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Evolution of Host Associations in Symbiotic Zoanthidea

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EVOLUTION OF HOST ASSOCIATIONS IN SYMBIOTIC ZOANTHIDEA

By

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To my wife Lisbeth and our daughters Laura and Audrey,
my parents Martha and Paul Swain,
and in memory of my grandparents Irene and Paul Scheuer

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ABSTRACT

Symbioses are pervasive in life and confer novel adaptive capabilities that enable ecological expansion into unexplored niches. Evolutionary transitions in symbiosis (terminations, origins, host shifts, or changes in relationship outcomes) can therefore have dramatic effects on the fitness, life history, and distribution of organisms. Because symbiotic interactions require coordination among traits that control recognition, colonization, and maintenance of symbiosis, transitions in symbiosis should generally be rare and conserved across evolutionary time. Cnidarians in the order Zoanthidea (class Anthozoa) are symbionts of taxa representing at least five invertebrate phyla and occur in most major benthic habitats from the intertidal to the deep sea. The Zoanthidea exhibit a startling array of evolutionary transitions in symbioses, and host associations and relationship outcomes appear to be highly homoplasious. To better understand these transitions and the effects of symbioses on Zoanthidea, I use a multifaceted approach that combines molecular phylogenetics and morphology with manipulative field experiments and surveys to clarify species delimitations, diversity and specificity of host associations, context-dependent relationship outcomes, and the evolution of symbioses. The results of this research indicate that our current understanding of symbiosis evolution in Zoanthidea is confounded by incomplete data on associations and relationships, and systematics that do not reflect evolutionary relationships; the data presented here indicate that host associations are largely conserved across evolutionary time.

INTRODUCTION

Symbioses are intimate and protracted interspecific associations that include the complete continuum of relationships ranging from mutualism to parasitism (Saffo 1992). Participating in symbiotic associations appears to be a general condition of life as there may not be truly axenic organisms. Although ubiquitous, symbioses are generally complex interactions that require coordination among multiple genomes for suites of traits that control recognition, colonization, and maintenance of symbiosis. In order for any of these traits to evolve the interacting traits must experience compensatory changes to retain any symbiotic interaction. Therefore evolutionary transitions in symbiosis should be relatively rare and the interactions should be conserved through evolutionary time (*e.g.* Peterson *et al.* 1999, Mouillot *et al.* 2006).

Cnidarians representing the order Zoanthidea (Anthozoa subclass Hexacorallia) form extraordinarily diverse symbiotic interactions that are heterogeneous in terms of species associations, relationship outcomes, functional roles, intimacy, degree of obligation, specificity, modes of transmission, endosymbionts, habitat, and biogeography. Much of this diversity is contained within suborder Macrocnemina, which is differentiated from suborder Brachycnemina by functionally inconsequential morphological features but fundamental ecological traits (Ryland *et al.* 2004). The Macrocnemina are symbionts of diverse invertebrates, infrequently zooxanthellate (genus *Symbiodinium*), and have global geographic and bathymetric distributions. Brachycnemina are rarely symbionts of invertebrates, usually (perhaps always) zooxanthellate and have tropical and subtropical photic zone distributions (Ryland *et al.* 2004). The dichotomy in symbioses of suborders represents an essential difference in how carbon budgets of Zoanthidea are balanced. Macrocnemina rely on the structure and behavior of their invertebrate hosts to provide greater access to environmental sources of energy through feeding. Brachycnemina rely on their symbiotic dinoflagellates to provide photosynthetically fixed carbon (*e.g.* Davy *et al.* 1996).

The research presented here will utilize the evolutionary transitions in host associations and relationship outcomes of the diverse Macrocnemina symbioses with invertebrates to examine the evolution of symbiosis, and use the fundamental ecological differences between the Zoanthidea suborders to explore the effects of disparate symbioses. The invertebrate symbioses of Macrocnemina appear to range from parasitism to mutualism, obligate to facultative (some

may be free-living), specialist to generalist, and intimate to contactual. The most common hosts are representatives of the Alcyonacea, Antipatharia, Hydrozoa, Demospongiae, Hexactinellida, Paguridae, Thoracica, and Polychaeta. It is generally believed that elevation out of stagnant waters into energy-supplying flow is the main benefit that Zoanthidea derive from symbiotic relationships with invertebrates, because zoanthids are generally incapable of building their own skeletal structures. Examples of Zoanthidea symbioses can be found in almost every recognizable benthic marine habitat including coral reefs, arctic hard-bottom, soft-sediments of the deep-sea, diverse intertidal substrata, and temperate rocky-shoals. The current systematics of Macrocnemina (following Fautin 2008) include 3 families and 6 genera (Epizoanthidae: *Epizoanthus*, *Palaeozoanthus* and *Thoracactis*; Gerardiidae: *Gerardia*; and Parazoanthidae: *Parazoanthus* and *Isozoanthus*) that are differentiated by subtle morphological features including the relative position of the marginal musculature and the morphology of mesogloeal canals.

The morphology-based systematics of Macrocnemina arranges many heterogeneous associations into each genus and family, and segregates many homogeneous associations into different genera and families, suggesting an evolutionary history that would necessitate multiple origins of symbiosis, host switching, convergent evolution, and loss of symbiosis. Using a single genus as an example to illustrate the diversity of interactions we find *Epizoanthus* species from the Caribbean as sponge symbionts (West 1979) and intertidal free-living zoanthids (Duerden 1898), in coastal China they form symbioses with echinoderms (Pei 1998), in the Mediterranean there are free-living pelagic species (Heberts 1972), on the Pacific coast of Mexico they parasitize gorgonian axial-skeletons (Cutress & Pequegnat 1960), and in the deep-sea they live on the stalks of hexactinellid sponges (Beaulieu 2001) and on gastropod shells used by pagurid-crabs (Ates 2003). Many of the symbioses of *Epizoanthus* appear to be identical to associations formed by zoanthid species representing other families and genera of Macrocnemina in an apparently haphazard organization such that Zoanthidea appear to display a challenge to the generally conserved patterns of symbiosis evolution observed in other systems.

The relationship outcomes of Zoanthidea have not attracted nearly as much attention, however there are two species of *Parazoanthus* that have been examined and apparently have opposing relationship outcomes (mutualism or parasitism) suggesting a transition in relationships within the genus and a further lack of conservation of symbioses through evolutionary time. Using a spongivorous reef fish, West (1976) demonstrated decreased consumption and faster

growth rates of sponges colonized with zoanthids relative to zoanthid-free fragments in aquaria or on unenclosed native reefs and concluded that the relationship is a host-predator mediated mutualism. Using a different spongivorous reef fish, Lewis (1982) detected no decrease in consumption of sponges, but a decrease in the variance of oscular pumping rates and concluded that the relationship is a resource-limiting parasitism. Therefore, Zoanthidea relationship outcomes also appear to display a challenge to the generally conserved patterns of symbiosis evolution observed in other systems.

Although there are data on some symbiotic interactions, the Zoanthidea are generally an understudied group that are seldom the subject of ecological studies and lack active taxonomic experts. Therefore the disparities in observed and expected patterns of symbiosis evolution may be the result of incomplete or flawed data on associations and relationships, and systematics that may not reflect species or evolutionary relationships. In order to verify the observed patterns of symbiosis evolution, I have examined a subset of regionally accessible symbioses in the Caribbean and reconstructed molecular phylogenies of Zoanthidea on regional (Caribbean) and global scales. Chapter 1, which was a collaborative effort coauthored with Janie Wulff, is a compilation of associations noted in the literature, captured in museum collections, and observed in field sites. These data expand the diversity of sponge species known to host Zoanthidea by more than four-fold and define the specificity of hosts and symbionts. The patterns in the observed associations are used to form hypotheses about relationship outcomes, the effects of photosymbionts, and higher-level systematics of sponge and zoanthid taxa. Chapter 2 is a molecular phylogeny-based assessment of morphological species and the evolution of host associations of Caribbean Zoanthidea symbioses. These phylogenetic analyses align the morphological descriptions of Caribbean zoanthids with delimitations apparent in the molecular data to expand the number of species in the region through new species description, identification of species not known to live in the region, and reassignment of species to a different order of Cnidaria; while simultaneously generating a new hypothesis of host association evolution. Chapter 3 is a series of manipulative field experiments conducted in different years, locations, and habitats to assess the relationship outcomes of some of the Caribbean Zoanthidea-Demospongiae symbioses and to determine if the outcomes may be context-dependent. These experiments reassess previously examined and unexamined relationships to determine outcomes over ecologically meaningful time periods and apply the results to phylogenetic hypotheses in

order to examine evolutionary transitions in outcomes. Chapter 4 is a comprehensive multi-gene global phylogeny of Zoanthidea that includes representatives from all major genera and symbiosis types. The phylogenetic analyses performed in this chapter test all previous molecular hypotheses of Zoanthidea phylogeny, reconstruct the ancestral history of host associations, and assess the effects of the loss of symbiosis with invertebrates and the gain of zooxanthellae symbioses.

CHAPTER 1

DIVERSITY AND SPECIFICITY OF CARIBBEAN DEMOSPONGIAE–ZOANTHIDEA SYMBIOSES

Introduction

Two related aspects of symbiotic interactions that can contribute to our understanding of the ecology and evolution of symbiotic species are the diversity of species involved in symbiotic relationships and the specificity of those species to their symbiotic partners. Specificity in symbiotic associations can be examined at the level of less-inclusive clades (*e.g.* genotypes, ecotypes, or species) and at the level of more-inclusive clades (*e.g.* genera, families, or orders), with each level of analysis being useful for revealing different information about the ecology and evolution of symbioses.

Examining specificity at the level of less-inclusive clades can give an indication of the adaptive significance of symbiosis and the mechanisms by which the association is mediated; for example, the specificity of gall forming wasps to distinct host trees suggests that biochemical interactions or other correlates of chemistry may be important to this parasitism (Abrahamson *et al.* 2003). Examining specificity at the level of more-inclusive clades may inform hypotheses about the evolutionary relationships of symbiotic species that cannot be inferred from other analyses; for example, different communities of gall-forming insects are associated with different hybrid species (Floate & Whitham 1995) and clades of species (Abrahamson *et al.* 1998).

Caribbean sponge–zoanthid associations provide a profitable system in which to study the diversity and specificity of symbioses because of the heterogeneity of species associations that suggest hypotheses about: (1) the adaptive significance of the symbioses and (2) the notoriously challenging (due to simple morphology) higher-level systematics of sponge and zoanthid taxa. Sponges (phylum Porifera, class Demospongiae), which perform unique functional roles in marine ecosystems independent of their symbionts, are known to form symbioses with a great diversity of taxa (Wulff 2006). However, sponge symbioses with zoanthids (phylum Cnidaria, class Anthozoa, order Zoanthidea, suborder Macrocnemina) are among the most common and widespread. Zoanthids can be found living on coral reef sponges

throughout the tropics, and in the wider Caribbean region the incidence rates can be very high (*i.e.* all individuals in a host-sponge population may be associated with zoanthids; Crocker & Reiswig 1981). However, the diversity of symbiotic species involved in sponge–zoanthid associations has only been reported from two locations, Puerto Rico (West 1979) and Barbados (Crocker & Reiswig 1981), with a combined total of 21 sponge and 6 zoanthid species.

The functional roles of sponge–zoanthid symbioses appear to vary with the particular species combination and the context of the interaction. Caribbean sponge-symbiotic zoanthids are obligate symbionts, although one species of zoanthid has been reported to rarely live on bare substratum (West 1979, Crocker & Reiswig 1981). Sponges are facultative hosts, although some sponges are only occasionally found without zoanthid symbionts (Crocker & Reiswig 1981). Zoanthids live embedded, to various degrees, in the pinacoderm of sponges (West 1979) and, in at least one species combination, the host coralline sponge physically reacts to the zoanthid by reorganizing skeletal elements around the base of polyps and coenenchyme (Willenz & Hartman 1994). In another combination of species, the zoanthid appears to be effective in reducing spongivorous fish predation on a host sponge (West 1976) but does not deter feeding by spongivorous seastars (Wulff 1995) or deter nonspongivorous fish from feeding on pelleted sponge (and zoanthid) extracts (Pawlik *et al.* 1995). In a third combination of species, the zoanthid does not reduce spongivorous fish predation on the host, but may reduce water flow through the host (Lewis 1982).

In the present study, we expand the diversity of species observed in sponge–zoanthid symbioses in the wider Caribbean to include a more than four-fold greater number of sponge species than previously reported, and use the observed specificity to less-inclusive clades to inform hypotheses about the adaptive significance of some species combinations, and the observed specificity to more-inclusive clades to inform hypotheses about the higher-order systematics of Demospongiae and Macrocnemina.

Material and Methods

To determine the diversity and specificity of sponge and zoanthid species involved in symbioses, we conducted roving diver surveys on coral reefs off of Holetown, Barbados (13°10′N, 59°38′W); Salisbury, Dominica (15°23′N, 61°25′W); Navassa Island, USA (18°24′N,

75°00′W); Bocas del Toro, Panamá (9°16′N, 82°14′W; 9°19′N, 82°13′W; 9°20′N, 82°12′W; 9°21′N, 82°16′W); Charlotteville, Tobago (11°19′N, 11°18′W; 11°18′N, 60°30′W); and on hard bottom communities off of the gulf coast of Florida, USA (29°39′N, 84°22′W; 29°53′N, 84°32′W) and Georgia, USA (31°36′N, 80°47′W). Additional specimens were sampled from the live collections at Gulf Specimen Marine Laboratory in Panacea, Florida, USA. From 2002 to 2005, we collected small samples of each sponge species observed hosting a zoanthid and isolated spicules using the sodium hypochlorite centrifugation protocol of Rützler (1978). We identified sponge species by microscopic examination of spicules and skeletal architecture, and zoanthid species by colony and polyp morphology. Field survey data were supplemented with species combinations published in the sponge and zoanthid literature, and captured in the Porifera and Cnidaria collections of the United States National Museum of Natural History (USNM).

We ranked the degree that zoanthids embed in the surface of sponges from a combination of species descriptions (West 1979), photographs and observations made during field surveys, and dissections of each zoanthid species sampled from associations with several different sponges. We estimated the size of zoanthid polyps by calculating the volume of a cylinder using the length and diameter of the polyp column as reported by West (1979). We assessed the similarity of sponge and zoanthid species in terms of their symbiotic associations by constructing similarity dendrograms based on the occurrences of their symbiotic partners, which we then compared with the recently published systematics of sponges and zoanthids to evaluate congruency between clades based on symbiotic associations and clades based on traditional taxonomy. We grouped sponges by their common zoanthid associations and zoanthids by their common sponge associations in distance analyses that are analogous to the hierarchical cluster analysis of Abrahamson *et al.* (1998). We created binary character matrices of the observed presence/absence of sponge and zoanthid taxa using MacClade 4.0 and treated the occurrence of species as characters in constructing similarity dendrograms.

Because zoanthid species associate with multiple sponge species, a small number of zoanthid ‘characters’ are sufficient to provide shared occurrences to calculate similarity. By contrast, each sponge species almost exclusively associates with a single zoanthid species and therefore zoanthids rarely share specific sponges, restricting our ability to estimate similarity by using sponge species as characters. The higher-level systematics of sponges provided additional

shared characters to assess similarities among zoanthids (*e.g.* two zoanthid species may share a genus or family of sponge hosts). However, an individual association between a zoanthid and sponge may be represented in multiple hierarchical taxonomic levels and therefore the characters (taxa) will not all be independent. We mitigated the effects of non-independent characters by disregarding more-inclusive sponge taxa with character states identical to their less-inclusive taxa in order to retain unique shared characters from all taxonomic levels while eliminating repeated characters and provide a more conservative estimate of similarity. Similarity among sponge genera is based on 5 symbiotic zoanthid species; and similarity among zoanthid species is based on 84 sponge taxa (species, genera, and families). The symbioses between an Edwardsiid Actinaria (previously reported as an undescribed *Epizoanthus* species by Crocker & Reiswig 1981) and Homosclephorida sponges were used as the root in these analyses. We constructed similarity dendrograms in PAUP 4.0b10 (Swofford 2002) using minimum evolution analyses with the total character difference as the distance criterion. Trees were found using a heuristic search algorithm, equal weight for all characters, and tree–bisection–reconnection branch swapping. Where computationally possible, we estimated support by 50,000 pseudoreplicates of nonparametric bootstrapping.

Results

Diversity

Eighty-nine species of sponges (Table 1.1) and five species of zoanthids [*Epizoanthus cutressi West*, *Parazoanthus catenularis* (Duchassaing & Michelotti), *Parazoanthus parasiticus* (Duchassaing & Michelotti), *Parazoanthus puertoricense* West, and *Parazoanthus swiftii* (Duchassaing & Michelotti)] were observed associated with sponges in the wider Caribbean region.

Specificity to Less-Inclusive Clades and the Adaptive Significance of Symbiosis

The surveys of zoanthid and sponge species combinations revealed that most sponge species host a single species of zoanthid, a few host two, and none host more. Zoanthid species were observed to associate with as few as 6 and as many as 51 different species of sponges (Table 1.1).

At least 9 species of host-sponges have photosynthetic endosymbionts (cyanobacteria or dinoflagellates) and 3 species of symbiotic-zoanthids have photosynthetic dinoflagellates (Table 1.1). The occurrence of zoanthid and sponge species combinations in which both partners either have or do not have photosynthetic endosymbionts outnumbered combinations in which only one partner had photosynthetic endosymbionts 53–20. A contingency table of the numbers of observed species-combinations in which partners have and do not have photosynthetic endosymbionts (Table 1.2) demonstrates that the occurrence of photosynthetic endosymbionts in sponge–zoanthid associations are not independent ($G = 14.53$, df = 1, $P < 0.001$). Additionally, the specificity of sponges with photosynthetic endosymbionts to zoanthids with photosynthetic endosymbionts is almost absolute, whereas the specificity of zoanthids with photosynthetic endosymbionts to sponges with photosynthetic endosymbionts is much less strict (Table 1.2).

The various degrees that zoanthids embed in the surface of sponges results in a wide range in intimacy of associations, from species that live entirely on the surface of sponges to species that live buried beneath the surface of sponges (Fig. 1.1A). The degree that zoanthids live embedded in sponges is inversely correlated (Spearman's rank correlation: $r_S = -0.975$, df = 4, $P = 0.017$) with number of host-sponge species observed for each zoanthid (Fig. 1.1B) (*i.e.* zoanthids that live deeply embedded in sponges have few hosts, and zoanthids that live on the surface of sponges have many hosts). The degree that zoanthid colonies are embedded in sponges is also inversely correlated (Spearman's rank correlation: $r_S = -0.921$, df = 4, $P = 0.017$) with the volume of zoanthid polyps (Fig. 1.1C) (*i.e.* zoanthids that live deeply embedded in sponges have smaller polyp volumes, and zoanthids that live on the surface of sponges have larger polyp volumes).

Specificity to More-Inclusive Clades and Similarity Among Associations

Sponge species associate with only one or two zoanthid species. When sponges associate with two zoanthids, the zoanthids tend to be congeners; with the exception of two sponge species (*Cribrochalina vasculum* and *Cribrochalina dura*) that associate with zoanthids that represent separate genera and families (Table 1.1).

Zoanthids colonize 6–51 different species of sponges and each zoanthid species colonizes a different taxonomic scope of sponges, ranging from specialists of a few sponge genera to more diffuse associations with several different sponge orders (Table 1.1). A G-test of the number of species combinations in a zoanthid species by sponge-order contingency table (Table 1.3)

demonstrates that zoanthid symbioses are not independent of sponge ordinal level systematics (G = 114, df = 16, $P << 0.001$) and each zoanthid species is restricted to a limited portion of the Caribbean sponge diversity.

Similarity dendrograms were used to group sponges and zoanthids based on the occurrence of their symbiotic partners. The dendrogram of sponge genera was constructed using 5 zoanthid species as characters and is the strict consensus of the 500,000 best trees. This analysis distinguished four clusters of sponge genera (Fig. 1.2) that closely correspond to the taxonomic orders of sponges as defined by Systema Porifera (Hooper & van Soest, 2002a): (1) Hadromerida with Haplosclerida (suborder Haplosclerina without genus Cribrochalina); (2) Haplosclerida (suborder Petrosina with the addition of Cribrochalina); (3) Poecilosclerida and Halichondrida (without genera *Svenzea* and *Hymeniacidon*); and (4) Agelasida (with Halichondrida genera Svenzea and Hymeniacidon). The genus *Plakortis* (order Homosclerophorida) was assigned to the outgroup because of its associations with Actiniaria and independent data that suggest that Homosclerophorida are different from all other orders of Demospongiae (Muricy & Díaz 2002, Boury-Esnault 2006).

The dendrogram of zoanthid species was constructed using 84 sponge-host taxa (species, genera, and families) and is the single best tree. Mitigating the effects of non-independent characters had no effect on the resulting topology of the zoanthid dendrogram, the identical topology was found if only species were included or if all 140 taxa ranging from species to orders were included. This analysis distinguished three clades of zoanthid species by their sponge-host taxa (Fig. 1.3): (1) *P. swiftii* with *P. puertoricense*; (2) *E. cutressi* with *P. catenularis*; and (3) *P. parasiticus* basal to the *E. cutressi* and *P. catenularis* group. Edwardsiidae sp. (BAR) was assigned to the outgroup because it is an Actiniaria.

Discussion

Diversity

Sponge species associated with zoanthids represent nearly half (5 out of 14) of the extant orders of Demospongiae (Hooper & van Soest 2002a) and 14% of the total described sponge species diversity of the region (640 sponge species from all depths and habitats within the Caribbean region; van Soest 1994). The 5 sponge-associated zoanthid species constitute all of

the previously reported Caribbean sponge-symbiotic zoanthids (Crocker & Reiswig 1981), except for a species originally thought to be an unidentified *Epizoanthus* which was later identified as an Edwardsiidae Actiniaria (Chapter 2).

Specificity to Less-Inclusive Clades and the Adaptive Significance of Symbiosis

Sponges are highly specific to zoanthid species and zoanthids are not specific to sponge species. The asymmetry between the specificity of facultative sponges and the specificity of obligate zoanthids suggests that zoanthids can obtain the benefit that they derive from associating with sponges from any of several different sponge species whereas the costs or benefits that sponges derive from associating with zoanthids are more particular, regardless of the exact effects of symbiosis on sponges.

In a distinct pattern that cuts across sponge and zoanthid taxonomic groups, sponges that host photosynthetic endosymbionts are almost exclusively associated with zoanthid species that also host photosynthetic endosymbionts (Table 1.2). The high degree of specificity of sponges to zoanthids with photosynthetic endosymbionts suggests a shared strategy for maximizing exposure to sunlight or more complex interactions between hosts and the endosymbionts of zoanthids (*e.g.* Saffo 1990) or between sponge and zoanthid endosymbionts. The high degree of specificity of sponges to zoanthids with photosynthetic endosymbionts is in contrast to the lack of specificity of zoanthids to sponges with photosynthetic endosymbionts. Slightly more than half of the species combinations in which zoanthids host photosynthetic endosymbionts are with sponges that do not (Table 1.2), suggesting that (in at least some species combinations) matching ecological strategies is not crucial for zoanthids to be successful symbionts of sponges.

Caribbean sponge-symbiotic zoanthids are obligate symbionts and therefore must receive some net benefit from forming associations with sponges. Sponges are facultative hosts of zoanthids and previous research has indicated that the relationships may include mutualisms (West 1976) and parasitisms (Lewis 1982, Willenz & Hartman 1994). Zoanthids appear to be able to successfully associate with many species of sponges, whereas sponges are quite specific about which zoanthid species are acceptable partners and about matching the presence of photosynthetic endosymbionts with their zoanthid partners. Specificity asymmetries are common and, at least in mutualistic symbioses, generally favor higher relative specificity of hosts for their symbionts (Smith & Douglas 1987). Reviews of specificity data by other authors have suggested a general trend for parasites to be highly specific (Adamson & Caira 1994),

mutualists to not be highly specific (Hoeksema & Bruna 2000), and parasites to be relatively more specific than mutualists (Law 1985, Smith 1992). The low degree of specificity of most zoanthid species to sponges and the asymmetry between the relative specificity of zoanthids and sponges suggest that most sponge–zoanthid symbioses are not likely to be parasitic associations; however, specificity can be determined by several other factors (*e.g.* Desdevises *et al.* 2002) and may be influenced by relative intimacy and size of zoanthids. The net outcomes of the actual interactions between sponges and zoanthids remain to be tested experimentally (see Chapter 3), but perhaps the associations at the extremes of specificity represent good comparisons with which to start.

Specificity among zoanthids positively correlates with the degree that zoanthids embed in the surface of sponges and negatively correlates with polyp size (Fig. 1.1). The hypothesis that we favor for this pattern is that the degree that zoanthids embed in sponges restricts the number of hosts (*i.e.* symbionts with more intimate relationships have fewer hosts; Borowicz & Juliano 1991) and the relative size of polyps (*i.e.* deeply embedded zoanthids occupy space within sponges and smaller zoanthids may require less reorganization of sponge skeletal elements). However, the alternative hypothesis that polyp size determines the number of hosts (*i.e.* large polyps may be better at adapting to novel hosts) and dictates the degree that zoanthids can embed in the surface of sponges (*i.e.* large polyps cannot embed in the surface of hosts) appears equally parsimonious.

The direct physical and chemical interactions between zoanthids and sponges have received little attention (but see Crocker & Reiswig 1981, Willenz & Hartman 1994); however, the interaction probably involves traits that are neither simple nor interchangeable for use with unfamiliar hosts and therefore restrict zoanthid species to groups of similar sponges. Host-specific traits involved in zoanthid–sponge symbioses may include traits that control recognition of hosts (larval chemotaxis), traits that control colonization of hosts (cell-surface structure and biochemistry), and traits that control the persistence of the symbiosis, regardless of the specific effects on sponges or zoanthids.

There are rare examples of nonspecific associations by *P. swiftii* with sponges that are not typical *P. swiftii* hosts (*e.g. Callyspongia* sp.), with sponges that are not normal hosts of any zoanthid [*e.g. Aplysina longissima* (Carter)], and of bare substratum (Crocker & Reiswig 1981). Nonspecific associations seem to be possible because of the apparently unique ability of *P.*

swiftii to migrate between adjacent hosts (Crocker & Reiswig 1981). However, because nonspecific associations are almost always observed when a typical host of *P. swiftii* (usually *Iotrochota birotulata*) is adherent to the unusual host (Crocker & Reiswig 1981), these associations may represent ephemeral expansions of a colony that are not independently viable.

The only other group of symbiotic zoanthids for which host/symbiont specificity data are available are the deep-sea zoanthid–pagurid crab symbioses. The patterns of specificity observed in the crab–zoanthid symbioses are the opposite of the sponge–zoanthid symbioses in that the zoanthids are relatively specific to crab species and crabs are less specific to zoanthid species (Ates 2003: table 1). The relatively low specificity of crabs to zoanthids may reflect the less intimate associations between pagurid crabs and their symbiotic-zoanthids which live on the surface of occupied gastropod shells, replace the shell with a carcinoecium, or are held near the carapace (with modified limbs) of crab-hosts. The relatively high specificity of zoanthids to pagurid crabs may also reflect host behavior-mediated mating opportunities that result from associations with mobile deep-sea crabs (similar examples are reviewed in Williams & McDermott 2004).

Specificity to More-Inclusive Clades

The diversity of zoanthids associated with any one sponge species is restricted by the relatively high specificity of sponges to zoanthids; however, when a sponge species is observed to associate with two different zoanthid species, they are usually congeneric. Closely-related sponges were also observed to associate with zoanthids that are congeneric, both in this and in previous morphological (Duerden 1898, West 1979) and molecular (Sinniger *et al.* 2005) studies. The only apparently distantly related zoanthids (from different genera and families) that we observed associated with a single sponge species are *P. catenularis* and *E. cutressi*.

The relatively diffuse specificity of zoanthids allows a high diversity of sponges to associate with individual zoanthid species. Each zoanthid species associates with a different taxonomic level of sponges, ranging from zoanthids that specialize on a few sponge genera to zoanthids that specialize on several sponge orders (Table 1.1).

Similarity Among Associations and Implications for Sponge Systematics

Although the grouping of sponges by their symbiotic associations (Fig. 1.2) is not a representation of phylogenetic relatedness *per se*, patterns of similar associations are almost perfectly congruent with the currently accepted systematics of sponges (Hooper & van Soest,

2002a) that are based on shared morphology, chemistry, cytology, or development. In addition, the few instances where the similarity of zoanthid symbioses differ from the current sponge systematics involve taxa in which there are documented uncertainties (discussed below) with respect to their systematic position; suggesting that zoanthid-symbioses may be informative for sponge systematics.

Zoanthid species distinguish between order Haplosclerida suborder Petrosina (with genus *Cribrochalina*) and orders Hadromerida and Haplosclerida suborder Haplosclerina (without genus *Cribrochalina*; Fig. 1.2). The concept of order Haplosclerida has undergone repeated revisions but, in the most recent configuration, this order encompasses two marine suborders: Haplosclerina and Petrosina (Hooper & van Soest 2002b). The two suborders are distinguished by viviparous reproduction and an 'organized' ectosomal skeleton in Haplosclerina, and oviparous reproduction and a 'confused' ectosomal skeleton in Petrosina (Hooper & van Soest 2002b). It has been suggested (Hooper & van Soest 2002b, McCormack *et al.* 2002) that reproduction and skeletal organization may be poor characters for distinguishing between Haplosclerina and Petrosina because each character is found in other distantly related sponges, the descriptions of skeletal characters are considered 'vague', and the suborders are not distinguished by chemical or molecular data. Similarly, genus *Cribrochalina* has had a controversial history and the current systematic position of this genus remains tentative (Desqueyroux-Faúndez & Valentine 2002). *Cribrochalina* was previously thought to be allied with suborder Petrosina; however, the current systematics places *Cribrochalina* in suborder Haplosclerina (with the caveat that some *Cribrochalina* species may more closely fit the concept of suborder Petrosina: Desqueyroux-Faúndez & Valentine 2002). *Cribrochalina dura* and *C. vasculum* host both *P. catenularis* and *E. cutressi*, which otherwise only associate with sponges in the suborder Petrosina. The specialization of *P. catenularis* and *E. cutressi* to sponges of suborder Petrosina supports the hypothesis that *C. dura* and *C. vasculum* also belong in suborder Petrosina, and supports the hypothesis of two marine suborders in order Haplosclerida (*i.e.* suborder Haplosclerina is exclusively associated with *P. parasiticus* and sponges of suborder Petrosina are the only hosts of *P. catenularis* and *E. cutressi*).

Zoanthid species also distinguish between order Agelasida (with order Halichondrida genera *Svenzea* and *Hymeniacidon*) and orders Poecilosclerida and Halichondrida (excluding *Svenzea* and *Hymeniacidon*; Fig. 1.2). The taxonomic history of all three orders contains

controversial reorganizations, with order Agelasida generally considered to be part of order Poecilosclerida until 1980 (van Soest & Hooper 2002a), and recent molecular and chemical evidence suggesting that parts of order Halichondrida are most closely related to species in order Agelasida (Borchiellini *et al.* 2004, Erpenbeck *et al.* 2005a, Erpenbeck *et al.* 2005b, Nichols 2005; Erpenbeck *et al.* 2006, van Soest & Hooper 2002b). The specificity of zoanthids supports the hypothesis that parts of order Halichondrida (genera *Svenzea* and *Hymeniacidon*) are more closely related to species of order Agelasida (hosts of *P. puertoricense* and *P. swiftii*), but does not distinguish between orders Poecilosclerida and Halichondrida (exclusively hosting *P. swiftii*).

Similarity Among Associations and Implications for Zoanthid Systematics

The associations of zoanthids with particular sponges have historically been used to inform zoanthid systematics because of the depauperate morphological character set of zoanthids; for example, Pax & Müller (1962) define the subspecies of *Parazoanthus axinellae* by the frequency of colonization of sponges in the genus *Thenea*. Recent molecular phylogenetics (Sinniger *et al.* 2005) also suggests that patterns of host taxa associations are informative for zoanthid systematics.

Sponge taxa distinguish between clades of zoanthid species (*P. swiftii* with *P. puertoricense*, and *P. parasiticus* basal to *E. cutressi* and *P. catenularis*), dividing the zoanthids by species that host endosymbiotic dinoflagellates and species that do not (Fig. 1.3). The grouping of *E. cutressi* with species of genus *Parazoanthus* is not congruent with the current morphology-based taxonomy, which arranges genera *Epizoanthus* and *Parazoanthus* into separate sister families (Epizoanthidae and Parazoanthidae) within the zoanthid suborder Macrocnemina (Ryland & Muirhead 1993). There is molecular evidence that the genus *Parazoanthus* may be paraphyletic; however, genus *Epizoanthus* and families Epizoanthidae and Parazoanthidae are apparently monophyletic (Sinniger *et al.* 2005). The zoanthid species included in the analysis of Sinniger *et al.* (2005) included examples of species with similar hosts across genera within family Parazoanthidae, but species with different hosts (or species which are generally thought to be asymbiotic) across families. If symbioses are informative about evolutionary relationships, then the diversity of symbioses sampled by Sinniger *et al.* (2005) would inadvertently bias the results to find monophyletic families and hide mixed family clades united by their symbioses. The similarity of sponge-hosts of *E. cutressi* and *P. catenularis* support the hypothesis that genus *Parazoanthus* is paraphyletic, but also suggests novel

hypotheses that genus *Epizoanthus* and the families Epizoanthidae and Parazoanthidae may be paraphyletic as well.

Conclusions

This study compiles data collected over 4 years of field surveys of the wider Caribbean, a review of the available literature, and a comprehensive examination of the Cnidaria and Porifera collections at the USNM; however, additional species combinations are certain to be discovered lurking in the vast literature of sponge biology, in new sponge species that are constantly being described, and in the unexplored regions and depths. With the data collected thus far, we offer the following conclusions:

1. Sponges representing at least 14% of the total described Caribbean sponge diversity and nearly half of the extant orders of Demospongiae associate with symbiotic-zoanthids.
2. Sponges are highly specific to zoanthid species (no one sponge species hosts more than two zoanthid species) and zoanthids are much less specific to sponge species (zoanthid species are associated with 6–51 different sponge species).
3. Sponges representing disparate taxonomic groups that host photosynthetic endosymbionts almost exclusively associate with zoanthids that also host photosynthetic endosymbionts, suggesting that the adaptive significance of this subset of symbioses includes a shared strategy for maximizing photosynthetic potential.
4. The low degree of specificity of most zoanthids to sponges and the asymmetries between zoanthid and sponge specificity may indicate that most sponge–zoanthid associations are generally not parasitic.
5. The degree that zoanthid species are embedded in sponges is negatively correlated with the number of host sponge species and the volume of zoanthid polyps, suggesting that intimacy with the host may constrain the specificity and size of zoanthids.
6. Although zoanthids form associations with many sponge species, they are specific to more-inclusive clades of sponges at various taxonomic levels (from one sponge genus to groups of sponge orders).
7. The similarity of symbiotic associations among sponge genera is almost entirely consistent with current sponge systematics. Zoanthid symbioses support generally accepted hypotheses dividing the sponge order Haplosclerida into suborders Petrosina and Haplosclerina, separating order Agelasida from order Poecilosclerida, and reassigning

parts of the order Halichondrida to order Agelasida; but also support the less accepted hypothesis that some species in genus *Cribrochalina* belong in suborder Petrosina.

8. The similarity of symbiotic associations among zoanthid species supports molecular evidence suggesting genus *Parazoanthus* is paraphyletic, but also supports the new hypothesis that genus *Epizoanthus* and families Epizoanthidae and Parazoanthidae are also paraphyletic.

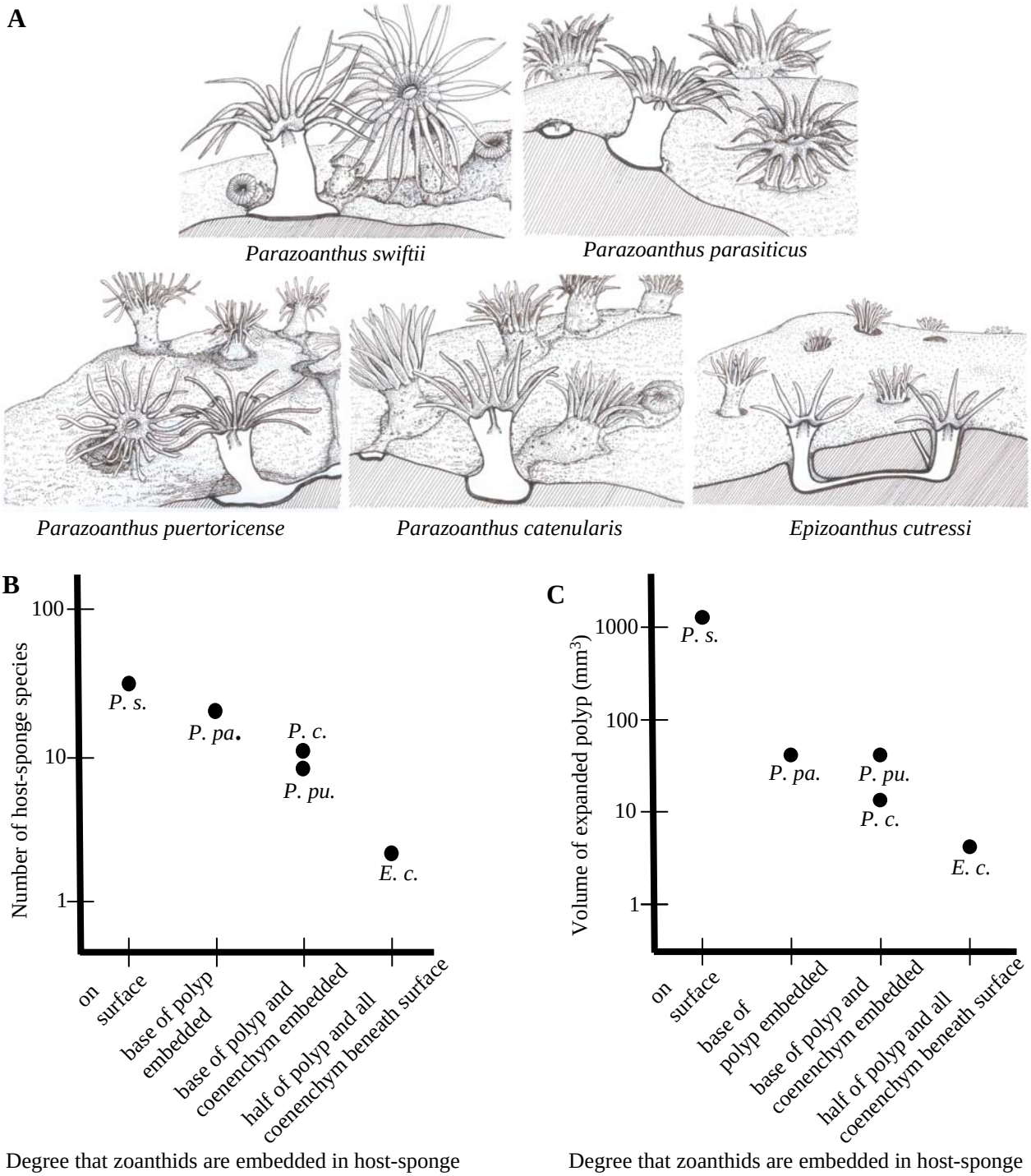

Figure 1.1. A, line drawings of symbiotic-zoanthids showing the degree that each species embeds in host sponges (intimacy). Species arranged according to the intimacy of the associations. Drawings by J. Putnam H. **B**, Correlation between the degree that zoanthids embed in sponges and the number of host-sponge species. **C**, correlation between the degree that zoanthids embed in sponges and the volume of expanded zoanthid polyps. *E. c.*, *Epizoanthus cutressi*; *P. c.*, *Parazoanthus catenularis*; *P. pa.*, *Parazoanthus parasiticus* ; *P. pu.*, *Parazoanthus puertoricense*; *P. s.*, *Parazoanthus swiftii*.

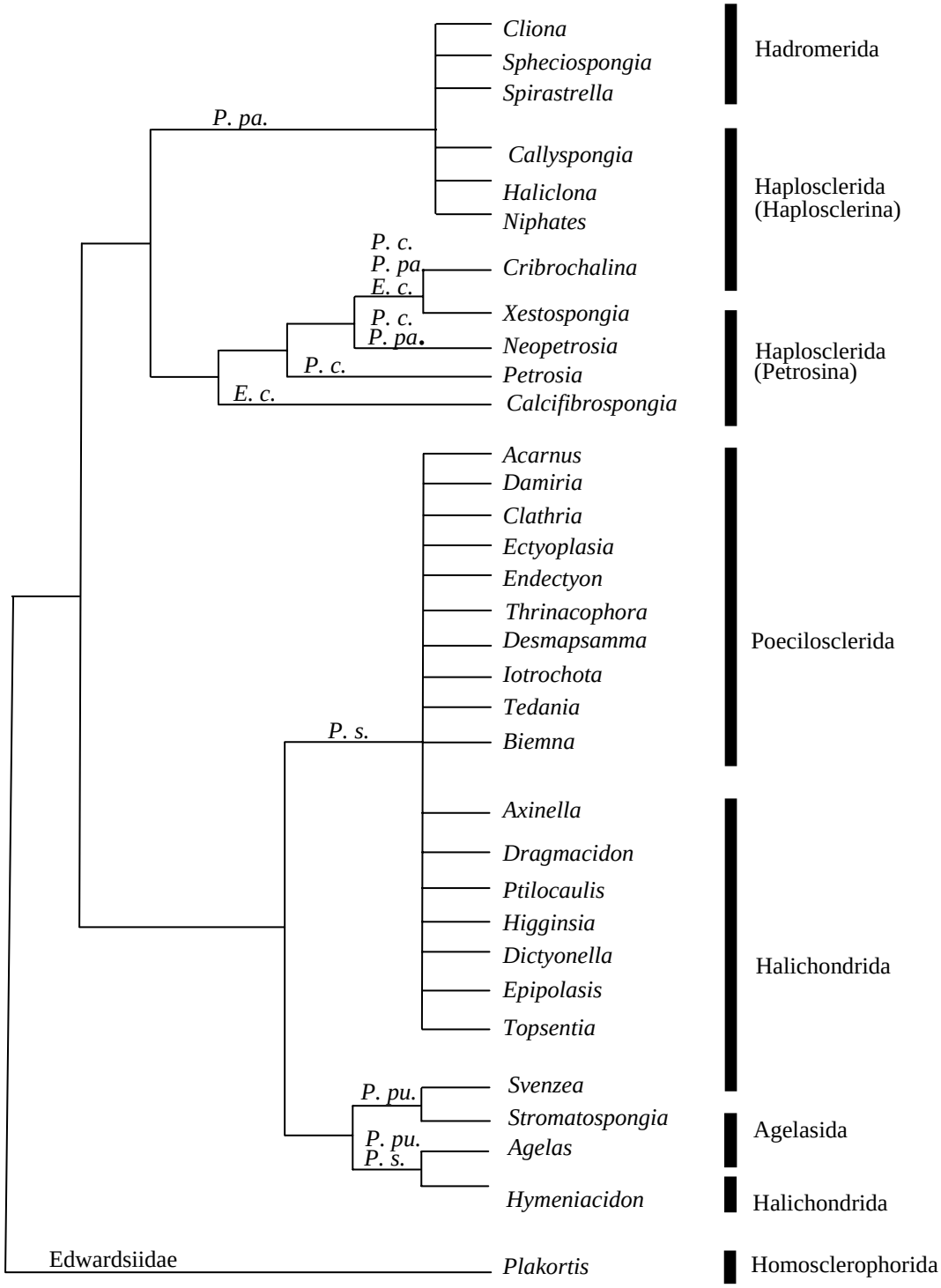

Figure 1.2. Sponge genera clustered by similarity of zoanthid symbioses. Similarity dendrogram of sponge genera based on binary presence/absence data for five zoanthid species and is the strict consensus of the 500,000 best trees. Zoanthid species abbreviations shown over branches of host-sponge clades. *E. c.*, *Epizoanthus cutressi*; *P. c.*, *Parazoanthus catenularis*; *P. pa.*, *Parazoanthus parasiticus*; *P. pu.*, *Parazoanthus puertoricense*; *P. s.*, *Parazoanthus swiftii*. An Edwardsiidae Actiniaria was used as the outgroup.

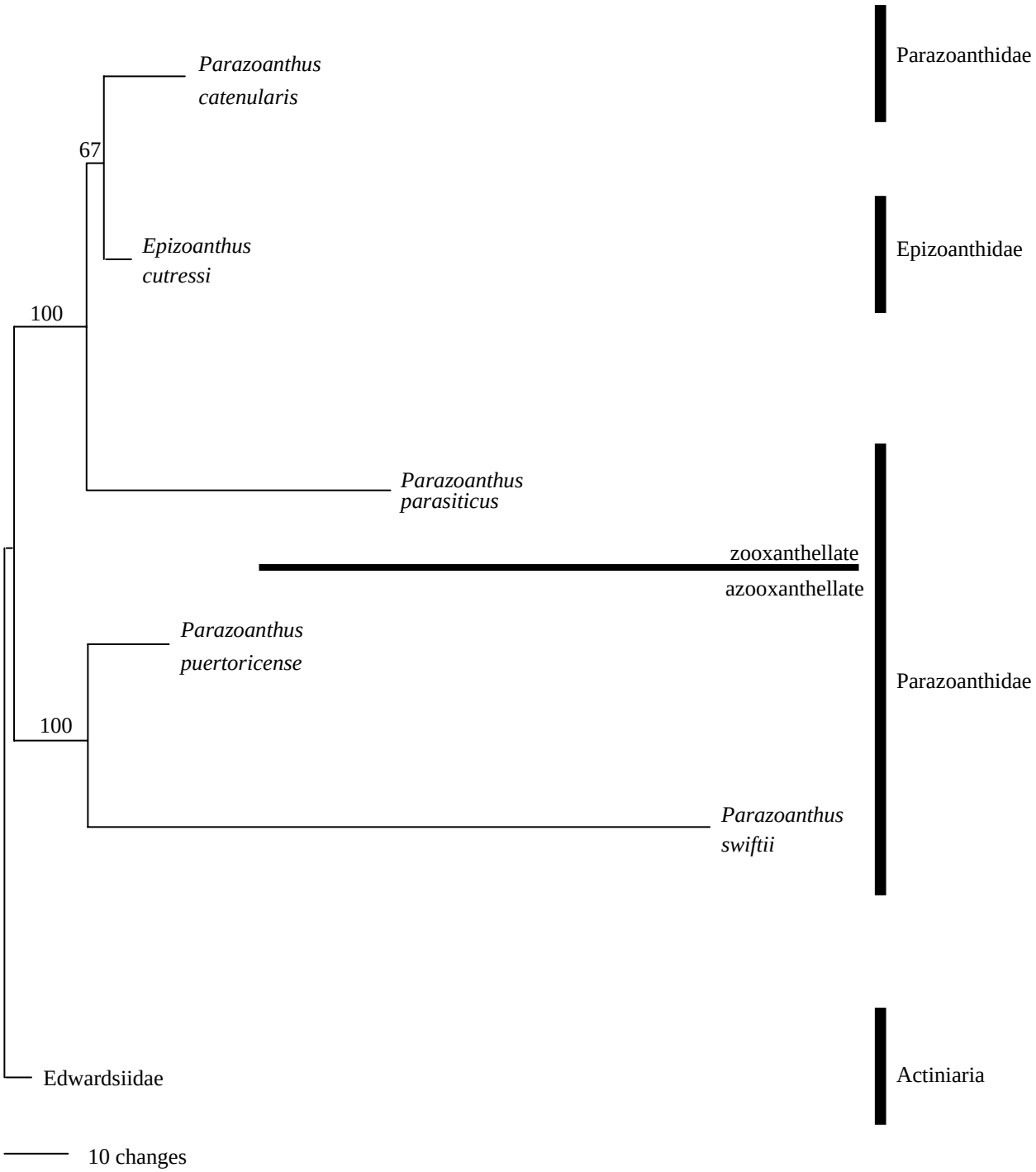

Figure 1.3. Zoanthid species clustered by similarity of sponge symbioses. Similarity dendrogram of zoanthid species based on binary presence/absence data for 84 unique sponge taxa (species, genera, and families) and is the single best tree with estimates of branch support calculated by 50,000 pseudoreplicates of nonparametric bootstrapping. An Edwardsiidae Actiniaria was used as the outgroup.

Table 1.1. Symbiotic associations of sponge and zoanthid species. Sponges arranged into higher taxa according to Systema Porifera (Hooper & van Soest 2002a). Sponge-zoanthid species combinations culled from the literature are listed by author and designated by a letter (A, Alvarez *et al.* 1998; C, Campos *et al.* 2005; C&R, Crocker & Reiswig 1981; D, Diaz *et al.* 1993; HI, Hill 1998; L&S, Lehnert & van Soest 1996; PA, Pang 1973; P, Pulitzer-Finali 1986; R, Rützler *et al.* 2003; S, van Soest 1980; S&W, van Soest & de Weerdt 2001; WE, West 1979; WI, Wiedenmayer 1977; W&H, Willenz & Hartman 1994; Z, Zea 1987; Z&W, Zea & Weil 2003), combinations observed in the field are listed by geographical location designated by a number (1, Panamá; 2, Dominica; 3, Tobago; 4, Navassa Island; 5, Barbados; 6, Florida; 7, Gulf Specimen Marine Laboratory; 8, Grey's Reef National Marine Sanctuary), and combinations observed in the collections of the USNM are designated by their museum specimen numbers. Parenthetical entries are our estimation of the zoanthid species identities from sources where the sponge species are expertly identified, but zoanthid species are incompletely described. The presence of photosynthetic endosymbionts in zoanthids or sponges is listed by publication designated by superscript letters after species names ([α]West, 1979; [β] Vicente, 1990; [γ] Rützler *et al.*, 2003).

Host Sponge Taxa	*Parazoanthus catenularis*[α]	*Parazoanthus parasiticus*[α]	*Parazoanthus puertoricense*	*Parazoanthus swiftii*	*Epizoanthus cutressi*[α]
Hadromerida					
Clionaidae					
Cliona aprica Pang [β]		(Z&W)			
Cliona caribbaea Carter [β]		PA, 4, USNM-31605			
Cliona celata Grant		USNM-39614			
Cliona delitrix Pang		C&R, 4, 5, USNM-49564			
Cliona lampa de Laubenfels		USNM-32890			
Cliona tenuis Zea & Weil		(Z&W)			
Cliona varians (Duchassaing & Michelotti) [β]		HI, USNM-48485			
Cliona cf. vermifera		4			
Cliona spp.		WE, USNM-34200			
Spheciospongia vesparium (Lamark) [β]		WE, 2, USNM-32955			
Spirastrellidae					
Spirastrella cf. coccinea		2			
Poecilosclerida					
Microcionina					
Acarnidae					
Acarnus annominatus Gray				C&R	
Damiria sp.				C&R	
Microcionidae					
Clathria (Axosuberites) obliqua (George & Wilson)				(USNM-33445)	

Table 1.1. Continued.

Host Sponge Taxa	*Parazoanthus catenularis*[α]	*Parazoanthus parasiticus*[α]	*Parazoanthus puertoricense*	*Parazoanthus swiftii*	*Epizoanthus cutressi*[α]
Clathria (Axosuberites) sp.				USNM-33389	
Clathria (Clathria) prolifera (Ellis & Solander)				7, 8	
Clathria (Microciona) spinosa (Wilson)				(USNM-33375)	
Clathria (Microciona) spp.				C&R, USNM-49156	
Clathria (Thalysias) juniperina (Lamarck)				WE, 5, USNM-31497	
Clathria (Thalysias) schoenus (de Laubenfels)				1	
Clathria (Thalysias) cf. schoenus				1	
Clathria (Thalysias) vasiformis (de Laubenfels)				(USNM-48219)	
Clathria spp.				3, (USNM-48224)	
Raspailiidae					
Ectyoplasia ferox (Duchassaing & Michelotti)				C&R	
Endectyon (Hemectyon) pearsei (Wells & Wells)				USNM-32183	
Thrinacophora funiformis Ridley & Dendy				USNM-1084839	
Myxillina					
Desmacididae					
Desmapsamma anchorata (Carter)				C&R, 2, 5	
Iotrochotidae					
Iotrochota birotulata (Higgin)				WE, 1-5, USNM-31599	
Iotrochota cf. birotulata				4	
Iotrochota imminuta Pulitzer-Finali				(P)	
Tedaniidae					
Tedania (Tedania) ignis (Duchassaing & Michelotti)				1	
Mycalina					
Desmacellidae					
Biemna sp.				(USNM-49089)	

Table 1.1. Continued.

Host Sponge Taxa	*Parazoanthus catenularis*[α]	*Parazoanthus parasiticus*[α]	*Parazoanthus puertoricense*	*Parazoanthus swiftii*	*Epizoanthus cutressi*[α]
Halichondrida					
Axinellidae					
Axinella corrugata (George & Wilson)				USNM-39875	
Axinella meandroides Alvarez *et al.*				(A), (USNM-42800)	
Axinella polycapella de Laubenfels				7	
Axinella waltonsmithi (de Laubenfels)				USNM-32202	
Axinella sp.				USNM-48017	
Dragmacidon reticulata (Ridley & Dendy)				(A), USNM-34155	
Dragmacidon lunecharta (Ridley & Dendy)				(P)	
Dragmacidon sp.				(NMNH-48262)	
Ptilocaulis walpersi (Duchassaing & Michelotti)				1	
Desmoxyidae					
Higginsia striglata (Lamarck)				USNM-33246	
Higginsia sp.				USNM-1015523	
Dictyonellidae					
Dictyonella cf. madeirensis				USNM-1084838	
Svenzea zeai (Alvarez *et al.*)[γ]			R, 2, 3, 4, USNM-42805		
Halichondriidae					
Epipolasis spp.				C&R, USNM-39378	
Hymeniacidon spp.			C&R, USNM-32321	C&R	
Topsentia bahamensis Diez *et al.*				(D)	
Topsentia ophiraphidites (de Laubenfels)				1, 3, (D)	
Topsentia cf. ophiraphidites				1	
Topsentia spp.				2, 3, 4, USNM-31606	
Agelasida					
Agelasiidae					
Agelas dispar Duchassaing & Michelotti			USNM-32345	2, 4	

Table 1.1. Continued.

Host Sponge Taxa	*Parazoanthus catenularis*[α]	*Parazoanthus parasiticus*[α]	*Parazoanthus puertoricense*	*Parazoanthus swiftii*	*Epizoanthus cutressi*[α]
Agelas clathrodes (Schmidt)				3	
Agelas conifera (Schmidt)			2, 4, USNM-31830		
Agelas inaequalis Pulitzer-Finali				(P)	
Agelas sceptrum (Lamark)			4		
Agelas sventres Lehnert & van Soest			(L&S)	3	
Agelas tubulata Lehnert & van Soest				(L&S)	
Agelas spp.			WE, C&R, 2, 3, 5	C&R, 2, 3	
Astroscleridae					
Stromatospongia vermicola Hartman			WE		
Haplosclerida					
Haplosclerina					
Callyspongiidae					
Callyspongia (Cladochalina) amigera (Duchassaing & Michelotti)		(P), 1			
Callyspongia (Cladochalina) vaginalis (Lamark)		WE, 1, 2, 6, USNM-31519			
Callyspongia (Cladochalina) villosa (Pallas)		USNM-31532			
Callyspongia spp.		C&R, USNM-31842			
Chalinidae					
Haliclona virdis (Duchassaing & Michelotti)		USNM-50286			
Haliclona sp.		USNM-49737			
Niphatidae					
Cribrochalina vasculum (Lamark)[β]	(WI), 4, 5,				4, 5
Cribrochalina dura (Wilson)[β]	3, USNM-31601				2, 4, USNM-31608
Niphates digitalis (Lamarck)		H, 2, 4, USNM-32233			
Niphates caycedoi (Zea & van Soest)		1			
Niphates erecta Duchassaing & Michelotti		C&R, 1-5, USNM-31900			

Table 1.1. Continued.

Host Sponge Taxa	*Parazoanthus catenularis*[α]	*Parazoanthus parasiticus*[α]	*Parazoanthus puertoricense*	*Parazoanthus swiftii*	*Epizoanthus cutressi*[α]
Petrosina					
Petrosiidae					
Neopetrosia proxima (Duchassaing & Michelloti)	(C), 1, 2	1			
Neopetrosia subtriangularis (Duchassaing)[β]	(S), 1, 2				
Petrosia pellasarca (de Laubenfels)	(Z), 2				
Petrosia weinbergi van Soest	(S)				
Petrosia sp.	3, 5				
Xestospongia deweerdtae Lehnert & van Soest	(S&W)				
Xestospongia dominicana Pulitzer-Finali	(P)				
Xestospongia muta (Schmidt)[β]	WE, 4, USNM-41535				WE
Xestospongia rampa (de Laubenfels)	(L&S)				
Xestospongia rosariensis Zea & Rützler[β]	1	1			
Xestospongia spp.	C&R, WE, USNM-32338				C&R, WE
Calcifibrospongiidae					
Calcifibrospongia actinostromarioides Hartman					(W&H)

Table 1.2. Contingency table of associations of zoanthid species with and without photosynthetic endosymbionts by sponge species with and without photosynthetic endosymbionts. Only sponges that could be identified to species were included.

Sponge Photosynthetic Endosymbionts	**Zoanthid Photosynthetic Endosymbionts**	
	symbionts reported	no symbionts reported
symbionts reported	14	1
no symbionts reported	18	40

Table 1.3. Contingency table of observed symbiotic associations arranged by zoanthid species and sponge order.

	Zoanthid Species				
Sponge Orders	*P. catenularis*	*P. parasiticus*	*P. puertoricense*	*P. swiftii*	*E. cutressi*
Hadromerida		12			
Poecilosclerida				23	
Halichondrida			3	21	
Agelasida			7	7	
Haplosclerida	14	12			6

CHAPTER 2

PHYLOGENY-BASED SPECIES DELIMITATIONS AND THE EVOLUTION OF HOST ASSOCIATIONS IN CARIBBEAN SYMBIOTIC ZOANTHIDEA

Introduction

The accurate and repeatable identification of species is the prelude to the study of any biological system. Our ability to recognize species as independent units of evolution will directly affect our assessment of how biological systems are structured, function, and evolve; especially in symbiotic systems, where particular interspecific interactions are linked to the fitness of associated species.

Although there are at least 22 different species concepts (Mayden 1997), the rise of molecular techniques has led to phylogenetic species concepts gaining prominence in addressing species questions (Knowlton 2000). Genetic studies of species delimitations have led to the synonymization of taxa that had been separated because of minor morphological differences, and to the splitting of other taxa where apparently minor variation has been demonstrated to be taxonomically important (reviewed in Knowlton 2000). Recent molecular phylogenetic analyses of Zoanthidea suggest similar conclusions, and provide data to support the synonymization of morphologically distinct species (*e.g.* Reimer *et al.* 2004) or separation of previously unrecognized species (*e.g.* Reimer *et al.* 2006), as well as supporting (or invalidating) other taxa at higher levels of the Linnean hierarchy (Reimer & Takishita *et al.* 2007).

Because of their simple morphology and variable coloration, delineating zoanthid species is a challenge that may require genetic techniques. The examination of genetic species delimitations has begun in Zoanthidea with the revision of free-living zoanthids (suborder Brachycnemina) of Japan (Reimer *et al.* 2006) and similar revisions may be necessary among symbiotic zoanthids (suborder Macrocnemina; Sinniger *et al.* 2005). Sinniger *et al.* (2005) have found a detectable genetic difference between light- and dark-colored zoanthids that are symbiotic with Caribbean hydroids. The original description (Duerden 1900), and a subsequent redescription (West 1979), of this hydroid symbiont disagree regarding morphology and

photoendosymbionts; however, they do agree about color. Intraspecific color variation is apparently common in both macrocnemic (*e.g.* Herberts 1972) and brachycnemic (*e.g.* Duerden 1898) zoanthids; therefore, knowing when color variation is informative for distinguishing between species may be useful, particularly for symbiotic associations that rely on aposematism (West 1976).

Conservatism of ecological niches between species through evolutionary time is predicted by theory (Peterson *et al.* 1999), and should include phylogenetic conservatism of specificity for hosts in symbiotic species (Mouillot *et al.* 2006), because hosts represent the niches of symbionts (Price 1990). Macrocnemic zoanthids associate with (among other invertebrates) gorgonians (*e.g.* Cutress & Pequegnat 1960), antipatharians (*e.g.* Ocaña & Brito 2003), hydroids (*e.g.* West 1979), demosponges (*e.g.* Crocker & Reiswig 1981), hexactinellid sponges (*e.g.* Beaulieu 2001), and pagurid crabs (*e.g.* Ates 2003); examples of similar associations are partitioned among different Zoanthidea genera and families. The extraordinary diversity of host associations among closely related zoanthids seems to be a direct challenge to phylogenetic conservatism in symbiosis evolution; however, initial analyses suggest that some higher taxa within Zoanthidea may not represent natural evolutionary clades. A phylogenetic analysis by Sinniger *et al.* (2005) found some genera, families, and suborders of zoanthids to be paraphyletic, but zoanthids with similar symbiotic associations appear to be closely related. An analysis of similarity among symbiotic zoanthid associations (Chapter 1) concluded that some heterogeneric zoanthids had greater similarity than congeneric zoanthids, suggesting further paraphyly in the current Zoanthidea systematics.

The analyses presented here use DNA sequences of the ribosomal RNA (rRNA) internal transcribed spacer (ITS) nuclear gene from individual colonies, representing the morphologic and chromatic range of taxa observed throughout the wider Caribbean, to reconstruct a regional phylogeny for symbiotic zoanthids. Phylogenetic analyses of DNA from multiple specimens collected across most of the natural distribution of each taxon are used to expose the diversity of species in the region, to clarify inconsistencies in descriptions of intraspecific morphologic and chromatic variability, and to elucidate the geographic distribution of taxa or morphotypes. Phylogenetic relationships inferred from the ITS nuclear gene and 16S rRNA mitochondrial gene sequences are used to evaluate phylogenetic conservatism in the evolution of host associations in symbiotic zoanthids, and to assess the morphology-based taxonomy of Zoanthidea.

Materials and Methods

DNA sequences were analysed from symbiotic zoanthids collected throughout the wider Caribbean region. The zoanthid species sampled included: *Epizoanthus cutressi* West 1979 (*E.c.*); *'Epizoanthus'* sp. nov. *sensu* Crocker & Reiswig 1981; *Parazoanthus catenularis* (Duchassaing & Michelotti 1860) (*P.c.*); *Parazoanthus parasiticus* (Duchassaing & Michelotti 1860) (*P.pa.*); *Parazoanthus puertoricense* West 1979 (*P.pu.*); *Parazoanthus swiftii* (Duchassaing & Michelotti 1860) (*P.s.*); and *Parazoanthus tunicans* Duerden 1900 (*P.t.*). The abbreviations given in parentheses are used in the figures. Between five and fifteen polyps from each morphologically and chromatically distinct colony were collected from the following locations: Búzios, Brazil (22°44′S, 41°51′W); Curaçao (12°03′N, 68°51′W); Flower Garden Banks National Marine Sanctuary, Galveston, TX, USA (28°09′N, 94°17′W); St. John, US Virgin Islands (18°18′N, 64°49′W); and at field sites described in chapter 1. Ancillary samples of *Parazoanthus axinellae* (Schmidt 1862) (*P.a.*) were collected from Mediterranean locations near the Medes Islands, Spain (42°02′N, 3°13′W), Banyuls-sur-Mer, France (42°29′N, 3°08′W), and from Omiš (43°26′N, 16°39′W), Vis Island (43°01′N, 16°12′W), and Fraškerić Island (44°49′N, 13°50′W), Croatia. Additional sequences culled from GenBank were included in the 16S analysis to provide the appropriate context for evaluating species groups. Four non-symbiotic zoanthids from the genus *Zoanthus* were used to represent the suborder Brachycnemina, two anemones (order Actiniaria) were used to represent the family Edwardsiidae, and a black coral (order Antipatharia) was used as the root (Table 2.1.), because independent evidence indicates that antipatharians are an appropriate outgroup (Berntson *et al.* 1999; Daly *et al.* 2003).

Amplification and Sequencing

Polyps were preserved in 100% ethanol following collection and, after several substitutions of fresh ethanol to counter dilution, stored at -80 °C. Total nucleic acid was extracted from individual polyps using a cetyl-trimethyl-ammonium bromide extraction technique (Doyle & Doyle 1987). Polymerase chain reaction (PCR) amplification was performed using Platinum® PCR Supermix (Invitrogen), the 16S primers of Sinniger *et al.* (2005), and the following novel primers: ITS-f 5′-CTAGTAAGCGCGAGTCATCAGC-3′; ITS-

r, 5′-GGTAGCCTTGCCTGATCTGA-3′; 16S-f 2824 5′TCGACTGTTTACCAAAAACATA GC-3′; 16S-r 3554 5′-CAATTCAACATCGAGGTCGCAA AC-3′. The thermal protocol used for all primers consisted of 94 °C for 3 min, 32 cycles of 94 °C for 30 s, 50 °C for 60 s, 72 °C for 90 s, with a final extension step of 72 °C for 10 min. PCR products were purified by enzymatic digestion (ExoSAP-IT®; USB Corporation) and were directly sequenced in both the forward and reverse directions using the amplification primers and Big-Dye® Terminator (Applied Biosystems) chemistry at the Florida State University Sequencing Facility.

Phylogenetic Analyses

Forward and reverse sequences were edited and assembled using SEQUENCHER 4.0.5 (Gene Codes Co.), and an initial alignment of all sequences was made using CLUSTAL X 1.81 (Thompson *et al.* 1997) with the default settings. The CLUSTAL X-derived alignment was adequate for 16S, 5.8S, the 3′ end of 18S, and the 5′ end of 28S for all sequences; however, the ITS1 and ITS2 regions could only be reasonably aligned by CLUSTAL X within groups of individuals that represented species or closely related species. Phylogenetic analyses of ITS regions often exclude large portions of ITS1 and ITS2 because of alignment difficulties (*e.g.* Reimer & Takishita *et al.* 2007). In order to include all nucleotides of the ITS genes in the phylogenetic analyses, blocks of unambiguously aligned sequences were shifted to create non-overlapping character sets in the alignment and the resulting gaps were coded as missing characters using BIOEDIT 7.0.5.2 (Hall 1999). The final ITS alignment contains the complete sequence of each individual, but regions that aligned among subsets of individuals were staggered throughout the alignment in an organization analogous to a concatenated multigene alignment with incomplete taxon sampling for each gene (see Fig. 2.1 for a schematic of ITS alignment). Exact duplicate haplotypes were removed from the ITS alignment (indicated by superscript notations in Table 2.1), and were not included in further analyses.

Model selection and parameter estimation were performed using the Akaike information criterion in MODELTEST 3.7 (Posada & Crandall 1998). The Tamura–Nei model (Tamura & Nei 1993) with invariable sites and gamma parameter (TrN + I + G) gave the best fit to the ITS data, with the following parameters: base frequencies, A = 0.2270, C = 0.2626, and G = 0.2704; substitution-rate matrix, rAC = 1.0000, rAG = 2.1157, rAT = 1.0000, rCG = 1.0000, and rCT = 2.8980; gamma shape parameter, 0.4557; proportion of invariable sites, 0.3616. The Tamura–Nei model (Tamura & Nei 1993) with gamma parameter (TrN + G) gave the best fit to the 16S

data, with the following parameters: base frequencies, A = 0.3112, C = 0.1900, and G = 0.2566; substitution-rate matrix, rAC = 1.0000, rAG = 4.5496, rAT = 1.0000, rCG = 1.0000, and rCT = 8.6916; gamma shape parameter, 0.3976. Phylogenetic analyses were conducted using PAUP 4.0 b10 (Swofford 2000) and MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001). Maximum likelihood (ML) searches were performed using a heuristic search algorithm with tree-bisection-reconnection branch swapping and five random-sequence taxon additions. Estimates of support were obtained by ML bootstrapping using the same likelihood parameters as the topology search, with 100 pseudoreplicates, and a Bayesian statistical approach using Markov chain Monte Carlo simulations (Huelsenbeck & Ronquist 2001). Bayesian analyses of the ITS data were performed on an alignment partitioned into three data subsets (ITS1; ITS2; and a concatenated 18S, 5.8S, and 28S), using models of molecular evolution empirically determined for each partition by MRBAYES. Every five-hundredth tree was sampled during a 5 million iteration chain and, after inspection for convergence using AWTY (Wilgenbusch *et al.* 2004), the first two million iterations were discarded as 'burn-in'. A 50% majority rule consensus tree was calculated from the remaining Bayesian iterations using PAUP.

Species Delimitations and Biogeography

Species delimitations were determined from the ITS phylogeny using a history-based phylogenetic species concept (Baum & Donoghue 1995) by identifying reciprocally monophyletic crown clades, which were then assessed by concordance with published descriptions of gross morphology (color, number of tentacles, number of scapular ridges, and size of polyps). Individual zoanthids were initially identified *in situ* and by macroscopic photography of zoanthid–host holobionts using a combination of polyp and colony morphology, and host specificity outlined in chapter 1 and as described by Duerden (1900), Pax & Müller (1962), West (1979), and Crocker & Reiswig (1981).

Species that did not match published morphological descriptions of Caribbean zoanthids were subjected to further microscopic examination of internal morphological structures. Individual polyps dissected from colonies were decalcified for 4 h in Formical-4™ (Decal Chemical Corporation; Tallman, NY) and desilicified for 4 h in 20% hydrofluoric acid, then stored in 70% ethanol. Polyps were dehydrated in ethanol, cleared with xylene, embedded in paraffin, and sectioned at the Florida State University Histology Facility. Serial 13-µm longitudinal and cross sections of polyps were stained with Harris' hematoxylin and eosin Y.

Type specimens were deposited at the U.S. National Museum of Natural History, Washington, DC, USA (USNM).

The color of individual colonies was mapped onto the ITS phylogeny to assess whether color could be used to distinguish species. The collection locations for zoanthid specimens were mapped on the ITS phylogeny to assess the effect of geography on the estimation of species delimitations. The geographic distributions of species were determined by compiling genetically verified species occurrence data from field collections, supplemented with occurrence data published in the sponge and zoanthid literature, and occurrence data transcribed from the labels of specimens in the Porifera and Cnidaria collections of the United States National Museum of Natural History (USNM).

Phylogenetic Relationships and the Evolution of Host Associations

The ITS phylogeny, constructed to examine species delimitations, also reveals the evolutionary relationships between species and is therefore useful in forming hypotheses about the evolution of symbioses in zoanthids and the validity of current zoanthid systematics. The host species of individual zoanthids were mapped onto the ITS phylogeny to assess the effects of particular host associations on zoanthid species clade topology.

The 16S phylogeny was constructed to provide an independent assessment of the clades of species inferred from the ITS analysis. The host associations of zoanthid species (as defined by Pax & Müller 1962; Herberts 1972; West 1979; and Chapter 1) were mapped onto the ITS and 16S phylogenies to assess phylogenetic conservatism in the evolution of zoanthid-host associations, and detect host switches. The occurrence of zoanthid photo-endosymbionts (*Symbiodinium*; as defined by West 1979) was also mapped onto the ITS and 16S phylogenies to assess phylogenetic conservatism in the evolution of zoanthid-*Symbiodinium* associations.

Results

Phylogenetic Analyses

Electrophoresis of ITS PCR products produced single compact bands of approximately 900 nucleotides in length, and direct sequencing produced forward and reverse sequences with no indication of prominent intragenomic nucleotide variation (Fig. 2.2) or length variation, except in haplotypes of *P. swiftii*. There is evidence of isolated intragenomic length variation in

all haplotypes of *P. swiftii*, which is apparently caused by a microsatellite composed of one to four repetitions of AGGG, located 36 nucleotides downstream from the 5′ end of ITS2 in all of the *P. swiftii* individuals examined. This microsatellite is excluded from further analyses because of uncertainty about the number of repeats within a genome. The sequences of the ITS region (ITS1, 5.8S, and ITS2) ranged from 656 to 930 nucleotides in length; however, the complete alignment (that also contained segments of 18S and 28S) consisted of 2266 characters because of the additional positions introduced by staggering hypervariable regions within ITS1 and ITS2. A search for the optimal ML tree (Fig. 2.2) resulted in three best trees (each with a score = -9854.54) that differed only in the relationships among individuals within crown clades, and therefore the differences between the trees are not relevant to the questions posed here.

Electrophoresis of 16S PCR products produced single compact bands of approximately 900 nucleotides in length. The sequences of the 16S region ranged from 884 to 941 nucleotides in length using the primers of Sinniger *et al.* (2005), and 623–655 nucleotides in length using the novel primers. The complete 16S alignment consisted of 1118 characters. A search for the optimal ML tree (Fig. 2.3) resulted in a single best tree (score = -4058.72).

Species Delimitations

The ML and Bayesian analyses of the ITS data found ten crown clades, and each clade is well supported by bootstrapping (> 70) and Bayesian posterior probabilities (> 80), except for the *P. catenularis* clade (Fig. 2.2). Crown clades of symbiotic species resolved in this analysis are congruent with the published descriptions of the gross morphology and host associations of named species (*P. axinellae*, *P. catenularis*, *P. parasiticus*, *P. puertoricense*, *P. tunicans*, and *E. cutressi*), except for three clades of individuals. Histological examination of the three unidentified species reveal an *Isozoanthus* species [the fifth septa complete (suborder Macrocnemina), marginal sphincter muscle entodermal (family Parazoanthidae), no conspicuous mesogloeal ring sinus (genus *Isozoanthus*)], and two species with affinity to the actiniarian family Edwardsiidae (eight coupled mesenteries, basilar and sphincter muscles absent, no pedal disc). These unidentified species are both genetically and morphologically distinguishable from their nearest relatives on the ITS phylogeny. The unidentified *Isozoanthus* has larger polyps, darker colored tissues, and significantly (Student's t-test: $t = 23.4$, df = 190, $P = 8.2 \times 10^{-58}$) more tentacles or scapular ridges in comparison with *P. tunicans* (30–38 tentacles and 22–30 tentacles, respectively). The polyps of Edwardsiidae sp. (BAR) have significantly (Student's t-test: $t =$

18.6, df = 56, $P = 1.2 \times 10^{-25}$) fewer tentacles (10–12 rather than 13–16) compared with Edwardsiidae sp. (CUR).

The color of individuals only indicated species-level differences when there were other morphological differences that were correlated with color. For example, white-, salmon-, yellow-, and orange-colored polyps were all genetically indistinguishable *P. swiftii* individuals of similar size and number of tentacles, whereas white *P. tunicans* (smaller, with a mode of 28 tentacles) and the seal-brown unidentified *Isozoanthus* (larger, with a mode of 32 tentacles) were genetically differentiated (Fig. 2.2).

Description of *Isozoanthus antumbrosus*, new species

Diagnosis—Zooxanthellate Parazoanthidae symbiotic with *Dentitheca dendritica* (Nutting, 1900). Expanded polyps dichromatic; coenenchyme, column, and oral disk seal brown with 30–38 golden tentacles. Coloration of oral disk and tentacles recalls an annular solar eclipse. Largest expanded polyp columns 8.9 mm long, 4.3 mm in diameter; oral disk diameter 4.8 mm. Contracted polyps monochromatic, with 15–19 distinct capitular ridges. Coenenchyme thin and encrusting, completely enveloping the central and secondary axial branches of *D. dendritica* colonies; usually not covering the finest pinnate branches, where the hydroid zooids are located (Fig. 2.4). Coenenchyme usually seal brown (but can appear dark olive green or nearly black) and densely infiltrated with calcareous sediment and siliceous spicules (and therefore appearing "flecked" with white). Fully expanded polyps dichromatic: capitulum and oral disk seal brown, tentacles translucent golden; color most saturated at the bases of tentacles (Fig. 2.4). Column 4.1–8.9 mm long, 2.2–4.3 mm in diameter, and infiltrated with calcareous sediments and siliceous spicules in a gradient that diminishes toward the bases of tentacles. Oral disk 2.7–4.8 mm in diameter, concave with obvious ridges corresponding to tentacles and internal mesenteries; a central, oval hypostome bears a slit-like mouth. Tentacles 30-38, in two cycles (alternating tentacles directed toward and away from the coenenchyme), 1.9–5.0 mm long and 0.4–0.7 mm in diameter at the point of insertion in the oral disk, and gradually tapered to rounded, nearly white tips.

Polyps at intervals of approximately 1.5–2.5 polyp diameters, often in an orthogonal or distichous (on the finest hydroid branches) arrangement with oral disks nearly parallel to the plane of pinnate hydroid branches. Tentacles of adjacent polyps nearly touching at tips but not interdigitating (Fig. 2.4). Contracted polyps seal brown, mammiform, 2.2–4.2 mm in diameter

and extending 3.3–9.9 mm above surrounding coenenchyme. Capitulum bearing 15–19 distinct ridges. Mesenteries 30–38, in typical macrocnemic arrangement (fifth mesentery complete; Fig. 2.5). Retractor muscles and mesoglea of macrocnemes minimal. Mesenterial filaments present. Marginal sphincter muscle endodermal and diffuse, supported by 18–25 pleats of mesoglea (Fig. 2.5). Ectoderm and mesoglea of column with many lacunae left behind by dissolved calcareous and siliceous particles (Fig. 2.5). Encircling sinus usually imperceptible.

Etymology—Antumbra is the astronomical term for the region from which an occulting body appears surrounded by the light source producing an annular eclipse. Coloration of the oral disk (seal brown) and tentacles (golden) recalls the appearance of an annular solar eclipse. From the Latin noun *umbra*, feminine, meaning shadow; used here as the masculine adjective, *antumbrosus*, to agree with the Latinized *Isozoanthus*, masculine, from the Greek *anthos*, neuter, meaning flower.

Type Specimens—Atlantic Ocean, Caribbean Sea, Netherlands Antilles, Curaçao, Spaanse Water Baai channel, 12°3′55″ N, 68°51′10″ W, 10 m, 1 December 2007, associated with *Dentitheca dendritica*, preserved in 4% formalin, stored in 70% ethanol, USNM 1113090, holotype. A second individual was collected at the same location and time, USNM 1113091, paratype. Atlantic Ocean, Caribbean Sea, Dominica, Salisbury, Whale Shark Reef, 15°26′24″ N, 61°27′26″ W, 21 m, 12 November 2003, preserved in 70% ethanol, consumed in analyses, paratype.

Biogeography of Symbiotic Zoanthids

Within the crown clades of the ITS phylogeny, the ML and Bayesian analyses cannot detect any phylogenetic structure that can be attributed to geographic location (Fig. 2.2 and Table 2.1). Individuals collected throughout the wider Caribbean region and across the Atlantic Ocean, separated by thousands of kilometers, share identical ITS haplotypes (Table 2.1). There is a geographic- and habitat-specific pattern to the color morphs of *P. swiftii*; which are exclusively white- to salmon-colored in the subtropical regions and (potentially) marginal tropical habitats (wave-swept reef crests and rocky overhangs), and pale yellow to bright orange on tropical coral reefs. However, this geographic pattern did not correspond to any phylogenetic pattern within the *P. swiftii* clade (Fig. 2.2).

The distribution of symbiotic zoanthids observed (or reported) in the wider Caribbean region thus far is characterized by relatively low species diversity in the subtropical regions (four

species observed on the Gulf and Atlantic coasts of the south-eastern USA, and two species from Brazil), and relatively high species diversity in the tropical Caribbean (six species in the eastern Caribbean – Belize, Honduras, and Panama – and seven species in the western Caribbean – Barbados, Curaçao, Dominica, and Tobago; Fig. 2.6). Although some species are nearly ubiquitous throughout the region (*P. swiftii* and *P. parasiticus*), the composition of species changes geographically, and some species have only been observed in the northern-most regions of the wider Caribbean (*P. axinellae*), or in the eastern Caribbean (*E. cutressi*; Fig. 2.6).

Phylogeny of Zoanthidea

Interpretation of the Zoanthidea ITS and 16S phylogenies must be tempered by regional taxonomic sampling, and weak bootstrap (< 70) and Bayesian (< 80) support values at some of the internal nodes. Phylogenetic analyses of ITS and 16S data recovered the same clades of symbiotic species with similar host associations (Figs 2.2, 2.3). *Parazoanthus axinellae* and *P. swiftii* form a clade of symbionts of sponges representing the order Halichondrida (and orders Poecilosclerida and Agelasida), *P. parasiticus*, *P. catenularis*, and *E. cutressi* form a clade of symbionts of sponges representing the order Haplosclerida (and order Hadromerida), and *P. tunicans* and *I. antumbrosus* form a clade of symbionts of hydroids representing the genus *Dentitheca*. The ITS and 16S data both support conservatism in the evolution of zoanthid host associations, with host switching an apparently rare event. A single host switch was detected within the crown clades: *P. puertoricense*, which is a symbiont of sponges representing the orders Agelasida and Halichondrida (similar to the host species of the *P. axinellae* and *P. swiftii* clade; Chapter 1), whereas the other members of this clade (*P. parasiticus*, *P. catenularis*, and *E. cutressi*) are symbionts of sponges representing the order Haplosclerida and Hadromerida.

The four zoanthid genera included in these analyses (*Epizoanthus*, *Parazoanthus*, *Isozoanthus*, and *Zoanthus*) represent three different families (Epizoanthidae, Parazoanthidae, and Zoanthidae) and two different suborders (Macrocnemina, which contains Epizoanthidae and Parazoanthidae; and Brachycnemina, which contains Zoanthidae) within the order Zoanthidea. Whereas some higher taxa (orders, suborders, families, and genera) were found to be monophyletic (Fig. 2.3), *Parazoanthus* and Parazoanthidae are paraphyletic in the ITS (Fig. 2.2) and 16S (Fig. 2.3) phylogenies, and *Epizoanthus* (Epizoanthidae) and *Isozoanthus* were nested within clades of *Parazoanthus*.

Discussion

Species Delimitations

ITS phylogeny-based species delimitations were congruent with the descriptions of gross morphology for *P. axinellae*, *P. catenularis*, *P. parasiticus*, *P. puertoricense*, *P. tunicans*, and *E. cutressi*, and detected three other species: *Isozoanthus antumbrosus*, Edwardsiidae sp. (BAR), and Edwardsiidae sp. (CUR). The presence (in the Caribbean) of *P. axinellae* and three unidentified species seems to have been previously overlooked, because of similarity with other species (*I. antumbrosus* and *P. axinellae*), or because they are extremely inconspicuous (transparent tissues, and small size of Edwardsiidae sp. (BAR) and Edwardsiidae sp. (CUR)).

The morphological and host similarities (Pax & Müller 1962) of *P. axinellae* may result in mistakenly recording *P. swiftii* when observing *P. axinellae* (a possibility we were aware of, and avoided in chapter 1). In the field, these two species may be particularly hard to distinguish: they are approximately the same size, the same color (and range of color variation), associate with the same groups of sponges, and occur sympatrically in the temperate northern Caribbean. The morphological similarity is so great that *P. swiftii* and *P. axinellae* were briefly synonymized (Pax 1910). However, the genetic differences between *P. axinellae* and *P. swiftii* are large (Fig. 2.2), and tentacle counts can be used to distinguish between these two species (*P. swiftii* has a maximum of 26 tentacles, whereas *P. axinellae* has a maximum of 38 tentacles). Furthermore, the ITS DNA sequences from specimens collected across the geographic distribution of both species (from Florida to Croatia for *P. axinellae*, and from Panamá to Barbados and Georgia to Brazil for *P. swiftii*) are nearly indistinguishable within species (Fig. 2.2 and Table 2.1), thereby providing a mechanism for reliable genetic verification of field identifications.

The host similarities of *P. tunicans* and *I. antumbrosus*, along with inconsistent descriptions in the literature, may have resulted in mistakenly identifying *P. tunicans* when observing *I. antumbrosus*. The only known hydroid host of both *P. tunicans* and *I. antumbrosus* is *D. dendritica*. The accepted diversity of morphology within *P. tunicans* has been in question since a redescription by West (1979) contained inconsistencies with the original Duerden (1900) description, and with the subsequent redescription by Pax (1910). Most notably, Duerden (1900) and Pax (1910) describe a species with 28–32 or 28–30 (respectively) tentacles that are colonized

by *Symbiodinium*, whereas West (1979) describes a species with a maximum of 36 tentacles and no *Symbiodinium*. The inconsistencies between descriptions may have led to the broad acceptance of variation in morphology and coloration within *P. tunicans* in popular field guides (*e.g.* Humann & DeLoach 2002) and scientific publications (*e.g.* Sinniger *et al.* 2005), which assign dark and light color morphs to *P. tunicans*. The ITS phylogeny supports separate species and confirms the results of mitochondrial data (Sinniger *et al.* 2005) that first detected a genetic difference between the putative color morphs. Observations of morphology and 'bleaching' in *P. tunicans* indicate congruence (22–30 tentacles, colored brown by *Symbiodinium* colonizations, with white polyp columns, and coenenchyme) with the original description of Duerden (1900). The morphology of *I. antumbrosus* is not congruent (30–38 tentacles, with seal-brown polyps and coenenchyme) with any Caribbean species and is therefore described above as a new species.

The only reports (Lewis 1965; Acosta *et al.* 2005) of a Caribbean hydroid-symbiotic zoanthid (other than *P. tunicans*) are referred to as '*Isozoanthus mirabilis* (Verrill)'. However, a published description of '*I. mirabilis*' has not been found, and therefore (under article 11 of the International Code of Zoological Nomenclature), this name is a *nomen nudum*. The museum specimens of '*I. mirabilis*' (USNM 17218, 50354, 50777, 50778, 50878, and 52526) include a specimen collected by Verrill in 1880 (USNM 17218), labeled as '*Synackis mirabilis*' and 'name change by Carlgren 1930'. '*Synackis mirabilis*' seems to be a misspelling of *Synathis mirabilis* Verrill, a junior synonym of the actiniarian *Amphianthus mirabilis* (Verrill 1879). No Carlgren publication from 1930 discusses a species with the specific epithet '*mirabilis*' (Carlgren 1930a, Carlgren 1930b), although Carlgren (1949) establishes *A. mirabilis* as the senior synonym of *S. mirabilis*. Histological preparations of USNM 50878 are indistinguishable from *I. antumbrosus*, and were collected from the same hydroid host species, indicating that '*I. mirabilis*' may (in part) be conspecific with *I. antumbrosus*.

The macroscopic size, transparent tissues, and ability to retract completely beneath the surface of host sponges are likely to have kept Edwardsiidae sp. (BAR) and Edwardsiidae sp. (CUR) from being noticed. The polyps of both species are difficult to observe in the field; however, their presence can be detected by the pores or volcano-shaped protuberances on the surface of host *Plakortis* spp. sponges that are otherwise absent (Fig. 2.7). The first specimens of Edwardsiidae sp. (BAR) were reported (as an unidentified *Epizoanthus* sp.) by Crocker &

Reiswig (1981) from Barbados, and (with the generous guidance of H. Reiswig, University of Victoria) the specimens reported here are from the same reef. Histological sections and *in situ* photographs loaned by H. Reiswig are indistinguishable from the material reviewed in this study. The two whorls of alternating tentacles (typical of Zoanthidea), symbioses with sponges (typical of *Epizoanthus* and *Parazoanthus*), macroscopic size, and notoriously simple morphology of the Edwardsiidae (Daly 2002) make the original identification of this species as *Epizoanthus* understandable. A second species, extremely similar to Edwardsiidae sp. (BAR), was collected in Curaçao and is genetically and morphologically (16 tentacles compared with 12) distinct from the Barbados species.

Biogeography of Symbiotic Zoanthids

The ITS phylogeny did not detect any phylogenetic structure that can be attributed to geographic location (Fig. 2.2; Table 2.1), although undetected intragenomic polymorphisms may distort the signal of population-level structure (*e.g.* Wörheide *et al.* 2004). The geographic distribution of symbionts are limited by the availability of suitable hosts; however, sponge distributions do not seem to be able to fully explain the distribution of symbiotic zoanthids. For example, *P. puertoricense* and *E. cutressi* associate with sponge species in the genera *Agelas* and *Xestospongia* (respectively), which are common in Bocas del Toro, Panama, but these zoanthid species have not been observed there (Fig. 2.6). *Parazoanthus swiftii* and *P. parasiticus* are present and conspicuously common in nearly all of the locations examined, whereas the other zoanthid species are usually rarer locally, and geographically less widespread (Fig. 2.6).

This is the first report of *P. axinellae* in the western Atlantic, which has been known from the northeastern Atlantic and Mediterranean for more than a century. A sponge (USNM 16870) collected from North Carolina, USA, in 1860 (two years before *P. axinellae* was first described by Schmidt in the Mediterranean), is colonized with zoanthids that are apparently *P. axinellae*, thereby indicating that the current distribution is not the result of a recent invasion. *Parazoanthus axinellae* may be particularly capable of obtaining large geographic distributions because it can flourish in the absence of hosts (Haddon & Shackleton 1891), produce thread-like asexual propagules, which have the potential to be dispersed by water currents (Ryland 1997), and because several representatives of its host sponge genera are found on both sides of the Atlantic (*e.g.* sponges representing the genus *Axinella*). Other pan-Atlantic macrocnemic zoanthids include the deep-sea sponge symbionts *Parazoanthus anguicomus* (Norman 1868),

reported by Verrill (1882) as '*Epizoanthus americanus*' *n.n.* (Haddon & Shackleton 1891; Carlgren 1913), and *Epizoanthus norvegicus* (Koren & Danielssen 1877), which are found on both the North American (USNM 22495) and European coasts. The deep-sea pagurid crab symbionts *Epizoanthus incrustatus* (Düeben & Koren 1847), *Epizoanthus paguriphilus* Verrill 1882, and *Epizoanthus abyssorum* Verrill 1885 are also known from both sides of the north Atlantic (Haddon & Shackleton 1891; Muirhead *et al.* 1986), although the mobility of the crab and relative continuity of their habitat may be an additional advantage for distant dispersal. Zoanthids from the sister suborder Brachycnemia also have pan-Atlantic distributions (*e.g. Isaurus tuberculatus*, Muirhear & Ryland 1985), but their dispersal abilities are thought to stem from long-lived larvae (Ryland *et al.* 2000). The larvae of macrocnemic zoanthids have not been described; however, they may share some of the same characteristics as their brachycnemic relatives (Ryland & Westphalen 2004) that may aid in long-distance dispersal.

Both *P. axinellae* and *P. swiftii* show extensive color variation over their distributions. In the Mediterranean, *P. axinellae* is reported to range in color from 'pale grayish-yellow to the brightest orange' (Herberts 1972), and to match the color of host sponges (Pax & Müller 1962) independent of habitat (Herberts 1972). I have observed similar color matching between *P. axinellae* and sponge hosts in the Gulf of Mexico, suggesting that color may serve to conceal *P. axinellae* in both populations. In temperate regions (and apparently marginal tropical habitats like wave-swept reef crests and walls), I have observed that *P. swiftii* is usually pale salmon or drab white. Whereas on tropical reefs, *P. swiftii* is usually bright yellow or orange, and often contrasts with the color of host sponges so strikingly that the color difference is thought to be aposematic (West 1976). The golden color of both species is likely to be created by parazoanthoxanthins: a fluorescent-yellow nitrogenous pigment that has been isolated from *P. axinellae* and several other zoanthids (Cariello *et al.* 1979), and is thought to serve as a chemical defense against predators (Sepčić *et al.* 1998, Pašić *et al*. 2001). Therefore, difference in color variation between *P. axinellae* and *P. swiftii* may reflect an adaptive response to differences in predation pressure in the two regions. In the temperate region where sponge predation is predominately by invertebrates (which have not been shown to influence the distribution of sponges; Wulff 2006), symbiotic zoanthids seem to disguise their presence with matching or dull coloration. In the tropical region, where predation is predominately by vertebrates (which have been shown to influence the distribution of sponges; Wulff 2006), symbiotic zoanthids seem to

advertise their presence with contrasting yellow/orange coloration. The predators of the symbiotic zoanthids themselves include both fishes of the genus *Chaetodon* and fireworms of the genus *Hermodice*; however, no experiments on the effect of predation on symbiotic zoanthid populations or distributions have yet been performed.

Phylogeny of Zoanthidea

Molecular phylogenies were constructed to examine species delimitations of Caribbean symbiotic zoanthids in a phylogenetic context, and any interpretation of the broader interspecific relationships of the Zoanthidea is limited by the regional taxonomic sampling. Clades of symbiotic zoanthid species recovered by both the ITS and 16S analyses are distinguishable by the symbioses that they form, rather than by the morphological characters (briefly reviewed in Walsh 1967) that have traditionally defined the zoanthid genera and families. With the exception of *P. puertoricense*, zoanthid symbionts of sponges representing the order Halichondrida (and orders Poecilosclerida and Agelasida), symbionts of sponges representing the order Haplosclerida (and order Hadromerida), and symbionts of hydroids representing the genus *Dentitheca*, are each monophyletic (Figs 2.2, 2.3). A previous mitochondria-based phylogenetic analysis (Sinniger *et al.* 2005) found clades of symbiotic zoanthid species that had similar host associations within the genus *Parazoanthus*. The repeated finding of monophyletic host associations suggests some degree of phylogenetic conservatism in the evolution of zoanthid host associations that was not predicted by the current systematics. The analyses reported here further suggest that there may be unrecognized phylogenetic structure within the order Zoanthidea that could provide a more parsimonious organization of the large diversity of associations currently observed within *Epizoanthus*, *Isozoanthus*, and *Parazoanthus*; and that new taxa may be required to clarify important phylogenetic relationships.

Although most symbiotic zoanthid species are members of phylogenetic clades that have similar host associations, *P. puertoricense* is conspicuously embedded in a clade of species that form different host associations. The hosts of *P. puertoricense* are sponges representing the order Halichondrida (similar to the hosts of *P. axinellae* and *P. swiftii*, Chapter 1), whereas *P. parasiticus*, *P. catenularis*, and *E. cutressi* all form associations with sponges representing the order Haplosclerida (Figs 2.2, 2.3). Furthermore, *P. puertoricense* is the only species in this clade that does not host *Symbiodinuim*. The most parsimonious explanation for the differences between *P. puertoricense* and other members of this clade is that *P. puertoricense* switched its

associations from sponges representing Haplosclerida to sponges representing Halichondrida, and lost its symbiosis with *Symbiodinuim*. An analyses of the specificity of Caribbean sponge–zoanthid symbioses demonstrated that if a sponge hosted photo-endosymbionts (either cyanobacteria or *Symbiodinuim*), then the associations that it formed were with zoanthids that also hosted photo-endosymbionts (*Symbiodinuim*) at a ratio of 13:1. If a sponge did not host photo-endosymbionts, then the associations that it formed were with zoanthids that also did not host photo-endosymbionts at a ratio of 2.2:1. These findings suggest that matching photo-endosymbionts between sponges and zoanthids are important to the symbiosis (Chapter 1). In support of this hypothesis, *Symbiodinuim*-hosting *P. parasiticus*, *P. catenularis*, and *E. cutressi* associate with sponges hosting photo-endosymbionts at a ratio of 1.2 : 1, whereas *Symbiodinuim*-free *P. puertoricense* associates with sponges free of photo-endosymbionts at a ratio of 5:1, suggesting that the loss of *Symbiodinuim* or the shift in host use of *P. puertoricense* may have been a compensatory shift in symbiotic state that maintained the match between sponge and zoanthid photoendosymbionts.

The ITS and 16S phylogenies recovered congruent clades, and found the zoanthid genus *Parazoanthus* and family Parazoanthidae to be paraphyletic, a result largely congruent with hypotheses presented in previous analyses based on symbiosis similarity (with the exception of host switching *P. puertoricense*; Chapter 1), and combined 12S and 16S mitochondrial DNA (Sinniger *et al.* 2005). The 16S analysis found all other multi-species orders, suborders, families, and genera to be consistent with classical taxonomy, but inconsistent with the previous combined 12S and 16S analysis of Sinniger *et al.* (2005), which recovered clades of zoanthids representing the suborder Brachycnemina within the suborder Macrocnemina in a clade with *P. tunicans*.

The genera of Macrocnemina are currently uncertain and include distinct subdivisions within genera and close evolutionary relationships among species in separate genera. The morphology of *I. antumbrosus* is consistent with the genus *Isozoanthus* (fifth mesentery complete, marginal sphincter muscle endodermal, and mesogloeal ring-sinus inconspicuous), but genetically related to representatives of the genus *Parazoanthus* (fifth mesentery complete, marginal sphincter muscle endodermal, and mesogloeal ring-sinus conspicuous). However, the clade that includes *I. antumbrosus* is distinct from the clade that includes the *Parazoanthus* type species (*Parazoanthus sensu stricto*: Reimer & Nonaka *et al.* 2008), suggesting that *I. antumbrosus* is not a representative of *Parazoanthus*. Because the inconsistency between

morphological and molecular data cannot be resolved with currently available data, I accept the morphological definition of *Isozoanthus* here, with the stipulation that it will probably change to a different (not yet described) genus in the future.

Key to hydroid and sponge-symbiotic zoanthids of the greater Caribbean region

(1) Host associations:
– Sponges (Demospongiae)..... 2
– Hydroids (Plumularidae).....3

(2) Color:
– Red/maroon polyps and coenenchyme 4
– Golden-brown polyps and coenenchyme.....5
– Orange, yellow, salmon, or off-white polyps and coenenchyme.....6

(3) Color:
– White coenenchyme with golden-brown tentacles 7
– Seal-brown coenenchyme with golden-brown tentacles 8

(4) Tentacles and mesenteries number to 24 and capitular ridges number to 12, polyps single or in small groups of 2–3. Symbiont of sponges representing the orders Halichondrida or Agelasida ..... *Parazoanthus puertoricense*

(5) Colony morphology:
– Polyps consistently single or in small groups of 2–3 9
– Polyps form chains early in ontogeny of the colony but may fragment into single polyps or small groups of 2–3 polyps in older colonies 10
– Coenenchyme stolon-like and buried beneath surface of host sponge. Polyps able to retract completely beneath surface of host sponge..... 11

(6) Polyp morphology:
– Tentacles and mesenteries number to 26 and capitular ridges to 13. Symbiont of sponges representing the orders Agelasida, Halichondrida, or Poecilosclerida…… ..... *Parazoanthus swiftii*
– Tentacles and mesenteries number to 32 and capitular ridges to 16. Symbiont of sponges representing the order Halichondrida..... *Parazoanthus axinellae*

(7) Tentacles and mesenteries number to 30 and capitular ridges to 15. Symbiont of the plumularid hydroid *Dentitheca dendritica*..... *Parazoanthus tunicans*

(8) Tentacles and mesenteries number to 38 and capitular ridges to 19. Symbiont of the plumularid hydroid *Dentitheca dendritica*..... *Isozoanthus antumbrosus*

(9) Tentacles and mesenteries number to 28 and capitular ridges to 14. Symbiont of sponges representing the orders Haplosclerida or Hadromerida……... ……...*Parazoanthus parasiticus*

(10) Tentacles and mesenteries number to 20 and capitular ridges to 10. Symbiont of sponges representing the order Haplosclerida..... *Parazoanthus catenularis*

(11) Tentacles and mesenteries number to 12 and capitular ridges to 12. Symbiont of sponges representing the order Haplosclerida..... *Epizoanthus cutressi*

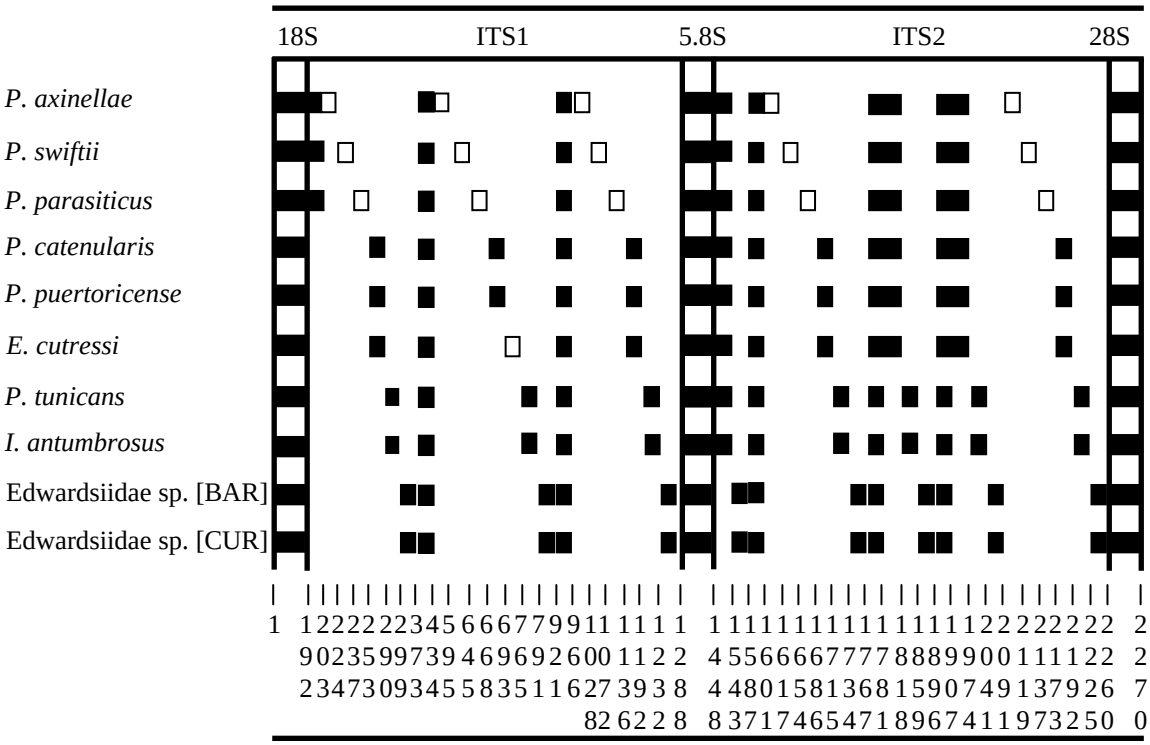

Figure 2.1. Schematic of the staggered alignment (an organization analogous to a concatenated multigene alignment with incomplete taxon sampling for each gene) used for the internal transcribed spacer region of the ribosomal RNA nuclear gene.

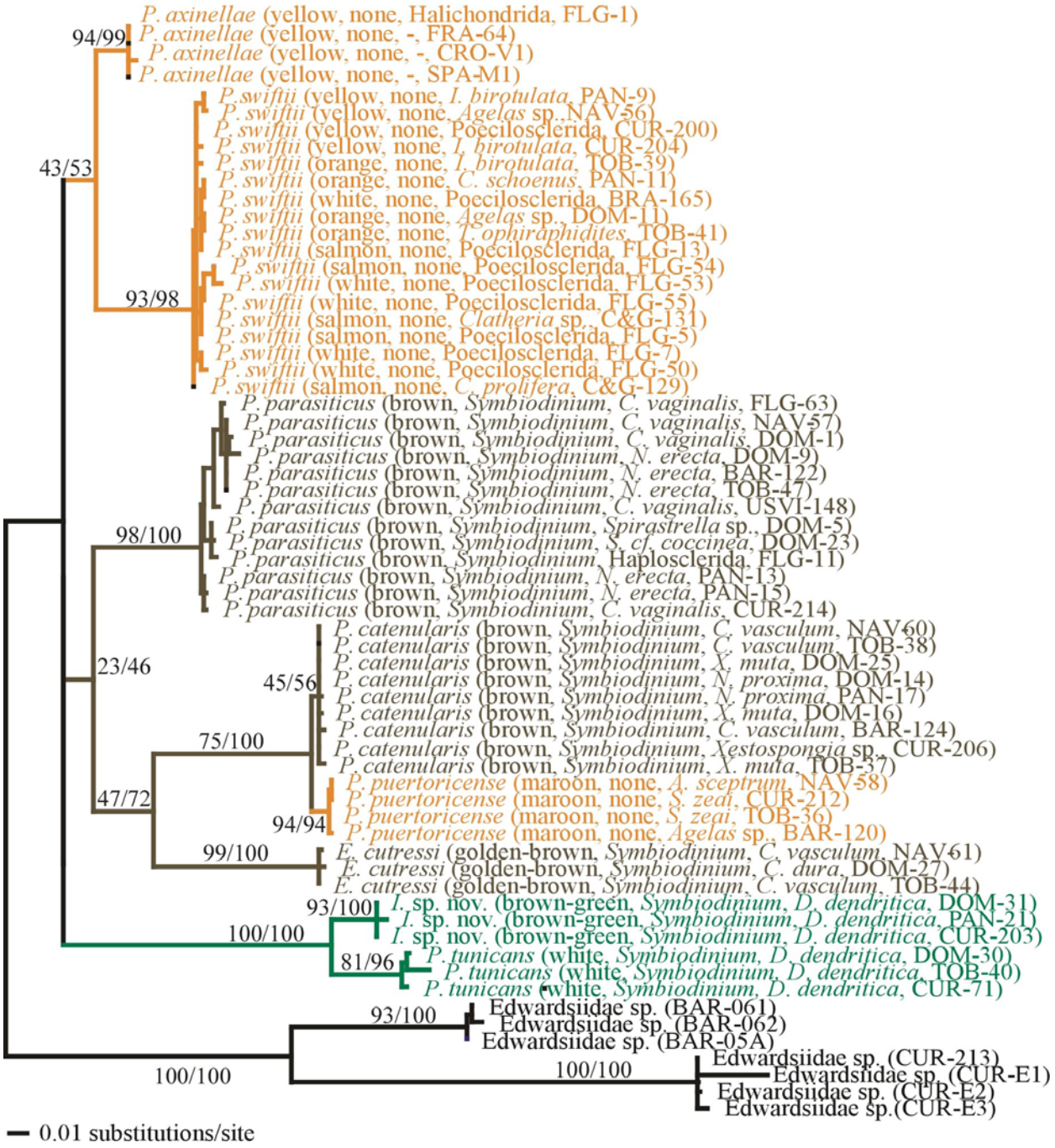

Figure 2.2. Phylogeny of Caribbean symbiotic zoanthids based on the internal transcribed spacer (ITS) region of the rRNA nuclear gene. Support values are 100 pseudoreplicate maximum likelihood (ML) bootstrap values followed by 3,000,000 iteration Bayesian posterior probabilities. The clades of symbiotic species are color coded according to their host associations. The information presented in parentheses after the specimens collected for this study includes: the color of the zoanthid, presence of *Symbiodinium*, host taxa, and individual identifier (which includes the collection location).

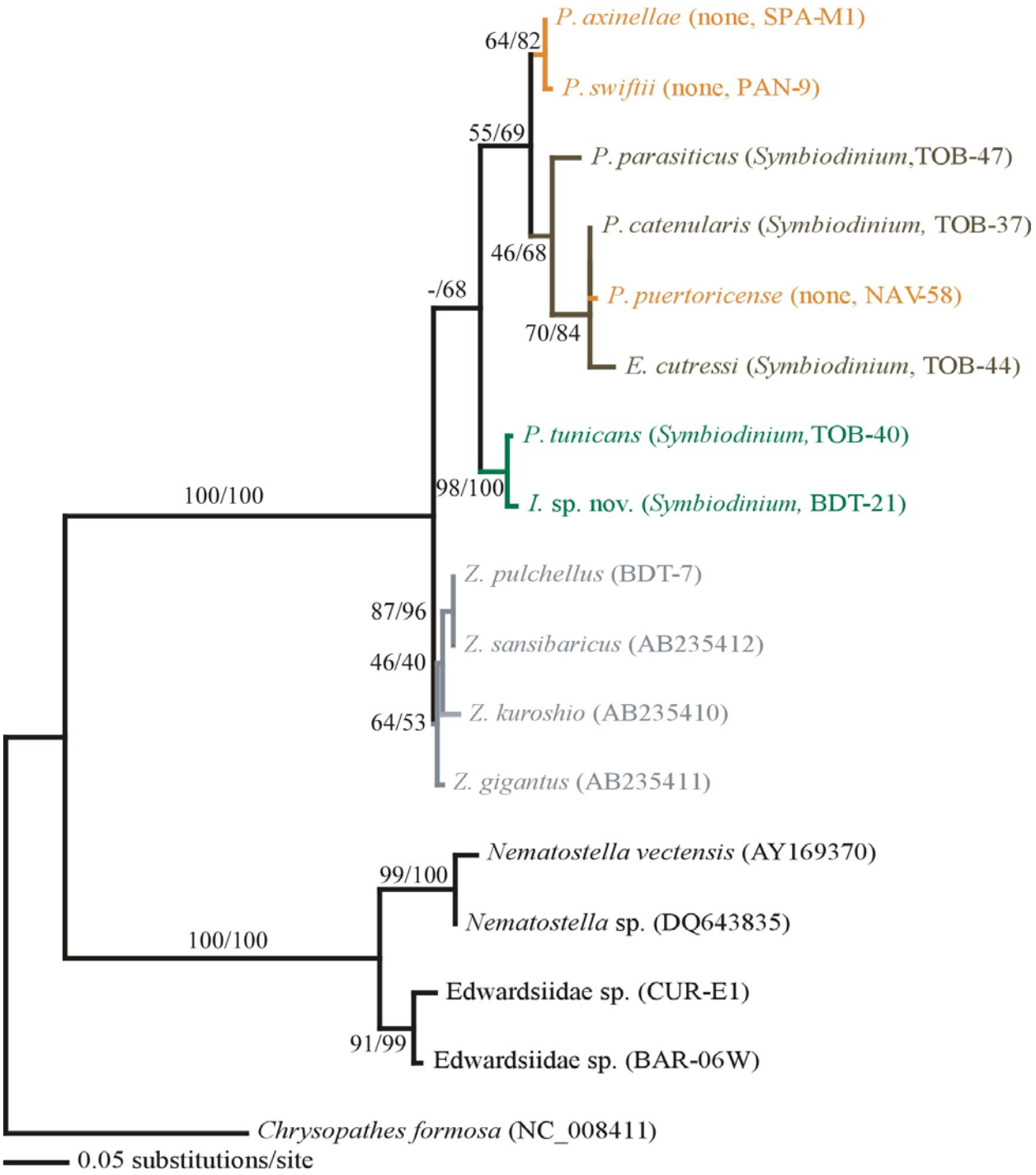

Figure 2.3. Phylogeny of Caribbean symbiotic zoanthids based on the 16S region of the rRNA mitochondrial gene. Support values are 100 pseudoreplicate maximum likelihood (ML) bootstrap values followed by 3,000,000 iteration Bayesian posterior probabilities. The clades of symbiotic species are color coded according to their host associations. The information presented in parentheses after the specimens collected for this study includes: presence of *Symbiodinium* and individual identifier (which includes the collection location). Sequences culled from GenBank only use the accession number.

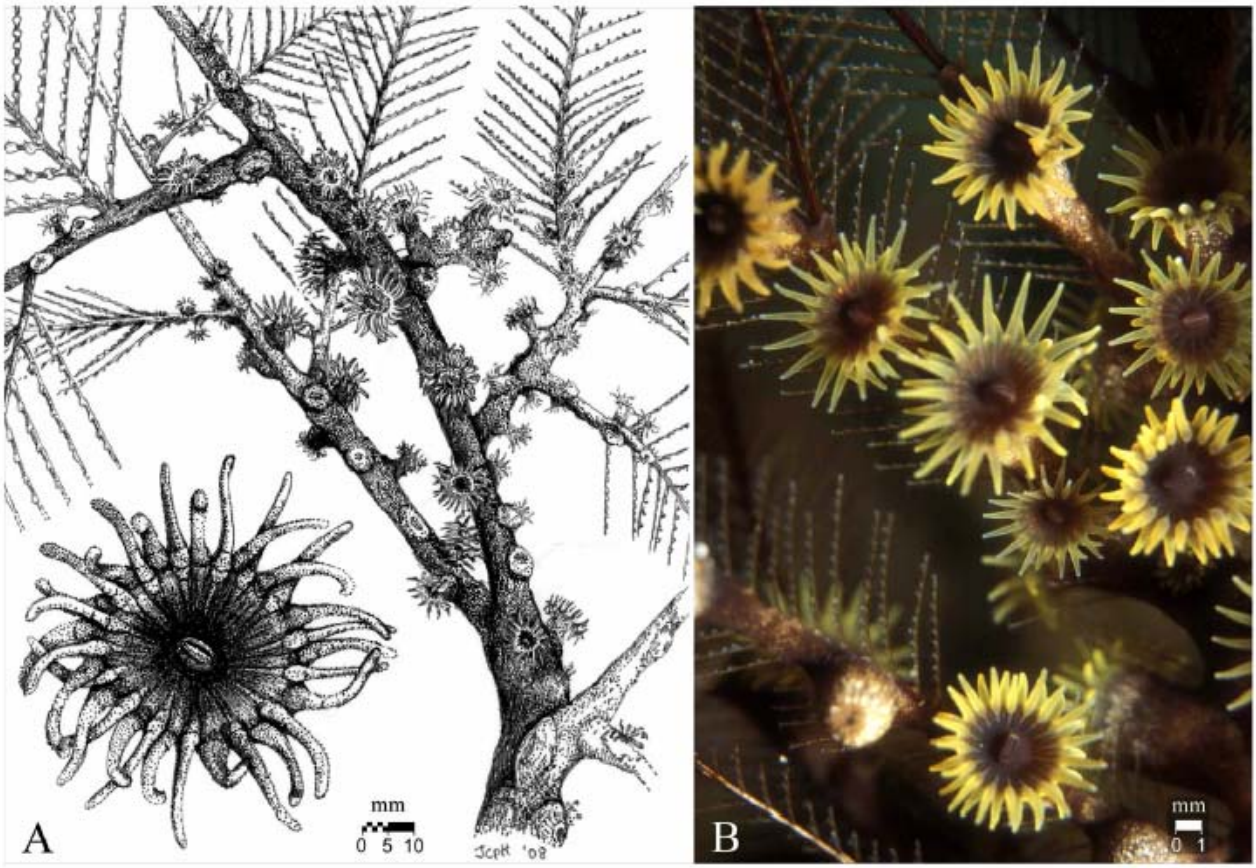

Figure 2.4. A, line drawing showing *Isozoanthus antumbrosus* colonized *Dentitheca dendritica*. Scale bar is solid for colony and checkered for polyp detail inset. Drawing by J. Putnam H. **B**, *In situ* macrophotograph of *Isozoanthus antumbrosus* with *Dentitheca dendritica* zooids visible in background.

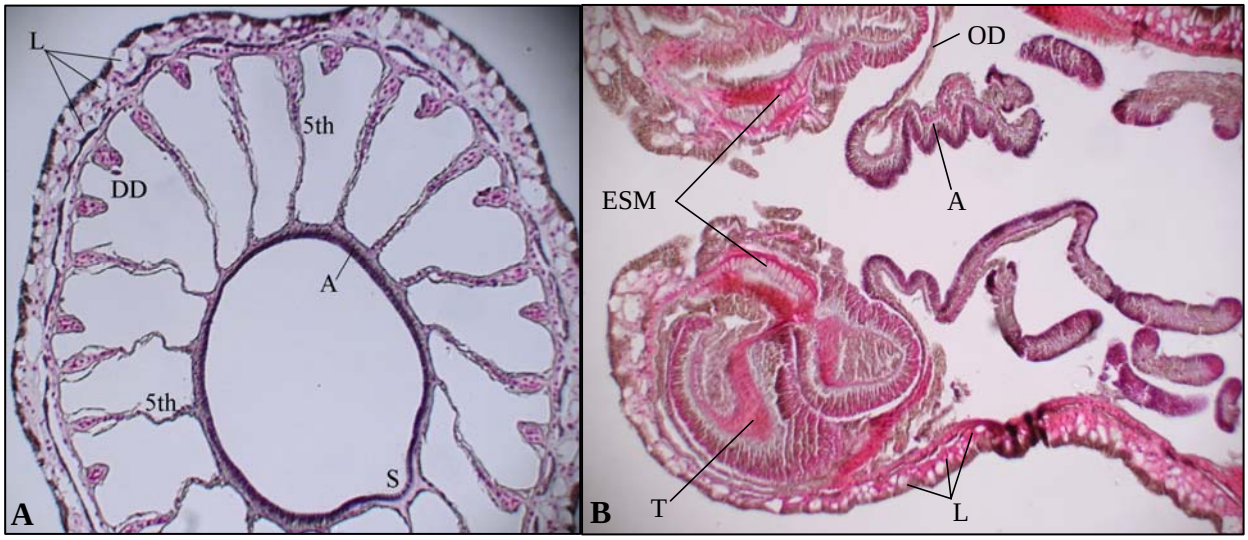

Figure 2.5. **A**, cross-section of *Isozoanthus antumbrosus* polyp at the region of the actinopharynx (A) showing the dorsal directives (DD), siphonoglyph (S) and the macrocnemic (complete) fifth mesenteries (5th). Note the abundant lacunae (L) in the mesoglea and ectoderm. **B**, longitudinal section of contracted *Isozoanthus antumbrosus* polyp at the region of the capitulum showing the endodermal sphincter muscle (ESM), actinopharynx (A), oral disk (OD) and tentacles (T). Note the abundant lacunae (L) in the mesoglea and ectoderm.

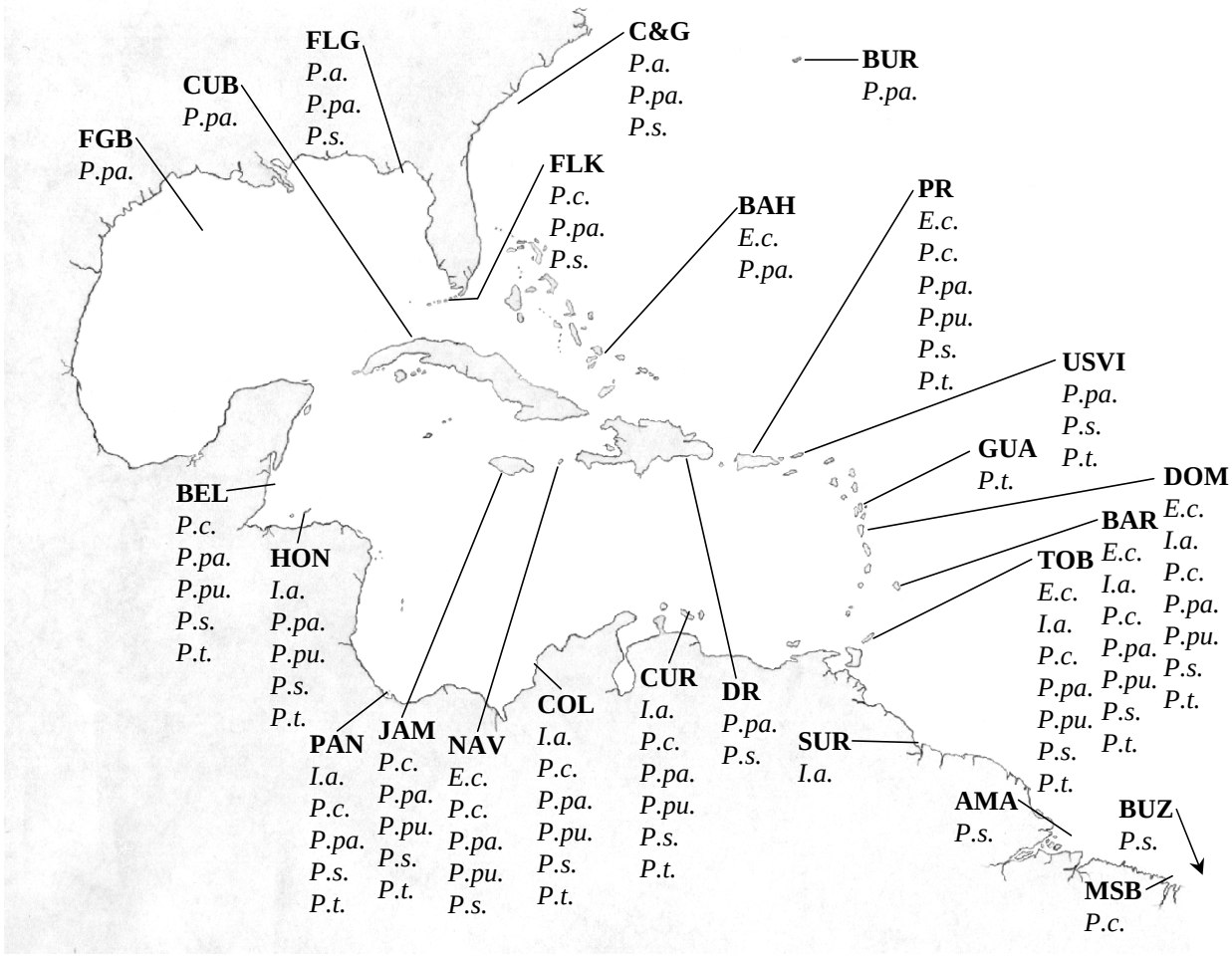

Figure 2.6. Map of the wider Caribbean region showing a compilation of observed symbiotic zoanthid species in each location. The following list defines the location abbreviations, and credits the source of observations. Species observations without citations are from the current study. Abbreviations: PR, La Parguera, Puerto Rico, West 1979; USVI, US Virgin Islands, Duchassaing & Michelotti 1860, this study, and (*P.t.*) Pax 1910; GUA, Guadeloupe, Pax & Müller 1956; DOM, Dominica; BAR, Barbados, Crocker & Reiswig 1981 and this study; TOB, Tobago; SUR, Suriname, USNM 50878; AMA, Amazon River outfall, Brazil, USNM 1084839; MSB, Maranhão State, Brazil, Campos *et al.*, 2005; BUZ, Búzios, Brazil; CUR, Curaçao; COL, Colombia, (Santa Marta, *P. pu.*) Alvarez *et al.* 1998, (Cartagena) J. Sanchez pers. comm.; PAN, Bocas del Toro, Panamá; HON, Utila, Honduras, Sinniger *et al.* 2005; BEL, Carrie Bow Cay, Belize, (*P.c.*) USNM 32338, (*P.pa.*) Lewis 1982, (*P.pu.*) USNM 32345, (*P.s.*) J. Wulff pers. comm.; CUB, Havana, Cuba, Varela *et al.* 2003; FGB, Flower Garden Banks, USA; FLG, Gulf coast of Florida, USA; FLK, Florida Keys, USA, (*P.c.*) USNM 41535; JAM, Jamaica, Duchassaing & Michelotti 1860, (*P.pu.* and *P.t.*) West 1979; NAV, Navassa Island, USA; BAH, Bahamas, Duchassaing & Michelotti 1860, (*E.c.*) Willenz & Hartman 1994; DR, Dominican Republic, Williams *et al.* 1983; C&G, Carolinas and Georgia, USA, (*P.a.*) USNM 16870, (*P.pa.*) USNM 51535, (*P.s.*) this study; BUR, Bermuda, Ryland & Westphlen 2004.

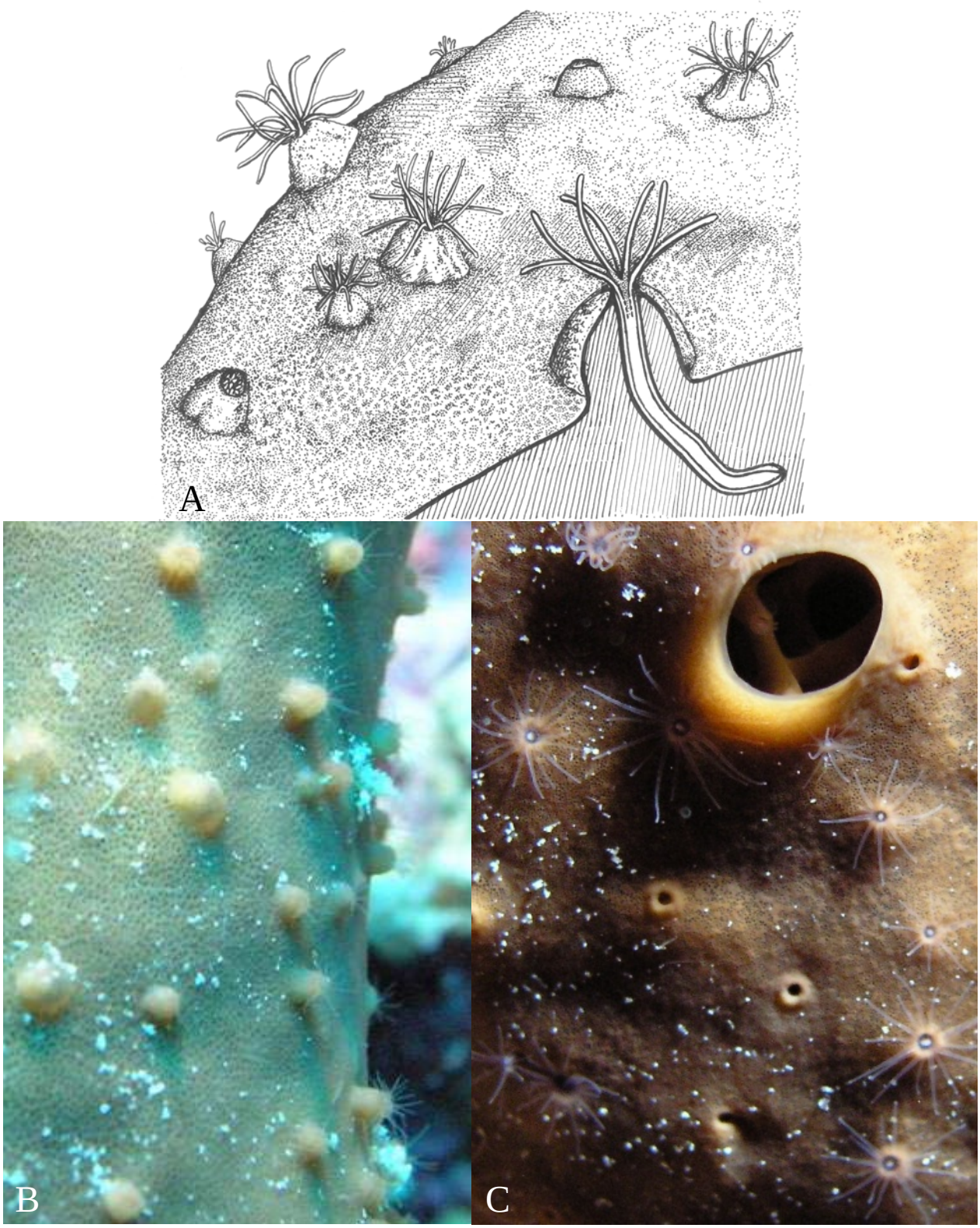

Figure 2.7. A, line drawing of symbiotic-Edwardsiidae embedded in a *Plakortis* sp. sponge from Barbados showing the morphology of the volcano-shaped protuberances on the surface of the host which only occur in the presence of the Edwardsiidae polyps. **B**, *In situ* photographs of the undescribed Edwardsiidae species and host *Plakortis* spp. from Barbados and (**C**) Curaçao.

Table 2.1. Genus and species, color, collection locality, host taxon, Genbank accession numbers, and individual identifier of individual zoanthids, actiniarians, and antipatharians used in this study. Individuals with identical sequences not included in the final internal transcribed spacer (ITS) analyses are indicated by a superscript of the individual identifier of the identical sequence that was included.

Genus and Species	Color	Collection Locality	Host	ITS Accession #	16S Accession #	Individual Identifier
Epizoanthus cutressi [TOB 44]	golden	Barbados	*Cribrochalina vasculum* (Lamark)	EU418264		BAR 123
Epizoanthus cutressi	golden	Dominica	*Cribrochalina dura* (Wilson)	EU418265		DOM 27
Epizoanthus cutressi	golden	Navassa, USA	*Cribrochalina vasculum* (Lamark)	EU418266		NAV 61
Epizoanthus cutressi	golden	Tobago	*Cribrochalina vasculum* (Lamark)	EU418267	EU828759	TOB 44
Isozoanthus sp. nov.	brown	Curaçao	*Dentitheca dendritica*	EU418275		CUR 203
Isozoanthus sp. nov.	brown	Dominica	*Dentitheca dendritica*	EU418276		DOM 31
Isozoanthus sp. nov.	brown	Bocas del Toro, Panamá	*Dentitheca dendritica*	EU418277	EU828761	PAN 21
Parazoanthus axinellae [FLG 1]	yellow	Fraškerić Island, Croatia		EU418278		CRO F11
Parazoanthus axinellae	yellow	Omiš, Croatia		EU418279		CRO V1
Parazoanthus axinellae [FLG 1]	yellow	Vis Island, Croatia		EU418280		CRO R1
Parazoanthus axinellae	yellow	Florida (gulf), USA	yellow Halichondrida	EU418281		FLG 1
Parazoanthus axinellae	yellow	Banyuls-sur-Mer, France		EU418282		FRA 64
Parazoanthus axinellae	yellow	Medes Islands, Spain		EU418283	EU828754	SPA M1
Parazoanthus catenularis	brown	Barbados	*Cribrochalina vasculum* (Lamark)	EU418284		BAR 124
Parazoanthus catenularis	brown	Curaçao	*Xestospongia* sp.	EU418285		CUR 206
Parazoanthus catenularis	brown	Dominica	*Neopetrosia proxima* (Duchassaing & Michelloti)	EU418286		DOM 14
Parazoanthus catenularis	brown	Dominica	*Xestospongia muta* (Schmidt)	EU418287		DOM 16
Parazoanthus catenularis	brown	Dominica	*Xestospongia muta* (Schmidt)	EU418288		DOM 25
Parazoanthus catenularis [NAV 60]	brown	Navassa, USA	purple encrusting Haplosclerida	EU418289		NAV 59
Parazoanthus catenularis	brown	Navassa, USA	*Cribrochalina vasculum* (Lamark)	EU418290		NAV 60

Table 2.1. Continued.

Genus and Species	Color	Collection Locality	Host	ITS Accession #	16S Accession #	Individual Identifier
Parazoanthus catenularis	brown	Bocas del Toro, Panamá	*Neopetrosia proxima* (Duchassaing & Michelloti)	EU418291		PAN 17
Parazoanthus catenularis	brown	Tobago	*Xestospongia muta* (Schmidt)	EU418292	EU828757	TOB 37
Parazoanthus catenularis	brown	Tobago	*Cribrochalina vasculum* (Lamark)	EU418293		TOB 38
Parazoanthus catenularis DOM 25	brown	Tobago	*Cribrochalina vasculum* (Lamark)	EU418294		TOB 46
Parazoanthus parasiticus	brown	Barbados	*Niphates erecta* Duchassaing & Michelloti	EU418295		BAR 122
Parazoanthus parasiticus	brown	Curaçao	*Callyspongia (Cladochalina) vaginalis* (Lamark)	EU418296		CUR 214
Parazoanthus parasiticus	brown	Dominica	*Callyspongia (Cladochalina) vaginalis* (Lamark)	EU418297		DOM 1
Parazoanthus parasiticus	brown	Dominica	*Spirastrella* sp.	EU418298		DOM 5
Parazoanthus parasiticus	brown	Dominica	*Niphates erecta* Duchassaing & Michelloti	EU418299		DOM 9
Parazoanthus parasiticus	brown	Dominica	*Spirastrella cf. coccinea*	EU418300		DOM 23
Parazoanthus parasiticus	brown	Florida (gulf), USA	tan Haplosclerida	EU418301		FLG 11
Parazoanthus parasiticus	brown	Florida (gulf), USA	*Callyspongia (Cladochalina) vaginalis* (Lamark)	EU418302		FLG 63
Parazoanthus parasiticus	brown	Navassa, USA	*Callyspongia (Cladochalina) vaginalis* (Lamark)	EU418305		NAV 57
Parazoanthus parasiticus	brown	Bocas del Toro, Panamá	*Niphates erecta* Duchassaing & Michelloti	EU418303		PAN 13
Parazoanthus parasiticus	brown	Bocas del Toro, Panamá	*Niphates erecta* Duchassaing & Michelloti	EU418304		PAN 15
Parazoanthus parasiticus	brown	Tobago	*Niphates erecta* Duchassaing & Michelloti	EU418306	EU828756	TOB 47
Parazoanthus parasiticus	brown	US Virgin Islands, USA	*Callyspongia (Cladochalina) vaginalis* (Lamark)	EU418307		USVI 148
Parazoanthus puertoricense	maroon	Barbados	*Agelas* sp.	EU418308		BAR 120
Parazoanthus puertoricense	maroon	Curaçao	*Svenzea zeai* (Alvarez *et al.*)	EU418309		CUR 212
Parazoanthus puertoricense	maroon	Dominica	*Svenzea zeai* (Alvarez *et al.*)	EU418310		DOM 7

Table 2.1. Continued.

Genus and Species	Color	Collection Locality	Host	ITS Accession #	16S Accession #	Individual Identifier
Parazoanthus puertoricense	maroon	Dominica	*Agelas conifera* (Schmidt)	EU418311		DOM 12
Parazoanthus puertoricense	maroon	Navassa, USA	*Agelas sceptrum* (Lamark)	EU418312	EU828758	NAV 58
Parazoanthus puertoricense TOB 36	maroon	Tobago	*Agelas conifera* (Schmidt)	EU418313		TOB 35
Parazoanthus puertoricense	maroon	Tobago	*Svenzea zeai* (Alvarez *et al.*)	EU418314		TOB 36
Parazoanthus swifti TOB 42	yellow	Barbados	*Iotrochota birotulata* (Higgin)	EU418315		BAR 121
Parazoanthus swifti BRA 165	salmon	Búzios, Brazil	red encrusting Poecilosclerida	EU418316		BRA 163
Parazoanthus swifti	white	Búzios, Brazil	red encrusting Poecilosclerida	EU418317		BRA 165
Parazoanthus swifti	salmon	Georgia, USA	*Clathria (Clathria) prolifera* (Ellis & Solander)	EU418318		C&G 129
Parazoanthus swifti	salmon	Georgia, USA	*Clathria* sp.	EU418319		C&G 131
Parazoanthus swifti	yellow	Curaçao	orange encrusting Poecilosclerida	EU418321		CUR 200
Parazoanthus swifti	yellow	Curaçao	*Iotrochota birotulata* (Higgin)	EU418320		CUR 204
Parazoanthus swifti	orange	Dominica	*Agelas* sp.	EU418322		DOM 11
Parazoanthus swifti	salmon	Florida (gulf), USA	Poecilosclerida	EU418323		FLG 5
Parazoanthus swifti	white	Florida (gulf), USA	Poecilosclerida	EU418324		FLG 7
Parazoanthus swifti FLG 54	white	Florida (gulf), USA	*Clathria* sp.	EU418325		FLG 9
Parazoanthus swifti	salmon	Florida (gulf), USA	orange Poecilosclerida	EU418326		FLG 13
Parazoanthus swifti	white	Florida (gulf), USA	orange encrust Poecilosclerida	EU418327		FLG 50
Parazoanthus swifti	white	Florida (gulf), USA	yellow branching Poecilosclerida	EU418328		FLG 53
Parazoanthus swifti	salmon	Florida (gulf), USA	black branching Poecilosclerida	EU418329		FLG 54
Parazoanthus swifti	white	Florida (gulf), USA	orange Poecilosclerida	EU418330		FLG 55
Parazoanthus swifti	yellow	Navassa, USA	*Agelas* sp.	EU418331		NAV 56
Parazoanthus swifti	yellow	Bocas del Toro, Panamá	*Iotrochota birotulata* (Higgin)	EU418332	EU828755	PAN 9
Parazoanthus swifti	orange	Bocas del Toro, Panamá	*Clathria (Thalysias) schoenus* (de Laubenfels)	EU418333		PAN 11
Parazoanthus swifti	orange	Tobago	*Iotrochota birotulata* (Higgin)	EU418334		TOB 39
Parazoanthus swifti	orange	Tobago	*Topsentia ophiraphidites* (de Laubenfels)	EU418335		TOB 41
Parazoanthus swifti CUR 200	orange	Tobago	*Agelas clathrodes* (Schmidt)	EU418336		TOB 42
Parazoanthus swifti TOB 42	yellow	Tobago	*Topsentia* sp.	EU418337		TOB 45
Parazoanthus swifti CUR 200	yellow	US Virgin Islands, USA	*Clathria (Thalysias) juniperina* (Lamark)	EU418338		USVI 151

Table 2.1. Continued.

Genus and Species	Color	Collection Locality	Host	ITS Accession #	16S Accession #	Individual Identifier
Parazoanthus tunicans	white	Curaçao	*Dentitheca dendritica*	EU418339		CUR 71
Parazoanthus tunicans	white	Dominica	*Dentitheca dendritica*.	EU418340		DOM 30
Parazoanthus tunicans	white	Tobago	*Dentitheca dendritica*	EU418341	EU828760	TOB 40
Zoanthus pulchellus		Bocas del Toro, Panamá			EU828762	PAN 7
Zoanthus sansibaricus		Japan			AB235412	
Zoanthus kuroshio		Japan			AB235410	
Zoanthus gigantus		Japan			AB235411	
Edwardsiidae sp. [BAR]	transparent	Barbados	*Plakortis* sp.	EU418268		BAR 05A
Edwardsiidae sp. [BAR]	transparent	Barbados	*Plakortis* sp.	EU418269	EU828764	BAR 06W
Edwardsiidae sp. [BAR]	transparent	Barbados	*Plakortis* sp.	EU418270		BAR 06Y
Edwardsiidae sp. [CUR]	transparent	Curaçao	*Plakortis* sp.	EU418271		CUR 213
Edwardsiidae sp. [CUR]	transparent	Curaçao	*Plakortis* sp.	EU418272	EU828763	CUR E1
Edwardsiidae sp. [CUR]	transparent	Curaçao	*Plakortis* sp.	EU418273		CUR E2
Edwardsiidae sp. [CUR]	transparent	Curaçao	*Plakortis* sp.	EU418274		CUR E3
Nematostella vectensis					AY169370	
Nematostella sp.					DQ643835	
Chrysopathes formosa		NE Pacific			NC008411	

CHAPTER 3

EFFECTS OF COLONIZATION ON HOST CONDITION FOR CARIBBEAN DEMOSPONGIAE-SYMBIOTIC ZOANTHIDEA

Introduction

Symbioses (intimate and prolonged interspecific associations, *sensu* Saffo 1992) are so pervasive in life that there may not be truly axenic organisms. While data on which species participate in associations have become more finely honed by the application of modern molecular tools (*e.g.* LaJeunesse *et al*. 2004), data on mechanisms and relationship outcomes (or types of symbiosis: mutualism, parasitism, *et cetera*) have greatly lagged behind. As a result, we know that associations occur but often cannot discern the effects of symbiotic relationships on the life histories of the participants. Our understanding of these relationships is obscured, in part, due to the difficulty of obtaining reliable data on the effects of symbiosis on each partner. Where the identities of species can often be determined by simple one-time observations of intact associations, the relationships are only revealed through effort-intensive manipulative experimentation and time-series measurements comparing the condition of intact holobionts to separated organisms. Although the relationship outcomes represent the net effect of the specific costs and benefits each organism experiences, identifying and quantifying those costs and benefits is an even greater challenge that requires clever experiments to minimize a cost or benefit without disrupting the relationship.

Understanding the transitions in both host associations and symbiotic relationships is critical to the study of symbiosis evolution. One could imagine that a suite of traits necessary for a symbiont to form a relationship with a specific host may be readily adaptable to form similar relationships among similar hosts. In this example the associations, but not the relationships, have transitioned. If that same symbiont then transitions to a different relationship, with or without a shift in host associations, the original suite of traits necessary to recognize and colonize hosts may remain useful; however the traits that control the maintenance of symbiosis and the evolutionary forces acting upon the new relationship may be dramatically different. For example, selection is thought to favor increased rates of molecular evolution in parasitic

symbioses (Red Queen's Hypothesis: van Valen 1973, van Valen 1974) and decreased rates in mutualistic symbioses (Red King effect: Bergstrom & Lachmann 2003), creating a diametric shift in the selective forces acting upon the interacting organisms after the transition to a different relationship. Therefore it would seem that transitions between hosts could be brought about by either relatively large or small evolutionary events (depending on the similarity of hosts), but transitions between symbiotic relationship outcomes should always be consequential and as a result more conserved than associations though evolutionary time.

Although relationship outcomes should be generally conserved (Peterson *et al.* 1999), ecological transitions between symbiotic relationships do occur. Associations can often be pushed along the continuum of outcomes by changing the conditions or context in which the associations are usually found, and the result may be sufficient to alter the magnitude or outcome of the relationship or disrupt the association. While such perturbations of relationships can be informative about specific costs and benefits and the underlying mechanisms of symbiosis, they are often ephemeral with nearly all associations maintaining the original relationship over time. Therefore a species association may have an unambiguous relationship outcome that has been has been honed by evolution and shared among closely related species and ancestors that can remain contextually plastic (*e.g.* coral bleaching).

Cnidarians in the order Zoanthidea (class Anthozoa) are symbionts of invertebrates representing at least five phyla and occur globally in benthic habitats ranging from the intertidal to the deep sea. These relationships are thought to benefit Zoanthidea by providing greater opportunity for feeding on environmental sources of fixed-carbon. Most Zoanthidea do not build their own skeletons (representatives of family Gerardiidae may be the only exception) and species that associate with invertebrates appear to rely on the structure and behavior of hosts to gain access to swifter water flow. Research on Zoanthidea symbioses has focused on the identification of host associations, while the outcomes of relationships remain almost entirely unexplored (but see West 1976, Lewis 1982, and Beaulieu 2001). The disparity in our understanding of the evolution of host associations and symbiotic relationship outcomes is also striking. While recent molecular analyses have increased our understanding of Zoanthidea phylogenetic relationships (Sinniger *et al.* 2005, Chapter 4) and have begun to unravel the evolution of host associations (Chapter 2, Chapter 4), no study has yet examined the evolution of Zoanthidea symbiotic relationship outcomes.

Caribbean Demospongiae-associated Zoanthidea are obligate symbionts that are specific to a subset of the diversity of sponges in the region (Chapter 1). These Zoanthidea live embedded in the inhalant surfaces of their hosts and can potentially benefit from both ambient and sponge-generated flows. The overall effect of Zoanthidea colonization on sponge host condition has not been examined and there are conflicting hypotheses about relationship outcomes based on observational and experimental data identifying some of the individual costs and benefits for hosts. The experimental research has focused on determining the effects of Zoanthidea colonization on the predators of host sponges (West 1976, Lewis 1982) and on the velocity of oscular flow (Lewis 1982). The observational data concerns the naturally occurring patterns of zoanthid colonization frequency (West 1976) and specificity (Chapter 1).

Using the spongivorous reef fish *Holacanthus tricolor* (rock beauty), West (1976) demonstrated significantly decreased consumption (metric: mean weight loss) of sponges (*Iotrochota birotulata*) colonized with zoanthids (*Parazoanthus swiftii*) relative to zoanthid-free fragments after 7 days in aquaria or on unenclosed native reefs. Zoanthid-colonized sponges also appeared to grow faster relative to the zoanthid-free fragments in predator-free control enclosures. West concluded that the presence of the zoanthid deterred the normal feeding of the sponge-predator and decided that the relationship was a host-predator mediated mutualism. The deterrence appears to be specific to the residential predators of this reef sponge as the presence of zoanthids does not effect predation by spongivorous seastars (*Oreaster reticulatus*) that are normally found in sea grass beds (Wulff 1995) or predation by nonspongivorous reef fish (*Thalassoma bifasciatum*) presented with pelleted sponge and zoanthid extracts (Pawlik *et al.* 1995). West (1976) also conducted field surveys of natural colonizations and recorded high occurrence rates for four sponge-symbiotic Zoanthidea species (*P. swiftii, Parazoanthus parasiticus, Parazoanthus catenularis,* and *Epizoanthus cutressi*) which were interpreted as a general indication of mutualism. This appears to be an appropriate hypothesis because uncolonized hosts are often rare in mutualistic systems (Smith 1992). Additionally, a review of species associations identified asymmetries in host and symbiont specificities (sponge hosts associate with 1 or 2 species; zoanthid symbionts associate with as many as 51 different species) that are often observed in mutualistic systems (Chapter 1).

Using the spongivorous reef fish *Pomacanthus arcuatus* (grey angel), Lewis (1982) demonstrated no significant decrease in consumption (metric: mean weight lost) of sponges

(*Callyspongia vaginalis*) colonized with zoanthids (*P. parasiticus*) relative to the zoanthid-free fragments after 12 days on enclosed native reefs. However, the zoanthid-colonized sponges grew significantly faster relative to the zoanthid-free fragments in predator-free control enclosures. Additionally, the presence of zoanthids significantly reduced variance (but not the mean) of volume-standardized pumping rates of a second sponge species (*Niphates digitalis*) relative to the zoanthid-free treatment which Lewis (1982) interpreted as an indication that the choanocytes of colonized hosts were operating at their physiological maximum to compensate for increased resistance to flow (created by the zoanthids). The presence of the zoanthids seemed to be increasing metabolic costs without effecting filtration rates, resulting in a resource-limiting parasitism.

The experimental data indicate opposing relationship outcomes (mutualism and parasitism) for congeneric species, suggesting that Zoanthidea relationships may not be highly conserved through evolutionary time. The observational data indicate similar relationship outcomes (mutualism) for the identical pair of species, suggesting phylogenetic conservatism of Zoanthidea relationships. The experiments presented here address the disparity between the experimental and observational data, and the apparent disagreement between the experimental data and the general expectation of conservation of symbiotic relationships, through a series of new experiments. Using the putative mutualist and parasitic associations, the condition (growth and survival) of zoanthid-colonized and zoanthid-free hosts were monitored over periods of 8 or 12 months. The experiments were repeated over space and time and some associations were also transplanted to novel habitats. The results indicate that zoanthid colonizations had positive (or insignificant) context-dependent effects on host-sponge condition and that the relationship outcomes were conserved across a transition in host associations.

Materials and Methods

Targeted Associations and Locations

Sponge species were chosen for experiments because they: (1) are the common hosts of the putative mutualist and parasite zoanthid species (Chapter 1) which represent two different phylogenetic clades of Demospongiae-symbiotic Zoanthidea and appear to have gone through a host shift in their recent evolutionary history (Chapter 4), (2) thrive and reattach after

manipulation and transplantation, and (3) naturally exist in sufficiently dense populations and are sufficiently colonized by zoanthids to be experimentally-useful. The sponge-zoanthid species combinations used are: *Iotrochota birotulata* / *Parazoanthus swiftii* (putative mutualism examined in West 1976), *Callyspongia vaginalis* / *P. parasiticus* (putative parasitism examined in Lewis 1982), *Niphates erecta* / *P. parasiticus*, *Neopetrosia proxima* / *P. parasiticus*, and *N. proxima* / *Parazoanthus catenularis*. Locations were chosen because they had experimentally-useful populations of sponges and zoanthids and presented reefs that experience a range of terrestrial influences (oceanic – estuarine). Experiments were conducted on reefs at four locations: near Holetown, Barbados (offshore oceanic: 15 m deep bank reef 1000 m from shore with average rainfall of 120 cm/yr); Director's Bay, Curaçao (nearshore mid-basin: 12 m deep fringing reef 15 m from shore with average rainfall of 50 cm/yr); Looe Key, Florida (offshore gulf with bay influences: 8 m deep patch reef 6000 m from shore with average rainfall of 120 cm/yr); and Bocas del Toro, Panamá (nearshore bay with river influences: 6 m deep fringing reef 10 m from shore with average rainfall of 400 cm/yr; see map and coordinates for all sites in Chapter 2). Transplants to the mangrove habitat were conducted in Spaanse Water Baai, Curaçao.

Experimental Design

The effects of colonization were assessed by comparing zoanthid-colonized and zoanthid-free explants (sample sizes indicated in Figures 3.1–3.4) using metrics of host sponge condition (growth and survival). Growth rates of hosts were determined by periodically measuring the volume (by geometric approximation *sensu* Wulff 2001) of explants standardized by initial length, genotype, and zoanthid colonization. Assessing volume rather than weight (as had been done in West 1976 and Lewis 1982) isolates changes in host growth from changes in symbiont growth. Single branches were cut from parent sponges with razor blades to obtain 8–10 cm long explants. Zoanthid-colonized and zoanthid-free explants were tips taken from the same individual sponges that were partially colonized by zoanthids, except for the experiments in Barbados because partially colonized hosts were not available. The colonizations needed for the Barbados experiments were created by attaching zoanthid-colonized or zoanthid-free 4 cm conspecific sponge fragments with thin nylon cable ties to non-tip explants. Although sponges will reject fragments that are not genetically identical (Wulff 1986), many genotypes will remain adherent long enough for successful zoanthid colonization which occurred within 7–14 days in

55% of the attempts. Cut explants were temporarily protected from spongivores in 1-liter nylon cages suspended above the reef. After 1–3 days, cut surfaces had visibly recovered and were removed from cages before being reattached to the substratum. Individual explants were attached with thin nylon cable ties to dried coral rubble anchored by sheathed (Tygon® R-3603) stainless steel wire inserted into the reef.

The effect of habitat on the outcome of relationships was assessed for *N. erecta* and *C. vaginalis* by transplanting replicate explants from coral reefs to non-native mangroves. Healed explants were attached with thin nylon cable ties to 30 cm lengths of 2.5 cm diameter chlorinated polyvinyl chloride (cpvc) pipes (to isolate reef sponges from mangrove sponges that are superior competitors: Wulff 2005) and suspended among sponge covered mangrove roots. Surviving explants in reef and mangrove habitats were counted and remeasured after incubation periods of 8 or 12 months. The incubation times were chosen to capture a broader portion of the effects of zoanthid colonization than had been assessed in previously published experiments (West 1976 and Lewis 1982) which had been incubated for 7-12 days.

Data Analyses

Growth of sponge explants was calculated by finding the change in volume standardized by the initial volume (Δ volume/initial volume = specific growth *sensu* Wulff 2008) and incubation time (specific growth/2 or 3 = 4 month specific growth). Within-site effects of colonization on host condition were assessed with the nonparametric Mann-Whitney Rank Sum Test using SigmaStat v 3.11 (Systat Software, Inc.) because all data sets did not meet the normality and equal variance assumptions of the parametric test. Survival was assessed with a contingency table comprised of the number of sponge explants recovered alive and the number that died for zoanthid-colonized and zoanthid-free explants, and analyzed with a Fisher's Exact Test using SigmaStat.

Results

Host condition was either not significantly different or significantly improved with the presence of zoanthid symbionts compared to zoanthid-free explants for all species combinations examined in native reef habitats. Host condition was either not significantly different or

significantly decreased with the presence of zoanthid symbionts compared to zoanthid-free explants for all species combinations examined in non-native mangrove habitats.

Putative mutualism: *Iotrochota birotulata* / *Parazoanthus swiftii*

Specific growth of *P. swiftii*-colonized *I. birotulata* was not different from zoanthid-free explants in any of the locations examined (Fig. 3.1). Survival of *P. swiftii*-colonized *I. birotulata* was significantly increased in Panamá ($p = 0.038$) and Barbados ($p = 0.048$), but not in Florida (Fig. 3.1). All of the experiments that resulted in non-significant differences in condition between *P. swiftii*-colonized and zoanthid-free *I. birotulata* trended toward increased growth and survival with zoanthid colonization (Fig. 3.1).

Putative parasitism: *Callyspongia vaginalis* / *Parazoanthus parasiticus*

Specific growth of *P. parasiticus*-colonized *C. vaginalis* increased ($p = 0.021$) compared to zoanthid-free explants in Curaçao, but not in Florida (Fig. 3.2). Survival of *P. parasiticus*-colonized *C. vaginalis* was not significantly different from zoanthid-free explants in any of the locations examined (Fig. 3.2). When transplanted to the mangrove habitat, *P. parasiticus*-colonized explants had decreased growth ($p = 0.049$) and survival ($p = 0.031$) compared to zoanthid-free explants.

Niphates erecta* / *Parazoanthus parasiticus

Specific growth of *P. parasiticus*-colonized *N. erecta* increased compared to zoanthid-free explants in Curaçao ($p = 0.010$), but not in Panamá (Fig. 3.3). Survival of *P. parasiticus*-colonized *N. erecta* was not different from zoanthid-free explants in any of the locations examined. When transplanted to the mangrove habitat, *P. parasiticus*-colonized explants had decreased growth ($p = 0.002$), but survival of *N. erecta* was not significantly different compared to zoanthid-free explants (Fig. 3.3).

Neopetrosia proxima* / *Parazoanthus parasiticus* or *Parazoanthus catenularis

Specific growth and survival of both *P. parasiticus* and *P. catenularis*-colonized *N. proxima* was not significantly different from zoanthid-free explants (Fig. 3.4).

Discussion

The series of experiments in native reef habitats indicate that there are positive effects of zoanthid-colonization on host-sponge condition over a period of at least 8 months. Three out of

five species associations had at least one comparison demonstrate statistically significant increases in zoanthid-colonized host condition; the two associations that did not were single experiments (not repeated across locations, years, or habitats) conducted in Panamá where the terrestrial influences appeared to be most similar to the mangroves and most other comparisons also showed no significant differences. Only 22% (4/18) of comparisons resulted in average decreases in zoanthid-colonized host condition in native reef habitats, most of which (75%) were conducted in locations with the greatest terrestrial influences (Panamá and Florida) and of none of which were significant. The only significant effects on host condition in native reef habitats are consistent with mutualistic relationship outcomes for *I. birotulata* / *P. swiftii*, *N. erecta* / *P. parasiticus*, and *C. vaginalis* / *P. parasiticus* sponge-zoanthid associations. No significant effect of zoanthid-colonization on sponge-host condition was detected for *N. proxima* / *P. parasiticus* and *N. proxima* /*P. catenularis* sponge-zoanthid associations in native reef habitats and the relationship outcomes remain unclear.

Iotrochota birotulata* / *Parazoanthus swiftii

The available observational and experimental data on *P. swiftii* / *I. birotulata* symbioses are consistent with mutualism. Previous experiments demonstrated spongivorous fish-mediated mutualism within seven days (West 1976) and surveys detected patterns of colonization frequency (West 1976) and specificity (Chapter 1) that are consistent with mutualism. The experiments presented here demonstrate mutualism over a longer time frame (12 months); however the mechanism for increases in host condition are not certain and it is not clear why survival was improved with *P. swiftii* colonization while growth was not. If the main benefit to hosts is a reduction in fish spongivory, then it is possible that smorgasbord feeding (common among sponge predators to take a few small bites and then move on: Randall & Hartman 1968, Wulff 1994) may only cause a small volume of sponge cells to be lost or damaged (undetectable by the growth metric), but exposes the internal cells that are normally protected by a continuous pinacoderm to diseases that increase mortality (detectable by the survival metric). The main cost to hosts may be greater resistance for pumping water (*i.e.* access to nutrients and dissolved oxygen is more costly) due to the colonization of zoanthids on the inhalant surfaces (which should be particularly acute for hosts of *P. swiftii* because of the band/sheet morphology of the coenenchyme), however the benefits derived from the association must be sufficiently large to hide the costs.

Callyspongia vaginalis* / *Parazoanthus parasiticus* and *Niphates erecta* / *Parazoanthus parasiticus

Most of the available observational and experimental data on *P. parasiticus* symbioses are also consistent with mutualism (Lewis 1982, West 1976, Chapter 1). Previous experiments with *C. vaginalis* / *P. parasiticus* symbioses did not demonstrate significant effects of zoanthid colonization on spongivorous fish feeding and experiments with *Niphates digitalis* / *P. parasiticus* symbioses did not demonstrate significant effects of zoanthid colonization on oscular flow rates; however *C. vaginalis* did grow significantly faster with *P. parasiticus* (Lewis 1982) within twelve days (suggesting mutualism) and surveys detected patterns of colonization frequency (West 1976) and specificity (Chapter 1) that are consistent with mutualism. The experiments presented here demonstrate mutualism over a longer time frame (at least 8 months); however the mechanisms for increased host condition are not known and it is not clear why growth of both host sponges was improved with *P. parasiticus* colonization while survival was not. The main benefit to hosts does not appear to be a reduction in fish spongivory (Lewis 1982) and therefore the mechanism of the symbiotic interaction is apparently different from *P. swiftii* while the relationship outcome (mutualism) is the same. The main cost to hosts may be greater resistance for pumping water, however this would seem to be less important than with *P. swiftii* because of the minimal or absent coenenchyme of *P. parasiticus*. An additional cost may be the skeletal reorganization necessary for sponges to host *P. parasiticus*. Similar to the coralline sponge that physically reacts to *Epizoanthus cutressi* colonization by reorganizing skeletal elements around the base of polyps and coenenchyme (Willenz & Hartman 1994), *C. vaginalis* and *N. erecta* form "cycts" of spicules and protein sheets around the base of *P. parasiticus* polyps.

Neopetrosia proxima* / *Parazoanthus parasiticus* or *Parazoanthus catenularis

Although none of the experiments or condition metrics demonstrated a significant difference between *N. proxima* with and without *P. parasiticus* or *P. catenularis*, they should have similar costs associated with greater resistance for pumping water and skeletal reorganization (particularly for *P. catenularis* because of its more persistent coenenchyme) and additional costs associated with shading the surfaces of hosts. *Neopetrosia proxima* hosts photosynthetic cyanobacteria endosymbionts that may provide host sponges with a portion of their fixed-carbon budgets (Steindler *et al.* 2005) and the presence of *P. parasiticus* or *P.*

catenularis colonizations could partially block or absorb ambient sunlight (both zoanthid species host photosynthetic dinoflagellates) and reduce irradiance levels for the bacterial symbionts. If these costs are large, the benefits of hosting *P. parasiticus* or *P. catenularis* must also be large as the net outcome appears to be no effect (at least at the Panamá site).

Evolution of relationship outcomes

Although there is much left to be learned about these symbioses (*e.g.* identifying and quantifying the mechanisms involved, comparing the relationship outcomes of less common host associations, assessing the relationships of the other zoanthid species), the experiments and observations of *P. swiftii* and *P. parasiticus* with their common sponge hosts are largely consistent with mutualism. The evolutionary history of these closely related zoanthids includes a transition in host associations between groups of Demospongiae orders (Halichondrida + Poecilosclerida and Hadromerida + Haplosclerida; Chapter 4), however the relationship outcomes of *P. swiftii* and *P. parasiticus* symbioses are conserved across this transition (hosts changed while the outcomes remained the same). Acknowledging that a single transition is insufficient to comment on the general evolutionary patterns of Zoanthidea, this is an example where relationship outcomes are more conserved than host associations.

Ecological transitions of relationship outcomes

The series of experiments in non-native mangrove habitats indicate that there are negative effects of zoanthid-colonization on host-sponge condition over a period of at least 8 months after transplantation. Three out of four comparisons demonstrated statistically significant decreases in zoanthid-colonized host condition. The only significant effects on host condition in non-native mangrove habitats are consistent with parasitic relationship outcomes for *N. erecta* / *P. parasiticus* and *C. vaginalis* / *P. parasiticus* sponge-zoanthid associations.

The relationship outcomes of *N. erecta* / *P. parasiticus* and *C. vaginalis* / *P. parasiticus* symbioses are generally mutualistic in native reef habitats and parasitic in non-native mangrove habitats. These experiments in reefs and mangroves were performed using genetically identical sponges collected from the same location and incubated during the same time period. The single aspect that differed is the habitat, suggesting that these relationships are pliable in ecological time and their outcomes are context-dependent. Because the mechanism of these symbioses are not understood it is impossible to discern how this transition occurs, but it seems reasonable that transplantation of these reef species has somehow shifted the cost-benefit equation of the

symbiosis such that the costs of symbiosis are revealed in the mangrove habitat when they were hidden by the benefits in the reef habitat.

The ability to alter the outcome of these relationships in ecological time through transplantation to novel habitats should not be interpreted as an indication they are not conserved though evolutionary time. Zoanthid-sponge associations are rarely found in mangrove habitats, often only forming associations with mangrove sponges in locations where the distinctions between reefs and mangroves have become blurred and mangroves are growing directly out of the reef crest (*e.g.* Pelican Cays, Belieze: Wulff 2000; or Bocas del Toro, Panamá). Nearly all the experiments and observations indicated mutualism across space and time in native reef habitats, even if the magnitude of the outcome was not consistent across the range of terrestrial influences.

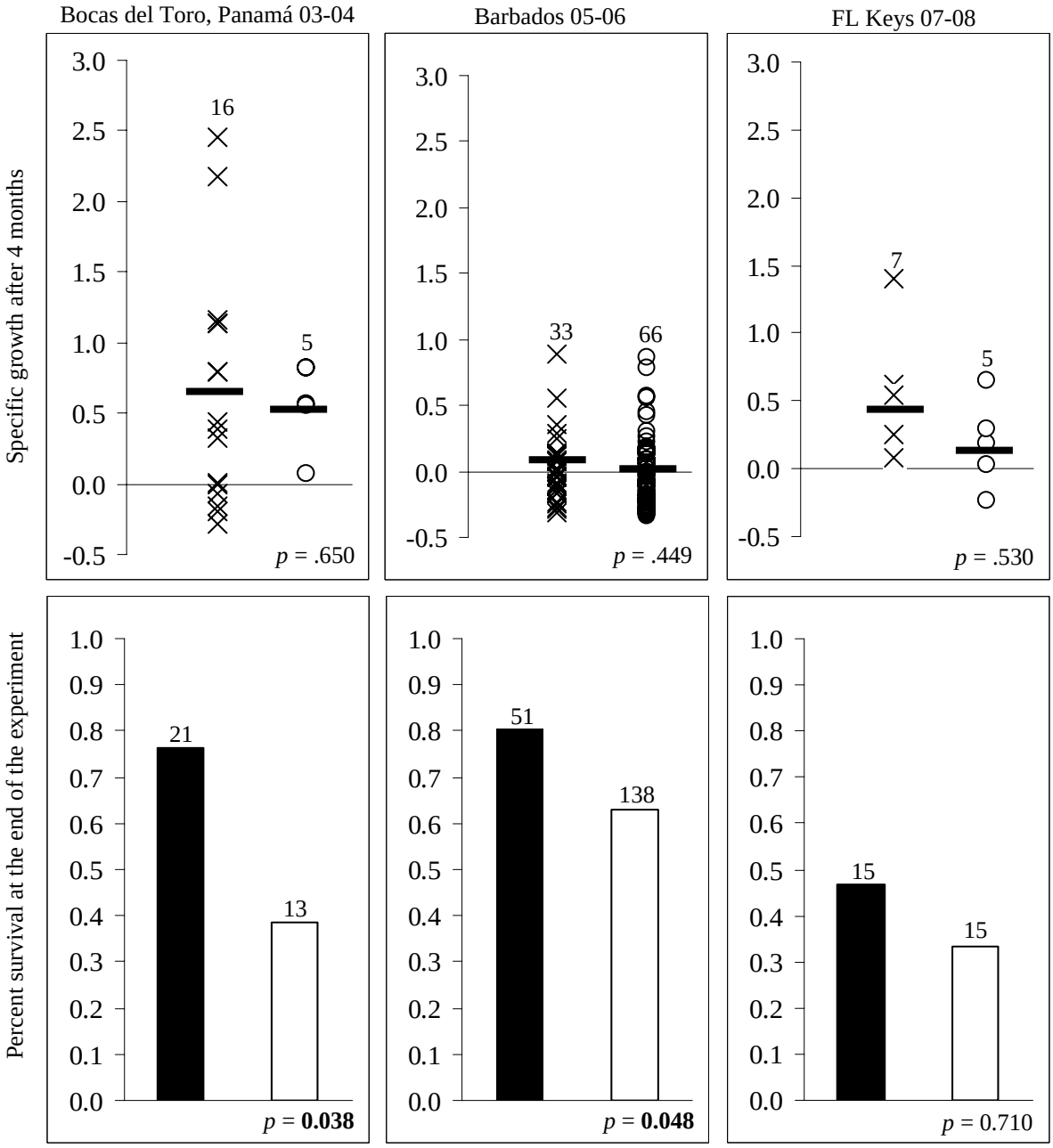

Figure 3.1. Growth and survival of *Iotrochota birotulata* with and without colonizations of *Parazoanthus swiftii*. Presence of zoanthids indicated by "X" and black columns. Absence of zoanthids indicated by open circles and columns. Horizontal bars indicate mean growth values. Sample sizes indicated above data points and columns; significant *p*-values are in bold.

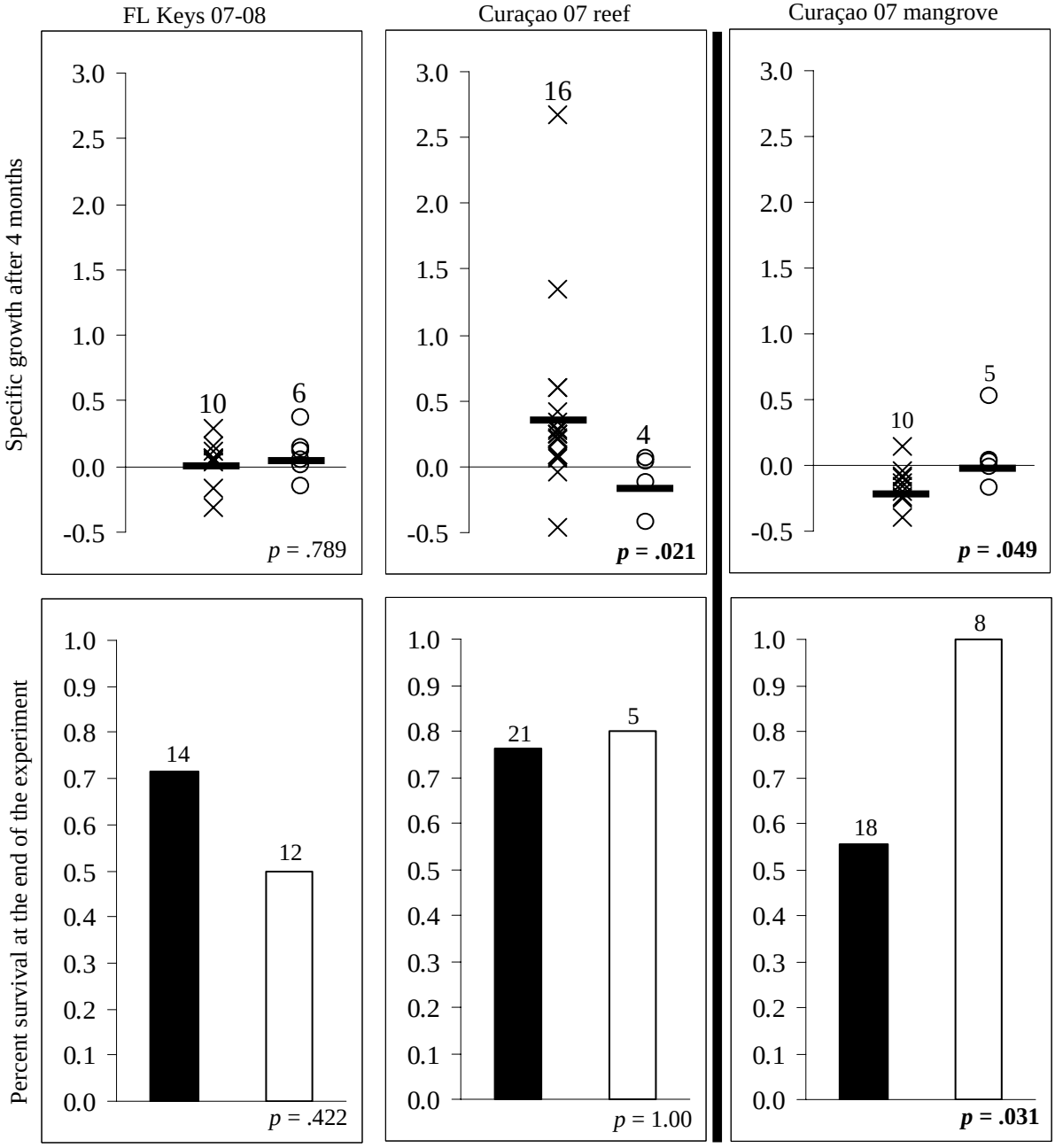

Figure 3.2. Growth and survival of *Callyspongia vaginalis* with and without colonizations of *Parazoanthus parasiticus*. Presence of zoanthids indicated by "X" and black columns. Absence of zoanthids indicated by open circles and columns. Horizontal bars indicate mean growth values. Vertical bar separates reef and mangrove experiments. Sample sizes indicated above data points and columns; significant *p*-values are in bold.

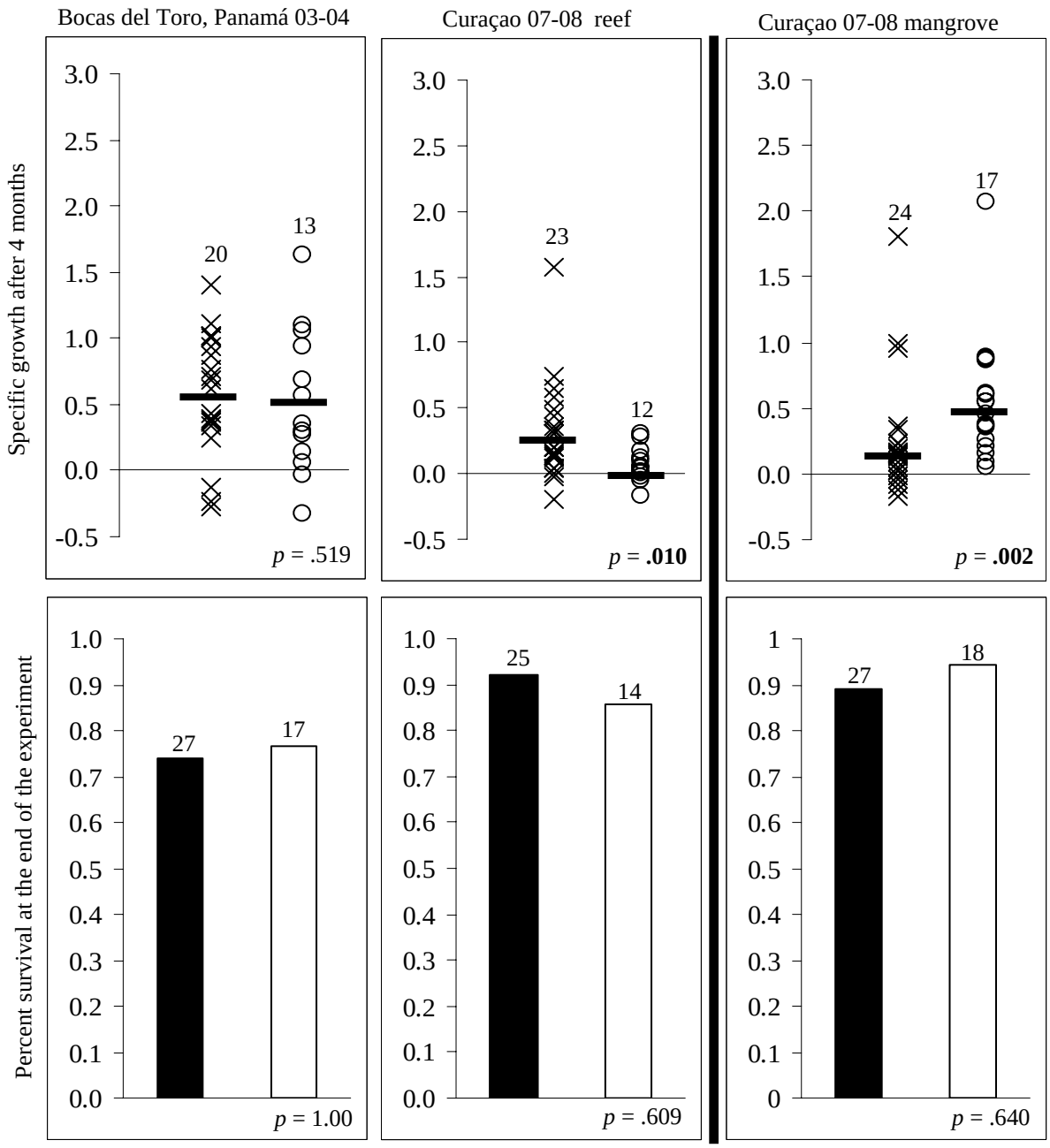

Figure 3.3. Growth and survival of *Niphates erecta* with and without colonizations of *Parazoanthus parasiticus.* Presence of zoanthids indicated by "X" and black columns. Absence of zoanthids indicated by open circles and columns. Horizontal bars indicate mean growth values. Vertical bar separates reef and mangrove experiments. Sample sizes indicated above data points and columns; significant *p*-values are in bold.

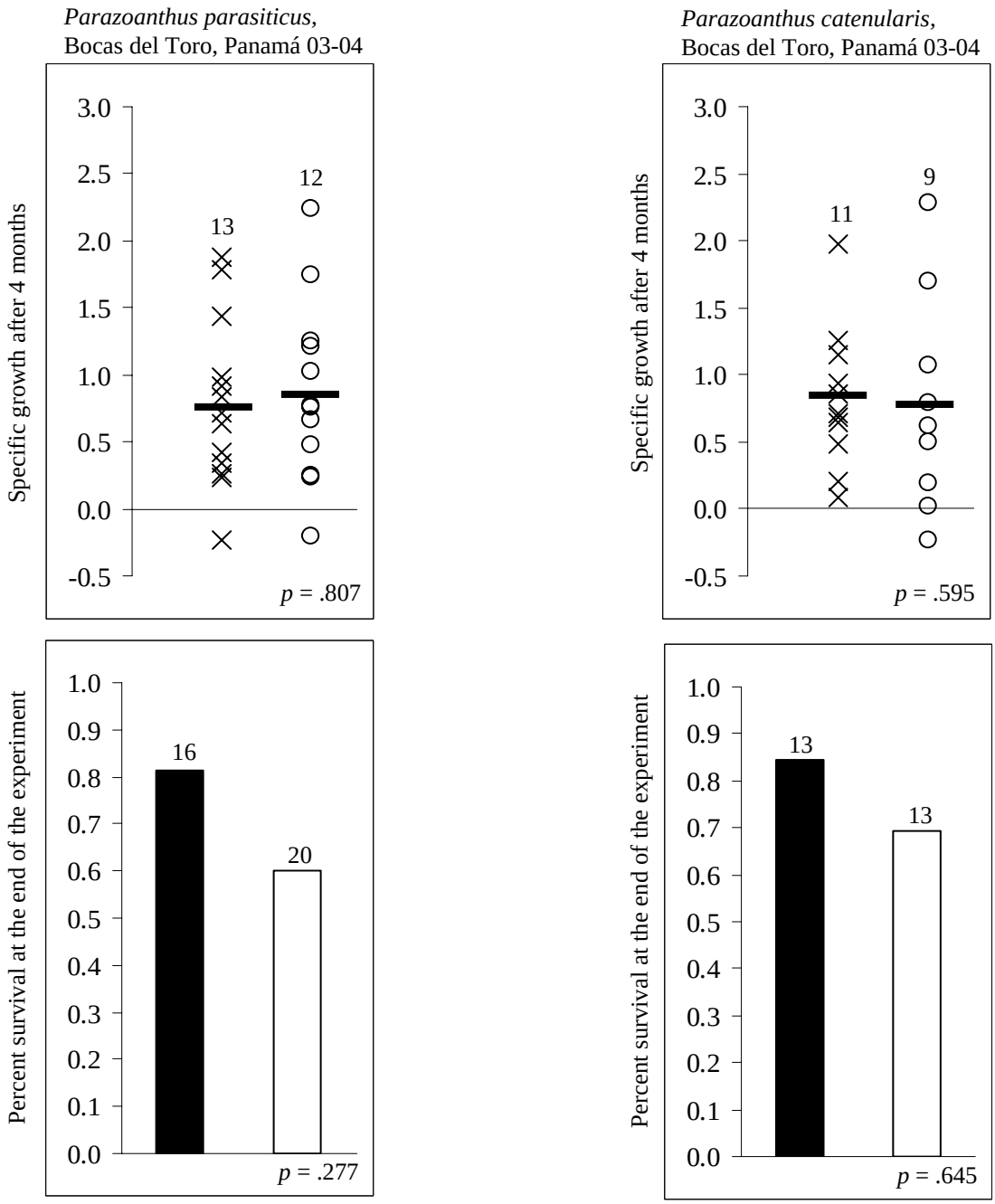

Figure 3.4. Growth and survival of *Neopetrosia proxima* with and without colonizations of *Parazoanthus parasiticus* or *Parazoanthus catenularis.* Presence of zoanthids indicated by "X" and black columns. Absence of zoanthids indicated by open circles and columns. Horizontal bars indicate mean growth values. Sample sizes indicated above data points and columns.

CHAPTER 4

EVOLUTIONARY TRANSITIONS IN ZOANTHIDEA SYMBIOSES: GLOBAL REDUCTIONS IN BATHYMETRIC AND GEOGRAPHIC RANGES COINCIDE WITH THE LOSS OF SYMBIOSES WITH INVERTEBRATES

Introduction

Symbioses (intimate and prolonged interspecific associations, *sensu* Saffo 1992) are pervasive in life and are largely responsible for the prevalence of organisms such as land plants, hermatypic corals, hydrothermal vent animals, phytophagous insects, and eukaryotic organisms in general. The evolution of symbiosis confers novel adaptive capabilities that enable ecological expansion into unexplored niches for one or both partners (Lewis 1973), and the availability of symbionts can be the deciding factor in overcoming barriers to ecological establishment (Richardson *et al.* 2000). Evolutionary transitions in symbiosis (terminations, origins, host shifts, or changes in specificity) can therefore have dramatic effects on the fitness, life history, and distribution of organisms.

Systems with many different types of associations will have undergone numerous and varied evolutionary transitions in symbioses, providing opportunities for understanding the causes and consequences of associations. Cnidarians representing order Zoanthidea (class Anthozoa) are symbionts of taxa representing at least five invertebrate phyla and occur in most major benthic habitats from the intertidal to the deep sea. The most common invertebrate hosts of Zoanthidea are representatives of the Alcyonacea, Antipatharia, Hydrozoa, Demospongiae, Hexactinellida, and Paguridae (Chapter 2), as well as Thoracica and Polychaeta. Although other invertebrates (*e.g.* representatives of Gastropoda, Echinodermata, and Bryozoa) have been collected with Zoanthidea, it is not clear if these represent characteristic symbioses or chance occurrences. It is generally believed that elevation out of stagnant waters into energy-supplying flow is the main benefit that Zoanthidea derive from symbiotic relationships with invertebrates, because they are generally incapable of building their own skeletal structures (representatives of family Gerardiidae may be the only exception; Ocaña *et al.* 1995). Species whose distributions

include photic zones may host symbiotic photosynthetic dinoflagellates (zooxanthellae; genus *Symbiodinium*) that alter their physiological requirements compared to heterotrophic Zoanthidea by providing photosynthetically fixed carbon (Davy *et al.* 1996). This results in a heterotrophic/symbiotic-autotrophic holobiont. Access to energy sources has been demonstrated to drive biodiversity and distributional ranges in coral reef cnidarians (Fabricius & De'ath 2008) and could therefore have similar effects on the global ranges of Zoanthidea.

The Zoanthidea are currently divided into the suborders Macrocnemina and Brachycnemina, which are defined by functionally insignificant morphological features but fundamental ecological differences (Ryland *et al.* 2004). Macrocnemina have complete fifth mesenteries (macrocnemes), global geographic and bathymetric distributions, are common symbionts of a wide array of invertebrates and are infrequently (~10% of species) zooxanthellate. Brachycnemina have incomplete fifth mesenteries (microcnemes), tropical and subtropical photic zone distributions, are rarely (~1% of species) symbionts of invertebrates and are usually (perhaps always) zooxanthellate (Ryland *et al.* 2004).

Although we recognize a distinction (through systematics) between symbiosis-aided heterotrophs (Macrocnemina) and heterotrophic/symbiotic-autotrophs (Brachycnemina), it is not clear why there is such an enormous disparity in distributions. The reliance on photosynthetic zooxanthellae could restrict a species to the photic zone, but not necessarily restrict that species to the tropics. Similar to the distribution patterns seen in sea anemones (Muller-Parker & Davey 2001), some zooxanthellate Zoanthidea (*e.g., Epizoanthus sabulosum*, *Isozoanthus sulcatus*, *Parazoanthus lividum*) have temperate distributions. If the evolution of zooxanthellae symbiosis is irreversible in the Zoanthidea, then clades of zoanthids should be entirely zooxanthellate and restricted to photic zones. This does not appear to be true as several genera in different Zoanthidea families host zooxanthellae; nor does it appear to be true in other anthozoan groups such as the Alcyonacea which have repeatedly gained and lost zooxanthellae symbioses over evolutionary time (van Oppen *et al.* 2005). However, the current systematics of Zoanthidea (Fautin 2008) may not be reflective of evolutionary relationships as several recent molecular phylogenies indicate that Macrocnemina may be ancestral to Brachycnemina and that some of the families and genera of Macrocnemina may not be monophyletic (Chapter 2, Sinniger & Häussermann 2009, Reimer & Nonaka *et al.* 2008, Reimer & Sinniger *et al.* 2008, Sinniger *et al.* 2008, Reimer & Sinniger *et al.* 2007, and Sinniger *et al.* 2005).

The phylogenetic analyses presented here use nuclear and mitochondrial nucleotide sequences of species representing the diversity of Zoanthidea to examine the evolutionary transitions of invertebrate and zooxanthellae symbioses with the goal of reconstructing the evolutionary events surrounding the rise of disparity in Zoanthidea distributions. Previously published phylogenetic analyses are used as *a priori* hypotheses in tests of monophyly to assess the putative morphological synapomorphies that define the current systematics and disparities in distributions, and the proposed relationships among and between types of symbioses. Ancestral host and Zoanthidea morphological character states are reconstructed to examine the evolutionary transitions of symbioses that coincide with the change in distributions.

Materials and Methods

All usable DNA sequence data available were combined with 127 new sequences to create a comprehensive phylogeny of Zoanthidea. Where possible, the morphology of new specimens was documented in MorphBank. The comprehensive phylogeny was used to evaluate the evolutionary relationships proposed by previously published molecular phylogenies and to reconstruct the evolution of morphology and symbioses of Zoanthidea in order to examine the origin of known distributional asymmetries.

Sampling Strategy

Species were selected to represent the geographic, bathymetric, symbiotic, and taxonomic ranges of extant Zoanthidea, including representatives of the major brachycnemic and macrocnemic genera and many of the major host associations (Table 4.1). Specimens were obtained with the help of colleagues, academic institutions, and museums (Table 4.2). Data from newly sampled species were combined with most of the ribosomal and protein coding (cytochrome oxidase I) DNA sequences available for Zoanthidea from GenBank (Table 4.3). Species were included if at least two of the five genes targeted in the analyses were available. Two anemone species were used to root the analyses because independent evidence indicates that Actinaria are an appropriate phylogenetic outgroup (Berntson *et al.* 1999, Daly *et al.* 2003).

Species Identification and Documentation

Zoanthids were identified to the species or genus level by comparing the original species descriptions and subsequent redescriptions to combinations of external polyp and colony

macroscopic morphology (number of tentacles and capitular ridges; height and diameter of polyps; color patterns of tentacles, oral disk, column, and coenenchyme; and host associations), and internal polyp microscopic anatomy (mesenterial number and arrangement; mesogleal lacunae and sinuses; position and structure of mesogleal pleats or loops supporting the marginal muscles). Calcareous and siliceous particles were removed from polyps by incubating in a formic acid fixative-decalcifier (Formical-4™; Decal Chemical Corporation) for 4 h (repeated with fresh Formical) and 20% hydrofluoric acid for 12 h. Specimens were dehydrated in ethanol, cleared with xylene, embedded in paraffin, and serial 10–15 μm longitudinal and cross sections were stained with Harris hematoxylin and eosin Y. All available *in situ*, intact specimen, dissection, histological, and host (*e.g.* hydroid zooid and sponge spicule) images used for species identifications are documented in MorphBank (publication collection number 514243; see Table 4.3 for species collection accessions).

DNA Amplification and Sequencing

Nuclear internal transcribed spacer (ITS) and large sub-unit (28S) ribosomal RNA (rRNA), and mitochondrial small and large sub-unit (12S and 16S) rRNA genes were targeted because they are commonly used to address evolutionary questions within Zoanthidea and Actiniaria. Nucleic acids were extracted using the cetyl-trimethyl-ammonium bromide (CTAB) technique of Doyle & Doyle (1987). Markers were selectively amplified by polymerase chain reaction (PCR) using Platinum® PCR Supermix (Invitrogen) and the primers and annealing temperatures listed in Table 4.4 (see Chapter 2 for complete PCR protocol). PCR products were purified by enzymatic digestion (ExoSAP-IT®; USB Corporation), and directly sequenced in the forward and reverse directions using the amplification primers and Big-Dye® Terminator (Applied Biosystems) chemistry.

Sequence Alignment and Phylogenetic Analysis

DNA sequences were assembled and edited using SEQUENCHER 4.0.5 (Gene Codes Co.), and manually aligned using BioEdit 7.0.5.2 (Hall 1999). Sequences obtained from GenBank were trimmed to remove primer sequences and single nucleotide insertions from protein coding genes. Ribosomal RNA contains hypervariable regions that are often excluded from phylogenetic analyses (*i.e.* data displaying high evolutionary rates are disregarded) because of difficulty in assessing homology (sequence similarity) within alignment positions. All nucleotides were included in these analyses (as in Chapter 2) by aligning homologous positions

identified in subsets of genetically similar taxa and treating non-homologous positions as missing data, such that blocks of unambiguously aligned sequences were staggered across hypervariable regions. DNA sequences have been deposited in GenBank (accession numbers GQ464848 – GQ464974, Table 4.3) and sequence alignments have been deposited in TreeBASE (http://purl. org/phylo/treebase/phylows/study/TB2:S10492).

To assess the similarity of the evolutionary history between nuclear and mitochondrial markers, and to reveal potentially misleading effects of undetected intragenomic variation, intergenomic phylogenetic congruence was tested using a likelihood-ratio test (LRT) implemented in Concaterpillar 1.4 (Leigh *et al.* 2008). Concaterpillar performed per-genome maximum likelihood (ML) analyses on identical taxon sets (71 taxa) using the General Time Reversible (GTR) model implemented in RAxML 7.0.4 (Stamatakis 2006a, Stamatakis 2006b). Concaterpillar does not yet allow data partitioning in the ML analyses and therefore the per-genome topological reconstructions are not at their optima (Li *et al.* 2008), artificially increasing incongruence between genomic data sets and making the LRT a more conservative estimation of congruence.

Per-genome and concatenated alignments were partitioned (following recommendations of Li *et al.* 2008) along boundaries of ribosomal subunits, hypervariable regions, and codons (12 total partitions); as delineated in Table 4.5. Optimal ML trees were identified for each genome (see TreeBASE submission) and the concatenated data using the GTR model with gamma (+Γ) and invariable site (+I) parameters in RAxML via the CIPRES Portal 1.15. Model parameters were estimated for each partition in RAxML (Table 4.5); however branch length optimization was linked due to incomplete per-partition taxon sampling. Nonparametric bootstrap support was estimated using GTR and a categorical per-site rate heterogeneity approximation (CAT) from 1000 pseudoreplicates in RAxML (Stamatakis *et al.* 2008).

Evolutionary Hypotheses Testing

Topological summaries of previously published phylogenies were constructed (Fig. 4.1) and used to generate hypotheses of the evolutionary relationships among zoanthids (Table 4.6). These hypotheses were then used to constrain the concatenated sequence data in ML analyses of RAxML. The constrained trees (see TreeBASE submission) were used as *a proiri* hypotheses in a partitioned (Table 4.5) Kishino–Hasegawa test (KH; Kishino & Hasegawa 1989) implemented by the ML analysis program BASEML in PAML 3.15 (Yang 2007) to assess the morphological

characters that define the current systematics and disparities in distributions, and the relationships among and between taxa with different host associations.

Ancestral Reconstructions and Character State Coding

Ancestral character states were reconstructed with the ML criterion using the single-parameter Markov model (Mk1) by tracing the current morphological and symbiosis character states over the ML tree using the StochChar module (Maddison & Maddison 2006) in Mesquite 2.6 (Maddison & Maddison 2008) to examine the historical evolutionary transitions in symbioses and morphology that coincide with the change in distributions. Individual species character assignments are listed in Table 4.7. The following character groups were chosen to assess the evolution of symbiosis with invertebrates and zooxanthellae, and the morphological features that define the suborders of Zoanthidea and families of Macrocnemina.

Fifth mesenteries—Assessed at the height of the actinopharynx and located five mesenteries from the microcnemic dorsal directives (opposite the siphonoglyph), the fifth mesenteries have two character states: 1) microcnemic (an incomplete mesentery that is little more than a slight protrusion of mesoglea and endoderm, never extending to the actinopharynx), or 2) macrocnemic (a complete mesentery that extends to the actinopharynx). These characters have defined the Zoanthidea suborders Brachycnemina and Macrocnemina since 1891 (Haddon & Shackleton). There are no known functional differences for the states of this character and it would seem unimportant; however there are substantial distributional and ecological differences between Zoanthidea that differ in this character (Ryland *et al.* 2004).

Marginal musculature—Assessed at the margin of the column (just beneath the base of the tentacles), circular muscles that form a sphincter to pull the margin over the tentacles during contraction are located in either of two positions: 1) endodermal (muscles are anchored to pleats of mesoglea that protrude into the endoderm) or 2) mesogleal (muscles are anchored within lacunae in the mesoglea). Endodermal and mesogleal marginal muscles have defined the Macrocnemina families Parazoanthidae and Epizoanthidae (respectively) since 1901 (Delage & Hérouard). Most of the marginal muscle of representatives of the Macrocnemina family Gerardiidae are endodermal (and therefore coded as such), but part of the muscle appears to be contained in a few mesogleal lacuna and is sometimes considered endo-mesodermal (Ocaña *et al.* 1995).

Zooxanthellae symbioses—Potentially critical for meeting the carbon budgets of zoanthids, this character has two states: 1) zooxanthellae or 2) zooxanthellae-free.

Symbiotic associations with invertebrates—This character was examined using a general (7 state) and detailed (13 state) assignment of states. The character states of the general assessment are: 1. free-living, 2. Demospongiae, 3. Hexactinellida, 4. Anthozoa, 5. Hydrozoa, 6. Crustacea, and 7. Polychaeta. The character states of the detailed assessment are: 1. free-living, 2. Petrosina (Demospongiae, Haplosclerida), 3. Agelasida (Demospongiae), 4. Hadromerida & Haplosclerida (Demospongiae), 5. Halichondrida & Poecilosclerida (Demospongiae), 6. Hexactinellida, 7. Alcyonacea (Anthozoa), 8. Alcyonacea & Antipatharia (Anthozoa), 9. Antipatharia (Anthozoa), 10. Plumularidae (Hydrozoa), 11. Paguridae (Crustacea) 12. Thoracica (Crustacea), 13. Eunicidae (Polychaeta), 14. Nereididae (Polychaeta). Although there are macrocnemic species that are not known to form symbioses with invertebrates, the current state of knowledge for most species is far too limited to be certain that they are not facultative symbionts and these species were coded as unknown in the analyses.

Results

Intergenomic Congruence and Phylogenetic Analysis

The LRT did not detect significant ($p = 0.14$; $\alpha = 0.05$) topological incongruence between the unpartitioned mitochondrial and nuclear data sets, even though the unpartitioned reconstructions were suboptimal. Because the mitochondrial and nuclear data sets are not significantly incongruent, they were combined in a concatenated alignment consisting of 11,269 positions divided into 12 partitions with independent sets of model parameter estimates (Table 4.5).

A search for the optimal ML tree using the partitioned data resulted in a best tree (Fig. 4.2) with a likelihood score of -35414.26. This analysis recovered clades of species that correspond to Brachycnemina and its subordinate taxa including monophyletic Sphenopidae, Zoanthidae, *Isaurus*, and *Acrozoanthus*; but did not find clades of species representing Brachycnemina genera *Zoanthus* and *Palythoa*, or suborder Macrocnemina and its subordinate taxa. Macrocnemina are divided into the Annelida/Arthropoda-symbiotic species and the Porifera/Cnidaria-symbiotic species with the Hydrozoa-symbiotic species as part of a clade with

Brachycnemina. The relationships between the Anthozoa, Hexactinellida, and Demospongiae-symbiotic species remain partially unresolved.

Evolutionary Hypothesis Testing

The partitioned K-H tests indicate that *a proiri* hypotheses A (monophyletic Brachycnemina), E (monophyletic host associations), H (monophyletic Hadromerida/Haplosclerida + Halichondrida/Poecilosclerida-symbiotic), I (monophyletic Petrosina + Agelasida-symbiotic), and J (monophyletic Alcyonacea/Antipatharia & Hadromerida/Haplosclerida + Halichondrida/Poecilosclerida-symbiotic) are significantly more likely (Table 4.8) than the alternative hypotheses (Table 4.6) given the concatenated sequence data.

Ancestral Character State Reconstruction

ML ancestral state reconstructions indicate a common ancestor of Zoanthidea that was likely macrocnemic (proportional likelihood = 0.9991) and a single transition to the microcnemic state (0.9897, node 4; Fig. 4.3). Mesogleal marginal muscles have at least five independent origins, but the reconstruction of a common ancestor is equivocal (0.5417 endodermal, 0.4583 mesogleal; node 1; Fig. 4.3). Zooxanthellae symbioses have at least three independent origins, with a possible transition to symbiosis at node 3 (0.5611; Fig. 4.4), prior to the evolution of Brachycnemina and the reduction in distributions. The general assessment of symbiosis evolution indicates a Crustacea (0.3089) or Polychaeta (0.3406) associated common ancestor of Zoanthidea (node 1) with host switches to Anthozoa (0.6955, node 2), Hydrozoa (0.4027, node 3), and a loss of symbiosis with invertebrates (0.9839, node 4; Fig. 4.4). The detailed assessment of symbiosis evolution indicates a Plumularidae (0.9986, node 5), Halichondrida and Poecilosclerida (0.5822, node 6), and Paguridae (0.4381) or Eunicidae (0.4958, node 7) associated ancestor at significantly supported (ML bootstrap values > 70) internal nodes (Fig. 4.5).

Discussion

Intergenomic Congruence and Phylogenetic Analysis

The lack of significant incongruence between mitochondrial and nuclear data sets indicates a shared evolutionary history between genomes and demonstrates that any undetected intragenomic variation within the multi-copy nuclear ribosomal genes provides insufficient noise to mask the phylogenetic signal of these data. Although topologically congruent, the two amplicons of nuclear DNA provide much higher resolution (two terminal polytomies) than the three amplicons of mitochondrial sequence (ten terminal polytomies; see TreeBASE submission). The data presented here and in Chapter 2 indicate that the ITS region may be at or near a species-level marker for Zoanthidea.

The concatenated data (Fig. 4.2) recovered nine significantly supported clades that largely circumscribe Brachycnemina and its subordinate taxa, and macrocnemic species associated with the same symbiotic hosts except: (1) Thoracica and Eunicidae-symbiotic species are within the Paguridae associated clade; (2) Agelasida-symbiotic species are interleaved with Petrosina-symbiotic species; and (3) Antipatharia-symbiotic Zoanthidea sp. [Mada 1] is within the Plumularidae associated clade. It is not yet clear if these exceptions represent true transitions in symbiosis or imperfections of phylogenetics as two of these symbiosis types are represented by single species and other associations (*e.g.*, with Mollusca or Echinodermata) have not yet been sampled. Macrocnemina and its subordinate taxa were not recovered; demonstrating that the morphological characters that define these taxa are plesiomorphic.

Evolutionary Hypothesis Testing

Most previous molecular phylogenies are consistent with the hypothesis of monophyletic Brachycnemina (Table 4.6), which was also found to be the most likely hypothesis here (Table 4.8). Topologies constrained under hypotheses of monophyletic Macrocnemina and monophyletic suborders are significantly less likely given the concatenated data. These results indicate that the macrocnemic mesenterial arrangement (macrocnemic fifth mesenteries) is symplesiomorphic while the microcnemic mesenterial arrangement (microcnemic fifth mesenteries) is synapomorphic. Although the anatomy of the fifth mesenteries appears to be functionally inconsequential, it belies substantial distributional and ecological attributes: Brachycnemina are restricted to tropical and subtropical photic zones, are zooxanthellate, and are not generally symbionts of invertebrates.

Most previous molecular phylogenies are consistent with the hypothesis of monophyletic Epizoanthidea (Table 4.6), but this was not supported here (Table 4.8). The topology

constrained under the hypothesis of monophyletic species with similar host associations is significantly more likely given the concatenated data, even though the unconstrained ML tree identified at least three deviations from monophyly amongst species with similar host associations. These results indicate low levels of homoplasy among host associations and greater phylogenetic conservatism (slower evolution) of symbioses than the relative position of the marginal muscle (the morphological basis of Macrocnemina families). This pattern of evolutionary relationships between the Epizoanthidae and Parazoanthidae was first predicted in an analysis of similarity among host associations in Chapter 1.

Previous molecular phylogenies are inconsistent in forming a general consensus about the relationships among Zoanthidea associated with Demospongiae and Anthozoa. The K-H test found significantly less likely topologies consistent with hypotheses of monophyly among Zoanthidea associated with Antipatharia + Demospongiae, favoring monophyly of species with associations within Demospongiae and associations with Alcyonacea and Antipatharia + Demospongiae given the concatenated sequence data (Table 4.8). Clades of Zoanthidea associated with Demospongiae orders Hadromerida & Haplosclerida and Halichondrida & Poecilosclerida are significantly supported in the unconstrained ML tree, but a clade of all Demospongiae-associated Zoanthidea is not significantly supported (Fig. 4.2). There are important evolutionary transitions within this group of Zoanthidea including emergence from the deep-sea, establishment of zooxanthellae symbioses, and host and specificity shifts within and between Demospongiae, Anthozoa, and Hexactinellida; however the relationships remain partly unresolved by these data.

Ancestral Character State Reconstruction

The ML ancestral state reconstruction identified a macrocnemic common ancestor of Zoanthidea (node 1) followed by a single shift to the microcnemic state (node 4; Fig. 4.3). A transition at this point represents a fundamental shift in the evolution of Zoanthidea and coincides with a severe reduction of bathymetric and geographic ranges. The range reduction could be explained by a shift in strategy for meeting carbon budgets (the gain of zooxanthellae symbiosis); however, reconstruction of zooxanthellae symbioses indicates that an origin of this association (node 3; Fig. 4.4) may have preceded the shift to the microcnemic state and the characteristic range restrictions (node 4; Fig. 4.3). Furthermore, there is no indication that restricted distributions are a general consequence of zooxanthellae symbiosis or that the

evolution of zooxanthellae symbiosis is irreversible in macrocnemic zoanthids. The Plumularidae-symbiotic Zoanthidea, sister to the Brachycnemina, have lost zooxanthellae symbiosis (Fig. 4.4) and are not restricted to tropical distributions. This indicates that the loss of symbiosis with invertebrates that coincides with the shift to the microcnemic state is a more likely mechanism for the dramatic reduction in the distribution of Brachycnemina. It has long been hypothesized that the main benefit that zoanthids derive from symbiosis with invertebrates is the exposure to flow and the fixed carbon that it delivers. The analyses presented here suggest that the loss of symbiosis with invertebrates restricted zoanthids to a fraction of their ancestral distribution and solidified their reliance on zooxanthellae symbioses.

Despite uncertainty at the ancestral origin of Zoanthidea, mesogleal marginal musculature is homoplasious with at least four independent origins (Fig. 4.3). These results should not be interpreted to indicate that relative positions of the marginal muscles are not useful for systematics, rather that they are not informative at the phylogenetic level we had originally imagined (binary state, delineating families). It seems that amongst Zoanthidea associated with Plumularidae, Alcyonacea, and possibly Petrosina there are clades of species which the most obvious morphological difference is a mesogleal rather than endodermal marginal muscle, and therefore the position of the marginal muscle may be informative when paired with other characters.

The evolution of Zoanthidea symbioses with invertebrates involves a combination of ancient and recent host shifts with a general pattern of close evolutionary relationships among species with similar host associations (Fig. 4.4 & 4.5). There are five potential host shifts detected among terminal taxa; however further sampling may alter this perception. Associations with representatives of Crustacea or Polychaeta and Hydrozoa are reconstructed as ancient and stable (Fig. 4.4), whereas the rise of associations with representatives of Anthozoa, Hexactinellida, and Demospongiae seem to be part of a rapid radiation with specialization to representatives of specific host orders (Fig. 4.5). The severe reduction in distribution coincident with the rise of Brachycnemina is independent of the evolution of zooxanthellae symbiosis and consistent with hypotheses of the benefits derived by zoanthid symbioses with invertebrates, indicating that the ability to persist in most habitats may have been lost with an evolutionary transition away from symbioses with invertebrates.

Implications for Zoanthidea systematics

Modern systematics seeks not only to group morphologically similar organisms, but also to reflect evolutionary history. Molecular data indicates that Macrocnemina, Epizoanthidae, Parazoanthidae, *Epizoanthus, Isozoanthus*, and *Parazoanthus* are not monophyletic (Fig. 4.2) and should therefore be considered invalid taxa. Although molecular characters may be essential to understanding evolutionary relationships among these anatomically simple organisms, molecular phylogenetics does not improve systematics without careful morphological identification and histological examination. We have not yet applied modern techniques to the majority of Zoanthidea species; therefore exclusively molecular approaches to the creation and revision of taxa are speculative at best. While we are beginning to understand its deficiencies, there is currently no viable morphological character set that can reliably replace the existing taxonomic system. A simple clarification that can be made here is the abandonment of the taxon Macrocnemina in favor of the phrase "non-brachycnemic Zoanthidea" to reflect the plesiomorphic macrocnemic mesenterial arrangement. Perhaps the histological examinations of this study documented in MorphBank (publication collection number 514243) will spur the identification of phylogenetically informative morphological characters. The ecological character set of symbiotic host associations with invertebrates does appear to be generally (if imperfectly) useful for predicting phylogenetic relationships and, when paired with as-of-yet-unknown informative morphological characters, may serve as the basis of systematics that are reflective of evolution. It should be noted that while the general assessment of symbiosis types (Fig. 4.4) provided the clearest reconstruction of ancestral character states, the detailed mapping (Fig. 4.5) appears to be at the level of specificity exhibited by the species themselves (*i.e.* useful in identifying terminal clades) and therefore identification of host phylum or class (*e.g.*, Porifera or Demospongiae) is insufficient for predicting closest relatives.

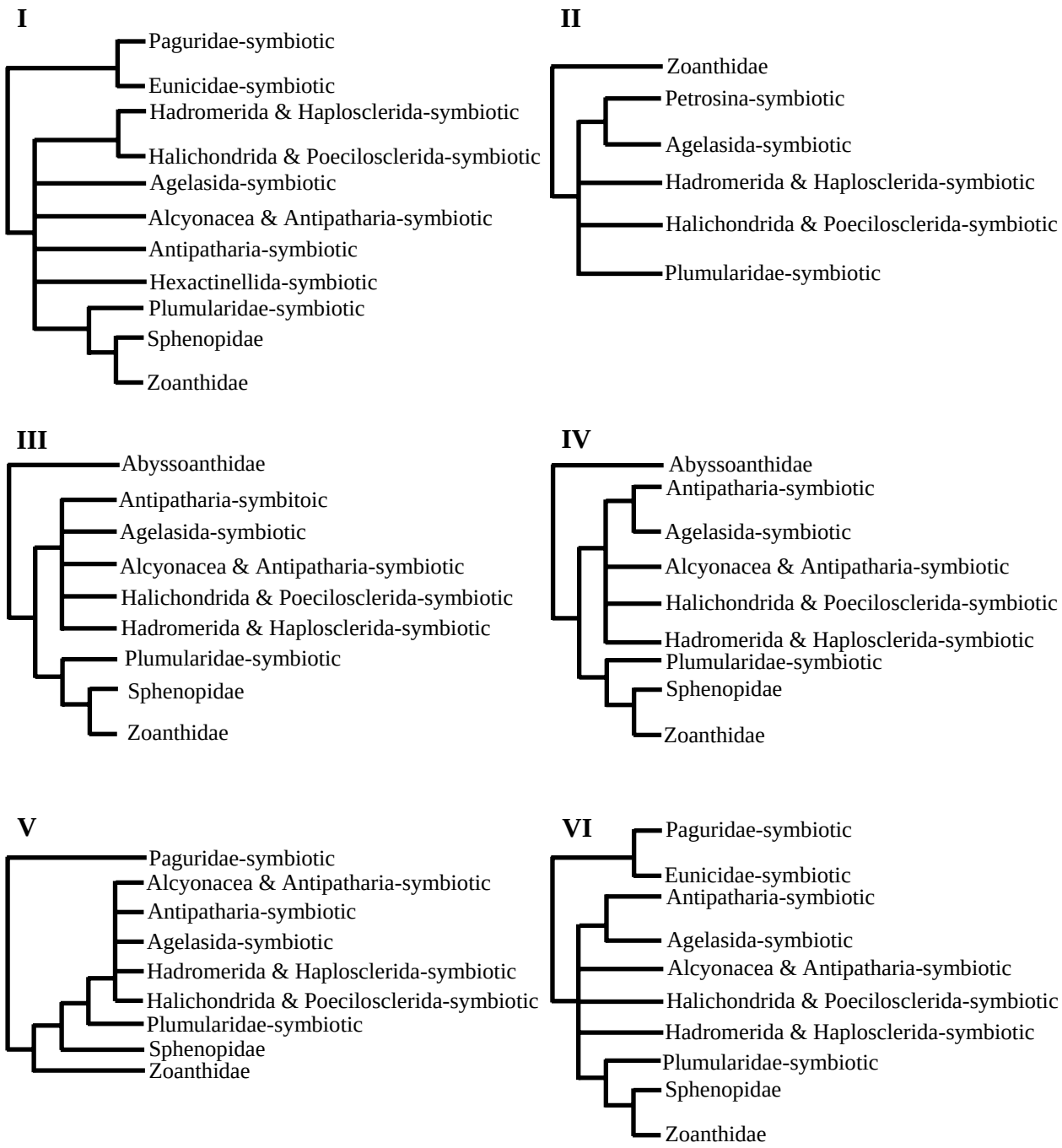

Figure 4.1. Summary topologies of phylogenetic hypotheses from previously published molecular analyses; used here as the basis of a priori hypotheses in tests of monoplyly. The literature sources of the phylogenies are: (I) Sinniger & Häussermann 2009, (II) Chapter 2, (III and IV) Reimer & Nonaka *et al.* 2008, (V and VI) Reimer & Sinniger *et al.* 2008, (VII and VIII) Sinniger *et al.* 2008, (IX and X) Reimer & Sinniger *et al.* 2007, and (XI) Sinniger *et al.* 2005.

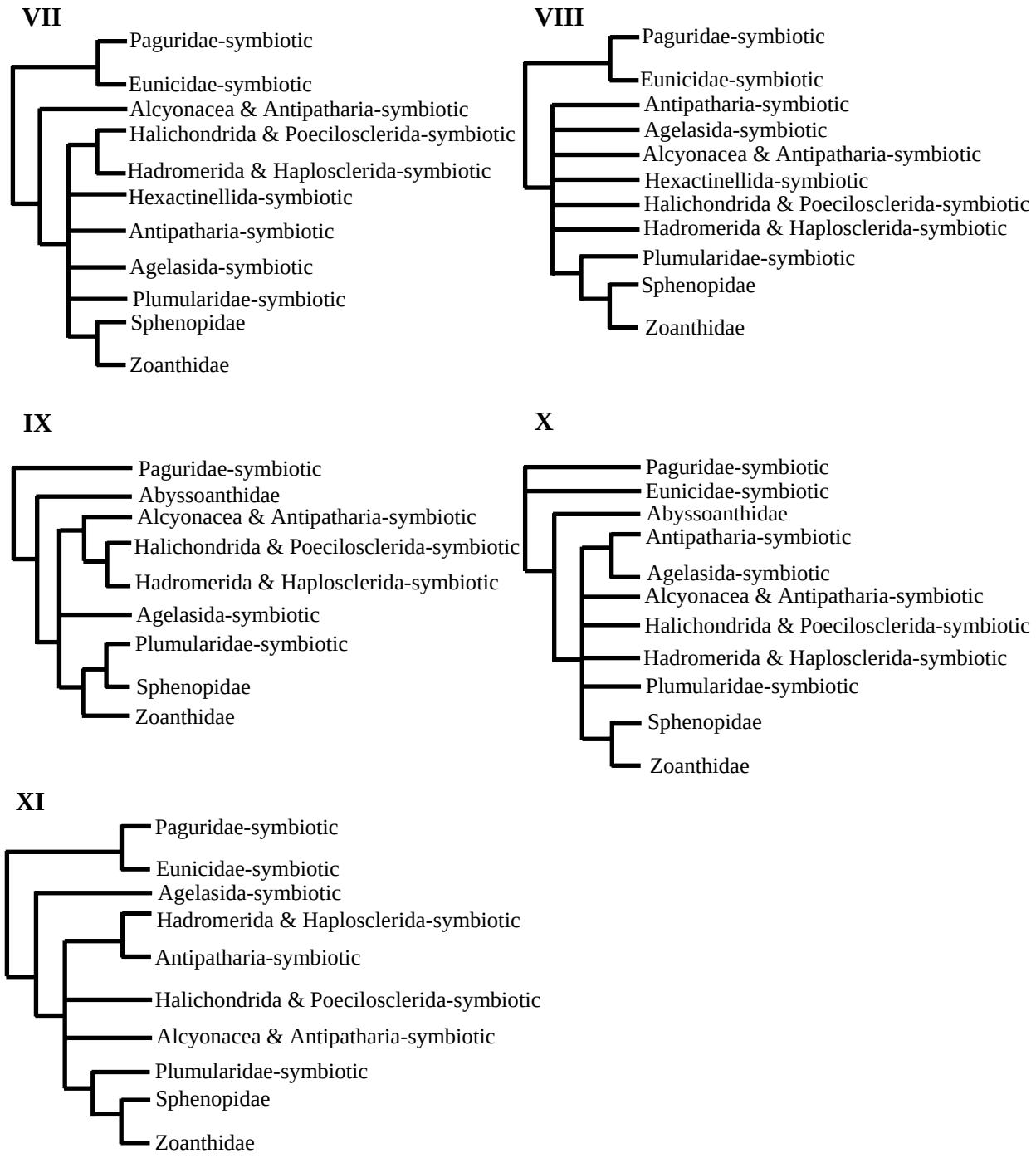

Figure 4.1. Continued.

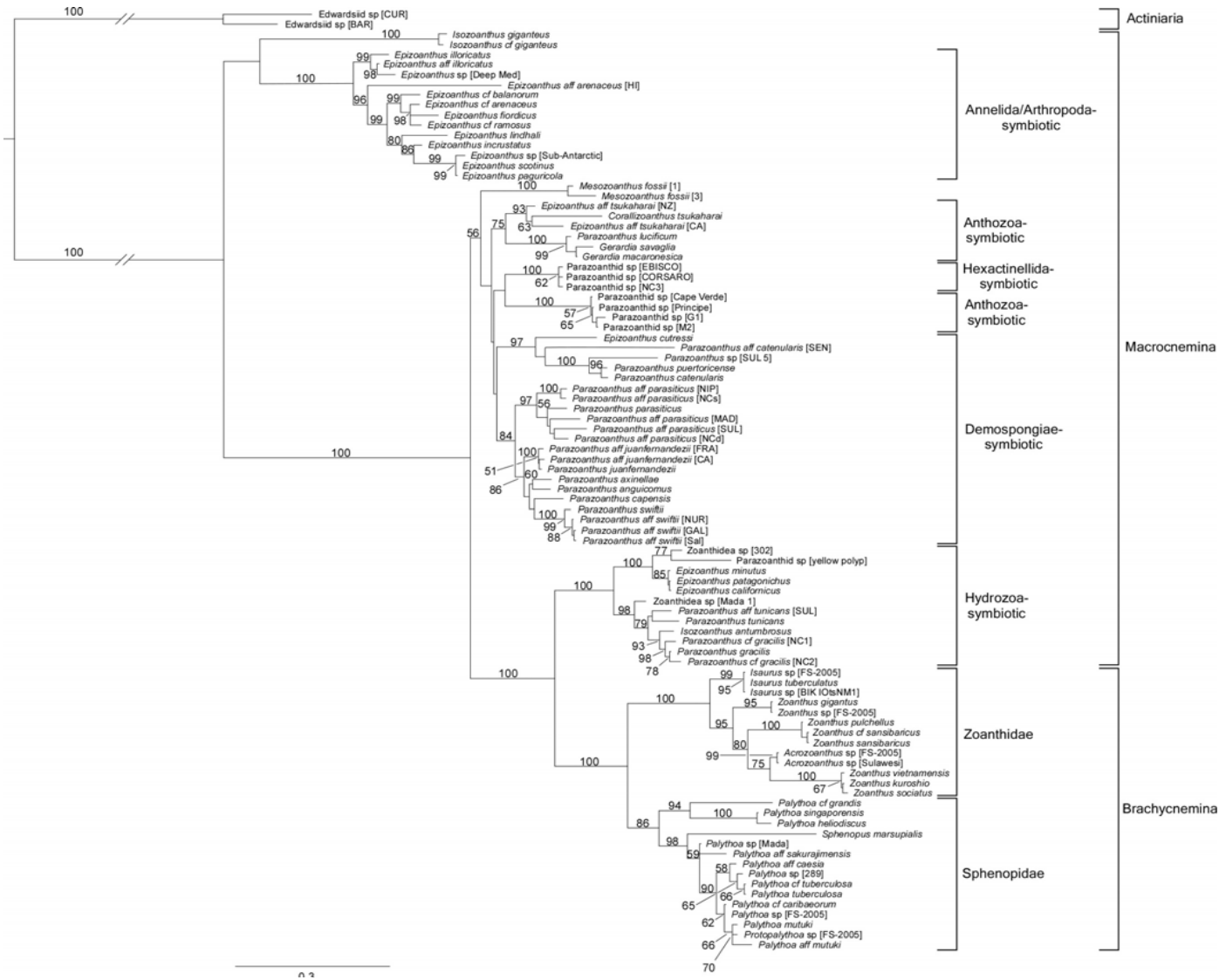

Figure 4.2. Maximum likelihood phylogeny of Zoanthidea based on concatenated nuclear (ITS & 28S) and mitochondrial (12S & 16S) ribosomal RNA and mitochondrial protein-coding (COI) nucleotide sequences. Support indicated (for values > 50) by 1000 pseudoreplicate maximum likelihood bootstrap values

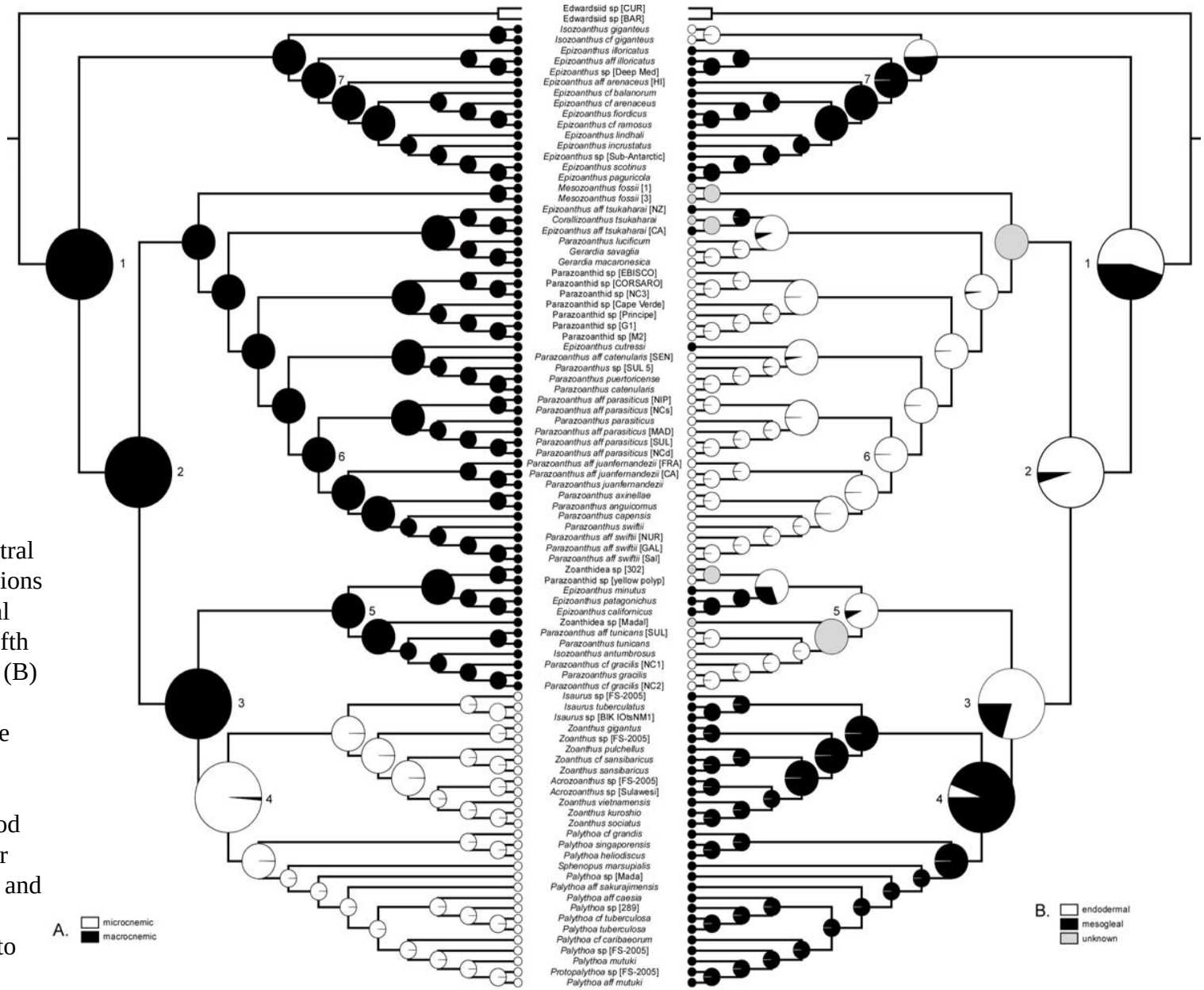

Figure 4.3. Maximum likelihood ancestral state reconstructions of morphological characters (A) fifth mesenteries and (B) marginal musculature. Pie chart sections represent the relative likelihood of each character state at the node and are enlarged at ancestral nodes to increase clarity.

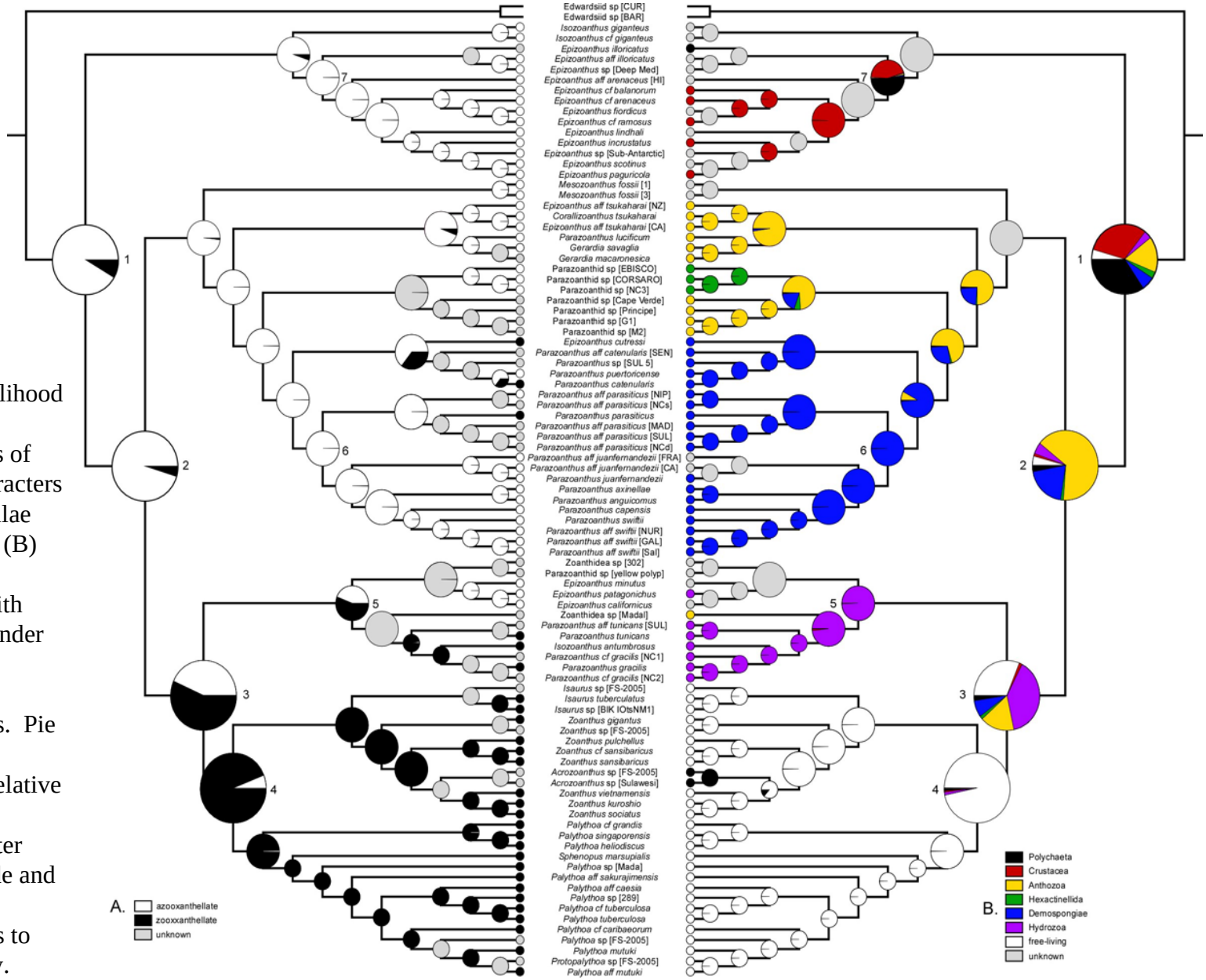

Figure 4.4. Maximum likelihood ancestral state reconstructions of ecological characters (A) zooxanthellae symbioses and (B) symbiotic associations with invertebrates under the general assignment of character states. Pie chart sections represent the relative likelihood of each character state at the node and are enlarged at ancestral nodes to increase clarity.

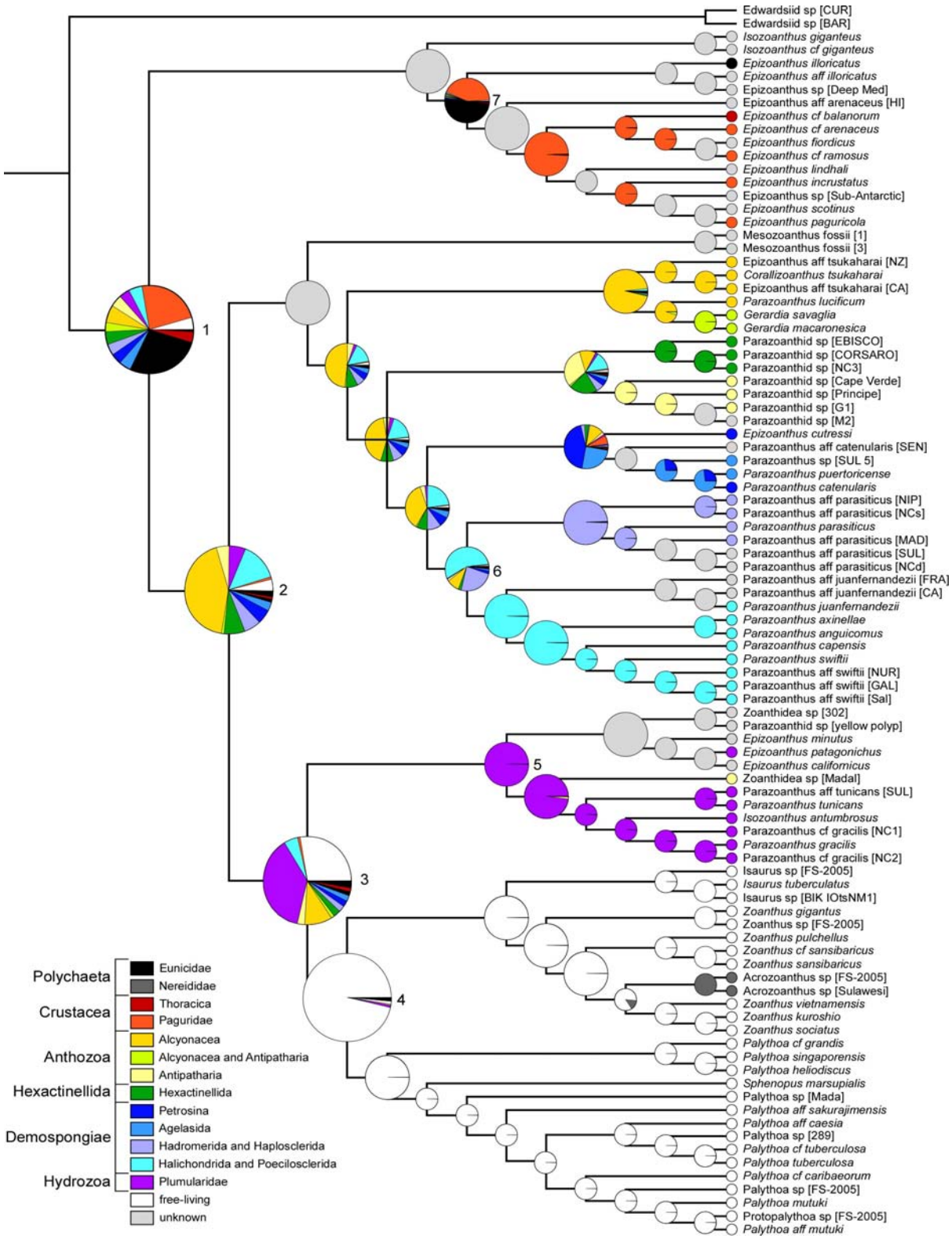

Figure 4.5. Maximum likelihood ancestral state reconstructions of the ecological character symbiotic associations with invertebrates under the detailed assignment of character states. Pie chart sections represent the relative likelihood of each character state at the node and are enlarged at ancestral nodes to increase clarity.

Table 4.1. Host associations of Zoanthidea families sampled for these analyses.

Host taxa	Zoanthidea family
Porifera	
Demospongiae orders Halichondrida and Poecilosclerida	Parazoanthidae
Demospongiae order Agelasida	Parazoanthidae
Demospongiae orders Hadromerida and Haplosclerida	Parazoanthidae
Haplosclerida suborder Petrosina	Parazoanthidae & Epizoanthidae
class Hexactinellida	Parazoanthidae
Cnidaria	
Anthozoa order Antipatharia	Parazoanthidae
Anthozoa orders Alcyonacea and Antipatharia	Gerardiidae
Anthozoa orders Alcyonacea	Parazoanthidae & Epizoanthidae
Hydrozoa family Plumularidae	Parazoanthidae & Epizoanthidae
Arthropoda	
Crustacea family Paguridae	Epizoanthidae
Crustacea superorder Thoracica	Epizoanthidae
Annelida	
Polychaeta family Eunicidae	Epizoanthidae
Polychaeta family Nereididae	Zoanthidae

Table 4.2. Collection information for non-Brachycnemina and Actiniaria specimens used in the analyses**.**

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Non-Brachycnemina								
Zoanthidea								
Isozoanthus giganteus	SA 259	-33.9821, 25.6912	Bell Buoy 1, Algoa Bay, Port Elizabeth	South Africa	20	3/10/08	free	E Rodriguez, Ohio State University
Isozoanthus cf giganteus	SA 263	-33.9807, 25.6601	Bell Buoy 2, Algoa Bay, Port Elizabeth	South Africa	18-22	3/13/08	free	E Rodriguez, Ohio State University
Epizoanthus illoricatus	Eill	–	North Sulawesi	Indonesia	22	9/03	Eunicidae	Sinniger & Häussermann 2009
Epizoanthus aff illoricatus	SIO 252	34.6952, -123.2095	Station M, CA	USA	4100	8/2/04	free	H Cha, Scripps Institute of Oceanography BIC-Co2124
Epizoanthus sp [Deep Med]	MedDeep	–	South Mediter-ranean Sea	–	1055	4/29/06	free	Sinniger *et al* 2008; Sinniger pers comm
Epizoanthus aff arenaceus [HI]	NMNH 100	20.9897 -157.3194	Penguin Bank, Moloka'i, HI,	USA	442	9/19/96	free	S France, University of Louisiana at Lafayette, Pisces DSR/V, USNM 98849
Epizoanthus cf balanorum	PER 239	-7.8088 - 79.4974	Islas Macabi	Peru	13	9/30/07	Thoracica	Ph Willenz, Royal Belgian Institute of Natural Sciences
Epizoanthus cf arenaceus	MED 65	42.4861, 3.1472	Banyuls-sur-Mer	France	80	04	free	Yves Desdevises, Université Pierre et Marie Curie
Epizoanthus fiordicus	Efio	-42.3439, -72.4572	Pta. Llonco, Comau Fjord	Chile	15	2/12/04	free	Sinniger *et al* 2008
Epizoanthus cf ramosus	NIP 154	–	Shirahama, Wakayama	Japan	~80	4/20/06	free	J Reimer, University of the Ryukyus, Kyoto University Marine Experimental Station
Epizoanthus lindhali	Elind	–	Arctic sea	–	572	9/10/05	free	Sinniger *et al* 2008; Sinniger pers comm
Epizoanthus incrustatus	ARC 269	77.5150, -14.9848	Danmark-shavn	Greenland	317	7/25/08	free	M Bergmann, Alfred Wegener Institute for Polar & Marine Research

Table 4.2. Continued.

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Epizoanthus sp [Sub-Antarctic]	SubAnt	–	SW Atlantic	–	450	6/16/58	free	Sinniger *et al* 2008; Sinniger pers comm
Epizoanthus scotinus	WA 166	48.5602 -123.0117	Point Caution, Friday Harbor, WA	USA	2-10	10/25/06	free	K Matterson, University of Washington
Epizoanthus paguricola	Epag	–	Tyrrhenian Sea	Italy	130	12/21/03	Paguridae	Sinniger *et al* 2005, Sinniger *et al* 2008; Sinniger pers comm
Mesozoanthus fossii [1]	MF 1	-48.4937, -74.0839	Bernardo Fjord	Chile	29	3/27/05	free	Sinniger *et al* 2008, Sinniger & Häussermann 2009
Mesozoanthus fossii [3]	MF 3	-42.3747, -72.4282	Punta Huinay, Comau Fjord	Chile	20	5/3/05	free	Sinniger *et al* 2008, Sinniger & Häussermann 2009
Epizoanthus aff tsukaharai [NZ]	NZ 66		–	New Zealand	–	–	Isididae	J Sanchez, Universidad de los Andes
Corallizoanthus tsukaharai	Ctsu	–	Ishigaki-jima, Okinawa	Japan	222	2/8/04	Paracorallium	Reimer *et al* 2008a
Epizoanthus aff tsukaharai [CA]	NMNH 258	35.735 -122.719	Davidson Seamount, CA	USA	1763	1/28/06	*Calyptrophora antilla*	Tiburon ROV, Monterey Bay Aquarium Research Institute, USNM 1102460
Parazoanthus lucificum	SAV 3	–	CA	USA	–	5/04	Alcyonacea	Sinniger *et al* 2008, Sinniger & Häussermann 2009
Gerardia savaglia	SAV 1	–	Marseille	France	41	5/03	Alcyonacea or Antipatharia	Sinniger *et al* 2005, Sinniger & Häussermann 2009
Gerardia macaronesica	Smac	–	Gran Canaria, Canary Islands	Spain	30	6/03	Alcyonacea or Antipatharia	Sinniger *et al* 2005, Sinniger *et al* 2008
Parazoanthid sp [EBISCO]	EBISCO	–	–	New Caledonia	~860	–	Hexactinellida	Sinniger & Häussermann 2009; Sinniger pers comm
Parazoanthid sp [CORSARO]	CORSARO	–	Mediter-ranean	–	690	2/5/06	Hexactinellida	Sinniger *et al* 2008, Sinniger & Häussermann 2009; Sinniger pers comm

Table 4.2. Continued.

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Epizoanthus sp [Sub-Antarctic]	SubAnt	–	SW Atlantic	–	450	6/16/58	free	Sinniger *et al* 2008; Sinniger pers comm
Epizoanthus scotinus	WA 166	48.5602 -123.0117	Point Caution, Friday Harbor, WA	USA	2-10	10/25/06	free	K Matterson, University of Washington
Epizoanthus paguricola	Epag	–	Tyrrhenian Sea	Italy	130	12/21/03	Paguridae	Sinniger *et al* 2005, Sinniger *et al* 2008; Sinniger pers comm
Mesozoanthus fossii [1]	MF 1	-48.4937, -74.0839	Bernardo Fjord	Chile	29	3/27/05	free	Sinniger *et al* 2008, Sinniger & Häussermann 2009
Mesozoanthus fossii [3]	MF 3	-42.3747, -72.4282	Punta Huinay, Comau Fjord	Chile	20	5/3/05	free	Sinniger *et al* 2008, Sinniger & Häussermann 2009
Epizoanthus aff tsukaharai [NZ]	NZ 66		–	New Zealand	–	–	Isididae	J Sanchez, Universidad de los Andes
Corallizoanthus tsukaharai	Ctsu	–	Ishigaki-jima, Okinawa	Japan	222	2/8/04	Paracorallium	Reimer *et al* 2008a
Epizoanthus aff tsukaharai [CA]	NMNH 258	35.735 -122.719	Davidson Seamount, CA	USA	1763	1/28/06	*Calyptrophora antilla*	Tiburon ROV, Monterey Bay Aquarium Research Institute, USNM 1102460
Parazoanthus lucificum	SAV 3	–	CA	USA	–	5/04	Alcyonacea	Sinniger *et al* 2008, Sinniger & Häussermann 2009
Gerardia savaglia	SAV 1	–	Marseille	France	41	5/03	Alcyonacea or Antipatharia	Sinniger *et al* 2005, Sinniger & Häussermann 2009
Gerardia macaronesica	Smac	–	Gran Canaria, Canary Islands	Spain	30	6/03	Alcyonacea or Antipatharia	Sinniger *et al* 2005, Sinniger *et al* 2008
Parazoanthid sp [EBISCO]	EBISCO	–	–	New Caledonia	~860	–	Hexactinellida	Sinniger & Häussermann 2009; Sinniger pers comm

Table 4.2. Continued.

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Parazoanthid sp [CORSARO]	CORSARO	–	Mediter-ranean	–	690	2/5/06	Hexactinellida	Sinniger *et al* 2008, Sinniger & Häussermann 2009; Sinniger pers comm
Parazoanthid sp [NC3]	NC 3	–	–	New Caledonia	~860	–	Hexactinellida	Sinniger & Häussermann 2009; Sinniger pers comm
Parazoanthid sp [Cape Verde]	CV	–	Sal Island	Cape Verde	17	–	Antipatharia	Sinniger *et al* 2005, Reimer *et al* 2007, Reimer *et al* 2008a, Sinniger & Häussermann 2009
Parazoanthid sp [Principe]	PRI	–	Príncipe	São Tomé and Príncipe	45	2/04	Antipatharia	Sinniger *et al* 2005, Sinniger & Häussermann 2009
Parazoanthus sp [G1]	GAL 1	-0.2370 -90.5731	Cousins Rock, Galapagos Bartolomé	Ecuador	23	11/11/03	*Antipathes galapagensis*	Reimer *et al* 2008b
Parazoanthid sp [M2]	MAD 2	–	North	Madagascar	10	–	Anthozoa	Sinniger & Häussermann 2009; Sinniger pers comm
Epizoanthus cutressi	TOB 44	11.294 -60.5059	Little Tobago	Trinidad & Tobago	3–20	6/12/04	*Cribrochalina vasculum*	TD Swain, Florida State University
Parazoanthus aff. cutressi [SEN]	SEN	–	–	Senegal	39	9/6/04	Demospongiae	Sinniger *et al* 2008, Sinniger & Häussermann 2009; Sinniger pers comm
Parazoanthus sp [SUL 5]	SUL 5	–	North Sulawesi	Indonesia	16	9/03	*Agelas* sp.	Sinniger *et al* 2005, Sinniger & Häussermann 2009
Parazoanthus puertoricense	NAV 58	18.3954 - 75.0174	Navassa Island	USA	12–16	11/4/04	*Agelas sceptrum*	TD Swain, Florida State University
Parazoanthus catenularis	TOB 37	11.2929 -60.4977	Blackjack	Trinidad & Tobago	10–25	6/12/04	*Xestospongia muta*	TD Swain, Florida State University
Parazoanthus aff. parasiticus [NIP]	NIP 155	31.7356 129.7513	Yura-shima, Shimo-Koshiki-Shima	Japan	1	11/3/06	Hadromerida	J Reimer, University of the Ryukyus
Parazoanthus aff. parasiticus [NCs]	NC Shal	–	south-west lagoon	New Caledonia	5	11/06	*Cliona* sp.	Sinniger 2006; Sinniger & Häussermann 2009

Table 4.2. Continued.

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Parazoanthus parasiticus	TOB 47	11.2896 -60.5105	Bookends	Trinidad & Tobago	10–25	6/12/04	*Niphates erecta*	TD Swain, Florida State University
Parazoanthus aff. parasiticus [MAD]	MAD 3	–	Nosy Sakatia	Madagascar	9	8/12/04	Hadromerida	Sinniger *et al* 2008, Sinniger & Häussermann 2009; Sinniger pers comm
Parazoanthus aff. parasiticus [SUL]	SUL 3	–	North Sulawesi	Indonesia	31	9/03	Demospongiae	Sinniger *et al* 2005
Parazoanthus aff. parasiticus [NCd]	NC Deep	–	–	New Caledonia	32	11/06	red encrusting Demospongiae	Sinniger 2006; Sinniger & Häussermann 2009
Parazoanthus aff. juanfernandezii [FRA]	FRA PC1	43.0191 6.3692	Montrémian, Port-Cros	France	6	9/13/05	none known	R Coma, Centre d'Estudis Avançats de Blanes
Parazoanthus aff. juanfernandezii [CA]	CA 128	33.4522 -118.4859	Catalina Bird Rock	CA, USA	18	5/21/05	free	M Martinez-Vergara, San Diego State University
Parazoanthus juanfernandezii	CHI 187	-42.5256 -72.6626	Renihué Fjord	Chile	14	5/24/07	Poecilosclerida	Ph Willenz, Royal Belgian Institute of Natural Sciences
Parazoanthus axinellae	SPA M1	42.0421 3.2269	Tasco Gran, Illes Medes	Spain	20	9/23/05	*Axinella* sp.	R Coma, Centre d'Estudis Avançats de Blanes
Parazoanthus anguicomus	IRE 266	55.3079 -6.2692	Ruecallan, Rathin Island, Northern Ireland	UK	32	4/14/07	Poecilosclerida	B Picton, Ulster Museum
Parazoanthus capensis	SA 262	-34.0061 25.7194	Algoa Bay, White Sands	South Africa	21–25	3/12/08	*Clathria* sp.	E Rodriguez, Ohio State University
Parazoanthus swiftii	PAN 9	9.3488 -82.2587	STRI point, Bocas del Toro	Panamá	1–4	8/6/03	*Iotrochota birotulata*	TD Swain, Florida State University
Parazoanthus aff. swiftii [Nur]	PER 249	-4.2243 -81.2059	El Nuro	Peru	8	10/18/07	*Clathria* sp.	Y Hooker, Universidad Peruana Cayetano Heredia
Parazoanthus aff. swiftii [GAL]	GAL 2	-0.0558 -91.5604	Punta Vicente Roca, Isabela, Galapagos,	Ecuador	2	1/16/03	free	Reimer *et al* 2008b
Parazoanthus aff swiftii [Sal]	PER 241	-3.9501 -80.9619	Punta Sal	Peru	11	10/16/07	Poecilosclerida	Y Hooker, Universidad Peruana Cayetano Heredia
Zoanthidea sp [302]	302	–	North	Madagascar	39	12/8/04	free	Sinniger *et al* 2008

Table 4.2. Continued.

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Parazoanthid sp. [yellow polyp]	YP	–	–	Indonesia	–	8/03	free	Sinniger *et al* 2005, Reimer *et al* 2007, Sinniger & Häussermann 2009; Sinniger pers comm
Epizoanthus minutus	GM 3	30.0263 -84.3862	Gulf of Mexico, Wakulla County, FL,	USA	1-10	10/5/03	free	V Spencer, Gulf Specimen Marine Lab
Epizoanthus patagonichus	PER 237	-6.9301 -80.7212	Islas Lobos de Afuera	Peru	8	10/9/07	free	P Willenz, Royal Belgian Institute of Natural Sciences
Epizoanthus californicus	PER 243	-3.9500 -80.9619	Punta Sal	Peru	11	10/16/07	free	Y Hooker, Universidad Peruana Cayetano Heredia
Zoanthidea sp [Mada1]	MAD 1	–	North	Madagascar	10	12/7/07	Antipatharia	Sinniger *et al* 2008; Sinniger pers comm
Parazoanthus aff gracilis [SUL]	SUL 1	–	North	Sulawesi, Indonesia	28	9/12/03	Hydrozoa	Sinniger *et al* 2005, Sinniger *et al* 2008, Sinniger & Häussermann 2009
Parazoanthus tunicans	TOB 40	11.294 -60.5059	Little Tobago	Trinidad & Tobago	3-20	6/12/04	*Dentitheca dendritica*	TD Swain, Florida State University
Isozoanthus antumbrosus	PAN 21	9.359 -82.2123	Bastimentos, Bocas del Toro	Panamá	3-10	8/12/03	*Dentitheca dendritica*	TD Swain, Florida State University
Parazoanthus aff gracilis [NC1]	NC 1.5	–	–	New Caledonia	33	–	Hydrozoa	Sinniger & Häussermann 2009; Sinniger pers comm
Parazoanthus gracilis	NIP 153	35.0764 140.1043	Igai-jima, Kamogawa	Chiba, Japan	17	10/06/06	Plumularidae	J Reimer, University of the Ryukyus
Parazoanthus aff. gracilis [NC2]	NC 2	–	–	New Calidonia	25	–	Hydrozoa	Sinniger & Häussermann 2009; Sinniger pers comm

Table 4.2. Continued.

Taxa	Unique ID	Coordinates	location	Country	Depth (m)	Date	Host	Source
Actiniaria								
Edwardsiid sp [BAR]	BAR 06X	13.1804, -59.6476	Dottins South	Barbados	27	6/11/06	*Plakortis* sp.	TD Swain, Florida State University
Edwardsiid sp [CUR]	CUR 213	12.0660, - 68.8601	Director's Bay, Curaçao	Netherlands Antilles	17	7/4/06	*Plakortis* sp.	TD Swain, Florida State University

Table 4.3. GenBank and MorphBank accession numbers of specimens used in the analyses. New accessions are in bold**.**

Taxa	Unique ID	Morph	ITS	28S	12S	16S	COI
Non-Brachycnemina Zoanthidea							
Isozoanthus giganteus	SA 259	**477929**	**GQ464896**	**GQ464931**	**GQ464964**	**GQ464867**	—
Isozoanthus cf giganteus	SA 263	**477928**	**GQ464897**	**GQ464932**	**GQ464965**	**GQ464868**	—
Epizoanthus illoricatus	Eill	—	EU591541	—	AY995901	AY995929	AB247349
Epizoanthus aff illoricatus	SIO 252	**477931**	**GQ464895**	**GQ464930**	**GQ464963**	**GQ464866**	—
Epizoanthus sp [Deep Med]	MedDeep	—	—	—	—	EF687817	EF672678
Epizoanthus aff arenaceus [HI]	NMNH 100	**477932**	**GQ464891**	**GQ464927**	**GQ464959**	**GQ464862**	—
Epizoanthus cf balanorum	PER 239	**477930**	**GQ464898**	**GQ464933**	**GQ464966**	**GQ464869**	—
Epizoanthus cf arenaceus	MED 65	—	**GQ464892**	**GQ464926**	**GQ464960**	**GQ464863**	EF672672
Epizoanthus fiordicus	Efio	—	—	—	—	EF687813	EF672674
Epizoanthus cf ramosus	NIP 154	**476250**	**GQ464893**	**GQ464928**	**GQ464961**	**GQ464864**	—
Epizoanthus lindhali	Elind	—	—	—	—	EF687816	EF672677
Epizoanthus incrustatus	ARC 269	**477927**	**GQ464894**	**GQ464929**	**GQ464962**	**GQ464865**	—
Epizoanthus sp [Sub-Antarctic]	SubAnt	—	—	—	—	EF687815	EF672676
Epizoanthus scotinus	WA 166	**475389**	**GQ464899**	**GQ464934**	**GQ464967**	**GQ464870**	—
Epizoanthus paguricola	Epag	—	EU591539	—	AY995902	AY995928	AB247347
Mesozoanthus fossii [1]	MF 1	—	EU591543	—	—	EF687821	EF672654
Mesozoanthus fossii [3]	MF 3	—	EU591545	—	—	EF687822	EF672653
Epizoanthus aff tsukaharai [NZ]	NZ 66	**476540**	**GQ464885**	**GQ464918**	**GQ464951**	**GQ464856**	—
Corallizoanthus tsukaharai	Ctsu	—	EU035621	—	—	EU035627	EU035633
Epizoanthus aff tsukaharai [CA]	NMNH 258	**476539**	**GQ464886**	**GQ464919**	**GQ464952**	**GQ464857**	—
Parazoanthus lucificum	SAV 3	—	EU591550	—	—	EF687819	EF672658
Gerardia savaglia	SAV 1	—	EU591548	—	AY995905	AY995925	AB247356
Gerardia macaronesica	Smac	—	—	—	AY995906	AY995930	EF672657
Parazoanthid sp [EBISCO]	EBISCO	—	EU591561	—	—	EU591601	EU591617
Parazoanthid sp [CORSARO]	CORSARO	—	EU591559	—	—	EF687824	EF672665
Parazoanthid sp [NC3]	NC 3	—	EU591558	—	—	EU591602	EU591616
Parazoanthid sp [Cape Verde]	CV	—	EU363365	—	AY995907	AY995931	AB247357
Parazoanthid sp [Principe]	PRI	—	EU591552	—	AY995908	AY995932	EU591618
Parazoanthus sp [G1]	GAL 1	—	EU333798	—	—	EU333756	EU333783
Parazoanthid sp [M2]	MAD 2	—	EU591554	—	—	EU591599	EU591619
Epizoanthus cutressi	TOB 44	**475839**	EU418267	**GQ464917**	**GQ464950**	EU828759	—
Parazoanthus aff catenularis [SEN]	SEN	—	EU591582	—	—	EF687820	EF672656
Parazoanthus sp [SUL 5]	SUL 5	—	EU591583	—	AY995917	AY995934	EU591627

Table 4.3. Continued.

Taxa	Unique ID	Morph	ITS	28S	12S	16S	COI
Parazoanthus puertoricense	NAV 58	**475843**	EU418312	**GQ464915**	**GQ464948**	EU828758	AB247351
Parazoanthus catenularis	TOB 37	**475842**	EU418292	**GQ464916**	**GQ464949**	EU828757	—
Parazoanthus aff parasiticus [NIP]	NIP 155	**476541**	**GQ464884**	**GQ464913**	**GQ464946**	**GQ464855**	AB247352
Parazoanthus aff parasiticus [NCs]	NC Shal	—	EU591568	—	—	EU591607	EU591626
Parazoanthus parasiticus	TOB 47	**474150**	EU418306	**GQ464914**	**GQ464947**	EU828756	EF672663
Parazoanthus aff parasiticus [MAD]	MAD 3	—	EU591576	—	—	EF687825	EF672664
Parazoanthus aff parasiticus [SUL]	SUL 3	—	EU591575	—	AY995911	AY995937	AB247354
Parazoanthus aff parasiticus [NCd]	NC Deep	—	EU591580	—	—	EU591605	EU591624
Parazoanthus aff juanfernandezii [FRA]	FRA PC1	**476543**	**GQ464877**	**GQ464904**	**GQ464937**	**GQ464848**	—
Parazoanthus aff juanfernandezii [CA]	CA 128	**476293**	**GQ464878**	**GQ464905**	**GQ464938**	**GQ464849**	—
Parazoanthus juanfernandezii	CHI 187	**475434**	**GQ464879**	**GQ464906**	**GQ464939**	**GQ464850**	—
Parazoanthus axinellae	SPA M1	**475885**	EU418283	**GQ464907**	**GQ464940**	EU828754	AB247355
Parazoanthus anguicomus	IRE 266	**475591**	**GQ464880**	**GQ464908**	**GQ464941**	**GQ464851**	EF672660
Parazoanthus capensis	SA 262	**475590**	**GQ464881**	**GQ464909**	**GQ464942**	**GQ464852**	—
Parazoanthus swiftii	PAN 9	**475844**	EU418332	**GQ464912**	**GQ464945**	EU828755	AB247350
Parazoanthus aff swiftii [NUR]	PER 249	**476289**	**GQ464883**	**GQ464911**	**GQ464944**	**GQ464854**	—
Parazoanthus aff swiftii [GAL]	GAL 2	—	EU333801	—	—	EU333749	EU333778
Parazoanthus aff swiftii [Sal]	PER 241	**476542**	**GQ464882**	**GQ464910**	**GQ464943**	**GQ464853**	—
Zoanthidea sp [302]	S302	—	—	—	—	EF687831	EF672666
Parazoanthid sp [yellow polyp]	YP	—	EU591595	—	AY995918	AY995939	AB247358
Epizoanthus minutus	GM 3	**475696**	**GQ464890**	**GQ464925**	**GQ464958**	**GQ464861**	—
Epizoanthus patagonichus	PER 237	**475886**	**GQ464888**	**GQ464923**	**GQ464956**	**GQ464859**	—
Epizoanthus californicus	PER 243	**476252**	**GQ464889**	**GQ464924**	**GQ464957**	**GQ464860**	—
Zoanthidea sp [Mada1]	MAD 1	—	—	—	—	EF687830	EF672669
Parazoanthus aff tunicans [SUL]	SUL 1	—	EU591590	—	AY995915	AY995942	EF672668
Parazoanthus tunicans	TOB 40	**475840**	EU418341	**GQ464922**	**GQ464955**	EU828760	EF672667
Isozoanthus antumbrosus	PAN 21	**475841**	EU418277	**GQ464921**	**GQ464954**	EU828761	AB247353
Parazoanthus cf gracilis [NC1]	NC 1	—	EU591592	—	—	EU591612	EU591629
Parazoanthus gracilis	NIP 153	**476251**	**GQ464887**	**GQ464920**	**GQ464953**	**GQ464858**	AB214178
Parazoanthus cf gracilis [NC2]	NC 2	—	EU591591	—	—	EU591611	EU591628
Brachycnemina							
Isaurus sp [FS-2005]	Isau05	—	—	—	AY995922	AY995945	—
Isaurus tuberculatus	IToM1	—	—	—	—	EF452253	EF452271
Isaurus sp [BIK IOtsNM1]	BIK	—	—	—	—	EF452247	AB247361

Table 4.3. Continued.

Taxa	Unique ID	Morph	ITS	28S	12S	16S	COI
Zoanthus gigantus	ZgYS1	—	AB214158	—	—	AB219192	AB214177
Zoanthus sp [FS-2005]	Zoan05	—	—	—	AY995920	AY995948	—
Zoanthus pulchellus	PAN 7	—	EU418345	—	**GQ464969**	EU828762	—
Zoanthus cf *sansibaricus*	Zcfsan	—	—	—	—	EU333744	EU333769
Zoanthus sansibaricus	ZAT7	—	—	—	—	AB219188	AB214173
Acrozoanthus sp [FS-2005]	Acro05	—	—	—	AY995921	AY995946	—
Acrozoanthus sp [Sulawesi]	Sul05	—	—	—	AY995919	AY995947	—
Zoanthus vietnamensis	ZvSH3	—	AB235397	—	—	AB235408	EU333696
Zoanthus kuroshio	ZkYS1	—	DQ442492	—	—	AB219191	AB214175
Zoanthus sociatus	SMG2	—	—	—	—	EU348605	EU348616
Palythoa cf grandis	DOM 18	—	**GQ464900**	**GQ464935**	**GQ464968**	**GQ464871**	—
Palythoa singaporensis	Psing	—	—	—	—	EU333660	EU333699
Palythoa heliodiscus	PhSaiLL1	—	DQ997881	—	—	AB219223	AB219214
Sphenopus marsupialis	Sphem	—	AB441420	—	—	—	AB441277
Palythoa sp [Mada]	Mada	—	—	—	—	EF687832	AB247360
Palythoa aff sakurajimensis	PWS1	—	DQ997887	—	—	DQ997863	—
Palythoa aff caesia	TOB 52	—	**GQ464901**	—	**GQ464970**	**GQ464872**	—
Palythoa sp [289]	PMad289	—	DQ997901	—	—	DQ997878	—
Palythoa cf tuberculosa	Pcftu	—	—	—	—	EU333746	EU333772
Palythoa tuberculosa	PtCN1	—	DQ997896	—	—	DQ997860	EU333704
Palythoa cf caribaeorum	TOB 33	—	—	—	**GQ464972**	**GQ464874**	—
Palythoa sp [FS-2005]	Pal05	—	—	—	AY995923	AY995943	—
Palythoa mutuki	PmYS2/K11	—	DQ997892	—	—	DQ997875	EU333698
Protopalythoa sp [FS-2005]	Pro05	—	—	—	AY995924	AY995944	—
Palythoa aff mutuki	TOB 51	—	**GQ464902**	—	**GQ464971**	**GQ464873**	—
Actiniaria							
Edwardsiid sp [BAR]	BAR 06X	—	**GQ464903**	**GQ464936**	**GQ464973**	**GQ464875**	—
Edwardsiid sp [CUR]	CUR 213	—	EU418271	—	**GQ464974**	**GQ464876**	—

Table 4.4. Description and corresponding amplification information for PCR primers used to generate the sequence data.

Gene	Primer	Sequence	Annealing temperature	Fragment size	Primer source
ITS	f	CTAGTAAGCGCGAGTCATCAGC	50°C	770-943	Swain 2009b
	r	GGTAGCCTTGCCTGATCTGA			
ITS	ext f	CACACCGCCCGTCGCTACTACCGATTGAATG	60°C	745-909	this publication
	ext r	CCCGCTTCACTCGCCGTTACTGGGGGAATCCTTGTTAG			
28S	f	CTTGACCTCAGATCAGGCAAGGCTACCCGCTGA	55–61°C	955-960	this publication
	r	AGCATAGTTCACCATCTTTCGGGTCCCATCGGACGCGCTC			
12S	1a f	TAAGTGCCAGCMGACGCGGT	50°C	676-709	Sinniger *et al*. 2005
	3 r	ACGGGCNATTTGTRCTAACA			
12S	ANTMT f	AGCCACACTTTCACTGAAACAAGG	50°C	910-974	Chen *et al*. 2002
	ANTMT r	GTTCCCYYWCYCTYACYATGTTACGAC			
16S	2824f	TCGACTGTTTACCAAAAACATAGC	50°C	623-655	Swain 2009b
	3554 r	CAATTCAACATCGAGGTCGCAAAC			
16S	ant1a f	GCCATGAGTATAGACGCACA	50°C	835-889	Sinniger *et al*. 2005
	bmoH r	CGAACAGCCAACCCTTGG			

Table 4.5. Partition definitions and per-partition parameter estimates used to model sequence evolution.

partition	concatenated alignment positions	base frequencies				substitution rates (G–T = 1)					rate heterogeneity	
		A	C	G	T	A–C	A–G	A–T	C–G	C–T	proportion of invariant sites	gamma shape
18S	1–197	0.2494	0.2344	0.2733	0.2429	0.4722	0.3499	0.2143	0.2354	3.0927	0.6226	0.9081
ITS1	198–4372	0.2440	0.2483	0.2502	0.2574	5.9282	10.8689	6.0234	7.0970	9.7225	0.0001	0.5501
5.8S	4373–4529	0.2352	0.2094	0.2795	0.2759	0.1835	0.5989	0.0795	0.1190	1.8367	0.6892	1150.9777
ITS2	4530–6920	0.2250	0.2645	0.2711	0.2393	7.1891	16.3897	8.7570	6.9802	16.2306	0.0001	0.5875
28S	6921–7971	0.2365	0.2456	0.3016	0.2163	0.6307	1.4294	0.3956	0.5975	5.9474	0.3532	0.4160
16S	7972–8056, 8203–8507, 9017–9122, 9343–9478	0.3073	0.1991	0.2566	0.2370	0.4075	1.0280	0.4905	0.1359	2.1930	0.6301	4.9990
16S-HV	8057–8202, 8508–9016, 9123–9342	0.2198	0.2878	0.3035	0.1889	0.4123	2.0060	1.9228	0.1922	3.0758	0.2044	0.8578
12S	9479–9656, 9731–9827, 9919–10142, 10213–10542, 10653–10664	0.3038	0.1920	0.2761	0.2281	0.4788	1.2640	0.7681	0.1732	1.8888	0.6576	5.3805
12S-HV	9657–9730, 9828–9918, 10143–10212, 10543–10652	0.1768	0.3699	0.2880	0.1653	1.3458	2.9147	4.3778	0.4412	4.5161	0.3336	2.8806
COI-1	10665–11296\3	0.1308	0.2556	0.1651	0.4485	0.0000	0.0000	0.0000	7.9798	2.3886	0.9659	1067.2275
COI-2	10666–11296\3	0.1894	0.2374	0.3161	0.2571	0.7606	3.5643	0.7404	0.1231	3.1358	0.3148	1228.5526
COI-3	10667–11296\3	0.2669	0.1803	0.3134	0.2394	0.5962	2.0471	0.3360	0.1531	3.7408	0.8265	1000.2995

Table 4.6. A priori hypotheses of the evolutionary relationships among zoanthids generated from topological summaries (Fig. 4.1) of previously published phylogenies. The literature sources of the phylogenies are: (I) Sinniger & Häussermann 2009, (II) Chapter 2, (III and IV) Reimer & Nonaka *et al.* 2008, (V and VI) Reimer & Sinniger *et al.* 2008, (VII and VIII) Sinniger *et al.* 2008, (IX and X) Reimer & Sinniger *et al.* 2007, and (XI) Sinniger *et al.* 2005.

Hypotheses of monophyly	**Literature source**
Suborders	
A) Brachycnemina	I, III, IV, VI, VII, VIII, X, XI
B) Macrocnemina	V
C) suborders	II & classical taxonomy
Families	
D) Epizoanthidae	I, III – XI & classical taxonomy
E) Host associations	II
Relationships among symbiosis types	
F) Antipatharia + Agelasida	IV, VI, X
G) Antipatharia + Hadromerida and Haplosclerida	XI
H) Hadromerida and Haplosclerida + Halichondrida and Poecilosclerida	I, VII
I) Petrosina + Agelasida	II
J) Alcyonacea and Antipatharia & Hadromerida and Haplosclerida + Halichondrida and Poecilosclerida	IX

Table 4.7. Morphologic and ecologic character state assignments of specimens used in the analyses**.**

Taxa	Unique ID	Fifth mesenteries	Marginal musculature	Zoox symbioses	Symbiotic associations (detailed)	Symbiotic associations (general)
Non-Brachycnemina Zoanthidea						
Isozoanthus giganteus	SA 259	macrocnemic	endodermal	no	?	?
Isozoanthus cf giganteus	SA 263	macrocnemic	endodermal	no	?	?
Epizoanthus illoricatus	Eill	macrocnemic	mesogleal	?	Eunicidae	Polycheatea
Epizoanthus aff illoricatus	SIO 252	macrocnemic	mesogleal	no	?	?
Epizoanthus sp [Deep Med]	MedDeep	macrocnemic	mesogleal	no	?	?
Epizoanthus aff arenaceus [HI]	NMNH 100	macrocnemic	mesogleal	no	?	?
Epizoanthus cf balanorum	PER 239	macrocnemic	mesogleal	no	Thoracica	Crustacea
Epizoanthus cf arenaceus	MED 65	macrocnemic	mesogleal	no	Paguridae	Crustacea
Epizoanthus fiordicus	Efio	macrocnemic	mesogleal	no	?	?
Epizoanthus cf ramosus	NIP 154	macrocnemic	mesogleal	no	Paguridae	Crustacea
Epizoanthus lindhali	Elind	macrocnemic	mesogleal	no	?	?
Epizoanthus incrustatus	ARC 269	macrocnemic	mesogleal	no	Paguridae	Crustacea
Epizoanthus sp [Sub-Antarctic]	SubAnt	macrocnemic	mesogleal	no	?	?
Epizoanthus scotinus	WA 166	macrocnemic	mesogleal	no	?	?
Epizoanthus paguricola	Epag	macrocnemic	mesogleal	no	Paguridae	Crustacea
Mesozoanthus fossii [1]	MF 1	macrocnemic	?	no	?	?
Mesozoanthus fossii [3]	MF 3	macrocnemic	?	no	?	?
Epizoanthus aff tsukaharai [NZ]	NZ 66	macrocnemic	mesogleal	no	Alcyonacea	Anthozoa
Corallizoanthus tsukaharai	Ctsu	macrocnemic	?	no	Alcyonacea	Anthozoa
Epizoanthus aff tsukaharai [CA]	NMNH 258	macrocnemic	mesogleal	no	Alcyonacea	Anthozoa
Parazoanthus lucificum	SAV 3	macrocnemic	endodermal	no	Alcyonacea	Anthozoa
Gerardia savaglia	SAV 1	macrocnemic	endodermal	no	Alcyonacea & Antipatharia	Anthozoa
Gerardia macaronesica	Smac	macrocnemic	endodermal	?	Alcyonacea & Antipatharia	Anthozoa
Parazoanthid sp [EBISCO]	EBISCO	macrocnemic	endodermal	no	Hexactinellida	Hexactinellida
Parazoanthid sp [CORSARO]	CORSARO	macrocnemic	endodermal	no	Hexactinellida	Hexactinellida
Parazoanthid sp [NC3]	NC 3	macrocnemic	endodermal	no	Hexactinellida	Hexactinellida
Parazoanthid sp [Cape Verde]	CV	macrocnemic	endodermal	?	Antipatharia	Anthozoa
Parazoanthid sp [Principe]	PRI	macrocnemic	endodermal	?	Antipatharia	Anthozoa
Parazoanthus sp [G1]	GAL 1	macrocnemic	endodermal	?	Antipatharia	Anthozoa
Parazoanthid sp [M2]	MAD 2	macrocnemic	endodermal	?	?	Anthozoa
Epizoanthus cutressi	TOB 44	macrocnemic	mesogleal	zoox	Petrosina	Demospongiae
Parazoanthus aff catenularis [SEN]	SEN	macrocnemic	endodermal	?	?	Demospongiae

Table 4.7. Continued.

Taxa	Unique ID	Fifth mesenteries	Marginal musculature	Zoox symbioses	Symbiotic associations (detailed)	Symbiotic associations (general)
Parazoanthus sp [SUL 5]	SUL 5	macrocnemic	endodermal	?	Agelasida	Demospongiae
Parazoanthus puertoricense	NAV 58	macrocnemic	endodermal	no	Agelasida	Demospongiae
Parazoanthus catenularis	TOB 37	macrocnemic	endodermal	zoox	Petrosina	Demospongiae
Parazoanthus aff parasiticus [NIP]	NIP 155	macrocnemic	endodermal	no	Hadromerida & Haplosclerida	Demospongiae
Parazoanthus aff parasiticus [NCs]	NC Shal	macrocnemic	endodermal	?	Hadromerida & Haplosclerida	Demospongiae
Parazoanthus parasiticus	TOB 47	macrocnemic	endodermal	zoox	Hadromerida & Haplosclerida	Demospongiae
Parazoanthus aff parasiticus [MAD]	MAD 3	macrocnemic	endodermal	?	Hadromerida & Haplosclerida	Demospongiae
Parazoanthus aff parasiticus [SUL]	SUL 3	macrocnemic	endodermal	?	?	Demospongiae
Parazoanthus aff parasiticus [NCd]	NC Deep	macrocnemic	endodermal	?	?	Demospongiae
Parazoanthus aff juanfernandezii [FRA]	FRA PC1	macrocnemic	endodermal	no	?	?
Parazoanthus aff juanfernandezii [CA]	CA 128	macrocnemic	endodermal	no	?	?
Parazoanthus juanfernandezii	CHI 187	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus axinellae	SPA M1	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus anguicomus	IRE 266	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus capensis	SA 262	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus swiftii	PAN 9	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus aff swiftii [NUR]	PER 249	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus aff swiftii [GAL]	GAL 2	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Parazoanthus aff swiftii [Sal]	PER 241	macrocnemic	endodermal	no	Halichondrida & Poecilosclerida	Demospongiae
Zoanthidea sp [302]	S302	macrocnemic	?	?	?	?
Parazoanthid sp [yellow polyp]	YP	macrocnemic	endodermal	?	?	?
Epizoanthus minutus	GM 3	macrocnemic	mesogleal	no	?	?
Epizoanthus patagonichus	PER 237	macrocnemic	mesogleal	no	Plumularidae	Hydrozoa
Epizoanthus californicus	PER 243	macrocnemic	mesogleal	no	?	?
Zoanthidea sp [Mada1]	MAD 1	macrocnemic	?	?	Antipatharia	Anthozoa
Parazoanthus aff tunicans [SUL]	SUL 1	macrocnemic	endodermal	?	Plumularidae	Hydrozoa
Parazoanthus tunicans	TOB 40	macrocnemic	endodermal	zoox	Plumularidae	Hydrozoa
Isozoanthus antumbrosus	PAN 21	macrocnemic	endodermal	zoox	Plumularidae	Hydrozoa
Parazoanthus cf gracilis [NC1]	NC 1	macrocnemic	endodermal	?	Plumularidae	Hydrozoa
Parazoanthus gracilis	NIP 153	macrocnemic	endodermal	zoox	Plumularidae	Hydrozoa
Parazoanthus cf gracilis [NC2]	NC 2	macrocnemic	endodermal	?	Plumularidae	Hydrozoa

Table 4.7. Continued.

Taxa	Unique ID	Fifth mesenteries	Marginal musculature	Zoox symbioses	Symbiotic associations (detailed)	Symbiotic associations (general)
Brachycnemina						
Isaurus sp [FS-2005]	Isau05	brachycnemic	mesogleal	?	free-living	free-living
Isaurus tuberculatus	IToM1	brachycnemic	mesogleal	zoox	free-living	free-living
Isaurus sp [BIK IOtsNM1]	BIK	brachycnemic	mesogleal	zoox	free-living	free-living
Zoanthus gigantus	ZgYS1	brachycnemic	mesogleal	zoox	free-living	free-living
Zoanthus sp [FS-2005]	Zoan05	brachycnemic	mesogleal	?	free-living	free-living
Zoanthus pulchellus	PAN 7	brachycnemic	mesogleal	zoox	free-living	free-living
Zoanthus cf *sansibaricus*	Zcfsan	brachycnemic	mesogleal	zoox	free-living	free-living
Zoanthus sansibaricus	ZAT7	brachycnemic	mesogleal	zoox	free-living	free-living
Acrozoanthus sp [FS-2005]	Acro05	brachycnemic	mesogleal	?	Nereididae	Polychaeta
Acrozoanthus sp [Sulawesi]	Sul05	brachycnemic	mesogleal	?	Nereididae	Polychaeta
Zoanthus vietnamensis	ZvSH3	brachycnemic	mesogleal	zoox	free-living	free-living
Zoanthus kuroshio	ZkYS1	brachycnemic	mesogleal	zoox	free-living	free-living
Zoanthus sociatus	SMG2	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa cf grandis	DOM 18	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa singaporensis	Psing	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa heliodiscus	PhSaiLL1	brachycnemic	mesogleal	zoox	free-living	free-living
Sphenopus marsupialis	Sphem	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa sp [Mada]	Mada	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa aff sakurajimensis	PWS1	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa aff caesia	TOB 52	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa sp [289]	PMad289	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa cf tuberculosa	Pcftu	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa tuberculosa	PtCN1	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa cf caribaeorum	TOB 33	brachycnemic	mesogleal	zoox	free-living	free-living
Palythoa sp [FS-2005]	Pal05	brachycnemic	mesogleal	?	free-living	free-living
Palythoa mutuki	PmYS2/K11	brachycnemic	mesogleal	zoox	free-living	free-living
Protopalythoa sp [FS-2005]	Pro05	brachycnemic	mesogleal	?	free-living	free-living
Palythoa aff mutuki	TOB 51	brachycnemic	mesogleal	zoox	free-living	free-living

Table 4.8. Results of the Kishino–Hasegawa test for significant differences in maximum likelihood scores of the concatenated nuclear and mitochondrial sequence data constrained by the a priori hypotheses detailed in Table 4.6.

Hypotheses of monophyly	ln likelihood	Δ ln likelihood	approximate standard error	pK-H
Suborders				
A) Brachycnemina	-36296.89	0.00	0.00	—
B) Macrocnemina	-36779.39	-482.50	36.24	< 0.01
C) suborders	-36482.47	-185.58	27.05	< 0.01
Families				
D) Epizoanthidae	-37392.46	-779.59	84.70	< 0.01
E) Host associations	-36612.87	0.00	0.00	—
Relationships among symbiosis types				
F) Antipatharia + Agelasida	-37061.70	-629.67	63.91	< 0.01
G) Antipatharia + Hadromerida and Haplosclerida	-36543.11	-111.08	32.56	< 0.01
H) Hadromerida and Haplosclerida + Halichondrida and Poecilosclerida	-36443.92	-11.89	17.06	0.32
I) Petrosina + Agelasida	-36432.03	0.00	0.00	—
J) Alcyonacea and Antipatharia & Hadromerida and Haplosclerida + Halichondrida and Poecilosclerida	-36437.57	-5.54	11.62	0.72

CONCLUSION

The patterns of evolutionary transitions in the symbioses of Zoanthidea demonstrate greater conservatism over evolutionary time than previously thought due to a combination of insufficient data on host associations and relationship outcomes, and a systematic scheme that is not reflective of evolutionary history. The Caribbean Zoanthidea associate with at least 89 species of hosts (a nearly five-fold increase on the previous estimates) representing 40% of the diversity of extant Demospongiae orders. The specificity of these Zoanthidea is at the taxonomic level of families–orders of Demospongiae; a much finer scale of host associations than is usually reported (currently assessed at the class–phylum level in most associations). Intimacy with hosts, polyp size, and the presence of host and zoanthid photosymbionts all appear to affect specificity; however the asymmetries in Zoanthidea and Demospongiae specificity are an indication that the observed associations are likely to be mutualisms. Experimental data are congruent with the hypothesis of mutualism for most of the associations assessed; however transplant experiments indicate that these associations can be pushed along the continuum of relationships in ecological time to produce parasitic outcomes in non-native habitats. Although two additional zoanthid species were identified in the Caribbean and two species were shown to be anemones, phylogenetic species delimitations are congruent with the original morphological descriptions and all Zoanthidea species in the region are recognizable by morphology alone. Regional phylogenies constructed for delimitating species recovered clades of heterogeneric species with similar host associations, indicating that host associations are largely conserved across evolutionary time even though the morphological features that define genera (and families) are not. The global multi-gene phylogeny recovered nearly identical clades of Demospongiae and Plumaridae symbionts and indicated a general pattern of conserved host associations with infrequent transitions between host groups. The same relationship outcome (mutualism) was identified in two clades of zoanthids that had undergone an ancestral transition in host associations, indicating conservatism in the evolution of host associations as well. Loss of symbiosis with invertebrates is coincident with reduction in ranges (rather than the rise of zooxanthellae symbioses) and appears to be a potential mechanism for the dramatic range reductions of Brachycnemina. The phylogenies are generally consistent with the conservation of

host associations and relationship outcomes which agree with the broader predictions of symbiosis evolution, but invalidate much of the current systematics of Zoanthidea.

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BIOGRAPHICAL SKETCH

Timothy Swain was born in Buffalo, New York and grew up on a small island in the Niagara River just north of the city. He learned to SCUBA dive in the lakes and rivers of the region with the intent to study marine science. In 1998, he earned a Bachelor of Science in Biological Sciences with a minor in Photography from the State University of New York at Buffalo under the direction of Howard Lasker and Mary Alice Coffroth. Continuing at SUNY Buffalo, he earned a Master of Science in Biological Sciences in 2002 under the direction of Derek Taylor. In 2010 he earned a Doctor of Philosophy in Biological Science at Florida State University under the direction of Janie Wulff. He has accepted a Postdoctoral Fellowship at Northwestern University in Chicago, Illinois.