornis* (MabT22): g = Gastropore, d = Dactylopore, 1. Diameter of gastropore, 2. Diameter of dactylopore, 3. Distance among dactylopores, 4. Distances from gastropore to nearest dactylopore, 5. Distances among gastropores.

2.4 Genetic Analyses

Genetic analyses were performed for the 30 colonies used in the morphological analysis, in addition 4 samples of *M. striata* and 2 samples of *M. squarrosa* were added (Table 1). Tissue was scraped from the skeletal surface of the sample and DNA extracted using the PureGene DNA isolation kit (Gentra) for fixed-tissue following the manufacturer's guidelines. A portion of the mitochondrial gene Cytochrome Oxidase Subunit I (COI) was amplified by PCR. Unlike scleractinian corals, where little variation at the mtDNA is found and is therefore unable to resolve microevolutionary processes (Romano and Palumbi 1996; Hellberg 2006), the COI gene proved to be highly variable for hydrozoans (Govindarajan et al. 2005). COI has also been

promoted by the Barcoding of Life initiative as the most appropriate gene to differentiate species of higher metazoans (Hebert et al. 2004; Moritz and Cicero 2004).

Initial gene amplifications were made with the COI primers designed by Fukami et al. (2004), but they were suboptimal. *Millepora*-specific primers COIF (5'- TAG-AAT-TAG-CTG-GGC-CAG-GA -3') and COIR (5'- CCT-GTC-TGT-AAG-CAG-CAT-GG -3') were designed from the initial COI sequences using the Primer 3 software. PCR cycling conditions consisted of an initial denaturation of 3 min at 95 °C followed by 35 cycles of 15 sec at 95 °C for denaturation, 30 sec at 50 °C of annealing, 60 sec at 72 °C for extension, and a final extension at 72 °C for 5 min. Successful PCR reactions were verified by running 5µl of the amplicon on a 1% TBE agarose gel stained with ethidium-bromide. PCR reactions were cleaned of excess dNTPs, primers, and other impurities by enzymatic treatment with the EXOSAP-IT method. Sequencing reactions with each of the primers were prepared with the 3.1 BigDye Termination Kit and were loaded in an ABI3130xl.

DNA sequencing trace files were processed with Codon Code Aligner (CodonCode, Dedham, MA, USA) for base calling, quality assessment, contig assembly, visualization and manual editing. Edited DNA sequences were imported to MacClade (Maddison and Maddison 1992) for alignment. Aligned sequences were imported to DnaSP (Rozas et al. 2003) for general summary statistics and population analysis. Summary statistics analyses included the nucleotide diversity estimations of π (Nei and Li 1979) and θ_w (Watterson 1975), number of haplotypes, number of parsimony informative sites, number of segregating sites and number of synonymous and non-synonymous substitutions. DNA neutrality tests such as Tajima's D (Tajima 1989) were computed to test for deviation from the neutral model of molecular evolution (Kimura 1968). Numbers of shared mutations, fixed differences and F_{ST} values were calculated in DnaSP

(Rozas et al. 2003).

Genealogies of the samples were constructed using Maximum Likelihood (ML) and Bayesian Inference (BI). ML analysis was performed in PAUP 4.10b (Swofford 2002) and BI was run in MrBayes (Ronquist and Huelsenbeck 2003). The AIC criterion in ModelTest (Posada and Crandall 1998) was used to identify the best substitution model for the COI sequences. Topology robustness was evaluated with 100 bootstrap replicates (Felsenstein 1985) and posterior probabilities. Genetic distances of F_{ST} were calculated in DnaSP and used to generate distance trees in MEGA (Tamura et al. 2007). Haplotype networks were constructed based with the parsimony method in TCS (Clement et al. 2000).

DNA sequences of the *Millepora* species and morphotypes sampled across the Caribbean were added to the sequences from the colonies used in the morphological and genetic analyses. An Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) was performed to test if the *M. complanata* and the two morphotypes of *M. alcicornis* were significantly differentiated. A total of seven populations were included in the analysis; Panama (MSPanama, McPanama, MabPanama), Grand Cayman (McGC, MabGC, MaeGC), Mona (McMona, MabMona, MaeMona), SWPR (McSWPR, MabSWPR, MaeSWPR), Vieques (McVieques, MabVieques, MaeVieques), Guadeloupe (McGd, MabGd, MaeGd), Curaçao (McCuraçao, MabCuraçao, MaeCuraçao); where MS = *M. striata*, Mc = *M. complanata*, Mab = *M. alcicornis* branched and Mae = *M. alcicornis* encrusted. AMOVA analysis was performed in Arlequin v3.11 (Excoffier et al. 2005) with 25,000 replications, corrected by the Tamura-Nei + ($\Gamma = 1.8$) distance. Genetic differences were partitioned among populations, among morphotypes within populations and within the Caribbean. Pairwise genetic comparisons of the morphotypes were corrected with the Tamura-Nei distance.

3. RESULTS

3.1 Variability of skeletal characters:

There were significant differences ($p<0.0001$; Kruskal-Wallis test, Figs 10-11) in gastropore diameters, dactylopore diameters, distances among dactylopores, distances among gastropores, and distances from gastropore to nearest dactylopore between *M. complanata* and the two morphotypes of *M. alcicornis*. These differences were noticed in the comparisons among the positions (base, center and tip) of the characters within the colony and among colonies of different morphologies.

Measurements of the skeletal traits (Table 3) were significantly different ($p<0.0001$) among *M. complanata*, M. *alcicornis* branched and *M. alcicornis* encrusted. The sizes of the gastropores (mm) were significantly larger ($p<0001$) in *M. complanata* (0.25 ± 0.03 mm) than in *M. alcicornis* (0.20 ± 0.04 mm), and gastropore size did not vary between the two morphotypes of *M. alcicornis*. Dactylopores sizes varied for the three morphotypes ($p< 0.0001$), *M. complanata* had the largest dactylopores (0.15 ± 0.02 mm), followed by *M. alcicornis* encrusted (0.13 ± 0.02 mm), and *M. alcicornis* branched (0.12 ± 0.02 mm). The diameters of the gastropores and dactylopores of *M. complanata* and *M. alcicornis* were similar to those previously reported by deWeerdt (1984).

Significant differences ($p<0.0001$) were found for the distances among gastropores and for the distances from gastropore to the nearest dactylopore. Distances among gastropores were significantly larger in *M. alcicornis* encrusted (1.45 ± 0.43 mm) compared to *M. alcicornis* branched (1.38 ± 0.38 mm), while *M. complanata* had the smallest values (1.10 ± 0.32 mm). The distances from gastropore to the nearest dactylopore were larger in *M. alcicornis* branched

(0.40 ± 0.09 mm) than in *M. complanata* and *M. alcicornis* encrusted which had similar distances (0.36 ± 0.09 mm) (Table 3).

Table 3. Descriptive statistics [mean, standard deviation (S.D.), minimum, maximum and median] of the measurements (mm) of gastropore diameter (G), dactylopore diameter (D), distances among dactylopores (D-D), distances from gastropore to nearest dactylopore (G-D) and distances among gastropores (G-G) of *M. complanata* (Mc), *M. alcicornis* branched morphotype (Mab) and *M. alcicornis* encrusted morphotype (Mae).

Morph	Variable	N	Mean	S.D.	Min	Max	Median
Mc	G	300	0.25	0.03	0.16	0.32	0.24
Mab	G	300	0.20	0.04	0.11	0.32	0.20
Mae	G	300	0.20	0.03	0.13	0.29	0.20
Mc	D	300	0.15	0.02	0.09	0.21	0.15
Mab	D	300	0.11	0.02	0.07	0.17	0.11
Mae	D	300	0.13	0.02	0.05	0.20	0.13
Mc	D-D	300	0.51	0.10	0.16	0.83	0.51
Mab	D-D	300	0.53	0.16	0.28	2.18	0.50
Mae	D-D	300	0.52	0.11	0.24	0.99	0.51
Mc	G-D	300	0.36	0.09	0.17	0.82	0.34
Mab	G-D	300	0.36	0.09	0.16	0.69	0.34
Mae	G-D	300	0.40	0.09	0.21	0.81	0.39
Mc	G-G	300	1.10	0.32	0.30	2.22	1.07
Mab	G-G	300	1.38	0.39	0.33	2.88	1.36
Mae	G-G	300	1.45	0.43	0.26	2.95	1.42

3.1.1 Within colony variation

Measurements of the skeletal characters on the base, center and tip of the colonies were significantly different ($p<0.0001$) within the colonies (Table 4). Overall, higher variation was found for the traits measured at the tip of the colonies (Tukey's test, $p<0.05$), whereas same characters in the base and center of the colony were less variable and thus used for species comparisons. However, significant differences ($p<0.0001$) were found among skeletal traits on the same position, among colonies of the same morphotype (Figs 5 to 9). Despite the high variance within traits, differences in diameter of gastropores and dactylopores, distances between gastropores, and distance from gastropore to nearest dactylopore were large enough to separate the morphotypes ($p<0.0001$; Figs 10 and 11).

Table 4. Comparisons of the measured morphological traits (in mm) at different positions on the colony. Trait: G: gastropore diameter, D: dactylopore diameter, D-D: distances between dactylopores, G-D: distance from gastropore to neaest dactylopore, G-G: distances between gastropores. H: Value of Kruskal-Wallis One-Way Anova, Different letters (A, B, C): significant values of Tukey's test.

Morphotype	Trait	position	N	Mean	S.D.	Median	H	*p*	Ranks	
M. complanata	G	base	120	0.23	0.03	0.23	24.03	<0.0001	152.34	A
M. complanata	G	center	120	0.26	0.05	0.25			172.45	A
M. complanata	G	tip	120	0.24	0.03	0.24			216.71	B
M. complanata	D	base	120	0.15	0.03	0.15	12.67	0.0018	157.2	A
M. complanata	D	center	120	0.16	0.02	0.16			179.33	AB
M. complanata	D	tip	120	0.16	0.02	0.16			204.98	B
M. complanata	D-D	base	120	0.46	0.08	0.46	5.57	0.0617		
M. complanata	D-D	center	120	0.46	0.08	0.44				
M. complanata	D-D	tip	120	0.50	0.13	0.47				
M. complanata	G-D	base	120	0.33	0.07	0.32	12.66	0.0018	162.47	A
M. complanata	G-D	center	120	0.33	0.07	0.32			171.41	A
M. complanata	G-D	tip	120	0.38	0.13	0.36			207.62	B
M. complanata	G-G	base	120	1.22	0.33	1.21	12.56	0.0019	160.13	A
M. complanata	G-G	center	120	1.13	0.45	1.14			174.70	A
M. complanata	G-G	tip	120	1.35	0.42	1.33			206.67	B
M. alcicornis b	G	base	120	0.16	0.03	0.15	122.65	<0.0001	113.37	A
M. alcicornis b	G	center	120	0.17	0.02	0.17			167.65	B
M. alcicornis b	G	tip	120	0.19	0.02	0.19			260.48	C
M. alcicornis b	D	base	120	0.08	0.02	0.07	139.70	<0.0001	100.73	A
M. alcicornis b	D	center	120	0.09	0.02	0.09			181.25	B
M. alcicornis b	D	tip	120	0.11	0.02	0.11			259.52	C
M. alcicornis b	D-D	base	120	0.49	0.10	0.48	10.43	0.0054	159.66	A
M. alcicornis b	D-D	center	120	0.47	0.07	0.46			178.89	AB
M. alcicornis b	D-D	tip	120	0.45	0.08	0.45			202.95	B
M. alcicornis b	G-D	base	120	0.38	0.08	0.37	20.70	<0.0001	154.78	A
M. alcicornis b	G-D	center	120	0.35	0.07	0.34			172.43	A
M. alcicornis b	G-D	tip	120	0.33	0.09	0.33			214.29	B
M. alcicornis b	G-G	base	120	1.47	0.41	1.45	56.14	<0.0001	124.07	A
M. alcicornis b	G-G	center	120	1.37	0.35	1.35			196.68	B
M. alcicornis b	G-G	tip	120	1.13	0.25	1.10			220.76	B
M. alcicornis e	G	base	120	0.19	0.03	0.20	85.15	<0.0001	137.63	A
M. alcicornis e	G	center	120	0.19	0.03	0.19			152.30	A
M. alcicornis e	G	tip	120	0.23	0.03	0.23			251.58	B
M. alcicornis e	D	base	120	0.11	0.02	0.11	177.92	<0.0001	108.18	A
M. alcicornis e	D	center	120	0.10	0.02	0.10			152.59	B
M. alcicornis e	D	tip	120	0.14	0.03	0.14			280.74	C
M. alcicornis e	D-D	base	120	0.50	0.09	0.50	8.78	0.0124	160.42	A
M. alcicornis e	D-D	center	120	0.48	0.09	0.48			180.85	AB
M. alcicornis e	D-D	tip	120	0.47	0.09	0.45			200.23	B
M. alcicornis e	G-D	base	120	0.38	0.10	0.37	7.68	0.0215	159.81	A
M. alcicornis e	G-D	center	120	0.39	0.08	0.38			185.82	AB
M. alcicornis e	G-D	tip	120	0.36	0.09	0.35			195.87	B
M. alcicornis e	G-G	base	120	1.48	0.47	1.43	3.13	0.2092		
M. alcicornis e	G-G	center	120	1.47	0.55	1.35				
M. alcicornis e	G-G	tip	120	1.38	0.43	1.33				

3.1.2 Across colony variation

Variation across colonies was significant ($p<0.001$) for all the traits measured (Figs 5 to 9). Differences in diameter and distances measured for the different traits were observed among colonies of different morphotypes but also between colonies of the same morphotype. Some colonies with different morphologies showed skeletal traits with similar dimensions.

Diameters of gastropores varied from 0.11 to 0.32 mm (Fig 5). There were significant differences ($p<0.0001$) among the gastropore diameters within *M. complanata* with measures varying between 0.16 mm and 0.32 mm. Six colonies (McC1, McMg1, McMg3, McE7 and McE8) presented mean diameters ranging from 0.22 to 0.25 mm, while three colonies (McMg6, McE5 and Mc123) had mean diameters of 0.26 to 0.27 mm. Gastropores diameters of colony McMg9 varied in size from 0.21 mm to 0.28 mm. The range of the gastropore diameters overlapped in the two *M. alcicornis* morphotypes and was highly variable in colonies within and between morphotypes. The size of the gastropores of colonies MabCO2, MabMg1, MaeLP9, MaeLP10, and MaeMg1 were significanlty different than the other *M. alcicornis* colonies, and very similar to the gastropore diameters of *M. complanata*.

Dactylopore diameter varied from 0.05 to 0.21 mm (Fig 6). *Millepora complanata* exhibited the largest mean dactylopore diameter while the smaller diameters were observed in the colonies of the branching *M. alcicornis*. The encrusted *M. alcicornis* had the most variable dactylopore size, some colonies, for example MaeLP2, MaeLP3, were very similar to *M. alcicornis* branched, while other colonies such as MaeLP9 were more similar to *M. complanata*. However, some colonies of the three morphotypes showed similar diameters such as McMg9, McE3, MabCO2, MaeLP7 and MaeT15.

Distances between dactylopores (Fig.7) varied from 0.16 to ~0.80 mm in the majority of the colonies from all the morphotypes, with the exception of colony MabT26 whose average distances were larger than the rest of the colonies. Colonies were divided in three main groups; in colonies MabT22, Mc123, MaeLP4 the distance among dactylopores ranged from 0.49 to 0.52 mm; in colonies McC1, McMg1, McMg3, MabMg1, MaeML1 the distance was intermediate with means among 0.55 to 0.59 mm, while in colonies MabT26 and MaeLP9, the distances among dactylopores attained a maximum distance of 2.18 and 0.99 mm, respectively.

The distances from gastropore to nearest dactylopore were also variable (Fig.8). There were significant differences among the measurements ($p<0.001$), however there were not clear differences among the morphotypes. For example, most colonies of *M. complanata* had equal distances among gastropores and dactylopores, however those values did not vary from the distances observed in branched *M. alcicornis*. Morphs of branched *M. alcicornis* were more similar to *M. complanata* than they were to the encrusted *M. alcicornis*.

Distances between gastropores were less variable among colonies of the same morphology than the aforementioned traits (Fig 9). The mean distance between gastropores (mm) was smaller in *M. complanata* (1.10 ± 0.32 mm) than in the *M. alcicornis* morphs. Colonies of branched *M. alcicornis* (1.38 ± 0.39 mm) were also distinct from colonies of encrusted *M. alcicornis* (1.45 ± 0.43 mm); however overlapping measurements were recorded among some colonies of the two morphotypes.

In summary, overlapping measurements were observed for all the studied traits among colonies of different morphotypes. In the three morphotypes (*M. alcicornis* branched, *M. alcicornis* encrusted and *M. complanata*) some colonies were generally more similar to colonies of other morphotypes than to their own. For example, all the traits measured for MabCO2 were,

on average, more similar to *M. complanata* than to colonies of *M. alcicornis* branched (Tukey's test was not significant).

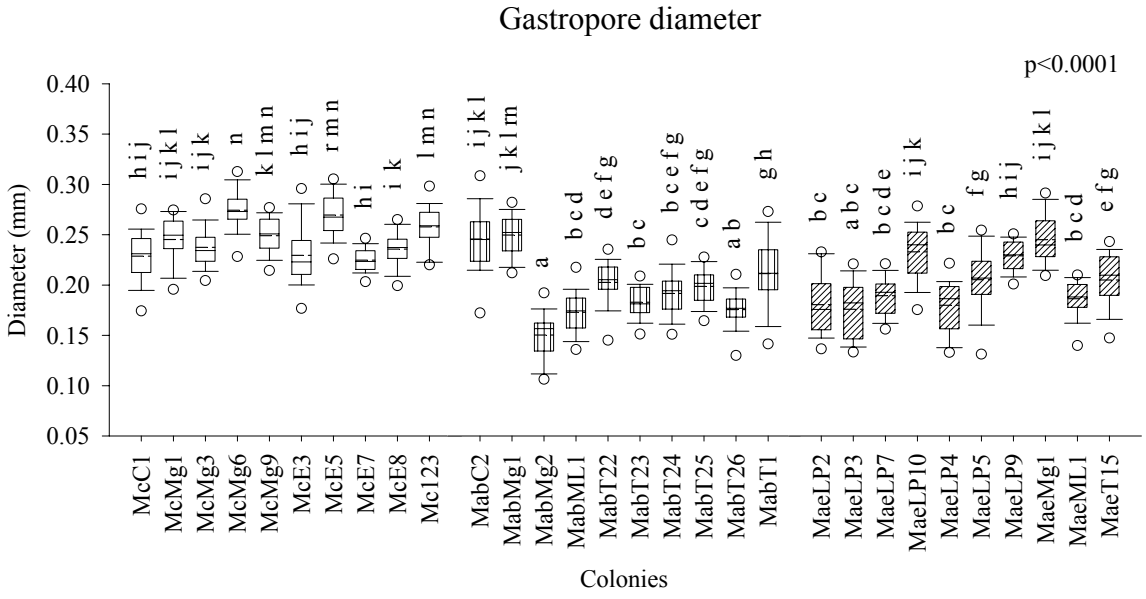

Figure 5. Variability (mean, median and 5th and the 95th percentiles) in gastropore diameters (mm). Colonies are plotted in the x-axis, diameter in mm in the y-axis. Solid line is the median, dashed line the mean. The white boxes are colonies of *M. complanata* (Mc), boxes with vertical lines are colonies from *M. alcicornis* branched (Mab), boxes with diagonal lines are colonies from *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among positions are denoted by different letters.

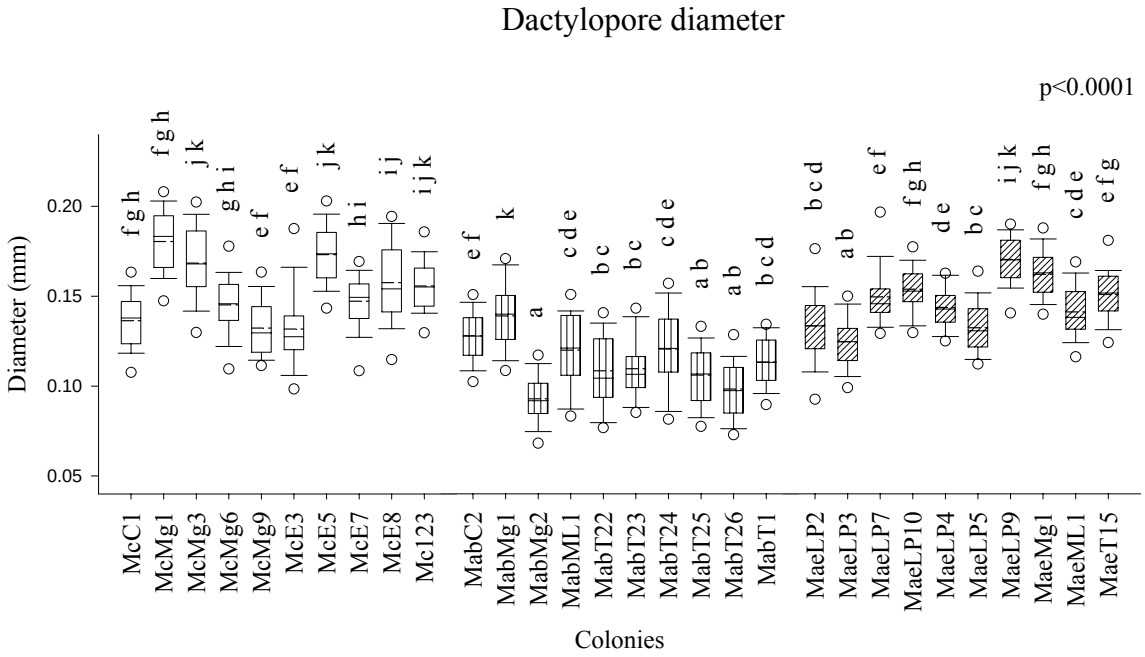

Figure 6. Variability (mean, median and 5th and the 95th percentiles) in dactylopore diameters (mm). Colonies are plotted in the x-axis, diameter in mm in the y-axis. Solid line is the median, dashed line the mean. The white boxes are colonies of *M. complanata* (Mc), boxes with vertical lines are colonies from *M. alcicornis* branched (Mab), boxes with diagonal lines are colonies from *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among positions are denoted by different letters.

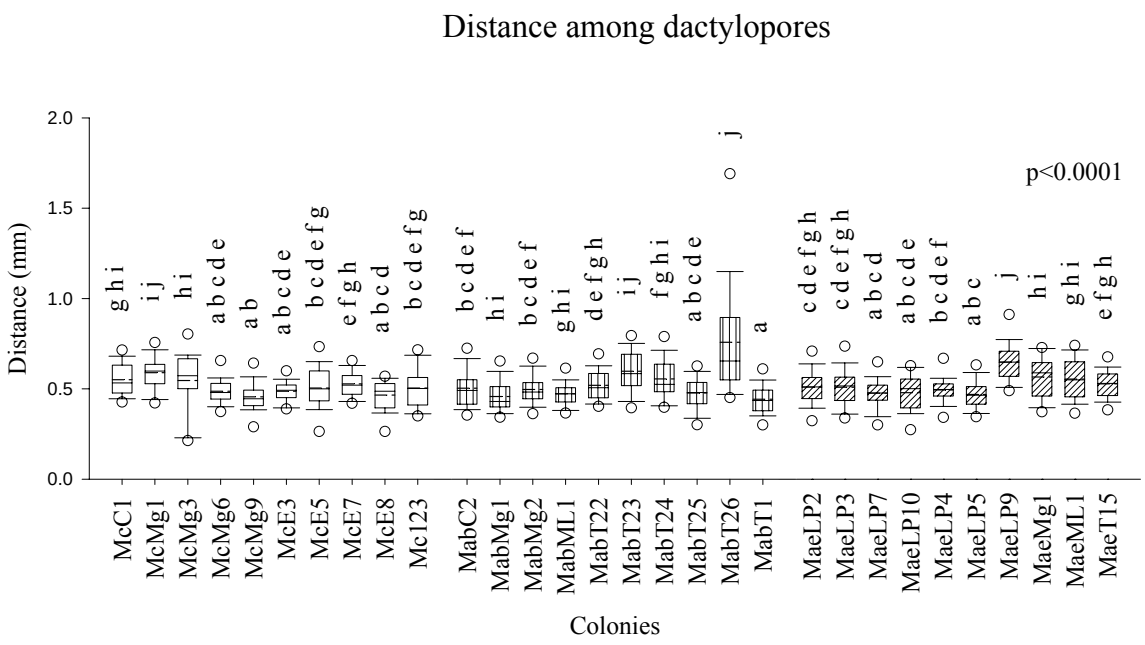

Figure 7. Variability (mean, median and 5th and the 95th percentiles) in the distances among dactylopores (mm). Colonies are plotted in the x-axis, distances in mm in the y-axis. Solid line is the median, dashed line the mean. The white boxes are colonies of *M. complanata* (Mc), boxes with vertical lines are colonies from *M. alcicornis* branched (Mab), boxes with diagonal lines are colonies from *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among positions are denoted by different letters.

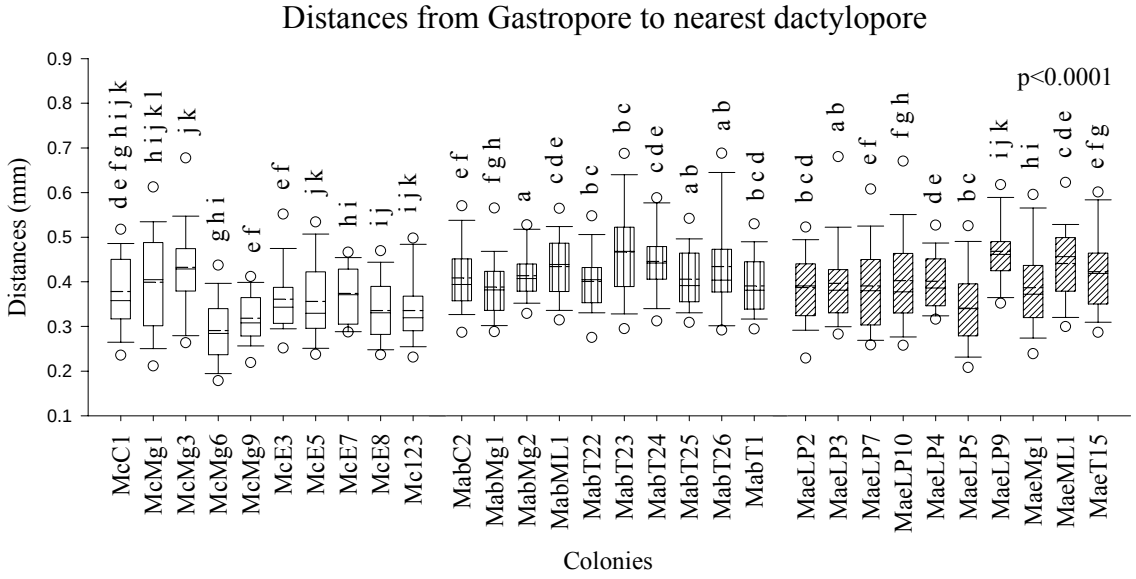

Figure 8. Variability (mean, median and 5th and the 95th percentiles) in the distances from gastropore to nearest dactylopore (mm). Colonies are plotted in the x-axis, distances in mm in the y-axis. Solid line is the median, dashed line the mean. The white boxes are colonies of *M. complanata* (Mc), boxes with vertical lines are colonies from *M. alcicornis* branched (Mab), boxes with diagonal lines are colonies from *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among positions are denoted by different letters.

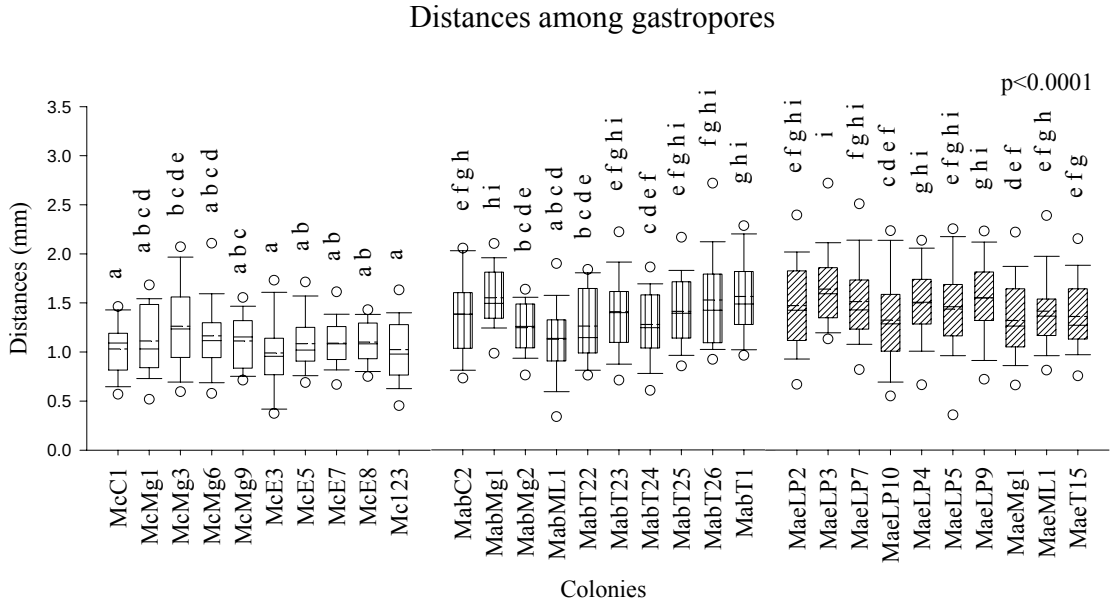

Fig 9. Variability (mean, median and 5th and the 95th percentiles) in the distances among gastropores (mm). Colonies are plotted in the x-axis, distances in mm in the y-axis. Solid line is the median, dashed line the mean. The white boxes are colonies of *M. complanata* (Mc), boxes with vertical lines are colonies from *M. alcicornis* branched (Mab), boxes with diagonal lines are colonies from *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among positions are denoted by different letters.

3.1.3 Variability among morphotypes

Despite some overlap among morphological traits, some characters were useful (diagnostic) to differentiate species. The diameters of the gastropores of *M. complanata* were significantly larger (Tukey's test, $p<0.0001$; Fig. 10) than the gastropores of the two morphotypes of *M. alcicornis*. There was no difference in gastropore diameters between the two morphotypes of *M. alcicornis*. Conversely, diameters of the dactylopores were different enough ($p<0.001$) to distinguish not only between *M. complanata* and *M. alcicornis* but also between the two morphs of *M. alcicornis*. Dactylopore diameters of *M. complanata* were significantly larger ($p<0.0001$) than the *M. alcicornis* morphotypes. The dactylopores of *M. alcicornis* encrusted were significantly larger than those of the branched morphotype (Fig.10).

The distances between dactylopores did not differ among *M. complanata*, *M. alcicornis* branched and *M. alcicornis* encrusted (Fig.11). While the distances from gastropore to nearest dactylopore were similar for *M. complanata* and branched *M. alcicornis* (Fig.11), they were shorter ($p<0.001$) than the encrusted morph of *M. alcicornis.* The measurements of the distances between gastropores were significantly different ($p<0.001$) for the three morphs (Fig.11). However, the distance between gastropores was highly variable within morphotypes ranging from 0.26 to 2.95 mm in the encrusted *M. alcicornis,* from 0.33 to 2.88 mm in the branched *M. alcicornis* and from 0.30 to 2.22 mm in *M. complanata*. Gastropores were more scattered in the encrusted *M. alcicornis*, than in the branched *M. alcicornis*, while the colonies of *M. complanata* had the smaller distances between gastropores.

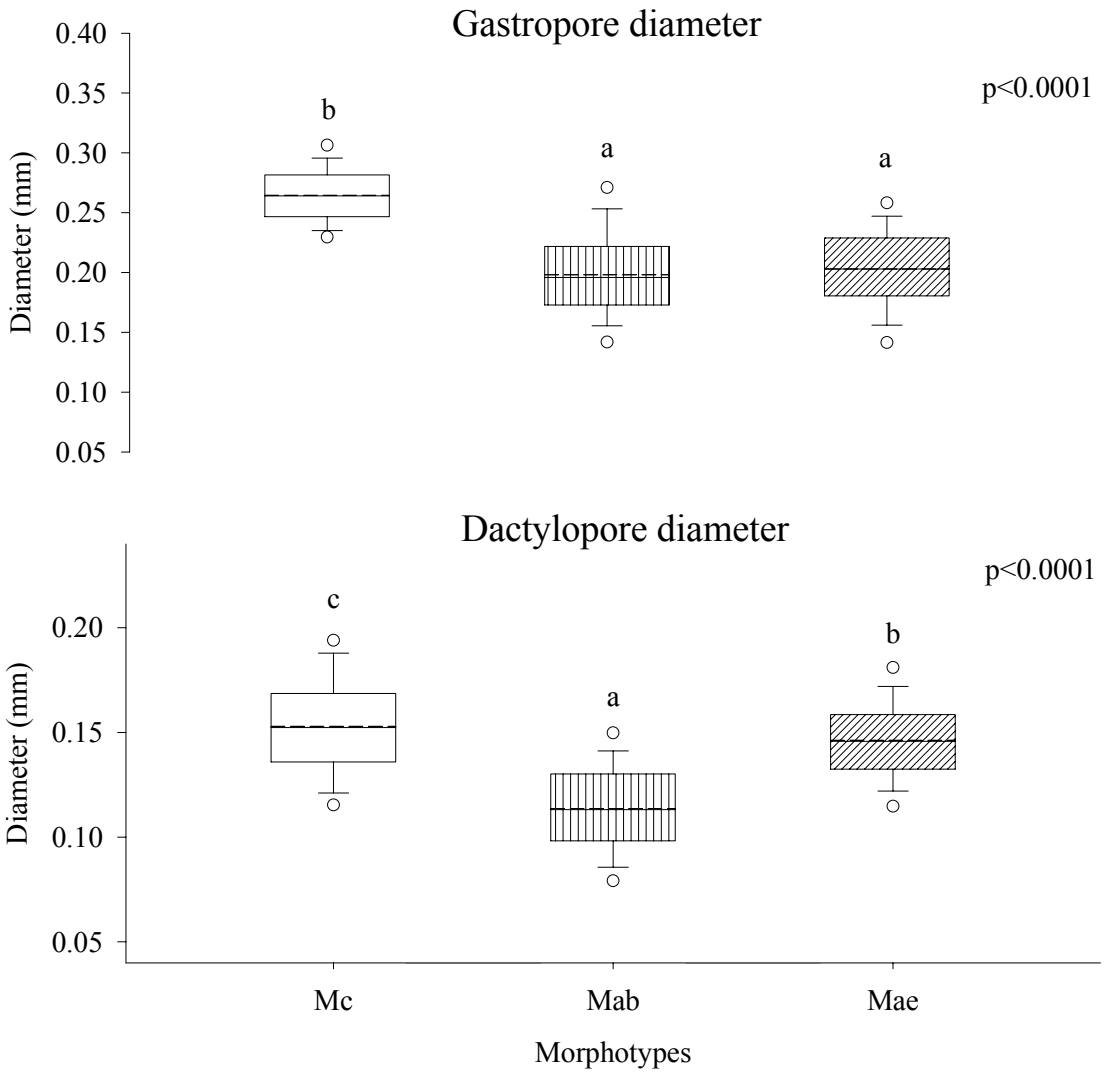

Figure 10. Variability (mean, median and 5th and the 95th percentiles) in the skeletal traits of the morphotypes (mm). Top graph shows variability in gastropore diameter, bottom graph shows variability in dactylopore diameters. Morphotypes are plotted in the x-axis, diameter the y-axis. The solid line is the median, dashed line the mean. The white boxes represent *M. complanata* (Mc), boxes with vertical lines is *M. alcicornis* branched (Mab), and boxes with diagonal lines is *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among morphotypes are denoted by different letters.

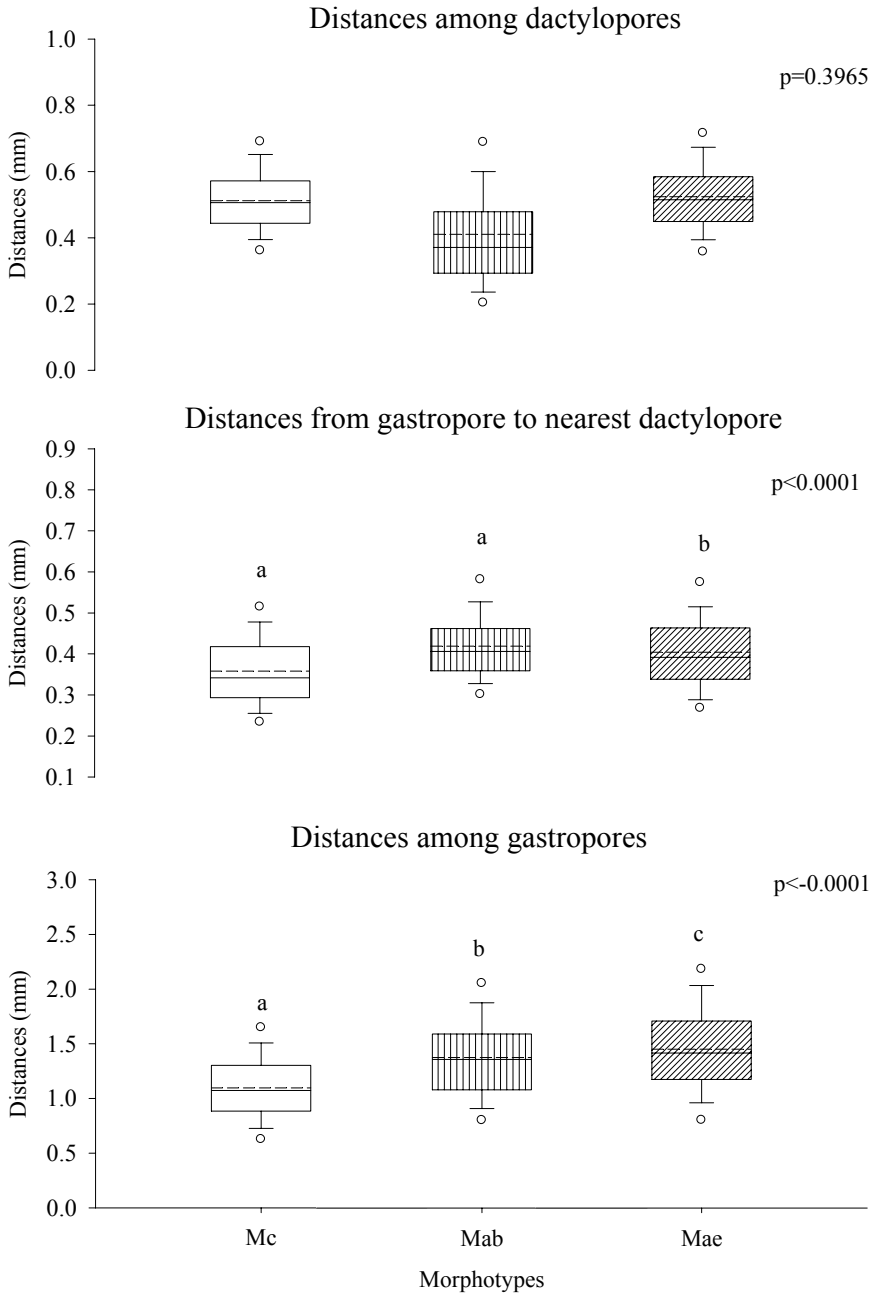

Fig 11. Variability (mean, median and 5th and the 95th percentiles) in the skeletal traits of the morphotypes (mm). Top graph shows variability in distances among dactylopores, center graph shows variability in distances from gastropore to nearest dactylopore, bottom graph shows variability in distances between gastropores. Morphotypes are plotted in the x-axis, distances in mm in the y-axis. The solid line is the median, dashed line the mean. The white boxes represent *M. complanata* (Mc), boxes with vertical lines is *M. alcicornis* branched (Mab), and boxes with diagonal lines is *M. alcicornis* encrusted (Mae). Letters above the boxes are values of Tukey's test, significant differences among positions are denoted by different letters.

The canonical Discriminant Function Analysis (Wilk's $\lambda = 0.145$, $F = 7.79$, $P < 0.0001$) , corroborates the *a priori* assigned groups, with 87.1% (n = 26) of the colonies correctly classified (Fig 12). Four colonies were misclassified; one belonging to *M. complanata*, one to the branched *M. alcicornis* and two to the encrusted *M. alcicornis*. The canonical plot showed three main groups corresponding to the three morphotypes. *Millepora complanata* was the most distinct morph, clustering at the left side of the plot. The two morphs of *M. alcicornis* were also differentiated in the plot, although several colonies of the two morphs (three of each morph) had similar values. A high spread among the values for the *M. alcicornis* morphotypes was also observed, illustrating the high trait variability of the colonies. Distances among dactylopores were the variable that better discriminated *M. complanata* from the *M. alcicornis* morphotypes; while distances from gastropore to nearest dactylopore distinguished the two morphotypes of *M. alcicornis*.

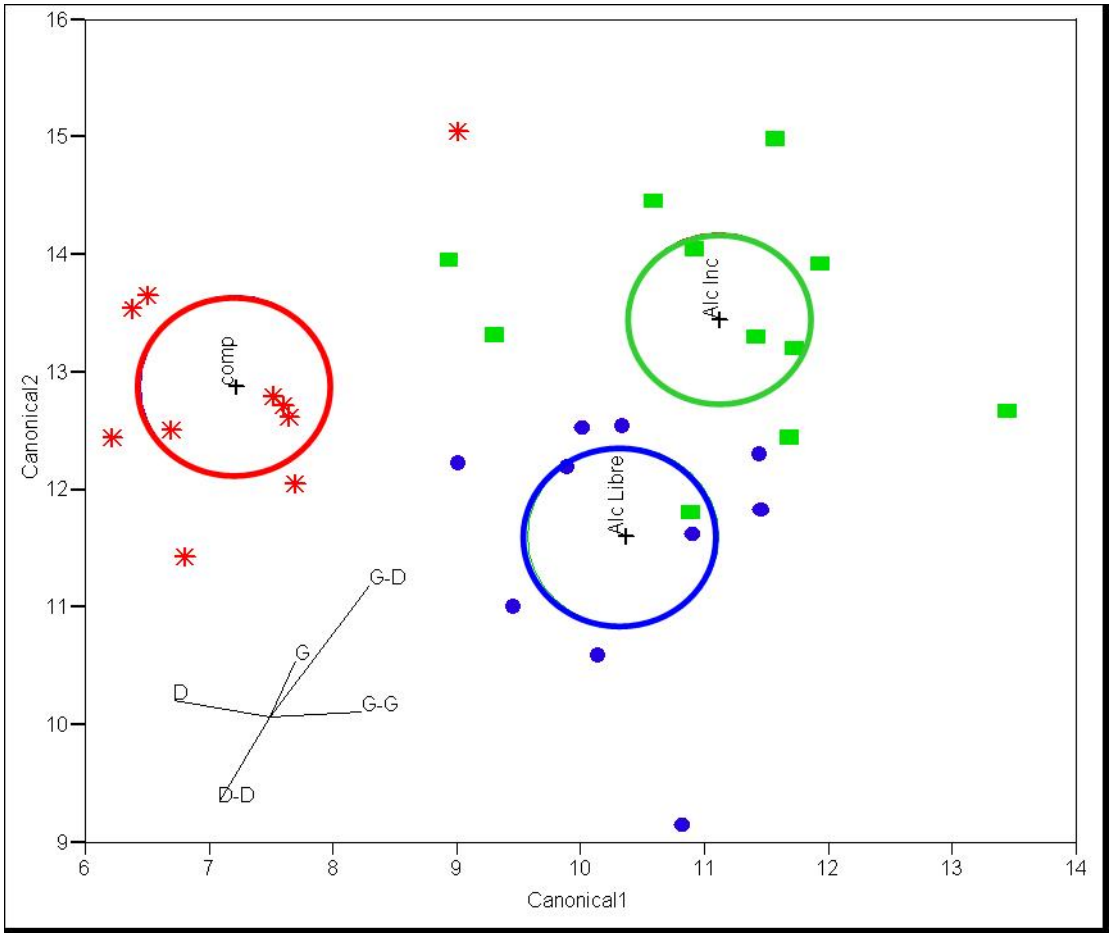

Figure 12. Discriminant function analysis canonical plot based on the morphological traits of the three *Millepora* morphotypes. Multivariate comparisons (fixed effects MANOVA) among morphotypes was significant (Wilk's $\lambda = 0.145$, $F = 7.79$, $P < 0.0001$). Misclassified colonies = 4 (13%). Red stars represent colonies of *M. complanata* (Mc), blue dots are colonies of *M. alcicornis* branched (Mab), green squares are colonies of *M. alcicornis* encrusted (Mae). Variables for comparison were D: dactylopore diameter, G: gastropore diameter, G-D: from gastropore to nearest dactylopore, G-G: distances among gastropores, D-D: distances among dactylopores. In canonical axis 1 and canonical axis 2, distances among dactylopores and gastropores, and gastropore diameters were the variables with more weight in the discrimination. The biplot rays (lower left) show the direction of the morphological trais in the canonical space. Each black cross represents the multivariate mean of each morphotype and is surrounded by a 95% confidence circle.

3.2 Molecular studies

After cleaning and end-trimming, a portion of the mitochondrial COI (385 base pairs) were used for the genetic analyses. Each colony used for the morphological study was also sequenced to measure levels of variation in COI. COI sequences of the other Caribbean species were added to the data set, for a total of 36 sequences (10 *M. complanata*, 10 *M. alcicornis* branched, 10 *M. alcicornis* encrusted, 4 *M. striata* and 2 *M. squarrosa*).

3.2.1 Summary Statistics

Overall, 25 haplotypes were identified from 36 sequences, with 70% of the sequences being unique. Of those haplotypes, six belong to *M. complanata*, four to *M. alcicornis* branched, nine to *M. alcicornis* encrusted, four to *M. striata* and two to *M. squarrosa*. All the *M. striata* and *M. squarrosa* haplotypes were unique and were not shared by any other species. One haplotype was shared among *M. complanata* and the branched morph of *M. alcicornis*. Nucleotide diversity (π) and Watterson's theta (θ_w) was similar for all the morphotypes/species, varying from 0.01 to 0.02 (Table 5). Tajima's D values suggested that the COI sequences did not deviate significantly from neutrality.

Table 5. Genetic diversity and summary statistics based on COI sequences. N: number of samples, S: segregating sites, M: mutations, S.S.: synonymous sites, N.S.: non-synonymous sites, H: number of haplotypes, Hd: haplotype diversity, π: nucleotide diversity, θ_w: Watterson's theta. SD = standard deviation.

Species (morph)	N	S	S.S.	N.S.	Position N.S.	H	Hd (SD)	π (SD)	θw (SD)	Tajima's D
M. complanata	10	20	18	2	221,324	6	0.91 (0.00)	0.02 (0.00)	0.02 (0.00)	-0.12
M. alcicornis (branched)	10	15	15	0	0	4	0.53 (0.18)	0.01 (0.00)	0.01 (0.00)	-1.32
M. alcicornis (encrusted)	10	19	18	1	65	9	0.98 (0.00)	0.02 (0.00)	0.02 (0.00)	-0.02
M. striata	4	9	9	0	0	4	1.00 (0.18)	0.01 (0.00)	0.01 (0.00)	-0.15
M. squarrosa	2	1	1	0	0	2	1.00 (0.50)	0.00 (0.00)	0.00 (0.00)	N/A

3.2.2 Genetic Divergence

Fifty five to 59 fixed differences were identified among *M. squarrosa* and the other species (Table 6) with about average of 63 nucleotide differences. Despite the absence of fixed differences among all pairwise comparisons (excluding *M. squarrosa*), *Millepora striata* and *M. complanata* had the highest average nucleotide difference (~12%). *Millepora striata* shared 4 mutations with *M. complanata* and the two morphs of *M. alcicornis*. On average, there were nine nucleotide differences among *M. striata* and both morphs of *M. alcicornis*. *Millepora complanata* shared seven mutations with *M. alcicornis* branched (average nucleotide difference of ~9.2%) and 11 mutations with the encrusted morph of *M. alcicornis* (average nucleotide difference of ~8.4%). The two morphotypes of *M. alcicornis* shared 9 mutations with average nucleotide difference of ~6.9%.

Table 6. Estimates of COI divergence among the species and morphotypes of *Millepora*: *M. striata*, *M. squarrosa*, *M. complanata* and *M. alcicornis* (b is the branching morphotype, e is the encrusted morphotype).

Comparisons between morphotypes	Fixed differences	Shared mutations	Average nucleotide differences between morphotypes (%)
M. striata_M. complanata	0	4	12.05
M. striata_M. alcicornis b	0	4	8.55
M. striata_M. alcicornis e	0	4	9.15
M. complanata_M. alcicornis b	0	7	9.18
M. alcicornis b_M. alcicornis e	0	9	6.88
M. complanata_M. alcicornis e	0	11	8.36
M. alcicornis e_M. Squarrosa	55	0	63.5
M. complanata_M. squarrosa	56	0	65.7
M. alcicornis b_M. squarrosa	58	0	61.3
M. striata_M. squarrosa	59	0	63

In the haplotype network analysis (Fig. 13), *M. squarrosa* sequences were excluded from the main network when the connection limit in the software TCS was set at 95% (Fig13: II.). Two main groups, unrelated by colony morphology, were recovered in the network (Fig13: B.

and C.). The TCS analysis suggested that the most common haplotype of *M. alcicornis* branched (Fig13: 1) was ancestral (square box) to the other sequences. However, one branched morph of *M. alcicornis* was an intermediate link (Fig13: 2) in the network while three sequences (Fig13: 3, 6, 26) were terminal nodes or recent haplotypes. A group (Fig13: B.) was composed by sequences of *M. complanata* and the two morphotypes of *M. alcicornis*. The putative ancestral haplotype of this group was shared among *M. complanata* and the branched *M. alcicornis* (Fig13: 4). The second group (Fig13: C.) was composed by all the sequences of *M. striata*, one *M. complanata*, one branched *M. alcicornis* and two encrusted *M. alcicornis*. One haplotype of encrusted *M. alcicornis* (Fig13: 16) was the putative ancestral sequence of this group. Two alternative connections or loops to each group were created, suggesting the presence of homoplasy in the data set.

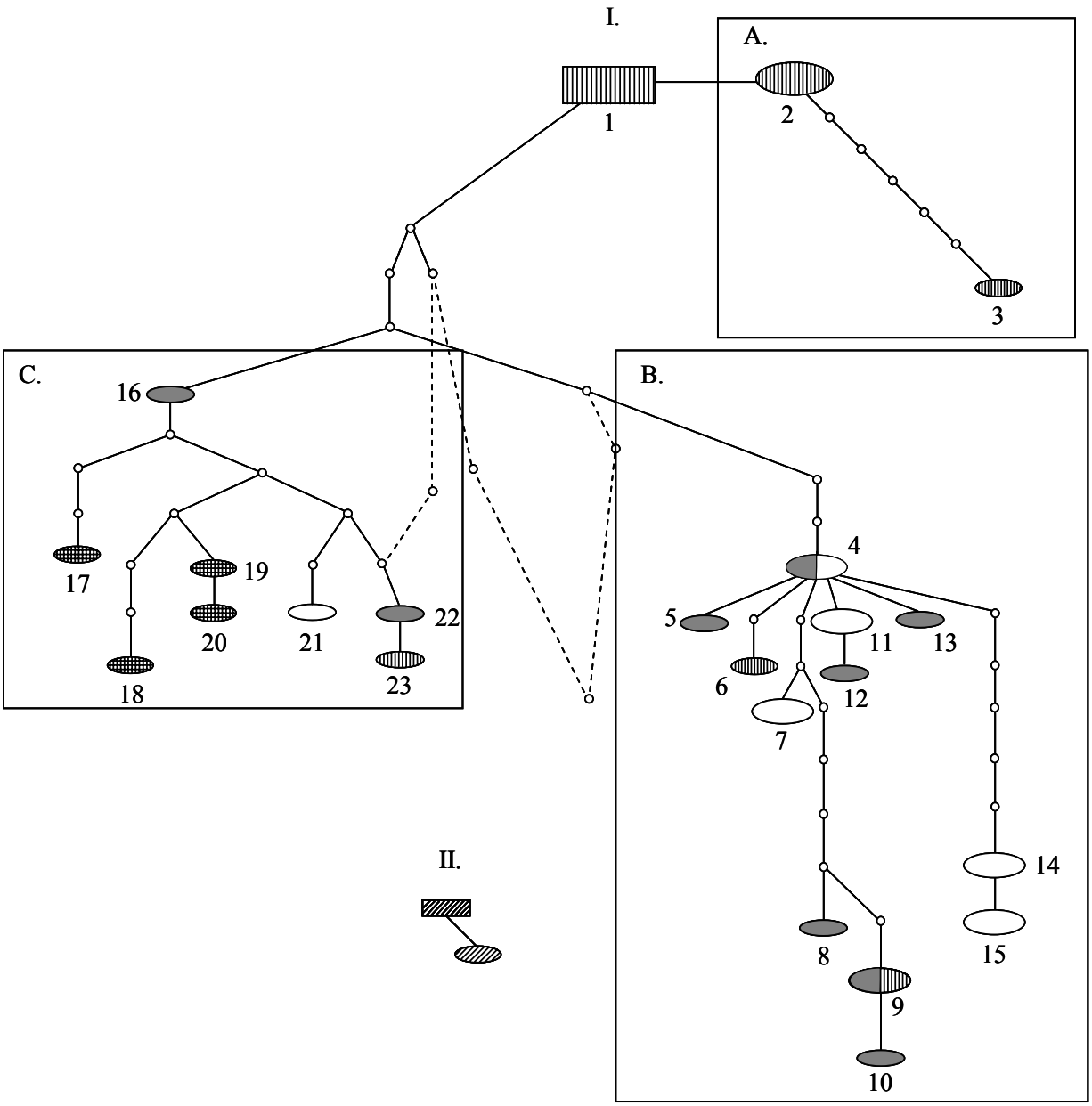

Figure 13. Parsimony haplotype network of the morphotypes based on COI. Patterns represent morphotypes: empty ovals: *M. complanata*, vertical lines: *M. alcicornis* branched morph,: solid: *M. alcicornis* encrusted morph, cross-stitched: *M. striata*, diagonal lines: *M. squarrosa*. Size of the ovals and squares is proportional to the observed number of sequences corresponding to the haplotype. Small circles are non-sampled or extinct haplotypes. Dashed lines represent alternative connections among the haplotypes, suggesting the presence of homoplasy.

3.2.3 Genealogy

Maximum likelihood (ML) genealogies (Fig 14) were constructed in PAUP with the Tamura-Nei substitution model as suggested by ModelTest, with unequal base frequencies (A = 0.22, C = 0.23, G = 0.18, T = 0.36), probability of invariable sites = 0.70 and gamma distribution parameter of α=1.768. The genealogical reconstruction of the COI haplotypes lacked resolution, mostly resembling a star topology. Two clades were recovered for *M. complanata*, one for encrusted *M. alcicornis*, and another clade was composed by one colony of branched *M. alcicornis* and one of encrusted *M. alcicornis*.

The genealogy based on Bayesian inference (Fig 15) was better resolved than the one based on maximum likelihood. All the colonies were assigned to four major clades, and were all polyphyletic. Eight colonies of the branched morph of *M. alcicornis* formed one clade; however two colonies of *M. alcicornis* were embedded in the other clades. Most recent groups were composed by members of same morphology; however the low posterior probabilities values render a weak inference. These groups corresponded to the ones recovered with the maximum likelihood analysis. The groups recovered with Bayesian analysis did not correspond with the classification of the morphological characters. For example, in the species *M. complanata* the group of colonies McE8, McMg6, McMg9 and Mc123 had dactylopores with similar diameters with the exception of colony McMg9. Colonies McMg1 and McMg3 had different diameters of dactylopores, both forming a clade in the COI tree. In the clade composed by colonies MaeLP3, MaeLP5 and MaeT15; colonies MaeLP5 and MaeLP3 had dactylopores of similar diameters while the diameters of colony MaeT15 differ. On the other hand the clade formed by different morphs of *M. alcicornis* (MabML1 and MaeLP2) had dactylopores with equal diameter. Analysis performed using Neighbor Joining yielded results similar to the Bayesian analysis.

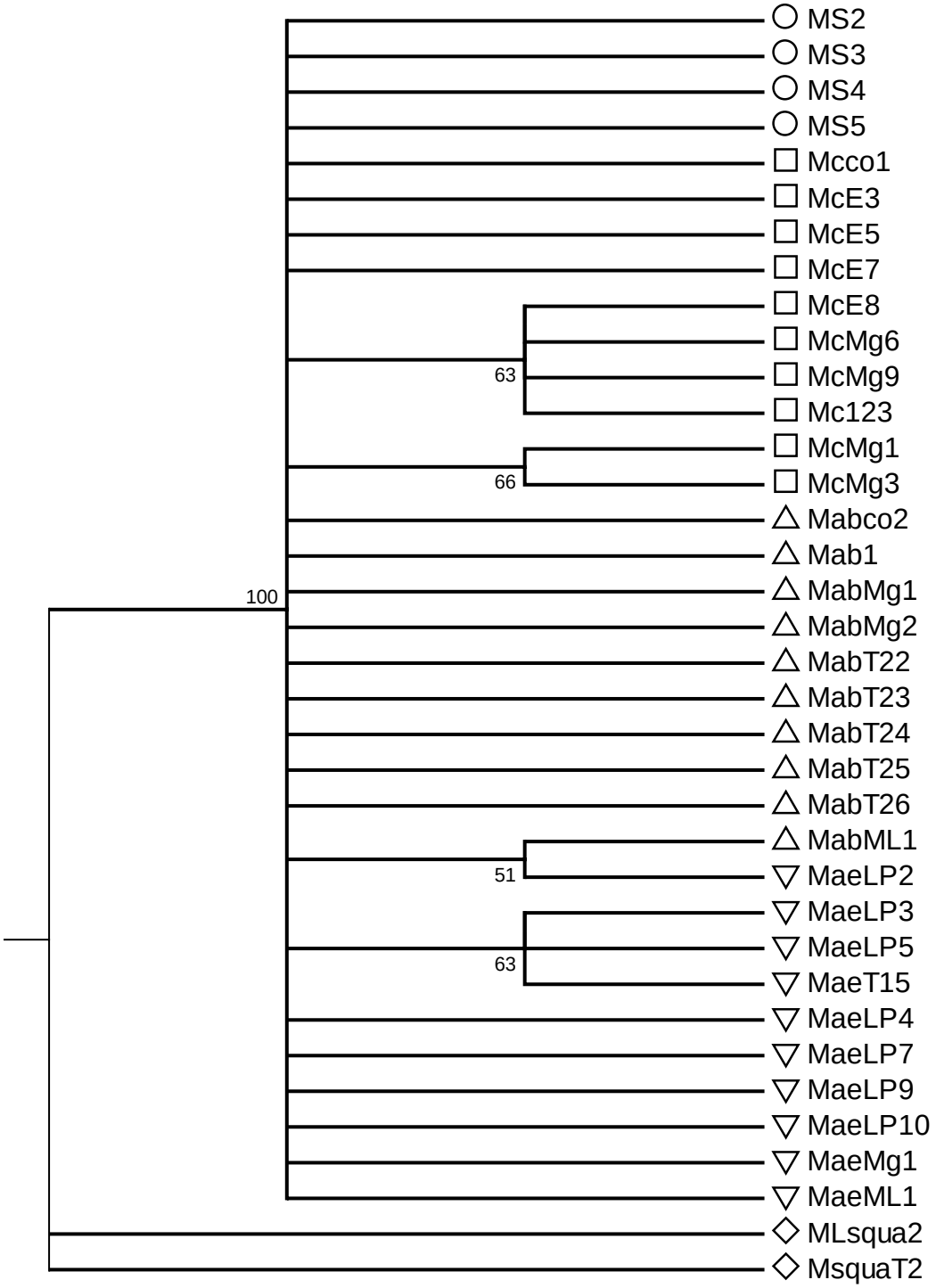

Figure 14. Maximum likelihood genealogy of *Millepora* based on COI. Bootstrap values (100 replicates) over 50% are shown. Shapes represent morphotypes: square: *M. complanata*, upside triangle: *M. alcicornis* branched morph, downside triangle: *M. alcicornis* encrusted morph, circle: *M. striata,* diamond: *M. squarrosa*. *Millepora squarrosa* is used as the outgroup.

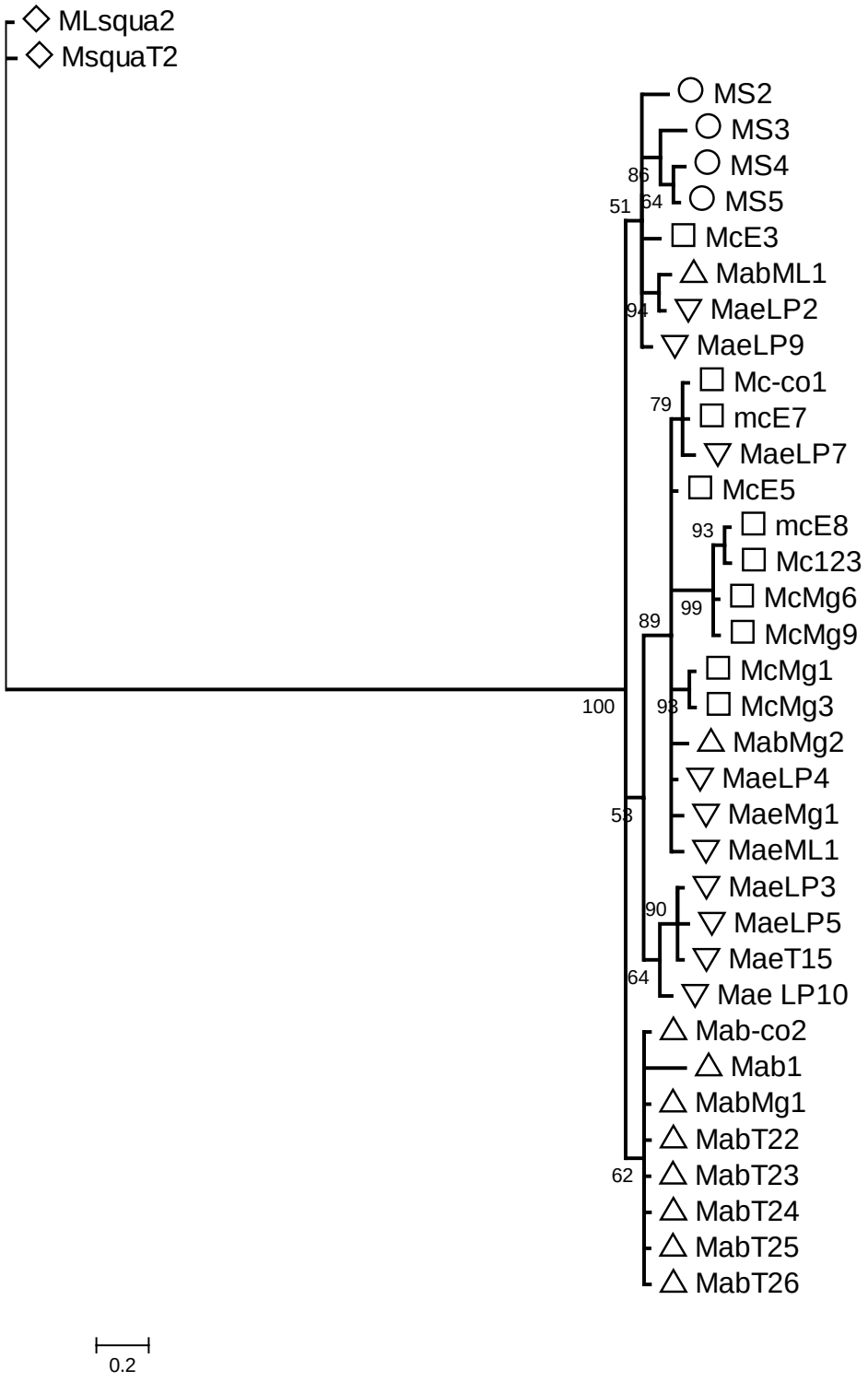

Figure 15. Bayesian genealogy of *Millepora* based on COI. Posterior probabilities over 50% are shown. Shapes represent morphotypes: square: *M. complanata*, upside triangle: *M. alcicornis* branched morph, downside triangle: *M. alcicornis* encrusted morph, circle: *M. striata,* diamond: *M. squarrosa*. *Millepora squarrosa* is used as the outgroup.

3.2.4 Caribbean wide genealogy

The Caribbean wide genealogy was estimated from *Millepora* colonies used for the morphological measurements and 342 additional sequences of *M. complanata* and the two morphs of *M. alcicornis* (Fig 16). Genealogies were built by Bayesian inference and the neighbor joining algorithm using the Tamura-Nei model of evolution with base frequency of A= 0.19, C= 0.25, G= 0.22, T= 0.34, proportion of invariable site = 0.55 and Gamma distribution α shape of 0.81. A total of 178 haplotypes were recovered from the sampled *Millepora* colonies, and 68% of them (121) were unique sequences. Twenty-one haplotypes (Fig 16) were shared among two or more morphotypes or species. Of the 10 most common haplotypes, five were shared among different morphotypes or species. Nine main clades were recovered, and as in the previous genealogies, none of the species were monophyletic, except *M. striata* (Fig 16). The resulting genealogy was not concordant with morphologically-based species assignments of the colonies; neither with the geographic origin of the samples.

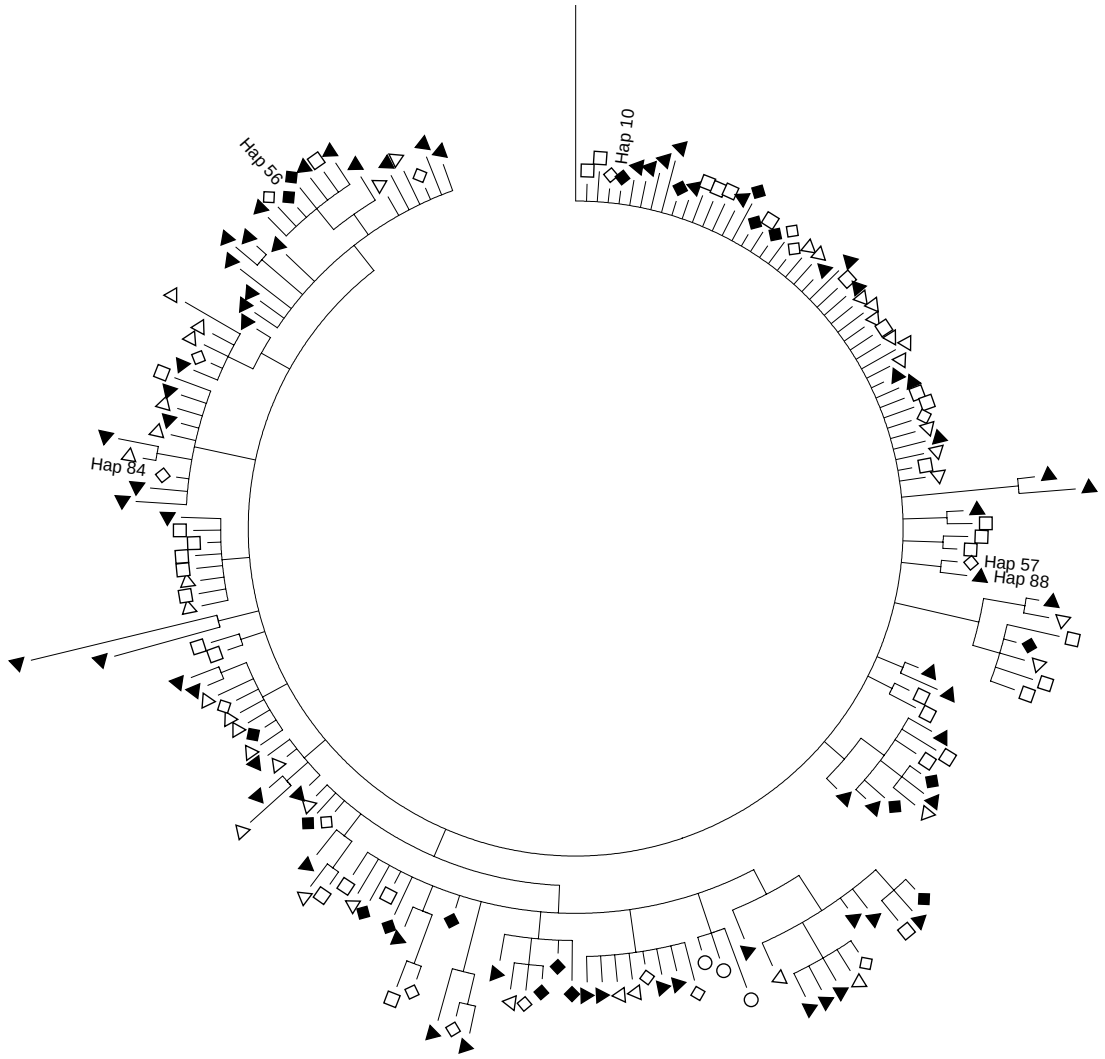

Figure 16. Caribbean wide genealogy of *Millepora* based on Bayesian analysis of COI haplotypes. Posterior probabilities over 50% are shown. Shapes represent morphotypes: square: ***M. complanata***, upside black triangle: ***M. alcicornis*** branched morph, downside triangle: ***M. alcicornis*** encrusted morph, circle: ***M. striata,*** diamond: haplotypes shared among ***M. alcicornis*** branched and ***M. alcicornis*** encrusted, black diamond: haplotypes shared among all the morphotypes. ***Millepora squarrosa*** is used as the outgroup.

3.2.5 Analysis of Molecular Variances (AMOVA)

An AMOVA test was applied to a portion of the Caribbean data used for the genealogical analysis (Table 7). Samples across the Caribbean from the three studied morphotypes were selected for a total of seven populations (Mona, La Parguera reef system, Vieques, Panama, Guadeloupe, Grand Cayman, Curaçao). Significant differentiation was detected among morphotypes within populations (Φ_{SC}: 0.067), among populations (Φ_{CT}: 0.037) and within the Caribbean (Φ_{ST}: 0.101). Variation among morphotypes within populations was almost two times larger (6.43%) than among populations (3.7%). The highest percentage of variation was found within the Caribbean region (89.89%). Although significant differentiation was detected for all the comparisons, it was higher among morphotypes of different geographic regions. This result is best illustrated in the pairwise comparison of the groups (Table 8); none of the different morphotypes within a population were significantly different from each other. However, populations were significantly different across the Caribbean, with some of the populations, such as Vieques and Culebra being only 14 kilometers apart.

Table 7. Analysis of molecular variances (AMOVA) for the morphotypes of *Millepora*. Comparison of morphotypes were made within the Caribbean basin, among populations and among morphotypes within populations. Populations were sampled from Panama, Grand Cayman, Mona, La Parguera reef system, Vieques, Guadeloupe and Curaçao. Φ_{ST} values were obtained by randomization of 25,000 permutations Bonferroni corrected. * $p < 0.002$, ** $p<0.0001$. Fixation Indices Φ_{SC}: 0.066**, Φ_{ST}: 0.101**, Φ_{CT}: 0.037*

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among populations	6	79.70	0.16 Va*	3.70
Among morphotypes within populations	14	102.16	0.28 Vb**	6.37
Within Caribbean	253	996.73	3.94 Vc**	89.94
Total	273	1177.99	4.38	

Table 8. Pairwise comparisons (Tamura-Nei distance) of *Millepora* populations assigned by morphotypes. Mc: *M. complanata*, Ms: *M. striata*, Mab: *M. alcicornis* branched, Mae: *M. alcicornis* encrusted. Pa: Panama, GC: Grand Cayman, Mo: Mona, SW: La Parguera Reef System, Vi: Vieques, Gd: Guadeloupe, Cu: Curaçao. Significant values ($p<0.01$) in bold.

	Ms Pa	Mab Pa	Mc Pa	Mc GC	Mab GC	Mae GC	Mab Mo	Mc Mo	Mae Mo	Mab SW	Mae SW	Mc SW	Mc Vi	Mab Vi	Mae Vi	Mab Gd	Mae Gd	Mc Gd	Mc Cu	Mae Cu	Mab Cu
MsPa																					
MabPa	**0.20**																				
McPa	0.09	0.05																			
McGC	0.33	0.05	0.06																		
MabGC	0.25	0.12	0.13	-0.02																	
MaeGC	0.23	0.05	0.05	-0.07	-0.09																
MabMo	**0.31**	**0.11**	**0.14**	-0.04	0.01	0.02															
McMo	**0.49**	**0.22**	0.20	0.00	**0.19**	0.16	0.08														
MaeMo	**0.29**	**0.09**	**0.12**	-0.05	0.02	0.02	-0.03	0.02													
MabSW	**0.26**	**0.08**	**0.14**	-0.01	0.06	0.02	0.00	0.09	0.01												
MaeSW	**0.32**	**0.12**	**0.14**	-0.03	0.05	0.02	-0.02	0.09	0.00	0.02											
McSW	**0.31**	**0.14**	0.11	-0.03	0.07	0.02	0.02	0.05	0.03	**0.07**	0.02										
McVi	0.16	0.12	0.11	0.11	0.00	-0.02	0.10	0.32	0.12	0.10	0.12	0.11									
MabVi	**0.28**	0.11	0.13	0.06	0.04	0.04	0.06	**0.27**	0.09	0.08	0.07	0.07	0.00								
MaeVi	**0.39**	0.15	0.12	-0.03	0.03	0.03	-0.05	0.12	-0.02	0.03	-0.06	-0.02	0.14	0.08							
MabGd	0.25	0.08	0.15	-0.01	-0.02	0.02	0.00	0.15	0.00	0.00	0.06	0.13	0.05	0.08	0.06						
MaeGd	0.71	**0.40**	0.28	0.26	0.31	0.34	**0.30**	0.27	0.21	0.31	0.28	**0.25**	0.51	0.48	0.36	0.25					
McGd	0.46	0.15	0.13	-0.08	0.10	0.06	0.00	-0.12	-0.05	0.02	0.02	0.03	0.24	0.20	0.05	0.02	0.11				
McCu	**0.57**	**0.30**	**0.26**	0.07	**0.23**	**0.22**	**0.14**	**0.16**	**0.12**	**0.18**	**0.11**	0.07	**0.37**	**0.26**	0.12	**0.33**	**0.41**	0.13			
MaeCu	0.53	**0.25**	**0.19**	0.01	0.10	0.11	0.05	0.13	0.05	0.13	0.05	0.01	0.27	0.15	0.04	0.21	**0.44**	0.08	-0.03		
MabCu	**0.44**	**0.18**	**0.26**	0.08	**0.20**	0.20	0.10	0.11	0.06	0.07	**0.14**	**0.19**	0.29	0.23	0.21	0.05	0.42	0.02	**0.34**	0.26	

4. DISCUSSION

There was no congruence between the morphological and molecular data. The molecular data suggests that two species of *Millepora* exist in the Caribbean: *M. squarrosa*, and the complex composed of *M. alcicornis*, *M. complanata* and *M. striata*. Analysis of variances of micro-morphological traits showed high variation between *M. complanata*, *M. alcicornis* branched and *M. alcicornis* encrusted, but also within and among colonies of the same morphotypes. Dactylopore diameter and distances between gastropore were the only measurements in which variation among morphotypes were higher than the variation within morphotypes, and therefore distinguishing the three morphotypes. The Discriminant Function Analysis of the skeletal traits assigned the colonies to their corresponding morphotype, suggesting a multivariate use of the skeletal characters can identify among species. The genetic data was characterized by unexpected high variability in the COI region, however the variation in this marker was unrelated to colony shape. The relationships among COI haplotypes recovered by Bayesian analysis were independent of the morphology.

These findings neither support nor discard that the diversity in forms in the Caribbean *Millepora* could be a consequence of phenotypic or genetic plasticity within the species. The presence of intermediate forms and lack of genetic monophyly suggests *M. alcicornis*, *M. complanata* and *M. striata* are not discrete species. Whether these morphotypes are ecotypes of one species, species in the process of divergence or hybrids requires further study. In contrast, *M. squarrosa* stands as a distinct species and its basal position in the genealogy suggest that this species is ancestral to the other Caribbean species.

For the genetic analysis, *Millepora* colonies were collected from Panama (*M. striata*, *M. complanata* and the two *M. alcicornis* morphs), Puerto Rico (all except *M. striata*),

Curaçao, Guadeloupe and Gran Cayman (all except *M. striata* and *M. squarrosa*), while the morphometric analysis included *Millepora* colonies from one location (La Parguera, Puerto Rico). Ideally, a comprehensive study of morphological variation in Caribbean *Millepora* should include several locations encompassing the known distribution of each species. In very closely related species, the species boundaries may vary according to the geographic origin of the samples, as it has been demonstrated in the *Montasteaea annularis* complex (Fukami et al. 2004). *Montastaea faveolata*, *M. annularis* and *M franksi* were genetically and morphologically different in Panama whereas in the Bahamas, all three species were indistinguishable morphologically and genetically (except *M. faveolata*). However, our current samples cover a good portion of the current distribution of the species, and still the species boundaries are unclear. We predict that additional geographic samples will further strengthen our conclusions. Additional samples from Bermuda, Bahamas, St. Thomas, St. Kitts and Honduras have been collected exclusively for genetic analysis and will be included in a future publication.

4.1. Morphological variation

High intra-specific variability in the micro-morphological traits of *Millepora* has been noted by previous authors. Boshma (1948) suggested that colony shape was the only character distinct enough to be useful in the taxonomic classification of genus and other studies (Martínez-Estalella 1982) concluded that the use of skeletal traits was impractical due to their high intra-specific variation. However, deWeerdt (1984) suggested that the size and density of dactylopores could have some taxonomic value when used in combination with colony shape. This study present similar results to those found by deWeerdt (1984), when only one morphological character was used, high variability among colonies within species

and colonies across species was evident. The only distinct characters among the three morphotypes of *Millepora* were diameter of dactylopores and distances between gastropores. A multivariate approach (DFA) of the five skeletal traits clustered 87% of the colonies into three groups (*M. complanata* and the two *M. alcicornis* morphs), indicating the taxonomic value of these characters. Nevertheless, caution is advisable as the traits were very variable and some overlapping was observed among colonies of different morphologies. Additionally, some of the five characters used in the analysis may covary, therefore violating the assumption of characters independence.

4.2. Phenotypic plasticity and the environment

The environment in which the organism develops could have a significant influence in the phenotype of the individual. In marine benthic organisms like cnidarians, water energy, light availability and sediment transport have been shown to influence the development and survival of the organisms (Yoshioka and Yoshioka 1989; Yoshioka and Yoshioka 1991). Water energy and light availability are the primary forces inducing morphological changes in the reef community. In places with high water flow regimes, scleractinian colonies tends to have thicker calcium carbonate skeletons to resist drag force exerted by the waves (Kaandorp 1999). While, in the absence of light, scleractinian corals tend to be more plate-like to increase the surface area available for light; for example, most colonies will become more tabular with increasing depths (Todd 2008).

Ecotypes along changing environmental conditions are well known in the scleractinians; *Pocillopora damicornis*, present a gradient of morphotypes corresponding to the gradient in physical parameters encountered along the reef slope (Veron 1995, Kaandorp 1999) but no genetic data were used to corroborate these observations. Overlap of

morphological characters, presence of colonies with intermediate forms and changes in growth strategy is not uncommon in the *Millepora* (Wahle 1980; deWeerdt 1981; Meroz-Fine et al. 2003; Razak and Hoeksema 2003). The different morphologies of *Millepora* have been attributed to the different environmental regimes along the reef structure (deWeerdt 1981; Vago et al. 1994; Kaandorp 1999). Like *Pocillopora damnicornis*, *Millepora alcicornis* shows similar responses to changes in water movement; branch thickness and colony compactness varied along water energy gradients (Kaandorp 1999). Generally, delicate branching types are distributed in calm or deep waters, while blade-like and encrusting morphs are conspicuous in high energy environments such as the reef crest (Lewis 2006). In the reef crest, where few species survive the high wave energy, *Millepora* is a good colonizer of the substratum; a bladed morphology will limit the water impact and drag force, while attaining more surface to trap light and plankton. In calm waters where there is more competition for substrate, a branch morphology will facilitate the exploitation of the resources in the water column increasing the surface area for feeding and light exposure (Coates and Jackson 1985; Harper 1985; Todd 2008). In high energy environments, branches are advantageous for fast growing corals, because fragmentation helps the organism to colonize and exploit new areas (Harper 1985; Jackson 1985). Other corals with more delicate branches and lower calcification rates tend to inhabit deeper environments or calmer waters (Kaandorp 1999). In the case of *M. alcicornis,* branches may also help to localize, reach and overgrow weaker space competitors, such as *Gorgonia ventalina* (Wahle 1980). The overgrowth of *G. ventalina* by *M. alcicornis* may be associated with an increase of the surface of the hydrozoan colony and, perhaps, a decrease of the energy input in calcification.

The individual zooids may be affected by the micro-environmental conditions, showing variability in the size of the zooids within a colony. For example, the amount of light received by the zooids in the upper and lower parts of the colonies can be different and as a consequence the growth rates of the zooids may also differ. In addition, the shape and size of the zooids in encrusted forms can be determined by the type of the substrate being covered.

In this study, the morphotypes of *M. complanata*, *M. alcicornis* branched and *M. alcicornis* encrusted were recovered with the Discriminant Function Analysis, suggesting a relationship between microskeletal characters and colony shape. Whether the morphotypes are influenced by the environment needs further investigation. Previous transplantation experiments have provided evidence for the ability of *Millepora* to change, to some extent, the colony shape when different environmental conditions are encountered (deWeerdt 1981; Meroz-Fine et al. 2003). However, the presence of different morphotypes occurring side by side in the reef suggests that part of this morphological plasticity may be genetically controlled.

4.3 Genetic variation

High intraspecific variability was not only observed at the phenotypic level but also at the molecular level; COI sequences showed high levels of polymorphism with 68% of the sequences being unique haplotypes. Despite the high number of haplotypes, sequences were not divergent enough to distinguish among species. *Millepora squarrosa* was an exception, as it was very different from the other morphs, with an average of 56 fixed mutations (25% difference). The lack of genetic differentiation among morphotypes was observed across all the sampled populations in the Caribbean; genetic differentiation was higher within Caribbean

populations than among the different morphotypes, providing evidence for a species complex consisted of the morphologies expressed by *M. alcicornis*, *M. complanata* and *M. striata*.

Previous genetic studies in the Red Sea, *M. dichotoma* exhibited four recognized morphotypes: delicate branched morph, blade-like branched, encrusting and box-work morphs (Vago 1998). Molecular studies (Meroz-Fine, et al. 2003) showed that the four morphotypes consisted of two species. Each species was represented by two different morphotypes: one species containing delicate and bladed growth morphs, the other species existing as encrusting and box-work. Similar results were found for the *Millepora* species occurring in Vietnam (Mancheko et al 1993). Our study revealed similar patterns in the Caribbean, where the cross-blade (*M. striata*), branched (*M. alcicornis*) and bladed (*M. complanata*) morphs were genetically indistinguishable with a neutral molecular marker, while the thick box-work *M. squarrosa* was the only distinct species.

Alternatively, the lack of genetic divergence among *M. alcicornis*, *M. complanata*, and *M. striata* may be explained by the low levels of variability observed in mitochondrial markers in cnidarians. Little or no useful mtDNA variation has been found in population or species level studies in Anthozoa, attributed to slow rates of evolution (Medina et al. 1999; Shearer et al. 2002; Hellberg 2006). For example, the COI gene sequences were identical among the *Montastraea* species (Medina et al. 1999; Fukami et al. 2004a). However, the opposite is true for the sister group of the Anthozoa, the Medusozoa (Scyphozoans + Cubozoans + Hydrozoans; *sensu* Petersen 1979), to which *Millepora* belongs. The fast rate of mitochondrial DNA evolution in comparison to the nuclear DNA in the Medusozoa is similar to those found in the Bilateria (Govindarajan et al. 2005, Hellberg 2006). The COI gene has been successfully used to identify siblings species within the Medusozoa (Dawson and Jacobs

2001; Govindarajan et al. 2005) and variation in this marker is comparable to that founded in *Millepora.* For example, the COI gene of *Obelia geniculata* contained 73 variables sites of which 52 were parsimony informative; such values are comparable with those for *Millepora* where 75 out of 84 variable sites were parsimony informative.

None of the mutations were fixed among *M. alcicornis*, *M. complanata* and *M. striata*, however >50 mutations were fixed in *M. squarrosa*. Sequence divergence ranged from 22 to 25% among *M. squarrosa* and the other morphotypes, and from 0 to 3% between *M. alcicornis*, *M. complanata* and *M. striata.* Values of sequence divergence ≥10% in the mtDNA have been considered as a rule of thumb for the distinction among some species (Dawson and Jacobs 2001, Moritz and Cicero 2004), but in other groups, sequence divergences as low as 4% was considered enough to distinguish among species of snapping shrimp (Knowlton and Weigt 1998), while divergences of 0.7 and 4.6 % were found in sister species of birds (Johnson and Cicero 2004). These last values would be enough to differentiate *M. striata*, however sequence divergence among *M. striata* and *M. complanata* varied from 0 to 4%, suggesting, morphology is not a good indicator of genetic differentiation. All suggestions about sequence divergence and speciation should be viewed cautiously since the threshold of divergence varies greatly from taxa to taxa and single mitochondrial genes are unreliable proxies for predicting speciation (Hudson and Turelli 2003). .

4.4. Taxonomical and genetic incongruence

Millepora is not the only cnidarian presenting taxonomic confusion; cryptic species, species complexes and morphological ecotypes are widely found in the phylum (Knowlton and Jackson 1994; Knowlton 2000). Also, the systematic relationships among the Cnidaria

are not well understood, and phylogenetic confusion prevails (Fautin and Lowenstein 1992; Romano and Palumbi 1996; Medina et al. 2001; Collins 2002; Fukami et al. 2008; Nunes et al. 2008). Given that most type species of corals were deposited in museums as remains of the calcareous skeletons (Knowlton and Jackson 1994; Veron 1995), the amount taxonomic misclassification due to phenotypic plasticity or convergent morphology is not surprising. In scleractinian corals, members of the same species have been placed in different genera; for example, the columnar and tabular morphs of *Pavona maldivensis* were previously described as *Pavona policata* and *Siderastrea madivensis* (Veron 1995). In other cases members of different species, genera or families have been placed together due to morphological convergence (Fukami et al. 2004b; Govindarajan et al. 2005; Fukami et al. 2008). A multi-gene approach by Fukami and colleagues (2004) showed that members of the coral family Faviidae in the Atlantic Ocean are more related to the Atlantic Mussidae than to the Pacific faviids. Obviously, the phylogenetic status of some Caribbean coral families is in flux and additional data will delineate the taxonomic boundaries.

Oftentimes, the number of species in a genus is greatly underestimated and only after a detailed molecular/morphological taxonomic revision is followed, a better estimate of species is revealed. The number of species of the genus *Obelia* (Hydrozoa) and *Aurelia* (Scyphozoa) have been underestimated given the lack of morphological characters (Dawson and Jacobs 2001, Govindajaran et al 2005). On the other hand, the scleractinians *Pocillopora damicornis* and *Pavona cactus* exist in such dissimilar ecotypes within a reef that members of the same species were thought to belong to different species or genera (Veron 1995). The multicharacter approach (e.g. morphological, molecular, behavioral, life history strategy, time of reproduction) in corals has been shown to successfully differentiate species, which could

not be distinguished with one set of characters (Knowlton et al. 1992; VanVeghel and Bak 1993; Weil and Knowlton 1994; Levitan et al. 2004). However, our multicharacter approach (morphology and genetics) yielded contrasting results; the morphological characters agreed with the prevailing taxonomic status (i.e. four species) but the COI gene differentiated only *M. squarrosa*.

Most of the COI sequences were unique haplotypes and when a haplotype was frequent in the population it was shared among different morphologies and species. The high numbers of unique haplotypes may indicate the presence of one *Millepora* species with a wide range of phenotypic plasticity that has undergone past population expansion. However, the scenario of past population expansion is not well supported by our current data since Tajima's D did not deviate significantly from neutrality (Table 5). The presence of such variety of phenotypes and limited number of characters may lead to a prolific description of new species, resulting in an artificially inflated speciose genus. Alternatively, if the time since the *Millepora* species diverged is short, the species may still carry similar sequences because of incomplete lineage sorting. (i.e. some of the genetic traits of the ancestor have not segregated in the different descendants). Alternatively, evolutionary processes such as hybridization can result in entities known as species complexes. One species is in the process of diverging in two or more species; or various species are hybridizing in their way to form one new species. Whether the complex is one or more species, only depends of the scale of time and their relative position in the speciation process.

4.5. The species problem in *Millepora*

Among the most widely accepted species concepts are the biological and the phylogenetic concepts (deQueiroz and Donaghue 1990; deQueiroz 2005; Mallet 2006). In the biological species concept, reproductive isolation among species is required (Mayr 1942; Dobzhansky 1950); while for the phylogenetic species concept, monophyly is the main condition (deQueiroz and Donaghue 1990).

Studies in corals highlight the limitation of applying a particular species concept in a group of recently divergent taxa (e.g. *Montastraea*, Pacific *Acropora*) that occupy similar habitats, exhibit phenotypic plasticity and have no temporal and physical reproductive barriers (i.e. during mass spawning). *Montastraea* species are morphologically distinct, but intermediate morphs are also common (Weil and Knowlton 1994). Studies on Panama populations provided evidence for three sibling species (Weil and Knowlton 1994), while similar studies in Curaçao and Florida provided evidence for a single species (VanVeghel and Bak 1993; Medina et al. 1999). Medina et al (1999) concluded that the species *M. faveolata*, *M. annularis* and *M. franksi* are indeed a single evolutionary entity. Later studies showed the geographical differences in the complex were due to a latitudinal hybridization gradient through the Western Atlantic (Fukami et al. 2004a).

The application of a species concept in *Millepora complanata* and *M. alcicornis*, two widely distributed milleporids in the Caribbean is equally problematic. Due to lack of studies on the reproduction of *Millepora* the biological species concept cannot be tested, yet. *Millepora complanata* and *M. alcicornis* formed paraphyletic clusters in the genealogical tree (except for *M. squarrosa*), therefore the taxa did not meet the species criteria according to the phylogenetic species concept.

Whether the *Millepora* species are a single evolutionary entity or just a case of incipient speciation is unknown. The presence of intermediate morphs and the paraphyly of *Millepora* species indicate the possibility of hybridization, which is a common phenomenon in corals (Veron 1995; Hatta et al. 1999; VanOppen et al. 2000; Richards et al. 2008). If speciation is occurring between *M. alcicornis* and *M. complanata*, lineage sorting of the COI region is still incomplete, suggesting a recent event of divergence. However, studies on the reproductive biology of *Millepora* and additional molecular markers should be surveyed to test for these hypotheses.

5. CONCLUSIONS

The goal of this study was to genetically and morphologically test the delineation of the four recognized species of Caribbean *Millepora* (*M. alcicornis*, *M. complanata*, *M. striata* and *M. squarrosa*) Our sampling design favored comparisons between *M. alcicornis* and *M. complanata* because they were the dominant milleporids in our sampling locations. Five skeletal traits of the two most common morphotypes of *M. alcicornis* (branched and encrusted) and *M. complanata* were compared and overlap among the skeletal traits of all studied morphotypes was observed. The diameter of dactylopores was the only measurement sensitive enough to distinguish the three morphotypes. In addition, gastropore diameter was useful to discriminate among *M. alcicornis* and *M. complanata*. The genetic differentiation among the four Caribbean species of *Millepora* was assessed with a portion of the mitochondrial gene COI. High haplotypic variation was detected and the most common haplotypes were shared among morphotypes of *M. alcicornis* and *M. complanata*, across the Caribbean. Divergence among *M. alcicornis*, *M. complanata* and *M. striata* was low, with *M. striata* more genetically different from *M. complanata* than from *M. alcicornis*. *Millepora squarrosa* was the most distinct species with about 25% of sequence divergence from the other two species. These results suggest that, genetically, the Caribbean milleporids include two species, *M. squarrosa* and the morphospecies complex of *M. alcicornis*-*M. complanata*-*M. striata*, while morphologically, the branched *M. alcicornis,* encrusted *M. alcicornis* and *M. complanata* can be differentiated. Nevertheless, transplant experiments, reproductive studies as well as the analysis of more genetic markers are needed to decipher if the species complex in *Millepora* is the result of hybridization, incipient speciation or phenotypic plasticity.

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APPENDICES:

Appendix 1: Summary of measurements of gastropore diameters in *Millepora* morphotypes. N: number of measurements, S.D.: Standard Deviation. H: Values of Kruskal-Wallis One Way Anova, Different letters significant values of Tukey's test. Morphotypes: Mc: *M. complanata*, Mab: *M. alcicornis* branched, Mae: *M. alcicornis* encrusted.

Morphotype	Trait	N	Mean	S.D.	Median	H	*p*	Morphotype	Ranks	Comparisons									
Mc123	G	30	0.26	0.02	0.26	613.21	<0.0001	MabMg2	71.68	A									
McC1	G	30	0.23	0.03	0.23			MabML1	158.48	A	B								
McE3	G	30	0.23	0.03	0.22			MabT26	168.88	A	B								
McE5	G	30	0.27	0.02	0.27			MaeLP3	195.52	A	B	C							
McE7	G	30	0.22	0.01	0.22			MabT23	206.68		B	C							
McE8	G	30	0.24	0.02	0.24			MaeLP4	207.88		B	C							
McMg1	G	30	0.25	0.02	0.25			MaeLP2	218.28		B	C							
McMg3	G	30	0.24	0.02	0.23			MaeML1	235.75		B	C	D						
McMg6	G	30	0.27	0.02	0.27			MaeLP7	251.63		B	C	D	E					
McMg9	G	30	0.25	0.02	0.25			MabT24	272.80		B	C	D	E	F				
MabC2	G	30	0.25	0.03	0.25			MabT25	317.83			C	D	E	F	G			
MabMg1	G	30	0.25	0.02	0.25			MabT22	355.32				D	E	F	G			
MabMg2	G	30	0.15	0.02	0.16			MaeT15	378.63					E	F	G			
MabML1	G	30	0.17	0.02	0.17			MaeLP5	386.75						F	G			
MabT1	G	30	0.21	0.03	0.21			MabT1	423.98							G	H		
MabT22	G	30	0.20	0.02	0.21			McE7	522.95								H	I	
MabT23	G	30	0.18	0.02	0.18			McE3	539.07								H	I	J
MabT24	G	30	0.19	0.02	0.19			McC1	545.28								H	I	J
MabT25	G	30	0.20	0.02	0.20			MaeLP9	554.52								H	I	J
MabT26	G	30	0.18	0.02	0.18			MaeLP10	576.93									I	J	K
MaeLP10	G	30	0.23	0.03	0.24			McE8	603.57									I	J	K
MaeLP2	G	30	0.18	0.03	0.18			McMg3	609.87									I	J	K
MaeLP3	G	30	0.18	0.03	0.18			MaeMg1	652.07									I	J	K	L
MaeLP4	G	30	0.18	0.03	0.19			MabC2	652.20									I	J	K	L
MaeLP5	G	30	0.21	0.03	0.21			McMg1	666.17										J	K	L	M
MaeLP7	G	30	0.19	0.02	0.19			McMg9	694.15											K	L	M	N
MaeLP9	G	30	0.23	0.01	0.23			MabMg1	694.30											K	L	M	N
MaeMg1	G	30	0.25	0.03	0.24			Mc123	742.88												L	M	N
MaeML1	G	30	0.19	0.02	0.19			McE5	793.95													M	N
MaeT15	G	30	0.21	0.03	0.21			McMg6	816.98														N

Appendix 2. Summary of measurements of dactylopore diameters in *Millepora* morphotypes. N: number of measurements, S.D.: Standar Deviation. H: Values of Kruskal-Wallis One Way Anova, Different letters significant values of Tukey's test. Morphotypes: Mc: M. complanata, Mab: M. alcicornis branched, Mae: M. alcicornis encrusted.

Morphotype	**Trait**	**N**	**Mean**	**S.D.**	**Median**	**H**	***p***	**Morphotype**	**Ranks**											
Mc123	D	30	0.16	0.01	0.16	561.09	<0.0001	MabMg2	95.87	A										
McC1	D	30	0.14	0.02	0.14			MabT26	143.75	A	B									
McE3	D	30	0.13	0.02	0.13			MaeLP3	167.52	A	B									
McE5	D	30	0.17	0.02	0.17			MabT25	202.12	A	B									
McE7	D	30	0.15	0.02	0.15			MabT23	233.50		B	C								
McE8	D	30	0.16	0.02	0.15			MabT22	236.23		B	C								
McMg1	D	30	0.18	0.02	0.18			MaeLP5	244.73		B	C								
McMg3	D	30	0.17	0.02	0.17			MabT1	263.15		B	C	D							
McMg6	D	30	0.15	0.02	0.15			MaeLP2	269.87		B	C	D							
McMg9	D	30	0.13	0.02	0.13			MabML1	349.42			C	D	E						
MabC2	D	30	0.13	0.01	0.13			MaeML1	354.62			C	D	E						
MabMg1	D	30	0.14	0.02	0.14			MabT24	355.15			C	D	E						
MabMg2	D	30	0.09	0.01	0.09			MaeLP4	382.90				D	E						
MabML1	D	30	0.12	0.02	0.12			MabC2	425.65					E	F					
MabT1	D	30	0.11	0.01	0.11			MaeLP7	446.38					E	F					
MabT22	D	30	0.11	0.02	0.10			McE3	450.72					E	F					
MabT23	D	30	0.11	0.02	0.11			McMg9	469.17					E	F					
MabT24	D	30	0.12	0.02	0.12			MaeT15	478.65					E	F	G				
MabT25	D	30	0.11	0.02	0.11			MaeLP10	518.55						F	G	H			
MabT26	D	30	0.10	0.02	0.10			McC1	518.72						F	G	H			
MaeLP10	D	30	0.14	0.02	0.14			MabMg1	541.58						F	G	H			
MaeLP2	D	30	0.11	0.02	0.11			McMg6	606.55							G	H	I		
MaeLP3	D	30	0.10	0.02	0.10			MaeMg1	618.55								H	I		
MaeLP4	D	30	0.12	0.01	0.12			McE7	633.23								H	I		
MaeLP5	D	30	0.11	0.02	0.11			McE8	692.43									I	J	
MaeLP7	D	30	0.13	0.02	0.13			MaeLP9	704.58									I	J	K
MaeLP9	D	30	0.16	0.02	0.16			Mc123	705.28									I	J	K
MaeMg1	D	30	0.15	0.02	0.15			McMg3	769.48										J	K
MaeML1	D	30	0.12	0.02	0.12			McE5	804.45										J	K
MaeT15	D	30	0.13	0.02	0.13			McMg1	832.20											K

Appendix 3. Summary of measurements of distances among dactylopores in *Millepora* morphotypes. N: number of measurements, S.D.: Standar Deviation. H: Values of Kruskal-Wallis One Way Anova, Different letters significant values of Tukey's test. Morphotypes: Mc: M. complanata, Mab: M. alcicornis branched, Mae: M. alcicornis encrusted.

Morphotype	Trait	N	Mean	S.D.	Median	H	*p*	Morphotype	Ranks									
Mc123	D-D	30	0.50	0.11	0.50	197.49	<0.0001	MabT1	257.20	A								
McC1	D-D	30	0.55	0.09	0.53			MabMg1	287.87	A	B							
McE3	D-D	30	0.49	0.06	0.49			McMg9	290.97	A	B							
McE5	D-D	30	0.50	0.12	0.50			MaeLP5	323.98	A	B	C						
McE7	D-D	30	0.53	0.07	0.52			MabML1	330.58	A	B	C	D					
McE8	D-D	30	0.47	0.09	0.49			McE8	346.37	A	B	C	D					
McMg1	D-D	30	0.59	0.09	0.60			MaeLP7	355.67	A	B	C	D					
McMg3	D-D	30	0.55	0.16	0.57			MabT25	368.05	A	B	C	D	E				
McMg6	D-D	30	0.49	0.07	0.48			McMg6	371.53	A	B	C	D	E				
McMg9	D-D	30	0.46	0.08	0.45			MaeLP10	373.12	A	B	C	D	E				
MabC2	D-D	30	0.50	0.11	0.49			McE3	376.55	A	B	C	D	E				
MabMg1	D-D	30	0.46	0.09	0.43			MaeLP4	400.33		B	C	D	E	F			
MabMg2	D-D	30	0.50	0.08	0.48			MabMg2	400.45		B	C	D	E	F			
MabML1	D-D	30	0.47	0.06	0.47			MabC2	406.13		B	C	D	E	F			
MabT1	D-D	30	0.44	0.08	0.44			Mc123	416.98		B	C	D	E	F	G		
MabT22	D-D	30	0.52	0.08	0.51			McE5	419.32		B	C	D	E	F	G		
MabT23	D-D	30	0.60	0.11	0.58			MaeLP3	435.52			C	D	E	F	G	H	
MabT24	D-D	30	0.56	0.11	0.52			MaeLP2	447.08			C	D	E	F	G	H	
MabT25	D-D	30	0.48	0.09	0.48			MalT22	460.65				D	E	F	G	H	
MabT26	D-D	30	0.76	0.34	0.65			McE7	493.00					E	F	G	H	
MaeLP10	D-D	30	0.48	0.10	0.50			MaeT15	496.75					E	F	G	H	
MaeLP2	D-D	30	0.51	0.09	0.51			MalT24	531.08						F	G	H	I
MaeLP3	D-D	30	0.51	0.11	0.52			McC1	537.72							G	H	I
MaeLP4	D-D	30	0.49	0.07	0.50			MaeML1	537.93							G	H	I
MaeLP5	D-D	30	0.47	0.08	0.47			McMg3	562.53								H	I
MaeLP7	D-D	30	0.48	0.09	0.48			MaeMg1	565.27								H	I
MaeLP9	D-D	30	0.65	0.11	0.65			MalT23	631.80									I
MaeMg1	D-D	30	0.57	0.11	0.59			McMg1	639.07									I
MaeML1	D-D	30	0.55	0.11	0.55			MalT26	719.23									
MaeT15	D-D	30	0.53	0.08	0.53			MaeLP9	732.27									

Apendix 4. Summary of measurements of distance from gastropore to nearest dactylopore in *Millepora* morphotypes. N: number of measurements, S.D.: Standar Deviation. H: Values of Kruskal-Wallis One Way Anova, Different letters significant values of Tukey's test. Morphotypes: Mc: M. complanata, Mab: M. alcicornis branched, Mae: M. alcicornis encrusted.

Morphotype	Trait	N	Mean	S.D.	Median	H	*p*	Morphotype	Ranks											
Mc123	G-D	30	0.34	0.07	0.32	167.60	<0.0001	McMg6	217.15	A										
McC1	G-D	30	0.38	0.08	0.36			McMg9	287.32	A	B									
McE3	G-D	30	0.36	0.08	0.34			MabMg1	307.83	A	B	C								
McE5	G-D	30	0.36	0.09	0.33			MabT1	314.30	A	B	C								
McE7	G-D	30	0.37	0.06	0.37			Mc123	336.80	A	B	C								
McE8	G-D	30	0.34	0.07	0.33			McE8	344.20	A	B	C	D							
McMg1	G-D	30	0.40	0.11	0.40			MabT22	357.05		B	C	D	E						
McMg3	G-D	30	0.43	0.11	0.43			MaeLP5	357.68		B	C	D	E						
McMg6	G-D	30	0.29	0.07	0.28			MabT25	366.13		B	C	D	E	F					
McMg9	G-D	30	0.32	0.05	0.31			MabC2	377.67		B	C	D	E	F	G				
MabC2	G-D	30	0.35	0.09	0.33			McE5	397.43		B	C	D	E	F	G	H			
MabMg1	G-D	30	0.32	0.08	0.32			MabMg2	402.62		B	C	D	E	F	G	H	I		
MabMg2	G-D	30	0.35	0.06	0.35			McE3	415.93		B	C	D	E	F	G	H	I		
MabML1	G-D	30	0.38	0.08	0.38			MabT26	424.00			C	D	E	F	G	H	I	J	
MabT1	G-D	30	0.33	0.08	0.32			MabML1	472.77				D	E	F	G	H	I	J	K
MabT22	G-D	30	0.34	0.08	0.34			McE7	472.90				D	E	F	G	H	I	J	K
MabT23	G-D	30	0.41	0.12	0.41			McC1	474.87				D	E	F	G	H	I	J	K
MabT24	G-D	30	0.39	0.08	0.38			MaeMg1	485.52					E	F	G	H	I	J	K
MabT25	G-D	30	0.34	0.07	0.33			MaeLP7	494.20						F	G	H	I	J	K
MabT26	G-D	30	0.38	0.12	0.34			MaeLP3	503.68							G	H	I	J	K
MaeLP10	G-D	30	0.40	0.11	0.38			MabT24	508.45							G	H	I	J	K
MaeLP2	G-D	30	0.39	0.08	0.39			MaeLP2	512.62								H	I	J	K
MaeLP3	G-D	30	0.40	0.10	0.38			McMg1	515.40								H	I	J	K	L	
MaeLP4	G-D	30	0.40	0.06	0.39			MaeLP10	516.00								H	I	J	K	L	
MaeLP5	G-D	30	0.34	0.09	0.34			MabT23	532.75									I	J	K	L	
MaeLP7	G-D	30	0.39	0.10	0.38			MaeLP4	555.52										J	K	L	
MaeLP9	G-D	30	0.47	0.07	0.46			MaeT15	593.12											K	L	M
MaeMg1	G-D	30	0.39	0.10	0.37			McMg3	603.93											K	L	M
MaeML1	G-D	30	0.44	0.09	0.46			MaeML1	645.35												L	M
MaeT15	G-D	30	0.42	0.09	0.42			MaeLP9	721.82													M

Appendix 5. Summary of measurements of distances among gastropores in *Millepora* morphotypes. N: number of measurements, S.D.: Standar Deviation. H: Values of Kruskal-Wallis One Way Anova, Different letters significant values of Tukey's test. Morphotypes: Mc: M. complanata, Mab: M. alcicornis branched, Mae: M. alcicornis encrusted.

Morphotype	Trait	N	Mean	S.D.	Median	H	*p*	Morphotype	Ranks								
Mc123	G-G	30	1.02	0.32	0.98	193.46	<0.0001	McE3	250.43	A							
McC1	G-G	30	1.03	0.27	1.09			Mc123	264.13	A							
McE3	G-G	30	0.99	0.36	0.96			McC1	270.10	A							
McE5	G-G	30	1.09	0.28	1.02			McE5	292.57	A	B						
McE7	G-G	30	1.09	0.24	1.08			McE7	298.07	A	B						
McE8	G-G	30	1.10	0.21	1.08			McE8	307.20	A	B						
McMg1	G-G	30	1.11	0.34	1.03			McMg9	322.87	A	B	C					
McMg3	G-G	30	1.26	0.42	1.23			McMg1	330.10	A	B	C	D				
McMg6	G-G	30	1.17	0.37	1.12			MabML1	344.03	A	B	C	D				
McMg9	G-G	30	1.11	0.27	1.15			McMg6	353.77	A	B	C	D				
MabC2	G-G	30	1.38	0.40	1.38			MabT22	418.13		B	C	D	E			
MabMg1	G-G	30	1.55	0.30	1.49			McMg3	419.87		B	C	D	E			
MabMg2	G-G	30	1.25	0.25	1.26			MabMg2	424.02		B	C	D	E			
MabML1	G-G	30	1.13	0.37	1.14			MabT24	447.97			C	D	E	F		
MabT1	G-G	30	1.56	0.39	1.49			MaeLP10	449.40			C	D	E	F		
MabT22	G-G	30	1.26	0.36	1.14			MaeMg1	455.73				D	E	F		
MabT23	G-G	30	1.41	0.39	1.40			MaeT15	488.62					E	F	G	
MabT24	G-G	30	1.28	0.36	1.24			MabC2	502.13					E	F	G	H
MabT25	G-G	30	1.41	0.35	1.39			MaeML1	515.15					E	F	G	H
MabT26	G-G	30	1.53	0.48	1.42			MabT23	522.97					E	F	G	H	I
MaeLP10	G-G	30	1.32	0.47	1.28			MabT25	523.80					E	F	G	H	I
MaeLP2	G-G	30	1.47	0.47	1.42			MaeLP5	543.73					E	F	G	H	I
MaeLP3	G-G	30	1.64	0.42	1.59			MaeLP2	546.23					E	F	G	H	I
MaeLP4	G-G	30	1.50	0.40	1.51			MabT26	565.53						F	G	H	I
MaeLP5	G-G	30	1.44	0.47	1.46			MaeLP7	575.65						F	G	H	I
MaeLP7	G-G	30	1.51	0.42	1.43			MaeLP4	590.77							G	H	I
MaeLP9	G-G	30	1.55	0.41	1.55			MaeLP9	608.07							G	H	I
MaeMg1	G-G	30	1.32	0.41	1.26			MabT1	609.10							G	H	I
MaeML1	G-G	30	1.41	0.40	1.36			MabMg1	624.73								H	I
MaeT15	G-G	30	1.36	0.36	1.27			MaeLP3	650.13									I

Appendix 6. Comparisons of morphological traits in *Millepora* morphotypes. N: number of measurements, S.D.: Standar Deviation. H: Values of Kruskal-Wallis One Way Anova, Different letters significant values of Tukey's test. Morphotypes: Mc: M. complanata, Mab: M. alcicornis branched, Mae: M. alcicornis encrusted.

Morphotype	Trait	N	Mean	S.D.	Median	H	*p*	Ranks	
M. complanata	G	300	0.25	0.03	0.24	276.89	<0.0001	653.49	A
M. alcicornis branched	G	300	0.20	0.04	0.20			332.22	B
M. alcicornis encrusted	G	300	0.20	0.03	0.20			365.80	B
M. complanata	D	300	0.15	0.02	0.15	300.19	<0.0001	648.22	A
M. alcicornis branched	D	300	0.11	0.02	0.11			284.64	B
M. alcicornis encrusted	D	300	0.13	0.02	0.13			418.64	C
M. complanata	D-D	300	0.51	0.10	0.51	1.85	0.3965		
M. alcicornis branched	D-D	300	0.53	0.16	0.51				
M. alcicornis encrusted	D-D	300	0.52	0.11	0.50				
M. complanata	G-D	300	0.36	0.09	0.34	51.63	0.0001	406.59	A
M. alcicornis branched	G-D	300	0.36	0.09	0.34			406.36	A
M. alcicornis encrusted	G-D	300	0.40	0.09	0.39			538.55	B
M. complanata	G-G	300	1.10	0.32	1.07	134.08	<0.0001	310.91	A
M. alcicornis branched	G-G	300	1.38	0.39	1.36			498.24	B
M. alcicornis encrusted	G-G	300	1.45	0.43	1.42			542.35	C